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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	2715.1350003/JUK/JHH
First Inventor	**Stephen Robert CHERINKO**
Title	**Extraction of Lipid From Cells and Products Therefrom**
Express Mail Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☐ **Fee Transmittal Form** (e.g., PTO/SB/17)
2. ☐ **Applicant claims small entity status.** See 37 CFR 1.27.
3. ☒ **Specification** [*Total Pages* 155]
   Both the claims and abstract must start on a new page
   (For information on the preferred arrangement, see MPEP 608.01(a))
4. ☒ **Drawing(s)** (*35 U.S.C. 113*) [*Total Sheets* 5]
5. **Oath or Declaration** [*Total Sheets* _____]
   a. ☐ Newly executed (original or copy)
   b. ☐ A copy from a prior application (37 CFR 1.63(d))
   (for continuation/divisional with Box 18 completed)
   i. ☐ DELETION OF INVENTOR(S)
   Signed statement attached deleting inventor(s) name in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. ☒ **Application Data Sheet.** See 37 CFR 1.76
7. ☐ **CD-ROM or CD-R** in duplicate, large table or Computer Program *(Appendix)*
   ☐ Landscape Table on CD
8. **Nucleotide and/or Amino Acid Sequence Submission**
   (if applicable, items a. – c. are required)
   a. ☐ Computer Readable Form (CRF)
   b. Specification Sequence Listing on:
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ACCOMPANYING APPLICATION PARTS

9. ☐ **Assignment Papers** (cover sheet (PTO-1595) & document(s))
   Name of Assignee _____
10. ☐ **37 CFR 3.73(b) Statement** *(when there is an assignee)* ☐ **Power of Attorney**
11. ☐ **English Translation Document** *(if applicable)*
12. ☐ **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    ☐ Copies of foreign patent documents, publications, & other information
13. ☐ **Preliminary Amendment**
14. ☐ **Return Receipt Postcard** (MPEP 503)
    (*Should be specifically itemized*)
15. ☐ **Certified Copy of Priority Document(s)**
    (*if foreign priority is claimed*)
16. ☐ **Nonpublication Request** under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
17. ☒ Other: **Authorization under 37 CFR 1.136(a)(3)**

18. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76:*

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: .....

Prior application information: *Examiner* _____ *Art Unit:* _____

19. CORRESPONDENCE ADDRESS

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Name (Print/Type)	**John T. Haran**	Registration No. (Attorney/Agent)	**58,010**

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: To Be Assigned
CHERINKO *et al.*	Art Unit: To Be Assigned
Appl. No.: To Be Assigned	Examiner: To Be Assigned
Filed: Herewith	Atty. Docket: 2715.1350003/JUK/JHH
For: **Extraction of Lipid From Cells and Products Therefrom**	

Authorization to Treat a Reply as Incorporating an Extension of Time Under 37 C.F.R. § 1.136(a)(3)

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Sir:

The U.S. Patent and Trademark Office is hereby authorized to treat any concurrent or future reply that requires a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. The U.S. Patent and Trademark Office is hereby authorized to charge all required extension of time fees to our Deposit Account No. 19-0036, if such fees are not otherwise provided for in such reply.

Respectfully submitted,

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SKGF_1367893.1

EXTRACTION OF LIPID FROM CELLS AND PRODUCTS THEREFROM

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Application No. 61/350,363, filed on June 1, 2010, U.S. Application No. 61/378,923, filed August 31, 2010, and U.S. Application No. 61/452,721, filed March 15, 2011, each of which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present invention relates to processes for obtaining a lipid from a cell by lysing the cell, raising a pH of the cell and/or contacting the cell with a salt, and separating the lipid. The present invention is also directed to lipids prepared by the processes of the present invention. The present invention is also directed to microbial lipids having a particular anisidine value, peroxide value, and/or phosphorus content.

Background Art

[0003] A typical process for obtaining lipids from a microbial cell, such as polyunsaturated fatty acids, involves growing microorganisms that are capable of producing the desired lipid in a fermentor, pond or bioreactor, separating the fermentation broth comprising a microbial cell biomass, drying the microbial cell biomass, and separating the lipids by solvent extraction. Steps in the separation can include diluting a fermentation broth with water, centrifuging the diluted broth, lysing the microbial cells, and extracting an intracellular lipid from the lysed cells by adding a water-immiscible solvent to the mixture in which the lipid is soluble, e.g., hexane.

[0004] Another method of extraction to remove a lipid from a microbial cell is lysing a cell in a fermentation broth using mechanical force (e.g., homogenization), enzymatic treatment, or chemical treatment to disrupt the cell walls. Lipid can be extracted from the resulting composition comprising lipids, microbial cell biomass, and water using an organic solvent, e.g., isopropyl alcohol. The lipid can be separated mechanically from the composition and the alcohol

must be removed from both the lipid and the aqueous biomass waste stream. *See*, e.g., International Pub. Nos. WO 01/76385 and WO 01/76715.

[0005] However, industrial scale production of lipids using either of the above processes requires a large amount of volatile and flammable organic solvent, thereby creating hazardous operating conditions. The use of organic solvents in the extraction process can also necessitate using an explosion-proof lipid recovery system, thereby adding to the cost of lipid recovery. Moreover, use of an organic solvent in extracting lipid from a microbial cell can generate an organic solvent waste stream that requires a complete solvent recovery system or a proper method of disposal, which further increases the overall production cost of lipid extraction. For example, strict limits on volatile organic compound (VOC) emissions require greater manpower and added cost to vessels and other equipment.

[0006] Therefore, there is a need for a process for obtaining lipids from a cell which does not use an organic solvent. Several processes have been proposed for separating a lipid from a cell without the use of an organic solvent. For example, U.S. Patent No. 6,750,048 discloses an aqueous washing process whereby an emulsion is washed with aqueous washing solutions until a substantially non-emulsified lipid is obtained. However, in some embodiments, this process requires multiple washing steps, which require substantial cost and time. U.S. Patent No. 7,431,952 discloses a process whereby lysed cells are centrifuged to remove cell wall debris and then oils are extracted and purified. However, this process provides a crude oil that requires extensive further purification. Thus, what is needed is a process that does not utilize a volatile solvent to extract a lipid from a cell, and which can be performed using readily available equipment and a minimum number of steps to provide a highly pure lipid.

BRIEF SUMMARY OF THE INVENTION

[0007] The present invention is directed to a process for obtaining a lipid from a microbial cell composition, the process comprising raising the pH of the cell composition to 8 or above, and separating a lipid from the cell composition, wherein the lipid optionally contains less than 5% by weight or volume of an organic solvent.

[0008] In some embodiments, the raising the pH lyses the cell composition. In some embodiments, the raising the pH demulsifies the cell composition.

[0009] In some embodiments, the process comprises adding a salt to the cell composition to demulsify the cell composition. In some embodiments, the adding a salt is performed after the raising the pH.

[0010] In some embodiments, the process further comprises heating the lysed cell composition to demulsify the cell composition. In some embodiments, the heating is performed after the raising the pH.

[0011] In some embodiments, the process further comprises raising the pH of the cell composition a second time to demulsify the cell composition. In some embodiments, the raising the pH a second time is performed after the adding a salt or the heating.

[0012] The present invention is also directed to a process for obtaining a lipid from a cell, the process comprising lysing a cell to form a lysed cell composition, raising the pH of the lysed cell composition to 8 or above to demulsify the cell composition, adding a salt to the lysed cell composition to demulsify the cell composition, and separating a lipid from the demulsified cell composition, wherein the lipid optionally contains less than 5% by weight or volume of an organic solvent.

[0013] The present invention is directed to a process for obtaining a lipid from a cell composition, the process comprising raising the pH of the cell composition to 8 or above to lyse the cell composition and demulsify the cell composition, adding a salt to the cell composition, and separating a lipid from the demulsified cell composition, wherein the lipid optionally contains less than 5% by weight or volume of an organic solvent.

[0014] The present invention is also directed to a process for obtaining a lipid from a cell, the process comprising lysing a cell to form a lysed cell composition, agitating the cell composition to demulsify the cell composition, and separating a lipid from the demulsified cell composition, wherein the lipid optionally contains less than 5% by weight or volume of an organic solvent.

[0015] In some embodiments, the process further comprises heating the lysed cell composition to demulsify the cell composition. In some embodiments, the heating is performed after the adding a salt.

[0016] In some embodiments, the process further comprises agitating the lysed cell composition to demulsify the cell composition. In some embodiments, the agitating is for 5 minutes to 96 hours.

[0017] In some embodiments, the agitating comprises agitating the cell composition with an impeller having a tip speed of 350 centimeters per second to 900 centimeters per second.

[0018] In some embodiments, the process further comprises raising the pH of the lysed cell composition to demulsify the cell composition. In some embodiments, raising the pH of the lysed cell composition to demulsify the cell composition comprises adding a base. In some embodiments, a second base is added after the adding of a salt or the heating.

[0019] In some embodiments, the heating is for 10 minutes to 96 hours.

[0020] In some embodiments, the cell composition is heated to a temperature of 60° C to 100° C. In some embodiments, the cell composition is heated to a temperature of 90° C to 100° C.

[0021] In some embodiments, raising the pH comprises adding a base. In some embodiments, the base has a pK_b of 1 to 12.

[0022] In some embodiments, the separating a lipid occurs at a temperature of 10° C to 100° C.

[0023] In some embodiments, the process comprises agitating the lysed cell composition by stirring, mixing, blending, shaking, vibrating, or a combination thereof. In some embodiments, the process comprises agitating the lysed cell composition at 0.1 hp/1000 gal to 10 hp/1000 gal of lysed cell composition. In some embodiments, the process comprises agitating the lysed cell composition with an agitator having an impeller tip speed of 200 ft/min to 1,000 ft/min.

[0024] In some embodiments, the lysing comprises mechanical treatment, physical treatment, chemical treatment, enzymatic treatment, or a combination thereof. In some embodiments, the mechanical treatment is homogenization.

[0025] In some embodiments, the salt is added in an amount of 0.1% to 20% by weight of the lysed cell composition. In some embodiments, the salt is added to the lysed cell composition in an amount of 0.5% to 15% by weight of the lysed cell composition. In some embodiments, the salt is added to the lysed cell composition in an amount of 2% to 10% by weight of the lysed cell composition.

[0026] In some embodiments, the salt is selected from the group consisting of: alkali metal salts, alkali earth metal salts, sulfate salts, and combinations thereof. In some embodiments, the salt is sodium chloride. In some embodiments, the salt is sodium sulfate.

[0027] In some embodiments, the separating comprises centrifuging. In some embodiments, the separating comprises centrifuging at a temperature of 30° C to 90° C.

[0028] In some embodiments, the process provides a lipid comprising at least 50% by weight triglyceride.

[0029] In some embodiments, the process provides a lipid having an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less.

[0030] In some embodiments, the process provides a lipid having a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less.

[0031] In some embodiments, the process provides a lipid having a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less.

[0032] In some embodiments, the process provides a lipid having at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of a desired polyunsaturated fatty acid (PUFA). In some embodiments, the process provides a lipid having at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of docosahexaenoic acid ("DHA"), and/or at least 10%, at least 15%, or at least 20% by weight of docosapentaenoic acid ("DPA n-6"), and/or at least 10%, at least 15%, or at least 20% by weight of eicosapentaenoic acid ("EPA"), and/or at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of arachidonic acid ("ARA").

[0033] In some embodiments, the cell is a microbial cell. In some embodiments, the process comprises concentrating a fermentation broth comprising the microbial cell.

[0034] In some embodiments, the cell is an oilseed. In some embodiments, the oilseed is selected from the group consisting of sunflower seeds, canola seeds, rapeseeds, linseeds, castor oil seeds, coriander seeds, calendula seeds, and genetically modified variants thereof.

[0035] In some embodiments, the process comprises washing the cell or cell composition.

[0036] In some embodiments, the process comprises pasteurizing the cell or cell composition.

[0037] In some embodiments, the process comprises concentrating the lysed cell composition.

[0038] In some embodiments, the process comprises refining the lipid. In some embodiments, the refining is selected from the group consisting of: caustic refining, degumming, acid treatment, alkali treatment, cooling, heating, bleaching, deodorizing, deacidification, and combinations thereof.

[0039] In some embodiments, the process comprises harvesting the lipid, wherein the harvesting comprises pumping the lipid without agitation.

[0040] The present invention is also directed to a lipid obtained by any of the processes of the present invention.

[0041] In some embodiments, the lipid comprises one or more polyunsaturated fatty acids. In some embodiments, the lipid has at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of a desired PUFA. In some embodiments, the lipid has at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of DHA, and/or at least 10%, at least 15%, or at least 20% by weight of DPA n-6, and/or at least 10%, at least 15%, or at least 20% by weight of EPA, and/or at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of ARA.

[0042] In some embodiments, the lipid has an overall aroma intensity of 3 or less. In some embodiments, the lipid has an overall aromatic intensity of 2 or less.

[0043] In some embodiments, the lipid comprises a triacylglycerol fraction of at least 10% by weight, wherein at least 12% by weight of the fatty acids in the triacylglycerol fraction is eicosapentaenoic acid, wherein at least 25% by weight of the fatty acids in the triacylglycerol fraction is docosahexaenoic acid, and wherein less than 5% by weight of the fatty acids in the triacylglycerol fraction is arachidonic acid.

[0044] In some embodiments, the lipid comprises at least 20% by weight eicosapentaenoic acid and less than 5% by weight each of arachidonic acid, docosapentaenoic acid n-6, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, erucic acid, and stearidonic acid.

[0045] In some embodiments, the lipid has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less, and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less.

[0046] In some embodiments, the lipid is a crude lipid. In some embodiments, the crude lipid optionally has less than 5% by weight or volume of an organic solvent.

[0047] The present invention is also directed to a crude microbial lipid having an anisidine value of 26 or less, a peroxide value of 5 or less, a phosphorus content of 100 ppm or less, and optionally less than 5% by weight or volume of an organic solvent.

[0048] In some embodiments, the crude microbial lipid has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less, and/or a peroxide value of 5

or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less.

[0049] In some embodiments, the crude microbial lipid has at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of a desired PUFA. In some embodiments, the crude microbial lipid has at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of DHA, and/or at least 10%, at least 15%, or at least 20% by weight of DPA n-6, and/or at least 10%, at least 15%, or at least 20% by weight of EPA, and/or at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of ARA.

[0050] The present invention is also directed to an extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, and wherein the oil has an anisidine value of 26 or less.

[0051] The present invention is also directed to an extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1, and wherein the oil has an anisidine value of 26 or less.

[0052] The present invention is also directed to an extracted microbial lipid comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight and wherein the oil has an anisidine value of 26 or less.

[0053] In some embodiments, the extracted lipid has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less, and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90

ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less.

[0054] In some embodiments, the extracted microbial lipid is a crude lipid or a crude oil. In some embodiments, the crude lipid optionally has less than 5% by weight or volume of an organic solvent.

[0055] The present invention is also directed to a process for obtaining a lipid, the process comprising refining a crude lipid of the present invention. In some embodiments, the refining is selected from the group consisting of: caustic refining, degumming, acid treatment, alkali treatment, cooling, heating, bleaching, deodorizing, deacidification, and combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0056] The accompanying drawings, which are incorporated herein and form a part of the specification, illustrate the present invention and, together with the description, further serve to explain the principles of the invention and to enable a person skilled in the pertinent art to make and use the invention.

[0057] **FIGs. 1-4** provide schematic flow charts describing processes of the present invention.

[0058] **FIG. 5** is a graph providing the electron paramagnetic resonance (EPR) over time of lysed cells compositions at various pHs.

[0059] The present invention will now be described with reference to the accompanying drawings. In the drawings, like reference numbers indicate identical or functionally similar elements. Additionally, the left-most digit(s) of a reference number can identify the drawing in which the reference number first appears.

DETAILED DESCRIPTION OF THE INVENTION

[0060] The present invention is directed to a process for obtaining a lipid from a microbial cell composition, the process comprising raising the pH of the cell composition to 8 or above and separating a lipid from the cell composition, wherein the lipid optionally contains less than 5% by weight or volume of an organic solvent. In some embodiments, the process further comprises one or more of adding a salt to the cell composition to demulsify the cell composition, heating the cell to demulsify the cell composition, agitating the cell composition to demulsify the cell

composition, and raising the pH of the cell composition a second time to demulsify the cell composition.

[0061] The present invention is also directed to a process for obtaining a lipid from a cell, the process comprising lysing a cell to form a lysed cell composition, raising the pH of the lysed cell composition to 8 or above to demulsify the cell composition, adding a salt to the lysed cell composition to demulsify the cell composition, and separating a lipid from the demulsified cell composition, wherein the lipid optionally contains less than 5% by weight or volume of an organic solvent. The cell can be a microbial cell or an oilseed cell. In some embodiments, the process further comprises one or more of: heating the lysed cell composition to demulsify the cell composition, agitating the lysed cell composition to demulsify the cell composition, and raising the pH of the lysed cell composition a second time to demulsify the cell composition.

[0062] The present invention is directed to a process for obtaining a lipid from a cell composition, the process comprising raising the pH of the cell composition to 8 or above to lyse the cell composition and demulsify the cell composition, adding a salt to the cell composition, and separating a lipid from the demulsified cell composition, wherein the lipid optionally contains less than 5% by weight or volume of an organic solvent. In some embodiments, the process further comprises one or more of heating the cell composition to demulsify the cell composition, agitating the cell composition to demulsify the cell composition, and raising the pH of the cell composition a second time to demulsify the cell composition.

[0063] The present invention is directed to a process for obtaining a lipid from a microbial cell, the process comprising lysing a microbial cell to form a lysed cell composition, adding a base to the lysed cell composition to demulsify the cell composition, and separating a lipid from the demulsified cell composition, wherein the lipid optionally contains less than 5% by weight or volume of an organic solvent. In some embodiments, the process further comprises one or more of: adding a salt to the lysed cell composition to demulsify the cell composition, heating the lysed cell composition to demulsify the cell composition, agitating the lysed cell composition to demulsify the cell composition, and adding a second base to the lysed cell composition to demulsify the cell composition.

[0064] The present invention is also directed to a process for obtaining a lipid from a cell, the process comprising lysing a cell to form a lysed cell composition, adding a base to the lysed cell composition to demulsify the cell composition, adding a salt to the lysed cell composition to demulsify the cell composition, and separating a lipid from the demulsified cell composition, wherein the lipid optionally contains less than 5% by weight or volume of an organic solvent.

The cell can be a microbial cell or an oilseed cell. In some embodiments, the process further comprises one or more of: heating the lysed cell composition to demulsify the cell composition, agitating the lysed cell composition to demulsify the cell composition, and adding a second base to the lysed cell composition to demulsify the cell composition.

[0065] The present invention is also directed to a process for obtaining a lipid from a cell, the process comprising lysing a cell to form a lysed cell composition, agitating the cell composition to demulsify the cell composition, and separating a lipid from the demulsified cell composition, wherein the lipid optionally contains less than 5% by weight or volume of an organic solvent.

[0066] The present invention is also directed to a lipid obtained by any of the processes of the present invention.

[0067] The present invention is also directed to an extraction process for obtaining a lipid from a cell, the process comprising lysing the cell to form a lysed cell composition, contacting the lysed cell composition with a first base, contacting the lysed cell composition with a salt, heating the lysed cell composition for 5 minutes to 96 hours, contacting the lysed cell composition with a second base, and separating a lipid from the lysed cell composition at a temperature of 10° C to 100° C.

[0068] The present invention is also directed to an extraction process for obtaining a lipid from a cell, the process comprising lysing the cell to form a lysed cell composition, contacting the lysed cell composition with a salt, and agitating the lysed cell composition for 5 minutes to 96 hours to provide a treated lysed cell composition, and separating a lipid from the treated lysed cell composition at a temperature of 10° C to 100° C.

[0069] The present invention is also directed to an extraction process for obtaining a lipid from a cell, the process comprising lysing the cell to form a lysed cell composition, contacting the lysed cell composition with a salt, and separating a lipid from the lysed cell composition at a temperature of 10° C to 100° C.

[0070] In some embodiments, the base or second base have a pK_b of 1 to 12. In some embodiments, the base or second base have a pK_b of 3 to 5.

[0071] In some embodiments, a process comprises agitating the lysed cell composition for 5 minutes to 96 hours, 10 minutes to 96 hours, 10 minutes to 4 hours, 12 hours to 84 hours, or 24 hours to 72 hours.

[0072] In some embodiments, the process comprises agitating the lysed cell composition by stirring, mixing, blending, shaking, vibrating, or a combination thereof. In some embodiments, the process comprises agitating the lysed cell composition at 0.1 hp/1000 gal to 10 hp/1000 gal of

lysed cell composition. In some embodiments, the process comprises agitating the lysed cell composition with an agitator having an impeller tip speed of 200 ft/min to 1000 ft/min.

[0073] In some embodiments, lysing comprises a process selected from: mechanically treating, physically treating, chemically treating, enzymatically treating, or a combination thereof.

[0074] In some embodiments, the lysed cell composition is contacted with a salt in an amount of 0.1% to 20% by weight, 0.5% to 15% by weight, or 2% to 10% by weight of the lysed cell composition.

[0075] In some embodiments, the salt is selected from the group consisting of: alkali metal salts, alkali earth metal salts, sulfate salts and combinations thereof. In some embodiments, the salt is sodium chloride. In some embodiments, the salt is sodium sulfate.

[0076] In some embodiments, the process comprises heating the lysed cell composition for 5 minutes to 96 hours, 10 minutes to 4 hours, 12 hours to 84 hours, or 24 hours to 72 hours.

[0077] In some embodiments, the separating comprises centrifuging. In some embodiments, the separating comprises centrifuging at a temperature of 10° C to 100° C.

[0078] In some embodiments, the process comprises prior to the lysing: washing, centrifuging, evaporating, or a combination thereof, a broth that includes the cell.

[0079] In some embodiments, the process provides a lipid having an anisidine value of 15 or less. In some embodiments, the process provides a lipid comprising at least 50% by weight triglycerides.

[0080] In some embodiments, the process does not add an organic solvent to the lysed cell composition. Organic solvents include polar solvents, non-polar solvents, water-miscible solvents, water-immiscible solvents, and combinations thereof.

[0081] In some embodiments, the process comprises concentrating a broth comprising a cell. In some embodiments, the process comprises concentrating the lysed cell composition.

[0082] The present invention is also directed to the lipid prepared by a process described herein. In some embodiments, the lipid comprises one or more polyunsaturated fatty acids. In some embodiments, the lipid has at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of a desired PUFA. In some embodiments, the lipid has at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of DHA, and/or at least 10%, at least 15%, or at least 20% by weight of DPA n-6, and/or at least 10%, at least 15%, or at least 20% by weight of EPA, and/or at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of ARA. In some

embodiments, the lipid has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less, and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less.

[0083] In some embodiments, the lipid comprises a triacylglycerol fraction of at least 10% by weight, wherein at least 12% by weight of the fatty acids in the triacylglycerol fraction is eicosapentaenoic acid, wherein at least 25% by weight of the fatty acids in the triacylglycerol fraction is docosahexaenoic acid, and wherein less than 5% by weight of the fatty acids in the triacylglycerol fraction is arachidonic acid. In some embodiments, the lipid has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less, and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less. In some embodiments, the lipid is a crude lipid.

[0084] In some embodiments, the lipid comprises at least 20% by weight eicosapentaenoic acid and less than 5% by weight each of arachidonic acid, docosapentaenoic acid n-6, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, erucic acid, and stearidonic acid. In some embodiments, the lipid has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less, and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less. In some embodiments, the lipid is a crude oil.

[0085] The present invention is also directed to a crude microbial lipid having an anisidine value of 26 or less, a peroxide value of 5 or less, a phosphorus content of 100 ppm or less, and optionally less than 5% by weight or volume of an organic solvent. In some embodiments, the crude microbial lipid has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less or 1 or less, and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less. In some embodiments, the crude microbial lipid has at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of a desired PUFA. In some embodiments, the crude microbial lipid has at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of DHA, and/or at least 10%, at least 15%, or at least 20% by weight of DPA n-6, and/or at least 10%, at least 15%, or at least 20% by weight of EPA, and/or at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of ARA.

[0086] The present invention is also directed to an extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, and wherein the oil has an anisidine value of 26 or less. In some embodiments, the extracted lipid has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less, and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less. In some embodiments, the extracted lipid is a crude lipid.

[0087] The present invention is also directed to an extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the

triglyceride fraction is at least 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1, and wherein the oil has an anisidine value of 26 or less. In some embodiments, the extracted lipid has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less, and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less. In some embodiments, the extracted lipid is a crude lipid.

[0088] The present invention is also directed to an extracted microbial lipid comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight and wherein the oil has an anisidine value of 26 or less. In some embodiments, the extracted lipid has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less, and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less. In some embodiments, the extracted lipid is a crude lipid.

[0089] The present invention is also directed to a crude lipid extracted from a microorganism of the species *Crypthecodinium cohnii*, having a phosphorus content of 100 ppm or less. In some embodiments, the crude lipid has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less, and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25

ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less.

[0090] The present invention is also directed to a process for obtaining a lipid, the process comprising refining a crude lipid of the present invention. In some embodiments, the refining is selected from the group consisting of: caustic refining, degumming, acid treatment, alkali treatment, cooling, heating, bleaching, deodorizing, deacidification, and combinations thereof.

Overview

[0091] Generally, the processes of the present invention do not utilize an organic solvent in order to extract or otherwise separate a lipid. Thus, in some embodiments, an organic solvent is not added to a cell broth comprising plant material or fermentation broth comprising a microbial cell, is not added to a cell composition, is not added to a lysed cell composition, or is not added to a lipid during a process of the present invention in an amount or concentration sufficient to extract a lipid. In some embodiments, an organic solvent can be added to a cell composition, a lysed cell composition, or a demulsified cell composition. In such embodiments, the organic solvent is added in a concentration less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.1%, or less than 0.05% by volume. As used herein, an "organic solvent" refers to a solvent that includes at least one carbon atom. As used herein, "solvent" refers to an agent that is hydrophobic or lipophilic, and is not a lipid. As used herein, "hydrophobic" refers to an agent that is repelled from a mass of water. As used herein, "lipophilic" refers to an agent that dissolves lipids. Organic solvents that are not used in a process of the present invention include, but are not limited to, polar solvents, non-polar solvents, water-miscible solvents, water-immiscible solvents, and combinations thereof. Non-limiting examples of organic solvents include substituted and unsubstituted C_4-C_8 alkyls (e.g., hexane and the like), C_5-C_{12} cylcolalkyls, C_4-C_{12} alkenes, C_1-C_8 alcohols (e.g., *iso*-propanol and the like), C_1-C_8 aldehydes, C_4-C_8 ethers, C_1-C_8 esters, C_6-C_{12} aryls, C_1-C_8 amides, C_5-C_{12} heteroaryls, and combinations thereof. An organic solvent as defined herein can be optionally added to a lysed cell composition, for example, as a component of a base and/or a salt for contacting with the lysed cell composition. However, in such embodiments the organic solvent is present in a concentration such that the lipid is not substantially extracted from the cell composition, lysed cell composition, or demulsified cell composition by the solvent (i.e., in a concentration of less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.1%, or less than 0.05% by volume or weight).

[0092] In some embodiments, a process of the present invention does not include washing, e.g., with water, or the process reduces the number of washings of, a lysed cell composition or a demulsified cell composition. "Washing" refers to a process of diluting a composition with, e.g., water or buffer and removing the water or buffer, e.g., by centrifugation. Washing a cell composition can decrease the overall yield of a lipid obtained from a cell. In the present invention, the washing can be decreased by 1 time, 2 times, 3 times or more.

Definitions

[0093] As used herein, "lipid" or "oil" refers to one or more fatty acids (including free fatty acids and esters of fatty acids), phospholipids, triacylglycerols (i.e. triglycerides), diacylglycerides, monoacylglycerides, lysophospholipids, soaps, phosphatides, waxes, sterols and sterol esters, carotenoids, xanthophylls, hydrocarbons, and other lipids known to one of ordinary skill in the art. Lipids include polar lipids and neutral lipids.

[0094] As used herein, "polar lipid" refers to lipids that contain a polar group and are more readily soluble in polar solvents. Polar lipids include phospholipids. As used herein, "phospholipid" refers to lipids having a phosphate group. As used herein, "neutral lipid" refers to lipids that do not contain areas of polarity and are more readily soluble in non-polar solvents. Neutral lipids include triacylglycerols (TAG).

[0095] Fatty acids are classified based on the length and saturation characteristics of the carbon chain. Fatty acids are termed short chain, medium chain, or long chain fatty acids based on the number of carbons present in the chain. Fatty acids are termed saturated fatty acids when no double bonds are present between the carbon atoms, and are termed unsaturated fatty acids when double bonds are present. Unsaturated long chain fatty acids are monounsaturated when only one double bond is present and are polyunsaturated when more than one double bond is present.

[0096] Fatty acids present in the lipid can have 4 to 28 carbon atoms. In some embodiments, a lipid comprises one or more polyunsaturated fatty acids. Polyunsaturated fatty acids (PUFAs) are classified based on the position of the first double bond from the methyl end of the fatty acid: omega-3 (n-3) fatty acids contain a first double bond at the third carbon, while omega-6 (n-6) fatty acids contain a first double bond at the sixth carbon. For example, docosahexaenoic acid ("DHA") is an omega-3 long chain polyunsaturated fatty acid (LC-PUFA) with a chain length of 22 carbons and 6 double bonds, often designated as "22:6 n-3." For the purposes of this application, long chain polyunsaturated fatty acids (LC-PUFAs) are defined as fatty acids of 18 and more carbon chain length, and are preferably fatty acids of 20 or more carbon chain length, containing 3 or more double bonds. LC-PUFAs of the omega-6 series include, but are not

limited to, di-homo-gammalinoleic acid (C20:3n-6), arachidonic acid (C20:4n-6) ("ARA"), docosatetraenoic acid or adrenic acid (C22:4n-6), and docosapentaenoic acid (C22:5n-6) ("DPA n-6"). The LC-PUFAs of the omega-3 series include, but are not limited to, eicosatrienoic acid (C20:3n-3), eicosatetraenoic acid (C20:4n-3), eicosapentaenoic acid (C20:5n-3) ("EPA"), docosapentaenoic acid (C22:5n-3), and docosahexaenoic acid (C22:6n-3). The LC-PUFAs also include fatty acids with greater than 22 carbons and 4 or more double bonds including, but not limited to, C24:6(n-3) and C28:8(n-3).

[0097] The terms "fatty acid," "polyunsaturated fatty acid," and "PUFA" include not only the free fatty acid form, but other forms as well, such as the triacylglycerol (TAG) form, the phospholipid (PL) form and other esterified forms. As used herein, the terms "ester" and "esterified" refer to the replacement of the hydrogen in the carboxylic acid group of a PUFA molecule with another substituent. Typical esters are known to those in the art, a discussion of which is provided by Higuchi, T. *et al.*, *Pro-drugs as Novel Delivery Systems, Vol. 14*, A.C.S. Symposium Series, *Bioreversible Carriers in Drug Design*, Edward B. Roche ed., Amer. Pharma. Assoc., Pergamon Press (1987), and *Protective Groups in Organic Chemistry*, McOmie ed., Plenum Press, New York (1973), each of which is incorporated herein by reference in its entirety. Examples of common esters include methyl, ethyl, trichloroethyl, propyl, butyl, pentyl, *tert*-butyl, benzyl, nitrobenzyl, methoxybenzyl and benzhydryl.

[0098] In some embodiments, a lipid comprises at least 10%, at least 20%, at least 30%, at least 35%, at least 40%, at least 50%, at least 60%, at least 70% or at least 80% by weight PUFA. In some embodiments, a lipid comprises at least 10%, at least 20%, at least 30%, at least 35%, at least 40%, at least 50%, at least 60%, at least 70% or at least 80% by weight DHA. In some embodiments, a lipid comprises less than 50%, less than 40%, less than 30%, less than 20%, less than 15%, less than 10%, or less than 5% by weight EPA. In some embodiments, a lipid comprises less than 10%, less than 5%, less than 2%, less than 1%, or less than 0.5% by weight sterols. In some embodiments, one or more PUFAs are present in a lipid in one or more forms, such as triglycerides, diglycerides, monoglycerides, phospholipids, free fatty acids, esterified fatty acids, alkali metal salts of fatty acids, alkali earth metal salts of fatty acids, and combinations thereof.

[0099] In some embodiments, a lipid separated after centrifuging in a process of the present invention comprises at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 50% to 95%, 50% to 90%, 50% to 85%, 50% to 80%, 50% to 75%, 60% to 95%, 60% to

90%, 60% to 85%, 70% to 95%, 70% to 90%, 70% to 85%, 75% to 95%, 75% to 90%, or 75% to 85%, by weight of triglycerides.

[0100] In some embodiments, the triglycerides comprise at least 10%, at least 20%, at least 30%, at least 35%, at least 40%, at least 50%, at least 60%, at least 70% or at least 80% by weight DHA. In some embodiments, the triglycerides comprise at least 50%, at least 40%, at least 30%, at least 20%, at least 15%, at least 10%, or at least 5% by weight EPA.

[0101] As discussed herein, additional refining of a lipid after the centrifuging can provide a lipid comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 80% to 99.5%, 80% to 99%, 80% to 97%, 80% to 95%, 80% to 90%, 85% to 99.5%, 85% to 99%, 85% to 97%, 85% to 95%, 85% to 90%, 90% to 99.5%, 90% to 99%, 90% to 97%, 90% to 95%, 95% to 99.5%, 95% to 99%, 95% to 97%, 97% to 99.5%, or 98% to 99.5% triglyceride by weight.

[0102] As used herein, a "cell" refers to a lipid-containing biomaterial, such as biomaterial derived from plants or microorganisms. In some embodiments, suitable plant material includes, but is not limited to, plant parts and oilseeds. Oilseeds include, but are not limited to, sunflower seeds, canola seeds, rapeseeds, linseeds, castor oil seeds, coriander seeds, calendula seeds or the like, and genetically modified variants thereof. Oil produced from plant material and/or microorganisms, such as oleaginous microorganisms, according to the processes described herein, is also referred to as vegetable oil. Oil produced from algae and/or fungi is also referred to as algal and/or fungal oil, respectively.

[0103] As used herein, a "microbial cell" or "microorganism" refers to organisms such as algae, bacteria, fungi, protist, and combinations thereof, e.g., unicellular organisms. In some embodiments, a microbial cell is a eukaryotic cell. A microbial cell suitable for use with the present invention includes, but is not limited to, golden algae (e.g., microorganisms of the kingdom *Stramenopiles*), green algae, diatoms, dinoflagellates (e.g., microorganisms of the order *Dinophyceae* including members of the genus *Crypthecodinium* such as, for example, *Crypthecodinium cohnii* or *C. cohnii*), yeast (Ascomycetes or Basidiomycetes), and fungi of the genera *Mucor* and *Mortierella*, including but not limited to *Mortierella alpina* and *Mortierella sect. schmuckeri*. A microbial cell suitable for use with the present invention can further include, but is not limited to genera found in the following groups of organisms: *Stramenopiles*, *Hamatores*, *Proteromonads*, *Opalines*, *Develpayella*, *Diplophrys*, *Labrinthulids*, *Thraustochytrids*, *Biosecids*, *Oomycetes*, *Hypochytridiomycetes*, *Commation*, *Reticulosphaera*, *Pelagomonas*, *Pelagococcus*, *Ollicola*, *Aureococcus*, *Parmales*, *Diatoms*, *Xanthophytes*, *Phaeophytes*, *Eustigmatophytes*, *Raphidophytes*, *Synurids*, *Axodines* (including

Rhizochromulinaales, *Pedinellales*, *Dictyochales*), *Chrysomeridales*, *Sarcinochrysidales*, *Hydrurales*, *Hibberdiales*, and *Chromulinales*.

[0104] In some embodiments, a microbial cell for use with the present invention is a microorganism of the phylum Labyrinthulomycota. In some embodiments, a microbial cell of the phylum Labyrinthulomycota is a thraustochytrid, such as a *Schizochytrium* or *Thraustochytrium*. According to the present invention, the term "thraustochytrid" refers to any member of the order Thraustochytriales, which includes the family Thraustochytriaceae, and the term "labyrinthulid" refers to any member of the order Labyrinthulales, which includes the family Labyrinthulaceae.

[0105] Members of the family Labyrinthulaceae were previously considered to be members of the order Thraustochytriales, but in more recent revisions of the taxonomic classification of such organisms, the family Labyrinthulaceae is now considered to be a member of the order Labyrinthulales. Both Labyrinthulales and Thraustochytriales are considered to be members of the phylum Labyrinthulomycota. Taxonomic theorists now generally place both of these groups of microorganisms with the algae or algae-like protists of the Stramenopile lineage. The current taxonomic placement of the thraustochytrids and labyrinthulids can be summarized as follows:

Realm: Stramenopila (Chromista)
Phylum: Labyrinthulomycota (Heterokonta)
Class: Labyrinthulomycetes (Labyrinthulae)
Order: Labyrinthulales
Family: Labyrinthulaceae
Order: Thraustochytriales
Family: Thraustochytriaceae

[0106] For purposes of the present invention, strains of microbial cells described as thraustochytrids include the following organisms: Order: Thraustochytriales; Family: Thraustochytriaceae; Genera: *Thraustochytrium* (Species: sp., *arudimentale*, *aureum*, *benthicola*, *globosum*, *kinnei*, *motivum*, *multirudimentale*, *pachydermum*, *proliferum*, *roseum*, and *striatum*), *Ulkenia* (Species: sp., *amoeboidea*, *kerguelensis*, *minuta*, *profunda*, *radiata*, *sailens*, *sarkariana*, *schizochytrops*, *visurgensis*, *yorkensis*, and *sp.* BP-5601), *Schizochytrium* (Species: sp., *aggregatum*, *limnaceum*, *mangrovei*, *minutum*, and *octosporum*), *Japonochytrium* (Species: sp., *marinum*), *Aplanochytrium* (Species: sp., *haliotidis*, *kerguelensis*, *profunda*, and *stocchinoi*), *Althornia* (Species: sp., *crouchii*), or *Elina* (Species: sp., *marisalba*, and *sinorifica*). For the purposes of this invention, species described within *Ulkenia* will be considered to be members of

the genus *Thraustochytrium*. *Aurantiacochytrium* and *Oblogospora* are two additional genuses encompassed by the phylum Labyrinthulomycota in the present invention. In some embodiments, a microbial cell is of the genus *Thraustochystrium*, *Schizochytrium*, and mixtures thereof.

[0107] Microbial cells suitable for use with the present invention include, but are not limited to, Labyrinthulids selected from: Order: Labyrinthulales, Family: Labyrinthulaceae, Genera: *Labyrinthula* (Species: sp., *algeriensis*, *coenocystis*, *chattonii*, *macrocystis*, *macrocystis atlantica*, *macrocystis macrocystis*, *marina*, *minuta*, *roscoffensis*, *valkanovii*, *vitellina*, *vitellina pacifica*, *vitellina vitellina*, and *zopfii*), *Labyrinthuloides* (Species: sp., *haliotidis*, and *yorkensis*), *Labyrinthomyxa* (Species: sp., *marina*), *Diplophrys* (Species: sp., *archeri*), *Pyrrhosorus* (Species: sp., *marinus*), *Sorodiplophrys* (Species: sp., *stercorea*), and *Chlamydomyxa* (Species: sp., *labyrinthuloides*, and *montana*) (although there is currently not a consensus on the exact taxonomic placement of *Pyrrhosorus*, *Sorodiplophrys*, and *Chlamydomyxa*).

[0108] Host cells of the phylum Labyrinthulomycota include, but are not limited to, deposited strains PTA-10212, PTA-10213, PTA-10214, PTA-10215, PTA-9695, PTA-9696, PTA-9697, PTA-9698, PTA-10208, PTA-10209, PTA-10210, PTA-10211, the microorganism deposited as SAM2179 (named "Ulkenia SAM2179" by the depositor), any *Thraustochytrium* species (including former *Ulkenia* species such as *U. visurgensis*, *U. amoeboida*, *U. sarkariana*, *U. profunda*, *U. radiata*, *U. minuta and Ulkenia sp.* BP-5601), and including *Thraustochytrium striatum*, *Thraustochytrium aureum*, *Thraustochytrium roseum*; and any *Japonochytrium* species. Strains of *Thraustochytriales* include, but are not limited to *Thraustochytrium* sp. (23B) (ATCC 20891); *Thraustochytrium striatum* (Schneider)(ATCC 24473); *Thraustochytrium aureum* (Goldstein) (ATCC 34304); *Thraustochytrium roseum* (Goldstein) (ATCC 28210); *Japonochytrium sp.* (L1) (ATCC 28207); ATCC 20890; ATCC 20892; a mutant strain derived from any of the aforementioned microorganisms; and mixtures thereof. *Schizochytrium* include, but are not limited to *Schizochytrium aggregatum*, *Schizochytrium limacinum*, *Schizochytrium sp.* (S31) (ATCC 20888), *Schizochytrium sp.* (S8) (ATCC 20889), *Schizochytrium* sp. (LC-RM) (ATCC 18915), *Schizochytrium sp.* (SR 21), deposited strain ATCC 28209, deposited *Schizochytrium limacinum* strain IFO 32693, a mutant strain derived from any of the aforementioned microorganisms, and mixtures thereof. In some embodiments, the host cell is a *Schizochytrium* or a *Thraustochytrium*. *Schizochytrium* can replicate both by successive bipartition and by forming sporangia, which ultimately release zoospores. *Thraustochytrium*,

however, replicate only by forming sporangia, which then release zoospores. In some embodiments, the host cell of the invention is a recombinant host cell.

[0109] Effective culture conditions for a microbial cell for use with the invention include, but are not limited to, effective media, bioreactor, temperature, pH, and oxygen conditions that permit lipid production. An effective medium refers to any medium in which a microbial cell, e.g., Thraustochytriales microbial cell, is typically cultured. Such media typically comprises an aqueous medium having assimilable carbon, nitrogen, and phosphate sources, as well as appropriate salts, minerals, metals, and other nutrients, such as vitamins. Microbial cells for use with the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. In some embodiments, culturing is carried out at a temperature, pH, and oxygen content appropriate for a recombinant cell.

[0110] In some embodiments, a microbial cell is capable of growth at a salinity level of 12 g/L or less, 5 g/L or less, or 3 g/L or less of sodium chloride.

[0111] In some embodiments, a microbial cell produces a lipid comprising omega-3 and/or omega-6 PUFAs. In some embodiments, a microbial cell produces a lipid comprising DHA, DPA (n-3), DPA (n-6), EPA, arachidonic acid (ARA), or the like, and combinations thereof. Non-limiting examples of microorganisms that produce a lipid comprising a PUFA are disclosed above and are also found in U.S. Pat. Nos. 5,340,594, 5,340,742 and 5,583,019, each of which is incorporated by reference herein in its entirety.

[0112] In some embodiments, a microbial cell comprises at least 30% by weight lipids, at least 35% by weight lipids, at least 40% by weight lipids, at least 50% by weight lipids, at least 60% by weight lipids, at least 70% by weight lipids, or at least 80% by weight lipids. In some embodiments, a microbial cell for use with the present invention is capable of producing at least 0.1 grams per liter per hour (g/L/h) of DHA, at least 0.2 g/L/h of DHA, at least 0.3 g/L/h of DHA, or at least 0.4 g/L/h of DHA.

Processes

[0113] The processes of the present invention comprises lysing a cell or cell biomass to form a lysed cell composition. As used herein, the term "cell biomass" refers to a population of plant or microbial cells. As used herein, the terms "lyse" and "lysing" refer to a process of rupturing the cell wall and/or cell membrane of a cell. In some embodiments, lysing comprises a process such as: mechanically treating, chemically treating, enzymatically treating, physically treating, or combinations thereof.

[0114] As used herein, mechanically treating includes, but is not limited to, homogenizing a cell, applying ultrasound to a cell, cold-pressing a cell, milling a cell or the like, and combinations thereof. In some embodiments, a process comprises lysing the cell by homogenization. In some embodiments, a process comprises lysing the cell with a homogenizer.

[0115] Homogenizing a cell can include, but is not limited to, processes utilizing a French pressure cell press, a sonicator, a homogenizer, a ball mill, a rod mill, a pebble mill, a bead mill, a high pressure grinding roll, a vertical shaft impactor, an industrial blender, a high shear mixer, a paddle mixer, a polytron homogenizer or the like, and combinations thereof. In some embodiments, a cell is flowed through a homogenizer that is optionally heated. In some embodiments, suitable homogenization can include 1 to 3 passes through a homogenizer at either high and/or low pressures. In some embodiments, a pressure during homogenization can be 150 bar to 1,400 bar, 150 bar to 1,200 bar, 150 bar to 900 bar, 150 bar to 300 bar, 300 bar to 1,400 bar, 300 bar to 1,200 bar, 300 bar to 900 bar, 400 bar to 800 bar, 500 bar to 700 bar, or 600 bar.

[0116] As used herein, physically treating can include, but is not limited to, heating a cell, drying a cell, or the like, and combinations thereof.

[0117] Heating a cell can include, but is not limited to, resistive heating, convection heating, steam heating, heating in a fluid bath, heating with solar energy, heating with focused solar energy, and the like, any of which can be performed in a tank, pool, tube, conduit, flask, or other containment device. In some embodiments, a cell is heated in a tank that includes resistive coils in/on its walls. In some embodiments, a cell is heated in a liquid bath that includes a tubing passing there through.

[0118] Drying a cell can include, but is not limited to, exposing to air flow, exposing to heat (e.g., convection heat, a heated surface, and the like), exposing to solar energy, freeze drying (lyophilizing), spray drying, and combinations thereof. In some embodiments, drying comprises applying a cell to a rotating drum that is optionally heated.

[0119] As used herein, chemically treating includes, but is not limited to, raising a pH of a cell, contacting a cell with a chemical or the like.

[0120] Raising a pH of a cell can include, but is not limited to, adding a base to a cell composition. In some embodiments, bases suitable for use with the present invention include, but are not limited to, hydroxide bases (e.g., LiOH, NaOH, KOH, $Ca(OH)_2$, and the like, and combinations thereof), carbonate bases (e.g., Na_2CO_3, K_2CO_3, $MgCO_3$, and the like, and combinations thereof), bicarbonate bases (e.g., $LiHCO_3$, $NaHCO_3$, $KHCO_3$, and the like, and combinations thereof), and combinations thereof. A base can be in the form of a solid (e.g.,

crystals, a granulate, pellets, and the like) or a liquid (e.g., an aqueous solution, an alcoholic solution such as a hydroxide base in methanol, ethanol, propanol, and the like), and combinations thereof. In some embodiments, the pH of the cell composition is raised to 8 or above, 9 or above, 10 or above, 11 or above, 12 or above, or a pH of 7 to 13, 7 to 12, 7 to 11, 7 to 10, 7 to 9, 8 to 13, 8 to 12, 8 to 11, 8 to 10, 8 to 9, 9 to 12, 9 to 11, 9 to 10, 10 to 12, or 10 to 11.

[0121] In some embodiments, raising a pH of a cell can include, but is not limited to, performing a chloralkali process. In some embodiments, a fermentation broth containing sodium chloride and a cell composition is subjected to electrolysis, which would result in the formation of sodium hydroxide. The formation of sodium hydroxide raises the pH of the cell. In some embodiments, a fermentation broth can include calcium chloride or potassium chloride in place of or in addition to sodium chloride. Subjecting such a fermentation broth to electrolysis results in the formation of calcium hydroxide or potassium hydroxide, respectively, thereby raising the pH of the cell.

[0122] Enzymatic lysing refers to lysis of a cell wall or cell membrane of a cell by contacting the cell with one or more enzymes. Enzymes suitable for use with the present invention include, but are not limited to, proteases, cellulases, hemicellulases, chitinases, pectinases, and combinations thereof. Non-limiting examples of proteases include serine proteases, theronine proteases, cysteine proteases, aspartate proteases, metalloproteases, glutamic acid proteases, alacase, and combinations thereof. Non-limiting examples of cellulases include sucrase, maltase, lactase, alpha-glucosidase, beta-glucosidase, amylase, lysozyme, neuraminidase, galactosidase, alpha-mannosidase, glucuronidase, hyaluronidase, pullulanase, glucocerebrosidase, galactosylceramidase, acetylgalactosaminidase, fucosidase, hexosaminidase, iduronidase, maltase-glucoamylase, and combinations thereof. A non-limiting example of a chitinase includes chitotriosidase. Non-limiting examples of pectinases include pectolyase, pectozyme, polygalacturonase, and combinations thereof. In some embodiments, some enzymes are activated by heating.

[0123] As used herein, a "lysed cell composition" refers to a composition comprising one or more lysed cells, including cell debris and other contents of the cell, in combination with a lipid (from the lysed cells), and optionally, broth that contains microbial cells or plant material. In some embodiments, plant material is contained in a broth or media comprising the plant material and water. In some embodiments, a microbial cell is contained in a fermentation broth or media comprising the microbial cell and water. In some embodiments, a lysed cell composition refers to a composition comprising one or more lysed cells, cell debris, a lipid, the natural contents of the cell, and aqueous components from a broth. In some embodiments, a lysed cell composition

is in the form of an oil-in-water emulsion comprising a mixture of a continuous aqueous phase and a dispersed lipid phase. In some embodiments, a dispersed lipid phase is present in a concentration of 1% to 60%, 1% to 50%, 1% to 40%, 1% to 30%, 1% to 20%, 5% to 60%, 5% to 50%, 5% to 40%, 5% to 30%, 5% to 20%, 10% to 60%, 10% to 50%, 10% to 40%, 20% to 60%, 20% to 50%, 20% to 40%, 30% to 60%, 30% to 50%, or 40% to 60% by weight of an emulsified lysed cell composition.

[0124] While not being bound to any particular theory, it is believed the processes of the present invention break up or demulsify an emulsified lysed cell composition, allowing a lipid to be separated from the lysed cell composition. As used herein, the terms "emulsion" and "emulsified" refers to a mixture of two or more immiscible phases or layers wherein one phase or layer is dispersed in another phase or layer. As used herein, the terms "break," "break up," "demulsify," "demulsification," "demulsifying," and "breaking" refer to a process of separating immiscible phases or layers of an emulsion. For example, demulsifying or breaking an emulsified lysed cell composition refers to a process by which an emulsified lysed cell composition changes from an emulsion having one or more phases or layers to a composition having two or more phases or layers. For example, in some embodiments, a process of the present invention breaks an emulsified lysed cell composition from a single-phase to two or more phases. In some embodiments, the two or more phases include a lipid phase and an aqueous phase. In some embodiments, a process of the present invention breaks an emulsified lysed cell compositions from one or more phases to at least three phases. In some embodiments, the three phases include a lipid phase, an aqueous phase, and a solid phase. In some embodiments, the three phases include a lipid phase, an emulsion phase, and an aqueous phase.

[0125] In some embodiments, the processes of the present invention demulsify a lysed cell composition to form a demulsified cell composition by removing or breaking at least 75% of the emulsion, at least 80% of the emulsion, at least 85% of the emulsion, at least 90% of the emulsion, at least 95% of the emulsion, at least 99% of the emulsion. In some embodiments, the process of the present invention demulsify a lysed cell composition by removing or breaking 75% of the emulsion to 99% of the emulsion, 75% of the emulsion to 95% of the emulsion, 75% of the emulsion to 90% of the emulsion, 75% of the emulsion to 85% of the emulsion, 75% of the emulsion to 80% of the emulsion, 80% of the emulsion to 99% of the emulsion, 80% of the emulsion to 95% of the emulsion, 80% of the emulsion to 90% of the emulsion, 80% of the emulsion to 85% of the emulsion, 85% of the emulsion to 99% of the emulsion, 85% of the emulsion to 95% of the emulsion, 85% of the emulsion to 90% of the emulsion, 90% of the

emulsion to 99% of the emulsion, 90% of the emulsion to 95% of the emulsion, or 95% of the emulsion to 99% of the emulsion by weight or volume.

[0126] In some embodiments, prior to lysing the cell, the cell can be washed and/or pasteurized. In some embodiments, washing the cell includes using an aqueous solution, such as water, to remove any extracellular water-soluble or water-dispersible compounds. In some embodiments, the cell can be washed once, twice, thrice, or more. In some embodiments, pasteurizing the cell includes heating the cell to inactivate any undesirable enzymes, for example any enzymes that might degrade lipid or reduce the yield of PUFAs. In some embodiments, the cell can be washed first and then pasteurized.

[0127] In some embodiments, the cell is plant biomaterial and the plant biomaterial is formed prior to lysing. In some embodiments, the plant biomaterial is formed by removing or extracting oilseeds from a plant. In some embodiments, an interior of an oilseed is removed from an outer hull of an oilseed by grinding, milling, extruding, aspirating, crushing, or combinations thereof. In some embodiments, the dehulled oilseeds can be homogenized or expelled using processes known in the art, such as by passing the oilseeds through a press to grind the dehulled oilseeds into a cake. In some embodiments, water can be added to the cake to form an emulsified lysed cell composition. In some embodiments, the emulsified lysed cell composition can be filtered using processes known in the art to remove any excess hull fragments from the lysed cell composition.

[0128] In some embodiments, treating a lysed cell composition with a first base breaks up (i.e., demulsifies) an emulsified lysed cell composition. In some embodiments, treating a lysed cell composition with a second base breaks (i.e., demulsifies) an emulsified lysed cell composition. In some embodiments, treating a lysed cell composition with a salt breaks (i.e., demulsifies) an emulsified lysed cell composition. In some embodiments, heating a lysed cell composition breaks (i.e., demulsifies) an emulsified lysed cell composition. In some embodiments, agitating a lysed cell composition breaks (i.e., demulsifies) an emulsified lysed cell composition. In some embodiments, simultaneous heating and agitating of a lysed cell composition breaks (i.e., demulsifies) an emulsified lysed cell composition. In some embodiments, one or more of the preceding treatments breaks up (i.e., demulsifies) an emulsified lysed cell composition.

[0129] In some embodiments, the process of the invention comprises raising the pH of a cell composition to lyse and/or demulsify the cell composition. In some embodiments, the process of the invention comprises raising the pH of a lysed cell composition to demulsify the lysed cell composition. In some embodiments, raising the pH comprises contacting a cell composition or

lysed cell composition with a base. In some embodiments, the process of the invention comprises contacting a lysed cell composition with a base to demulsify the lysed cell composition. As used herein, "contacting" refers to combining a cell composition or a lysed cell composition with a second composition (e.g., by adding a composition to a cell composition or a lysed cell composition, by adding a cell composition or a lysed cell composition to a composition, and the like). As used herein, a "composition" can comprise a pure material or include a combination of two or more materials, substances, excipients, portions, and the like. Contacting a lysed cell composition with a first base raises the pH of the lysed cell composition. In some embodiments, a lysed cell composition is contacted with a second base. In some embodiments, the pH of a lysed cell composition or a demulsified cell composition is raised a second time. In some embodiments, the second raising of the pH comprises contacting a lysed cell composition or demulsified cell composition with a second base. In some embodiments, a lysed cell composition is contacted with a first base, then heated, agitated, or a combination thereof, and subsequently contacted with a second base to provide a treated lysed cell emulsion.

[0130] In some embodiments, the first base and/or second base has a pK_b of 1 to 12, 1 to 10, 1 to 8, 1 to 6, 1 to 5, 2 to 12, 2 to 10, 2 to 8, 2 to 6, 2 to 5, 3 to 10, 3 to 6, 3 to 5, 4 to 10, 4 to 8, 4 to 6, 5 to 10, or 5 to 8. As used herein, the term "pK_b" refers to the negative logarithm of the base association constant, K_b, of the base. K_b refers to the equilibrium constant for the ionization of the base in water, wherein:

$$B + H_2O \rightleftharpoons HB^+ + OH^-; \text{ and}$$

the K_b of base, B, is defined as:

$$K_b = \frac{[HB^+][OH^-]}{[B]}.$$

[0131] Bases suitable for use with the present invention include, but are not limited to, hydroxide bases (e.g., LiOH, NaOH, KOH, $Ca(OH)_2$, and the like, and combinations thereof), carbonate bases (e.g., Na_2CO_3, K_2CO_3, $MgCO_3$, and the like, and combinations thereof), bicarbonate bases (e.g., $LiHCO_3$, $NaHCO_3$, $KHCO_3$, and the like, and combinations thereof), and combinations thereof. A base can be in the form of a solid (e.g., crystals, a granulate, pellets, and the like) or a liquid (e.g., an aqueous solution, an alcoholic solution such as a hydroxide base in methanol, ethanol, propanol, and the like), and combinations thereof. Thus, a solvent can be optionally present in a base for use with the present invention. As used herein, "solvent" refers to an agent that is hydrophobic or lipophilic. As used herein, "hydrophobic" refers to an agent that is

repelled from a mass of water. As used herein, "lipophilic" refers to an agent that dissolves in lipids.

[0132] In some embodiments, contacting a cell composition or a lysed cell composition with a base raises the pH of the lysed cell composition. In some embodiments, contacting a lysed cell composition with a base raises the pH of the lysed cell composition to 8 or above, 9 or above, 10 or above, 11 or above, 12 or above, or a pH of 7 to 13, 7 to 12, 7 to 11, 7 to 10, 7 to 9, 8 to 13, 8 to 12, 8 to 11, 8 to 10, 8 to 9, 9 to 12, 9 to 11, 9 to 10, 10 to 12, or 10 to 11. In some embodiments, contacting a lysed cell composition with a base provides a pH of 8 or below, 7 or below, 6 or below, or 5 or below to the composition.

[0133] In some embodiments, raising the pH of the cell composition or lysed cell composition with the addition of the base inhibits lipid oxidation, thereby minimizing the amount of free radicals in the lysed cell composition so that the crude lipid obtained from the processes of the invention has a low peroxide value (e.g., 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less) and/or a low anisidine value (e.g., 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less). As used herein, the terms "peroxide value" or "PV" refer to the measure of primary reaction products, such as peroxides and hydroperoxides, that occur during oxidation of the lipid. As used herein peroxide value is measured in meq/kg. As used herein, the terms "anisidine value" or "AV" refer to the measure of secondary reaction products, such as aldehydes and ketones, that occur during oxidation of the lipid.

[0134] In some embodiments, free radicals in the lysed cell composition after adjusting the pH with a base are detected using an Electron Paramagnetic Resonance spectrometer, e.g., Bruker BioSpin e-scan EPR (system number SC0274) (Bruker BioSpin, Billerica, MA). In some embodiments, a sample of the lysed cell composition is diluted in about 1:1 ratio with deionized water prior to measuring the EPR. In some embodiments, in order to measure the EPR, a spin trap chemical is added to a sample of the lysed cell composition. In some embodiments, the spin trap chemical is any spin trap chemical known in the art, including, but not limited to, POBN (α-(4-Pyridyl 1-oxide)-N-tert-butylnitrone) or DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide). In some embodiments, the spin trap chemical is about 1.25 M and about 50 µL is added to about 0.5 gram sample of the lysed cell composition. In some embodiments, a sample containing the spin trap chemical is incubated at room temperature (e.g., about 20° C). In some embodiments, the following spectrometer parameters are used: modulation frequency of about 86 Hz, modulation amplitude of about 2 gauss, microwave power of about 5 mW, time constant of about 20 seconds,

sweep time of about 10 seconds, sweep width of about 100 gauss, and a number of scans of about 8. The EPR is measured over time to determine the concentration of free radicals present in the lipid. In some embodiments, the EPR is measured hourly over a course of four hours. In some embodiments, the lysed cell composition has an EPR signal strength (intensity or amplitude) at the above listed parameters of less than 0.15×10^6, less than 0.14×10^6, less than 0.13×10^6, less than 0.12×10^6, less than 0.11×10^6, less than 0.1×10^6, less than 0.09×10^6, less than 0.08×10^6, less than 0.07×10^6, less than 0.06×10^6, or less than 0.05×10^6 after 4 hours. In some embodiments, the lysed cell composition has an EPR of 0.05×10^6 to 0.15×10^6, 0.05×10^6 to 0.14×10^6, 0.05×10^6 to 0.13×10^6, 0.05×10^6 to 0.12×10^6, 0.05×10^6 to 0.11×10^6, 0.05×10^6 to 0.1×10^6, 0.05×10^6 to 0.09×10^6, 0.07×10^6 to 0.15×10^6, 0.07×10^6 to 0.13×10^6, 0.07×10^6 to 0.11×10^6, 0.08×10^6 to 0.14×10^6, 0.08×10^6 to 0.12×10^6, 0.08×10^6 to 0.1×10^6, 0.09×10^6 to 0.13×10^6, or 0.09×10^6 to 0.11×10^6. In some embodiments, the pH of the lysed cell composition resulting in an EPR specified above is 8 to 12, 8 to 11, 8 to 10, 8 to 9, 9 to 12, 9 to 11, 9 to 10, 10 to 12, or 10 to 11. In some embodiments, a lysed cell composition having an EPR signal strength specified above results in a crude lipid having an AV of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less. In some embodiments, a lysed cell composition having an EPR specified above results in a crude lipid having a PV of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less.

[0135] In some embodiments, a process comprises contacting a cell composition or lysed cell composition with a salt to demulsify the lysed cell composition. As used herein, a "salt" refers to an ionic compound formed by replacing a hydrogen ion from an acid with a metal (e.g., an alkali metal, an alkali earth metal, a transition metal, and the like) or a positively charged compound (e.g., NH_4^+ and the like). Salts suitable for use with the present invention include, but are not limited to, alkali metal salts, alkali earth metal salts, or the like, and combinations thereof. Negatively charged ionic species present in a salt for use with the present include, but are not limited to, halides, sulfate, bisulfate, sulfite, phosphate, hydrogen phosphate, dihydrogen phosphate, carbonate, bicarbonate, or the like, and combinations thereof. In some embodiments, a salt for use with the present invention is selected from: sodium chloride, sodium sulfate, sodium carbonate, calcium chloride, potassium sulfate, magnesium sulfate, monosodium glutamate, ammonium sulfate, potassium chloride, iron chloride, iron sulfate, aluminum sulfate, and combinations thereof. In some embodiments, a salt does not include NaOH. A salt can be added as a solid (e.g., in crystalline, amorphous, pelletized, and/or granulated form), and/or as a

solution (e.g., a dilute solution, a saturated solution, or a super-saturated solution) containing, for example, water, an alcohol, and the like, and combinations thereof.

[0136] In some embodiments, the salt is added in an amount of 5 g/l to 25 g/l, 5 g/l to 10 g/l, 10 g/l to 15 g/l, 15 g/l to 20 g/l, 20 g/l to 25 g/l, or 10 g/l to 20 g/l.

[0137] In some embodiments, a temperature of a cell composition or a lysed cell composition is less than or equal to 60° C, less than or equal to 55° C, less than or equal to 45° C, less than or equal to 40° C, less than or equal to 35° C, less than or equal to 30° C, or less than or equal to 25° C when a salt is added to demulsify the cell composition or the lysed cell composition. In some embodiments, a temperature of a lysed cell composition is 0 °C to 60 °C, 0 °C to 55 °C, 0 °C to 50 °C, 0 °C to 45 °C, 0 °C to 40 °C, 0 °C to 35 °C, 0 °C to 30 °C, 0 °C to 25 °C, 20 °C to 60 °C, 20 °C to 55 °C, 20 °C to 50 °C, 20 °C to 45 °C, 20° C to 40° C, 20° C to 35° C, 20° C to 30° C, 30 °C to 60 °C, 30° C to 55° C, 30° C to 50° C, 30° C to 45° C, 30° C to 40° C, 30° C to 40° C, 40° C to 60° C, 40° C to 55° C, 40° C to 50° C, or 50° C to 60° C when a salt is added to demulsify the cell composition or the lysed cell composition.

[0138] In some embodiments, the process comprises contacting a cell composition or a lysed cell composition with 20% or less, 15% or less, 10% or less, 7.5% or less, 5% or less, or 2% or less salt by weight, of the lysed cell composition or the cell composition. In some embodiments, a process comprises contacting a cell composition or a lysed cell composition with 0.1% to 20%, 0.1% to 15%, 0.1% to 10%, 0.5% to 20%, 0.5% to 15%, 0.5% to 10%, 0.5% to 5%, 0.5% to 4%, 0.5% to 3%, 0.5% to 2.5%, 0.5% to 2%, 0.5% to 1.5%, 0.5% to 1%, 1% to 20%, 1% to 15%, 1% to 10%,1% to 5%, 1% to 4%, 1% to 3%, 1% to 2.5%, 1% to 2%, 1% to 1.5%, 1.5% to 5%, 1.5% to 4%, 1.5% to 3%, 1.5% to 2.5%, 1.5% to 2%, 2% to 20%, 2% to 15%, 2% to 10%, 2% to 5%, 2% to 4%, 2% to 3%, 2% to 2.5%, 2.5% to 5%, 2.5% to 4%, 2.5% to 3%, 3% to 5%, 3% to 4%, 4% to 5%, 5% to 20%, 5% to 15%, 5% to 10%, 10% to 20%, 10% to 15%, or 15% to 20% salt, by weight, of the cell composition or lysed cell composition (e.g., a total broth weight). For example, when a lysed cell composition weighs 1,000 kg, contacting with 0.5% to 20% salt, by weight, requires combining 5 kg to 200 kg of salt with the lysed cell composition.

[0139] In some embodiments, the process comprises heating a cell composition or a lysed cell composition to demulsify the lysed cell composition. In some embodiments the cell composition or the lysed cell composition is heated for a sufficient period of time for a base and/or a salt to demulsify a cell composition or a lysed cell composition. In some embodiments, the process comprises heating a cell composition or a lysed cell composition for at least 5 minutes, at least 10 minutes, at least 20 minutes, at least 30 minutes, at least 1 hour, at least 2 hours, at least 4 hours,

at least 8 hours, at least 12 hours, at least 18 hours, at least 24 hours, at least 30 hours, at least 36 hours, at least 42 hours, at least 48 hours, at least 54 hours, at least 60 hours, at least 66 hours, at least 72 hours, at least 78 hours, at least 84 hours, at least 90 hours or at least 96 hours. In some embodiments, the process comprises heating a lysed cell composition for 5 minutes to 96 hours, 5 minutes to 4 hours, 5 minutes to 2 hours, 5 minutes to 1 hour, 10 minutes to 4 hours, 10 minutes to 2 hours, 10 minutes to 1 hour, 1 hour to 96 hours, 1 hour to 84 hours, 1 hour to 72 hours, 1 hour to 60 hours, 1 hour to 48 hours, 1 hour to 36 hours, 1 hour to 24 hours, 1 hour to 4 hours, 4 hours to 96 hours, 4 hours to 84 hours, 4 hours to 72 hours, 4 hours to 60 hours, 4 hours to 48 hours, 4 hours to 36 hours, 4 hours to 24 hours, 8 hours to 96 hours, 8 hours to 84 hours, 8 hours to 72 hours, 8 hours to 60 hours, 8 hours to 48 hours, 8 hours to 36 hours, 8 hours to 24 hours, 8 hours to 12 hours, 12 hours to 96 hours, 12 hours to 84 hours, 12 hours to 72 hours, 12 hours to 60 hours, 12 hours to 48 hours, 12 hours to 36 hours, 12 hours to 24 hours, 24 hours to 96 hours, 24 hours to 84 hours, 24 hours to 72 hours, 24 hours to 60 hours, 24 hours to 48 hours, or 24 hours to 36 hours.

[0140] In some embodiments, a cell composition or a lysed cell composition can be heated at a temperature of at least 10 °C, at least 20 °C, at least 25 °C, at least 30 °C, at least 35° C, at least 40° C, at least 45° C, at least 50° C, at least 55° C, at least 60° C, at least 65° C, at least 70° C, at least 75° C, at least 80° C, at least 85° C, at least 90° C, at least 95° C, or at least 100° C. In some embodiments, a process comprises heating a cell composition or a lysed cell composition at a temperature of 10 °C to 100 °C, 10 °C to 90 °C, 10 °C to 80 °C, 10 °C to 70 °C, 20 °C to 100 °C, 20 °C to 90 °C, 20 °C to 80 °C, 20 °C to 70 °C, 30° C to 100° C, 30° C to 90° C, 30° C to 80° C, 30 °C to 70 °C, 40° C to 100° C, 40° C to 90° C, 40° C to 80° C, 50° C to 100° C, 50° C to 90° C, 50° C to 80° C, 50° C to 70° C, 60° C to 100° C, 60° C to 90° C, 60° C to 80° C, 70° C to 100° C, 70° C to 90° C, 80° C to 100° C, 80° C to 90° C, or 90° C to 100° C. In some embodiments, a salt can be added to the cell composition or the lysed cell composition during the heating.

[0141] In some embodiments, a cell composition or a lysed cell composition can be heated in a closed system or in a system with an evaporator. In some embodiments, a cell composition or lysed cell composition can be heated in a system with an evaporator such that a portion of the water present in the cell composition or the lysed cell composition is removed by evaporation. In some embodiments, a process comprises heating a cell composition or a lysed cell composition in a system with an evaporator to remove up to 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% or 50% by weight of water present in the cell composition or lysed cell composition. In some embodiments, a process comprises heating a cell composition or a lysed cell composition in

a system with an evaporator to remove 1% to 50%, 1% to 45%, 1% to 40%, 1% to 35%, 1% to 30%, 1% to 25%, 1% to 20%, 1% to 15%, 1% to 10%, 1% to 5%, 5% to 50%, 5% to 45%, 5% to 40%, 5% to 35%, 5% to 30%, 5% to 25%, 5% to 20%, 5% to 15%, 5% to 10%, 10% to 50%, 10% to 45%, 10% to 40%, 10% to 35%, 10% to 30%, 10% to 25%, 10% to 20%, 10% to 15%, 15% to 50%, 15% to 45%, 15% to 40%, 15% to 35%, 15% to 30%, 15% to 25%, 15% to 20%, 20% to 50%, 20% to 45%, 20% to 40%, 20% to 35%, 20% to 30%, 20% to 25%, 25% to 50%, 25% to 45%, 25% to 40%, 25% to 35%, 25% to 30%, 30% to 50%, 30% to 45%, 30% to 40%, 30% to 35%, 35% to 50%, 35% to 45%, 35% to 40%, 40% to 50%, 40% to 45%, or 45% to 50%.

[0142] In some embodiments, the process comprises holding a cell composition or a lysed cell composition in a vessel for a predetermined time to demulsify the lysed cell composition. In some embodiments, the process comprises holding a cell composition or a lysed cell composition in a vessel for at least 5 minutes, at least 10 minutes, at least 20 minutes, at least 30 minutes, at least 1 hour, at least 2 hours, at least 4 hours, at least 8 hours, at least 12 hours, at least 18 hours, at least 24 hours, at least 30 hours, at least 36 hours, at least 42 hours, at least 48 hours, at least 54 hours, at least 60 hours, at least 66 hours, at least 72 hours, at least 78 hours, at least 84 hours, at least 90 hours or at least 96 hours. In some embodiments, the process comprises holding a cell composition or a lysed cell composition for 5 minutes to 96 hours, 5 minutes to 4 hours, 5 minutes to 2 hours, 5 minutes to 1 hour, 10 minutes to 4 hours, 10 minutes to 2 hours, 10 minutes to 1 hour, 1 hour to 96 hours, 1 hour to 84 hours, 1 hour to 72 hours, 1 hour to 60 hours, 1 hour to 48 hours, 1 hour to 36 hours, 1 hour to 24 hours, 1 hour to 4 hours, 4 hours to 96 hours, 4 hours to 84 hours, 4 hours to 72 hours, 4 hours to 60 hours, 4 hours to 48 hours, 4 hours to 36 hours, 4 hours to 24 hours, 8 hours to 96 hours, 8 hours to 84 hours, 8 hours to 72 hours, 8 hours to 60 hours, 8 hours to 48 hours, 8 hours to 36 hours, 8 hours to 24 hours, 8 hours to 12 hours, 12 hours to 96 hours, 12 hours to 84 hours, 12 hours to 72 hours, 12 hours to 60 hours, 12 hours to 48 hours, 12 hours to 36 hours, 12 hours to 24 hours, 24 hours to 96 hours, 24 hours to 84 hours, 24 hours to 72 hours, 24 hours to 60 hours, 24 hours to 48 hours, or 24 hours to 36 hours.

[0143] In some embodiments, the process comprises contacting an antioxidant with a lysed cell emulsion. Antioxidants suitable for use with the present invention include, but are not limited to, a tocopherol, a tocotrienol, a polyphenol, resveratrol, a flavonoid, a carotenoid, lycopene, a carotene, lutein, ascorbic acid, ascorbyl palmitate, or the like, and combinations thereof.

[0144] In some embodiments, the process comprises allowing an emulsified lysed cell composition to stand, wherein the lipid is separated from the emulsified lysed cell composition using gravity.

[0145] As used herein, the terms "agitating" and "agitation" refer to a process of affecting motion in a lysed cell composition through an application of force. In some embodiments, the process of the invention comprises agitating a cell composition or a lysed cell composition by stirring, mixing, blending, shaking, vibrating, or a combination thereof. In some embodiments, the process of agitating a cell composition or a lysed cell composition demulsifies the cell composition or the lysed cell composition.

[0146] In some embodiments, the process of the invention comprises agitating a lysed cell composition at 0.1 hp/1,000 gal to 10 hp/1,000 gal, 0.5 hp/1,000 gal to 8 hp/1,000 gal, 1 hp/1,000 gal to 6 hp/1,000 gal, or 2 hp/1,000 gal to 5 hp/1,000 gal of lysed cell composition.

[0147] In some embodiments, the process of the invention comprises agitating a cell composition or a lysed cell composition using an agitator. In some embodiments, the agitator is a dispersion style agitator that disperses a base and/or salt in the cell composition or the lysed cell composition. In some embodiments, an agitator has one or more impellers. As used herein, "impeller" refers to a device arranged to impart motion to a cell composition or a lysed cell composition when rotated. Impellers suitable for use with the present invention include straight blade impellers, Rushton blade impellers, axial flow impellers, radial flow impellers, concave blade disc impellers, high-efficiency impellers, propellers, paddles, turbines, or the like, and combinations thereof. In some embodiments, a process includes agitating a cell composition or a lysed cell composition using an agitator having an impeller tip speed of 90 ft/min to 1,200 ft/min, 200 ft/min to 1,000 ft/min, 300 ft/min to 800 ft/min, 400 ft/min to 700 ft/min, or 500 ft/min to 600 ft/min. In some embodiments, a process includes agitating a cell composition or a lysed cell composition using an agitator having an impeller tip speed of 350 centimeters/second to 900 centimeters per second, 350 centimeters/second to 850 centimeters per second, 350 centimeters/second to 800 centimeters/second, 350 centimeters/second to 750 centimeters/second, 350 centimeters/second to 700 centimeters/second, 350 centimeters/second to 650 centimeters/second, 350 centimeters/second to 600 centimeters/second, 350 centimeters/second to 550 centimeters/second, 350 centimeters/second to 500 centimeters/second, 350 centimeters/second to 450 centimeters/second, 350 centimeters/second to 400 centimeters/second, 400 centimeters/second to 900 centimeters per second, 400 centimeters/second to 850 centimeters per second, 400 centimeters/second to 800 centimeters/second, 400 centimeters/second to 750 centimeters/second, 400 centimeters/second to 700 centimeters/second, 400 centimeters/second to 650 centimeters/second, 400 centimeters/second to 600 centimeters/second, 400 centimeters/second to 550

centimeters/second, 400 centimeters/second to 500 centimeters/second, 400 centimeters/second to 450 centimeters/second, 450 centimeters/second to 900 centimeters per second, 450 centimeters/second to 850 centimeters per second, 450 centimeters/second to 800 centimeters/second, 450 centimeters/second to 750 centimeters/second, 450 centimeters/second to 700 centimeters/second, 450 centimeters/second to 650 centimeters/second, 450 centimeters/second to 600 centimeters/second, 450 centimeters/second to 550 centimeters/second, 450 centimeters/second to 500 centimeters/second, 500 centimeters/second to 900 centimeters per second, 500 centimeters/second to 850 centimeters per second, 500 centimeters/second to 800 centimeters/second, 500 centimeters/second to 750 centimeters/second, 500 centimeters/second to 700 centimeters/second, 500 centimeters/second to 650 centimeters/second, 500 centimeters/second to 600 centimeters/second, 500 centimeters/second to 550 centimeters/second, 550 centimeters/second to 900 centimeters per second, 550 centimeters/second to 850 centimeters per second, 550 centimeters/second to 800 centimeters/second, 550 centimeters/second to 750 centimeters/second, 550 centimeters/second to 700 centimeters/second, 550 centimeters/second to 650 centimeters/second, 550 centimeters/second to 600 centimeters/second, 600 centimeters/second to 900 centimeters per second, 600 centimeters/second to 850 centimeters per second, 600 centimeters/second to 800 centimeters/second, 600 centimeters/second to 750 centimeters/second, 600 centimeters/second to 700 centimeters/second, 600 centimeters/second to 650 centimeters/second, 650 centimeters/second to 900 centimeters per second, 650 centimeters/second to 850 centimeters per second, 650 centimeters/second to 800 centimeters/second, 650 centimeters/second to 750 centimeters/second, 650 centimeters/second to 700 centimeters/second, 700 centimeters/second to 900 centimeters per second, 700 centimeters/second to 850 centimeters per second, 700 centimeters/second to 800 centimeters/second, 700 centimeters/second to 750 centimeters/second, 750 centimeters/second to 900 centimeters per second, 750 centimeters/second to 850 centimeters per second, 750 centimeters/second to 800 centimeters/second, 800 centimeters/second to 900 centimeters per second, 800 centimeters/second to 850 centimeters per second, or 850 centimeters/second to 900 centimeters/second. As used herein, "impeller tip speed" refers to the speed of the outer most portion of the impeller as it rotates around its central axis.

[0148] In some embodiments, the agitating (and optionally additional steps as described herein) is performed in a container comprising an impeller, wherein a ratio of the impeller diameter to the container volume is 0.1 to 0.5, 0.1 to 0.4, 0.2 to 0.5, 0.2 to 0.4, 0.3 to 0.5, or 0.3 to 0.4.

[0149] In some embodiments, the agitating (and optionally additional steps as described herein) is performed in a container comprising an impeller, wherein a ratio of the impeller diameter to the inner diameter of the container is at least 0.25, at least 0.34, at least 0.65, 0.25 to 0.65, 0.25 to 0.33, 0.3 to 0.6, 0.3 to 0.5, 0.3 to 0.4, 0.34 to 0.65, 0.34 to 0.6, 0.34 to 0.55, 0.37 to 0.55, 0.4 to 0.65, 0.4 to 0.6, 0.4 to 0.5, or 0.42 to 0.55.

[0150] In some embodiments, agitating comprises mixing a cell composition or a lysed cell composition such that the cell composition or the lysed cell composition is placed under flow conditions described by a Reynolds number of 10 to 10,000, 1,000 to 10,000, 1,500 to 10,000, or 2,000 to 10,000. In some embodiments, a lysed cell emulsion during the agitating has a Reynolds number of 2,000 or more, 3,000 or more, or 5,000 or more, or 2,000 to 10,000, 3,000 to 10,000, or 5,000 to 10,000.

[0151] In some embodiments, a process comprises agitating a cell composition or a lysed cell composition for at least 5 minutes, at least 10 minutes, at least 20 minutes, at least 30 minutes, at least 1 hour, at least 2 hours, at least 4 hours, at least 8 hours, at least 12 hours, at least 18 hours, at least 24 hours, at least 30 hours, at least 36 hours, at least 42 hours, at least 48 hours, at least 54 hours, at least 60 hours, at least 66 hours, at least 72 hours, at least 78 hours, at least 84 hours, at least 90 hours or at least 96 hours. In some embodiments, a process comprises agitating a cell composition or a lysed cell composition for 5 minutes to 96 hours, 5 minutes to 4 hours, 5 minutes to 2 hours, 5 minutes to 1 hour, 10 minutes to 4 hours, 10 minutes to 2 hours, 10 minutes to 1 hour, 1 hour to 96 hours, 1 hour to 84 hours, 1 hour to 72 hours, 1 hour to 60 hours, 1 hour to 48 hours, 1 hour to 36 hours, 1 hour to 24 hours, 1 hour to 4 hours, 4 hours to 96 hours, 4 hours to 84 hours, 4 hours to 72 hours, 4 hours to 60 hours, 4 hours to 48 hours, 4 hours to 36 hours, 4 hours to 24 hours, 8 hours to 96 hours, 8 hours to 84 hours, 8 hours to 72 hours, 8 hours to 60 hours, 8 hours to 48 hours, 8 hours to 36 hours, 8 hours to 24 hours, 8 hours to 12 hours, 12 hours to 96 hours, 12 hours to 84 hours, 12 hours to 72 hours, 12 hours to 60 hours, 12 hours to 48 hours, 12 hours to 36 hours, 12 hours to 24 hours, 20 hours to 40 hours, 24 hours to 96 hours, 24 hours to 84 hours, 24 hours to 72 hours, 24 hours to 60 hours, 24 hours to 48 hours, or 24 hours to 36 hours.

[0152] In some embodiments, a process comprises simultaneously agitating and heating a cell composition or a lysed cell composition to demulsify the cell composition or the lysed cell composition. In some embodiments, a process comprises agitating a cell composition or a lysed cell composition at a temperature of at least 10 °C, at least 20 °C, at least 25 °C, at least 30 °C, at least 35° C, at least 40° C, at least 45° C, at least 50° C, at least 55° C, at least 60° C, at least 65°

C, at least 70° C, at least 75° C, at least 80° C, at least 85° C, at least 90° C, at least 95° C, or at least 100° C. In some embodiments, a process comprises agitating a cell composition or a lysed cell composition at a temperature of 10 °C to 100 °C, 10 °C to 90 °C, 10 °C to 80 °C, 10 °C to 70 °C, 20 °C to 100 °C, 20 °C to 90 °C, 20 °C to 80 °C, 20 °C to 70 °C, 30° C to 100° C, 30° C to 90° C, 30° C to 80° C, 30 °C to 70 °C, 40° C to 100° C, 40° C to 90° C, 40° C to 80° C, 50° C to 100° C, 50° C to 90° C, 50° C to 80° C, 50° C to 70° C, 60° C to 100° C, 60° C to 90° C, 60° C to 80° C, 70° C to 100° C, 70° C to 90° C, 80° C to 1000° C, 80° C to 90° C, or 90° C to 100° C.

[0153] In some embodiments, the various combinations of forming a lysed cell composition, contacting a lysed cell composition with a base or raising the pH of a lysed cell composition, contacting a lysed cell composition with a salt, heating the lysed cell composition, and agitating a lysed cell composition can occur in a single vessel. In some embodiments, the various combinations of forming a cell composition, contacting a cell composition with a base or raising the pH of a cell composition, contacting a cell composition with a salt, heating the cell composition, and agitating a cell composition can occur in a single vessel. In some embodiments, the single vessel includes a fermentation vessel. In some embodiments, the fermentation vessel can have a volume of at least 20,000 liters, at least 50,000 liters, at least 100,000 liters, at least 120,000 liters, at least 150,000 liters, at least 200,000 liters, or at least 220,000 liters. In some embodiments, the fermentation vessel can have a volume of 20,000 liters to 220,000 liters, 20,000 liters to 100,000 liters, 20,000 liters to 50,000 liters, 50,000 liters to 220,000 liters, 50,000 liters to 150,000 liters, 50,000 liters to 100,000 liters, 100,000 liters to 220,000 liters, 100,000 liters to 150,000 liters, 100,000 liters to 120,000 liters, 150,000 liters to 220,000 liters, 150,000 liters to 200,000 liters, or 200,000 liters to 220,000 liters.

[0154] In some embodiments, a quantity of cell composition or lysed cell composition formed in a vessel can be transferred into one or more agitation vessels. In some embodiments, the agitation vessels can have a volume of at least 20,000 liters, at least 30,000 liters, at least 40,000 liters or at least 50,000 liters. In some embodiments, the agitation vessels can have a volume of 20,000 liters to 50,000 liters, 20,000 liters to 40,000 liters, 20,000 liters to 30,000 liters, 30,000 liters to 50,000 liters, 30,000 liters to 40,000 liters or 40,000 liters to 50,000 liters.

[0155] In some embodiments, the agitation vessels can have any combination of the following properties. In some embodiments, the agitation vessels can have two impellers. In some embodiments, the impellers are Rushton blade impellers. In some embodiments, the impellers are separated from each other by a distance at least equal to a diameter of the smallest impeller. In some embodiments, the impellers are 30 inches to 40 inches, 33 inches to 37 inches, 33 inches,

34 inches, 35 inches, 36 inches or 37 inches from tip to tip. In some embodiments, the agitation vessels have a volume of at least 10,000 liters, at least 20,000 liters, at least 30,000 liters, at least 40,000 liters or at least 50,000 liters. In some embodiments, the agitation vessels have an inner diameter of 90 inches to 110 inches, 95 inches to 105 inches, 98 inches, 99 inches, 100 inches, 101 inches, or 102 inches. In some embodiments, a first impeller is located 15 inches to 20 inches, 16 inches to 19 inches, or 17 inches to 18 inches from a bottom of the agitation vessel and a second impeller is located 60 inches to 80 inches, 65 inches to 75 inches, 68 inches, 69 inches, 70 inches, 71 inches, 72 inches, 73 inches, 74 inches, or 75 inches above the first impeller. In some embodiments, a lysed cell composition is agitated at least 50 rpm, at least 60 rpm, or at least 70 rpm. In some embodiments, a lysed cell composition is agitated at 50 rpm to 70 rpm, 50 rpm to 60 rpm, or 60 rpm to 70 rpm.

[0156] In some embodiments, the cell composition, the lysed cell composition, or the lipid are harvested from a vessel by pumping the cell composition, the lysed cell composition, or the lipid from the vessel. In some embodiments, the cell composition, the lysed cell composition, or the lipid are harvested from a vessel without agitating the vessel. In some embodiments, the cell composition, the lysed cell composition, or the lipid are harvested from a vessel by pumping, without agitation, the cell composition, the lysed cell composition, or the lipid from the vessel. In some embodiments, the cell composition, the lysed cell composition, or the lipid are harvested from a vessel without blowing air. In some embodiments, harvesting the cell composition, the lysed cell composition, or the lipid by the techniques described above results in a crude lipid having a low anisidine value (e.g., 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less) and/or a low phosphorus content (e.g., 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less).

[0157] As described herein, the present invention utilizes various combinations of contacting a lysed cell composition with a first base or raising the pH of a lysed cell composition, contacting a lysed cell composition with a salt, heating a lysed cell composition, and agitating a lysed cell composition to provide a treated lysed cell composition. As described herein, the present invention utilizes various combinations of contacting a cell composition with a first base or raising the pH of a cell composition, contacting a cell composition with a salt, heating a cell composition, and agitating a cell composition to provide a treated cell composition. The treated

cell composition or treated lysed cell composition is at least partially demulsified compared with an untreated cell composition or treated lysed cell composition. Thus, a treated cell composition or treated lysed cell composition can be placed in a centrifuge and a lipid can be separated therefrom.

[0158] In some embodiments, after raising the pH of a cell composition or lysed cell composition, e.g., by contacting with a first base, the heating the cell composition or lysed cell composition and/or the agitating the cell composition or lysed cell composition can decrease the pH of the treated cell composition or treated lysed cell composition. In order for a lipid to be more effectively separated from a treated cell composition or treated lysed cell composition by centrifuging, the pH of the treated cell composition or treated lysed cell composition is raised a second time, e.g., by contacting the treated cell composition or the treated lysed cell composition with a second base. In some embodiments, contacting a treated lysed cell composition with a second base raises the pH of the treated cell composition or the treated lysed cell composition. In some embodiments, a treated cell composition or a treated lysed cell composition is contacted with a second base to raise the pH of the treated cell composition or the treated lysed cell composition to 7 or above, 7.5 or above, 8 or above, 8.5 or above, 9 or above, 9.5 or above, 10 or above, 10.5 or above, 11 or above, 11.5 or above, or 12 or above. In some embodiments, a treated cell composition or a treated lysed cell composition is contacted with a second base to raise the pH of the treated lysed cell composition to 7 to 13, 7 to 12, 7 to 11, 7 to 10, 7 to 9, 7 to 8, 7 to 7.5, 7.5 to 8, 8 to 13, 8 to 12, 8 to 11, 8 to 10, 8 to 9, 8 to 8.5, 8.5 to 9, 9 to 12, 9 to 11, 9 to 10, 9 to 9.5, 9.5 to 10, 10 to 12, or 10 to 11.

[0159] In some embodiments, the pH of a treated cell composition or a treated lysed cell emulsion is 7 or less, 6 or less, 5 or less, 4 or less, or 3 or less.

[0160] The processes of the present invention comprise separating a lipid from a treated cell composition or a treated lysed cell composition. In some embodiments, a lipid is separated from a lysed cell emulsion after contacting a lysed cell emulsion with a second base, after the agitating a lysed cell emulsion, or after contacting a lysed cell emulsion with a salt by, for example, permitting the treated lysed cell emulsion to rest for a period of time sufficient for a lipid to separate from the treated lysed cell emulsion (e.g., as a separate layer). The lipid can be subsequently removed, for example, by decanting, skimming, vacuuming, pumping, sucking off, drawing off, siphoning, or otherwise removing the lipid from the surface of the treated lysed cell emulsion.

[0161] In some embodiments, the separating comprises centrifuging a treated cell composition or a treated lysed cell composition (e.g., at a temperature of 30° C to 100° C), whereby the centrifuging separates a lipid from the treated cell composition or the treated lysed cell composition.

[0162] In some embodiments, a process comprises centrifuging a treated cell composition or a treated lysed cell composition at a temperature of at least 10 °C, at least 20 °C, at least 25 °C, at least 30 °C, at least 35° C, at least 40° C, at least 45° C, at least 50° C, at least 55° C, at least 60° C, at least 65° C, at least 70° C, at least 75° C, at least 80° C, at least 85° C, at least 90° C, at least 95° C, or at least 100° C. In some embodiments, a process comprises centrifuging a treated cell composition or a treated lysed cell composition at a temperature of 10 °C to 100 ° C, 10 °C to 90 °C, 10 °C to 80 °C, 20 °C to 100 °C, 20 °C to 90 °C, 20 °C to 80 °C, 25 °C to 100 °C, 25 °C to 90 °C, 25 °C to 80 °C, 25 °C to 75 °C, 30° C to 100° C, 30° C to 90° C, 30° C to 80° C, 40° C to 100° C, 40° C to 90° C, 40° C to 80° C, 50° C to 100° C, 50° C to 90° C, 50° C to 80° C, 50° C to 70° C, 60° C to 100° C, 60° C to 90° C, 60° C to 80° C, 60° C to 70° C, 70° C to 100° C, or 70° C to 90° C.

[0163] In some embodiments, centrifuging is conducted at a feed rate (of a treated cell composition or a treated lysed cell composition into a centrifuge) of 1 kilogram per minute (kg/min) to 500 kg/min, 1 kg/min to 400 kg/min, 1 kg/min to 300 kg/min, 1 kg/min to 200 kg/min, 1 kg/min to 100 kg/min, 1 kg/min to 75 kg/min, 1 kg/min to 50 kg/min, 1 kg/min to 40 kg/min, 1 kg/min to 30 kg/min, 1 kg/min to 25 kg/min, 1 kg/min to 10 kg/min, 10 kg/min to 500 kg/min, 10 kg/min to 400 kg/min, 10 kg/min to 300 kg/min, 10 kg/min to 200 kg/min, 10 kg/min to 100 kg/min, 10 kg/min to 75 kg/min, 10 kg/min to 50 kg/min, 10 kg/min to 40 kg/min, 10 kg/min to 30 kg/min, 20 kg/min to 500 kg/min, 20 kg/min to 400 kg/min, 20 kg/min to 300 kg/min, 20 kg/min to 200 kg/min, 20 kg/min to 100 kg/min, 20 kg/min to 75 kg/min, 20 kg/min to 50 kg/min, 20 kg/min to 40 kg/min, 25 kg/min to 500 kg/min, 25 kg/min to 400 kg/min, 25 kg/min to 300 kg/min, 25 kg/min to 200 kg/min, 25 kg/min to 100 kg/min, 25 kg/min to 75 kg/min, 25 kg/min to 50 kg/min, 30 kg/min to 60 kg/min, 30 kg/min to 50 kg/min, 30 kg/min to 40 kg/min, 50 kg/min to 500 kg/min, 100 kg/min to 500 kg/min, or 200 kg/min to 500 kg/min.

[0164] The total time required for the separating can vary depending on the volume of the treated cell composition or the treated lysed cell composition. Typical total time for separation (e.g., centrifuge time) is at least 0.1 hour, at least 0.2 hour, at least 0.5 hour, at least 1 hour, at least 2 hours, at least 4 hours, at least 6 hours, at least 8 hours, at least 10 hours, at least 12 hours, or

0.1 hour to 24 hours, 0.5 hour to 24 hours, 1 hour to 12 hours, 2 hours to 10 hours, or 4 hours to 8 hours.

[0165] In some embodiments, a process of the invention comprises centrifuging a treated cell composition or a treated lysed cell composition at a centrifugal force of 1,000 g to 25,000 g, 1,000 g to 20,000 g, 1,000 g to 10,000 g, 2,000 g to 25,000 g, 2,000 g to 20,000 g, 2,000 g to 15,000 g, 3,000 g to 25,000 g, 3,000 g to 20,000 g, 5,000 g to 25,000 g, 5,000 g to 20,000 g, 5,000 g to 15,000 g, 5,000 g to 10,000 g, 5,000 g to 8,000 g, 10,000 g to 25,000 g, 15,000 g to 25,000 g, or at least 1,000 g, at least 2,000, g, at least 4,000 g, at least 5,000 g, at least 7,000 g, at least 8,000 g, at least 10,000 g, at least 15,000 g, at least 20,000 g, or at least 25,000 g. As used herein, "g" refers to standard gravity or approximately 9.8 m/s^2. In some embodiments, a process of the invention comprises centrifuging a treated cell composition or a treated lysed cell composition at 4,000 rpm to 14,000 rpm, 4,000 rpm to 10,000 rpm, 6,000 rpm to 14,000 rpm, 6,000 rpm to 12,000 rpm, 8,000 to 14,000 rpm, 8,000 rpm to 12,000 rpm, or 8,000 rpm to 10,000 rpm.

[0166] In some embodiments, a process of the invention comprises drying a lipid after separation of the lipid from a treated cell composition or a treated lysed cell composition in order to remove water from the lipid. In some embodiments, drying the lipid can include, but is not limited to, heating the lipid to evaporate water. In some embodiments, after drying, the lipid has a water content by weight percentage of lipid that is less than 3%, less than 2.5%, less than 2%, less than 1.5%, less than 1%, less than 0.5%, less than 0.1%, or 0%. In some embodiments, after drying, the lipid has a water content by weight percentage of lipid of 0% to 3%, 0% to 2.5%, 0% to 2%, 0% to 1.5%, 0% to 1%, 0% to 0.5%, 0.1% to 3%, 0.1% to 2.5%, 0.1% to 2%, 0.1% to 1.5%, 0.1% to 1%, 0.1% to 0.5%, 0.5% to 3%, 0.5% to 2.5%, 0.5% to 2%, 0.5% to 1.5%, 0.5% to 1%, 1% to 3%, 1% to 2.5%, 1% to 2%, 1% to 1.5%, 1.5% to 3%, 1.5% to 2.5%, 1.5% to 2%, 2% to 3%, 2% to 2.5%, or 2.5% to 3%.

[0167] In some embodiments, a process further comprises refining a lipid by one or more processes selected from caustic refining, degumming, alkali-refining, bleaching, deodorization, deacidification, or the like, and combinations thereof to remove one or more phospholipids, free fatty acids, phosphatides, color bodies, sterols, odors, and other impurities. As used herein, a "refined oil" is a crude lipid or crude oil that has been refined. As used herein, "a crude lipid" or "a crude oil" is a lipid or oil that has not been refined. In some embodiments, the lipid separated from a demulsified cell composition is a crude lipid.

[0168] Various exemplary processes of the present invention are described schematically in **FIGs. 1-4**. Referring to **FIG. 1**, in some embodiments, the present invention is directed to a process (**100**) for obtaining a lipid (**110**) from a cell (**101**), comprising lysing (**102**) the cell (**101**) to form a lysed cell composition (**103**). The lysed cell composition is contacted with a first base (**104**) to demulsify lysed cell composition (**103**), contacted with a salt (**105**) to demulsify lysed cell composition (**103**), and heated (**106**), e.g., for 10 minutes to 96 hours, to provide a treated lysed cell composition (**107**). The treated lysed cell composition (**107**) is contacted with a second base (**108**) and separated (**109**), e.g., at a temperature of 10° C to 100° C, to provide a lipid (**110**).

[0169] Referring to **FIG. 2**, in some embodiments, the present invention is directed to a process (**200**) for obtaining a lipid (**210**) from a cell, the process comprising lysing (**102**) a cell (**101**) to form a lysed cell composition (**103**). The lysed cell composition is then contacted with a base (**204**) to demulsify lysed cell composition (**103**) and to provide a treated lysed cell composition (**207**). The treated lysed cell composition (**207**) is separated (**209**), e.g., at a temperature of 10° C to 100° C, to provide a lipid (**210**).

[0170] Referring to **FIG. 3**, in some embodiments, the present invention is directed to a process (**300**) for obtaining a lipid (**310**) from a cell, the process comprising lysing (**102**) a cell (**101**) to form a lysed cell composition (**103**). The lysed cell composition is then contacted with a salt (**305**) to demulsify lysed cell composition (**103**) and to provide a treated lysed cell composition (**307**), which is separated (**309**), e.g., at a temperature of 10° C to 100° C, to provide a lipid (**310**).

[0171] Referring to **FIG. 4**, in some embodiments, the present invention is directed to a process (**400**) for obtaining a lipid (**410**) from a cell, the process comprising lysing (**102**) a cell (101) to form a lysed cell composition (**103**). The lysed cell composition is then contacted with a salt (**405**) to demulsify lysed cell composition (**103**) and agitated (**401**), e.g., for 5 minutes to 96 hours, and optionally heated (**402**), to provide a treated lysed cell composition (**407**). The treated lysed cell composition is then separated (**409**), e.g., at a temperature of 10° C to 100° C, to provide a lipid (**410**).

[0172] In some embodiments, a process of the present invention comprises concentrating a broth comprising a microbial cell, a broth comprising plant material and/or concentrating a lysed cell composition. As used herein, "concentrating" refers to removing water from a composition. Concentrating can include, but is not limited to, evaporating, chemical drying, centrifuging, and the like, and combinations thereof.

[0173] In some embodiments, a broth comprising a microbial cell or a broth comprising plant material is concentrated to provide a lipid concentration of at least 4%, at least 5%, at least 10%,

at least 15%, at least 20%, at least 25%, or at least 30% by weight of the broth. In some embodiments, a broth comprising a microbial cell or a broth comprising plant material is concentrated to provide a lipid concentration of 4% to 40%, 4% to 30%, 4% to 20%, 4% to 15%, 5% to 40%, 5% to 30%, 5% to 20%, 10% to 40%, 10% to 30%, 10% to 20%, 15% to 40%, 15% to 30%, 20% to 40%, 20% to 30%, 25% to 40%, or 30% to 40% by weight of the broth.

[0174] In some embodiments, a cell composition or a lysed cell composition is concentrated to provide a lipid concentration of at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, or at least 30% by weight of the lysed cell composition. In some embodiments, a cell composition or a lysed cell composition is concentrated to provide a lipid concentration of 4% to 40%, 4% to 30%, 4% to 20%, 4% to 15%, 5% to 40%, 5% to 30%, 5% to 20%, 10% to 40%, 10% to 30%, 10% to 20%, 15% to 40%, 15% to 30%, 20% to 40%, 20% to 30%, 25% to 40%, or 30% to 40% by weight of the lysed cell composition.

[0175] In some embodiments, a lipid prepared by a process of the present invention has an overall aroma intensity of 2 or less. As used herein, the term "overall aroma intensity" refers to the olfactory sensory rating given to the lipid by a panel of sensory analysts. As used herein, the term "sensory analyst" refers to a trained individual that provides feedback on and/or rates the sensory characteristics of a substance.

[0176] In some embodiments, a lipid prepared by a process of the present invention has an overall aromatic intensity of 3 or less. As used herein, the term "overall aromatic intensity" refers to the gustatory, or taste, sensory rating given to the lipid by a panel of sensory analysts. In some embodiments, the Universal Spectrum descriptive analysis method is used to assess the aroma and aromatic characteristics of samples. This method uses an intensity scale of 0 - 15, where 0 = none detected and 15 = very high intensity, to measure the aroma and aromatic attributes of the oils.

[0177] In some embodiments, a lipid prepared by a process of the present invention does not have an aftertaste characterized as fishy. As used herein, the term "aftertaste" refers to the persistence of a sensation of a flavor in the lipid, as characterized by a panel of sensory analysts.

[0178] In some embodiments, a process of the present invention provides a crude lipid having a peroxide value (PV) of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less. As used herein, the terms "peroxide value" or "PV" refer to the measure of primary reaction products, such as peroxides and hydroperoxides, that occur during oxidation of the lipid. In some embodiments, the PV is an indicator of the quality of the lipid and the extent of oxidation which has occurred in the lipid

having a low PV (i.e., 5 or less) demonstrates increased stability and sensory profiles than lipids having a PV greater than 5. In some embodiments, adding a base to a lysed cell composition, as discussed above, raises the pH of the lysed cell composition and inhibits lipid oxidation, thereby minimizing the amount of free radicals in the lysed cell composition so that the crude lipid obtained from the processes of the invention has a low PV (i.e., 5 or less).

[0179] In some embodiments, a process of the present invention provides a crude lipid having an anisidine value (AV) of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less. As used herein, the terms "anisidine value" or "AV" refer to the measure of secondary reaction products, such as aldehydes and ketones, that occur during oxidation of the lipid. In some embodiments, the AV is an indicator of the quality of the lipid and the extent of oxidation which has occurred in the lipid. A lipid having a low AV (i.e., 26 or less) demonstrates increased stability and sensory profiles than lipids having an AV greater than 26. In some embodiments, adding a base to a lysed cell composition, as discussed above, raises the pH of the lysed cell composition and inhibits lipid oxidation, thereby minimizing the amount of free radicals in the lysed cell composition so that the crude lipid obtained from the processes of the invention has a low AV (i.e., 26 or less).

[0180] In some embodiments, a process of the present invention provides a crude lipid having a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less.

[0181] In some embodiments, a process of the present invention provides a crude lipid that has a lower anisidine value, lower peroxide value, lower phosphorus content and/or a higher extraction yield than if extraction was performed using a solvent (e.g., atypical hexane extraction or a FRIOLEX® process (Westfalia Separator AG, Germany)). The FRIOLEX® process which is a process of extracting lipids with a water-soluble organic solvent as described in U.S. Patent No. 5,928,696 and International Pub. Nos. WO 01/76385 and WO 01/76715, each of which is incorporated by reference herein in its entirety.

[0182] In some embodiments, heating the lysed cell composition causes the secondary reaction products (e.g., aldehydes and ketones) to participate in a reaction similar to the Maillard reaction with proteins present in the lysed cell composition. The reaction is believed to create products that possess antioxidant activity, which reduces the oxidation of the lipid. In some embodiments,

additional protein, e.g., soy protein, can be added to the lysed cell composition to increase the antioxidant activity. The reduction in oxidation of the lipid reduces the AV of the lipid, reduces any aftertaste of the lipid and/or increases the stability of the lipid. In some embodiments, the stability is increased at least 5%, at least 10%, at least 15% or at least 20%.

[0183] In some embodiments, a lipid extracted by a process of the present invention, the biomass remaining after extraction of the lipid, or combinations thereof can be used directly as a food or food ingredient, such as an ingredient in baby food, infant formula, beverages, sauces, dairy based foods (such as milk, yogurt, cheese and ice-cream), oils (e.g., cooking oils or salad dressings), and baked goods; nutritional supplements (e.g., in capsule or tablet forms); feed or feed supplement for any non-human animal (e.g., those whose products (e.g., meat, milk, or eggs) are consumed by humans); food supplements; and pharmaceuticals (in direct or adjunct therapy application). The term "animal" refers to any organism belonging to the kingdom *Animalia* and includes any human animal, and non-human animal from which products (e.g., milk, eggs, poultry meat, beef, pork or lamb) are derived. In some embodiments, the lipid and/or biomass can be used in seafood. Seafood is derived from, without limitation, fish, shrimp and shellfish. The term "products" includes any product derived from such animals, including, without limitation, meat, eggs, milk or other products. When the lipid and/or biomass are fed to such animals, polyunsaturated lipids can be incorporated into the flesh, milk, eggs or other products of such animals to increase their content of these lipids.

Microbial Lipids

[0184] In some embodiments, the present invention is directed to a microbial lipid extracted according to the processes of the present invention. In some embodiments, a crude microbial lipid has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less, and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less. In some embodiments, the crude microbial lipid has less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% by weight or volume of an organic solvent. In some embodiments, the crude microbial lipid has at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of a desired

PUFA. In some embodiments, the crude microbial lipid has at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of DHA, and/or at least 10%, at least 15%, or at least 20% by weight of DPA n-6, and/or at least 10%, at least 15%, or at least 20% by weight of EPA, and/or at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by weight of ARA. In some embodiments a crude microbial lipid extracted according to the processes of the present invention result in a lower anisidine value, lower peroxide value, lower phosphorus content and/or a higher extraction yield than if extraction was performed using a solvent (e.g., a typical hexane extraction or a FRIOLEX® process (Westfalia Separator AG, Germany)).

Lipids Extracted From a First Set of Isolated Thraustochytrid Microorganisms

[0185] In some embodiments, the present invention is further directed to a microbial lipid extracted from a thraustochytrid as described in U.S. Pub. No. 2010/0239533 and Int'l. Pub. No. WO 2010/107415, each of which is incorporated by reference herein in its entirety. In some embodiments, the method comprises growing a thraustochytrid in a culture to produce a biomass and extracting a lipid comprising omega-3 fatty acids from the biomass. The lipid can be extracted from a freshly harvested biomass or can be extracted from a previously harvested biomass that has been stored under conditions that prevent spoilage. Known methods can be used to culture a thraustochytrid of the invention, to isolate a biomass from the culture, and to analyze the fatty acid profile of oils extracted from the biomass. *See*, e.g., U.S. Patent No. 5,130,242, incorporated by reference herein in its entirety. The lipid can be extracted according to the processes of the present invention.

[0186] A microbial lipid of the invention can be any lipid derived from a microorganism, including, for example: a crude oil extracted from the biomass of the microorganism without further processing; a refined oil that is obtained by treating a crude microbial oil with further processing steps such as refining, bleaching, and/or deodorizing; a diluted microbial oil obtained by diluting a crude or refined microbial oil; or an enriched oil that is obtained, for example, by treating a crude or refined microbial oil with further methods of purification to increase the concentration of a fatty acid (such as DHA) in the oil.

[0187] In some embodiments, the microbial lipid comprises a sterol esters fraction of 0%, at least 0.1%, at least 0.2%, at least 0.5%, at least about 1%, at least 1.5%, at least 2%, or at least 5% by weight. In some embodiments, the microbial lipid comprises a sterol esters fraction of from 0% to 1.5%, 0% to 2%, 0% to 5%, 1% to 1.5%, 0.2% to 1.5%, 0.2% to 2%, or 0.2% to 5% by weight.

In some embodiments, the microbial lipid comprises a sterol esters fraction of less than 5%, less than 4%, less than 3%, or less than 2% by weight.

[0188] In some embodiments, the microbial lipid comprises a triglyceride fraction of at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% by weight. In some embodiments, the microbial lipid comprises a triglyceride fraction of from 65% to 95%, 75% to 95%, or 80% to 95% by weight, or 97% by weight, or 98% by weight.

[0189] In some embodiments, the microbial lipid comprises a free fatty acid fraction of at least 0.5%, at least 1%, at least 1.5%, at least 2%, at least 2.5%, or at least 5% by weight. In some embodiments, the microbial lipid comprises a free fatty acid fraction of from 0.5% to 5%, 0.5% to 2.5%, 0.5% to 2%, 0.5% to 1.5%, 0.5% to 1%, 1% to 2.5%, 1% to 5%, 1.5% to 2.5%, 2% to 2.5%, or 2% to 5% by weight. In some embodiments, the microbial lipid comprises a free fatty acid fraction of less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% by weight.

[0190] In some embodiments, the microbial lipid comprises a sterol fraction of at least 0.5%, at least 1%, at least 1.5%, at least 2%, or at least 5% by weight. In some embodiments, the microbial lipid comprises a sterol fraction of from 0.5% to 1.5%, 1% to 1.5%, 0.5% to 2%, 0.5% to 5%, 1% to 2%, or 1% to 5% by weight. In some embodiments, the microbial lipid comprises a sterol fraction of less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% by weight.

[0191] In some embodiments, the microbial lipid comprises a diglyceride fraction of at least 1.5%, at least 2%, at least 2.5%, at least 3%, at least 3.5%, or at least 5% by weight. In some embodiments, the microbial lipid comprises a diglyceride fraction of from 1.5% to 3%, 2% to 3%, 1.5% to 3.5%, 1.5% to 5%, 2.5% to 3%, 2.5% to 3.5%, or 2.5% to 5% by weight.

[0192] In some embodiments, the microbial lipid comprises unsaponifiables of less than 2%, less than 1.5%, less than 1%, or less than 0.5% by weight of the oil.

[0193] The lipid classes present in the microbial oil, such as the triglyceride fraction, can be separated by flash chromatography and analyzed by thin layer chromatography (TLC), or separated and analyzed by other methods know in the art.

[0194] In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, and combinations thereof, comprises at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, or at least 80% by weight DHA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the

triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, and combinations thereof, comprises from 40% to 45%, 40% to 50%, 40% to 60%, 50% to 60%, 55% to 60%, 40% to 65%, 50% to 65%, 55% to 65%, 40% to 70%, 40% to 80%, 50% to 80%, 55% to 80%, 60% to 80%, or 70% to 80% by weight DHA. In some embodiments, the microbial lipid comprises a sterol esters fraction comprising 45% or less, 40% or less, 35% or less, 30% or less, 25% or less, 20% or less, 15% or less, or 13% or less by weight DHA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, and combinations thereof, comprises 10% or less, 9% or less, 8% or less, 7% or less, 6% or less, 5% or less, 4% or less, 3% or less, 2% or less, or 1% or less by weight EPA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, and combinations thereof, comprises from 2% to 3%, 2% to 3.5%, 2.5% to 3.5%, 2% to 6%, 2.5% to 6%, 3.0% to 6%, 3.5% to 6%, 5% to 6%, or 2% to 10% by weight EPA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, is substantially free of EPA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises a weight ratio of DHA to EPA of at least 5:1, at least 7:1, at least 9:1, at least 10:1, at least 15:1, at least 20:1, at least 25:1, at least 30:1, or at least 50:1, wherein the microbial lipid and/or one or more fractions thereof comprises 10% or less by weight of EPA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises a weight ratio of DHA to EPA of at least 5:1, but less than 20:1. In some embodiments, the weight ratio of DHA to EPA is from 5:1 to 18:1, from 7:1 to 16:1, or from 10:1 to 15:1. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof comprises from 0.1% to 0.25%, 0.2% to 0.25%, 0.1% to 0.5%, or 0.1% to 1.5% by weight ARA. In some embodiments, the microbial lipid and/or one or more fractions

thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises 1.5% or less, 1% or less, 0.5% or less, 0.2% or less, or 0.1% or less by weight ARA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, is substantially free of ARA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises a weight ratio of DHA to ARA of at least 20:1, at least 30:1, at least 35:1, at least 40:1, at least 60:1, at least 80:1, at least 100:1, at least 150:1, at least 200:1, at least 250:1, or at least 300:1. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises from 0.5% to 1%, 0.5% to 2%, 0.5% to 2.5%, 0.5% to 3%, 0.5% to 3.5%, 0.5% to 5%, 0.5% to 6%, 1% to 2%, 2% to 3%, 2% to 3.5%, 1% to 2.5%, 1% to 3%, 1% to 3.5%, 1% to 5%, or 1% to 6% by weight DPA n-6. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises 6% or less, 5% or less, 3% or less, 2.5% or less, 2% or less, 1% or less, or 0.5% or less by weight DPA n-6. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, is substantially free of DPA n-6. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises a weight ratio of DHA to DPA n-6 of greater than 6:1, of at least 8:1, at least 10:1, at least 15:1, at least 20:1, at least 25:1, at least 50:1, or at least 100:1. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the

phospholipid fraction), and combinations thereof, comprises 5% or less, 4% or less, 3% or less, 2% or less, 1.5% or less, 1% or less, or 0.5% or less by weight each of linoleic acid (18:2 n-6), linolenic acid (18:3 n-3), eicosenoic acid (20:1 n-9), and erucic acid (22:1 n-9). In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises 5% or less, 4% or less, 3% or less, 2% or less, 1.5% or less, or 1% or less by weight of heptadecanoic acid (17:0). In some embodiments, the microbial lipid and/or one or more fractions thereof comprise 0.01% to 5% by weight, 0.05% to 3% by weight, or 0.1% to 1% by weight of heptadecanoic acid.

[0195] In some embodiments, an extracted microbial lipid comprises a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, and wherein the oil has an anisidine value of 26 or less. In some embodiments, an extracted microbial lipid comprises a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1, and wherein the lipid has an anisidine value of 26 or less. In some embodiments, an extracted microbial lipid comprises a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight and wherein the lipid has an anisidine value of 26 or less. In some embodiments, an extracted microbial lipid having any of the above fatty acid profiles has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less. In some embodiments, an extracted microbial lipid having any of the above fatty acid profiles is extracted from an isolated thraustochytrid microorganism having the characteristics of the thraustochytrid species deposited under ATCC Accession No. PTA-9695, PTA-9696, PTA-9697,

or PTA-9698. In some embodiments, an extracted microbial lipid having any of the above fatty acid profiles is a crude lipid. In some embodiments, the crude lipid has less than 5% by weight or volume of an organic solvent. In some embodiments the microbial lipid extracted according to the processes of the present invention result in a lower anisidine value, lower peroxide value, lower phosphorus content and/or a higher extraction yield if extraction was performed using a solvent (e.g., atypical hexane extraction or a FRIOLEX® process (Westfalia Separator AG, Germany)).

Lipids Extracted From a Second Set of Isolated Thraustochytrid Microorganisms

[0196] In some embodiments, the present invention is further directed to a microbial lipid extracted from a thraustochytrid as described in U.S. Appl. No. 12/729,013 and PCT/US2010/028175, each of which is incorporated by reference herein in its entirety. In some embodiments, the method comprises growing a thraustochytrid in a culture to produce a biomass and extracting a lipid comprising omega-3 fatty acids from the biomass. The lipid can be extracted from a freshly harvested biomass or can be extracted from a previously harvested biomass that has been stored under conditions that prevent spoilage. Known methods can be used to culture a thraustochytrid of the invention, to isolate a biomass from the culture, and to analyze the fatty acid profile of oils extracted from the biomass. *See*, e.g., U.S. Patent No. 5,130,242, incorporated by reference herein in its entirety. The lipid can be extracted according to the processes of the present invention.

[0197] A microbial lipid of the invention can be any lipid derived from a microorganism, including, for example: a crude oil extracted from the biomass of the microorganism without further processing; a refined oil that is obtained by treating a crude microbial oil with further processing steps such as refining, bleaching, and/or deodorizing; a diluted microbial oil obtained by diluting a crude or refined microbial oil; or an enriched oil that is obtained, for example, by treating a crude or refined microbial oil with further methods of purification to increase the concentration of a fatty acid (such as DHA) in the oil.

[0198] In some embodiments, the microbial lipid comprises a sterol esters fraction of 0%, at least 0.1%, at least 0.2%, at least 0.5%, at least 1%, at least 1.5%, at least 2%, or at least 5% by weight. In some embodiments, the microbial lipid comprises a sterol esters fraction of 0% to 1.5%, 0% to 2%, 0% to 5%, 1% to 1.5%, 0.2% to 1.5%, 0.2% to 2%, or 0.2% to 5% by weight. In some embodiments, the microbial lipid comprises a sterol esters fraction of 5% or less, 4% or less, 3% or less, 2% or less, 1% or less, 0.5% or less, 0.3% or less, 0.2% or less, 0.5% or less, 0.4% or less, 0.3% or less, or 0.2% or less by weight.

[0199] In some embodiments, the microbial lipid comprises a triacylglycerol fraction of at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% by weight. In some embodiments, the microbial lipid comprises a triacylglycerol fraction of 35% to 98%, 35% to 90%, 35% to 80%, 35% to 70%, 35% to 70%, 35% to 65%, 40% to 70%, 40% to 65%, 40% to 55%, 40% to 50%, 65% to 95%, 75% to 95%, 75% to 98%, 80% to 95%, 80% to 98%, 90% to 96%, 90% to 97%, 90% to 98%, 90%, 95%, 97%, or 98% by weight.

[0200] In some embodiments, the microbial lipid comprises a diacylglycerol fraction of at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20% by weight. In some embodiments, the microbial lipid comprises a diacylglycerol fraction of 10% to 45%, 10% to 40%, 10% to 35%, 10% to 30%, 15% to 40%, 15% to 35%, or 15% to 30% by weight. In some embodiments, the microbial lipid comprises a 1,2-diacylglycerol fraction of at least 0.2%, at least 0.3%, at least 0.4%, at least 0.5%, at least 1%, at least 5%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20% by weight. In some embodiments, the microbial lipid comprises a diacylglycerol fraction of 0.2% to 45%, 0.2% to 30%, 0.2% to 20%, 0.2% to 10%, 0.2% to 5%, 0.2% to 1%, 0.2% to 0.8%, 0.4% to 45%, 0.4% to 30%, 0.4% to 20%, 0.4% to 10%, 0.4% to 5%, 0.4% to 1%, 0.4% to 0.8%, 0.5% to 1%, 0.5% to 0.8%, 10% to 45%, 10% to 40%, 10% to 35%, 10% to 30%, 15% to 40%, 15% to 35%, 15% to 30%, or 15% to 25% by weight. In some embodiments, the microbial lipid comprises a 1,3-diacylglycerol fraction of at least 0.1%, at least 0.2%, at least 0.5%, at least 1%, at least 2%, at least 2.5 %, or at least 3% by weight.

[0201] In some embodiments, the microbial lipid comprises a sterol fraction of at least 0.3%, at least 0.4%, at least 0.5%, at least 1%, at least 1.5%, at least 2%, or at least 5% by weight. In some embodiments, the microbial lipid comprises a sterol fraction of 0.3% to 5%, 0.3% to 2%, 0.3% to 1.5%, 0.5% to 1.5%, 1% to 1.5%, 0.5% to 2%, 0.5% to 5%, 1% to 2%, or 1% to 5% by weight. In some embodiments, the microbial lipid comprises a sterol fraction of 5% or less, 4% or less, 3% or less, 2% or less, 1.5% or less, or 1% or less by weight.

[0202] In some embodiments, the microbial lipid comprises a phospholipid fraction of at least 2%, at least 5%, or at least 8% by weight. In some embodiments, the microbial lipid comprises a phospholipid fraction of 2% to 25%, 2% to 20%, 2% to 15%, 2% to 10%, 5% to 25%, 5% to 20%, 5% to 20%, 5% to 10%, or 7% to 9% by weight. In some embodiments, the microbial lipid comprises a phospholipid fraction of less than 20%, less than 15%, less than 10%, less than 9%,

or less than 8% by weight. In some embodiments, the microbial lipid is substantially free of phospholipids.

[0203] In some embodiments, the microbial lipid comprises unsaponifiables of less than 2%, less than 1.5%, less than 1%, or less than 0.5% by weight of the oil.

[0204] The lipid classes present in the microbial lipid, such as a triacylglycerol fraction, can be separated by flash chromatography and analyzed by thin layer chromatography (TLC), or separated and analyzed by other methods known in the art.

[0205] In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the free fatty acid fraction, the sterol fraction, the diacylglycerol fraction, and combinations thereof, comprises at least 5%, at least 10%, more than 10%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 25%, at least 30%, least 35%, at least 40%, or at least 45% by weight EPA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the free fatty acid fraction, the sterol fraction, the diacylglycerol fraction, and combinations thereof, comprises 5% to 55%, 5% to 50%, 5% to 45%, 5% to 40%, 5% to 35%, 5% to 30%, 10% to 55%, 10% to 50%, 10% to 45%, 10% to 40%, 10% to 35%, 10% to 30%, at least 12% to 55%, at least 12% to 50%, at least 12% to 45%, at least 12% to 40%, at least 12% to 35%, or at least 12% to 30%, 15% to 55%, 15% to 50%, 15% to 45%, 15% to 40%, 15% to 35%, 15% to 30%, 15% to 25%, 15% to 20%, 20% to 55%, 20% to 50%, 20% to 45%, 20% to 40%, or 20% to 30% by weight EPA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the diacylglycerol fraction, the sterol fraction, the sterol esters fraction, the free fatty acids fraction, the phospholipid fraction, and combinations thereof, comprises at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, or at least 60% by weight DHA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the diacylglycerol fraction, the sterol fraction, the sterol esters fraction, the free fatty acids fraction, the phospholipid fraction, and combinations thereof, comprises 5% to 60%, 5% to 55%, 5% to 50%, 5% to 40%, 10% to 60%, 10% to 50%, 10% to 40%, 20% to 60%, 25% to 60%, 25% to 50%, 25% to 45%, 30% to 50%, 35% to 50%, or 30% to 40% by weight DHA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the diacylglycerol fraction, the sterol fraction, the sterol esters fraction, the free fatty acids fraction, the phospholipid fraction, and combinations thereof, comprises 10% or less, 9% or less, 8% or less, 7% or less, 6% or less, 5%

or less, 4% or less, 3% or less, 2% or less, or 1% or less by weight DHA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the diacylglycerol fraction, the sterol fraction, the sterol esters fraction, the free fatty acids fraction, the phospholipid fraction, and combinations thereof, comprises 1% to 10%, 1% to 5%, 2% to 5%, 3% to 5%, or 3% to 10% by weight of the fatty acids as DHA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the diacylglycerol fraction, the sterol fraction, the sterol esters fraction, the free fatty acids fraction, the phospholipid fraction, and combinations thereof, is substantially free of DHA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the diacylglycerol fraction, the sterol fraction, the sterol esters fraction, the free fatty acids fraction, the phospholipid fraction, and combinations thereof, comprises 0.1% to 5%, 0.1% to less than 5%, 0.1% to 4%, 0.1% to 3%, 0.1% to 2%, 0.2% to 5%, 0.2% to less than 5%, 0.2% to 4%, 0.2% to 3%, 0.2% to 2%, 0.3% to 2%, 0.1% to 0.5%, 0.2% to 0.5%, 0.1% to 0.4%, 0.2% to 0.4%, 0.5% to 2%, 1% to 2%, 0.5% to 1.5%, or 1% to 1.5% by weight ARA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the diacylglycerol fraction, the sterol fraction, the sterol esters fraction, the free fatty acids fraction, the phospholipid fraction, and combinations thereof, comprises 5% or less, less than 5%, 4% or less, 3% or less, 2% or less, 1.5% or less, 1% or less, 0.5% or less, 0.4% or less, 0.3% or less, 0.2% or less, or 0.1% or less by weight ARA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the diacylglycerol fraction, the sterol fraction, the sterol esters fraction, the free fatty acids fraction, the phospholipid fraction, and combinations thereof, is substantially free of ARA. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the diacylglycerol fraction, the sterol fraction, the sterol esters fraction, the free fatty acids fraction, the phospholipid fraction, and combinations thereof, comprises 0.4% to 2%, 0.4% to 3%, 0.4% to 4%, 0.4% to 5%, 0.4% to less than 5%, 0.5% to 1%, 0.5% to 2%, 0.5% to 3%, 0.5% to 4%, 0.5% to 5%, 0.5% to less than 5%, 1% to 2%, 1% to 3%, 1% to 4%, 1% to 5%, or 1% to less than 5% by weight DPA n-6. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the diacylglycerol fraction, the sterol fraction, the sterol esters fraction, the free fatty acids fraction, the phospholipid fraction, and combinations thereof, comprises 5%, less than 5%, 4% or less, 3% or less, 2% or less, 1% or less, 0.75% or less, 0.6% or less, or 0.5% or less by weight DPA n-6. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the

triacylglycerol fraction, the diacylglycerol fraction, the sterol fraction, the sterol esters fraction, the free fatty acids fraction, the phospholipid fraction, and combinations thereof, is substantially free of DPA n-6. In some embodiments, the microbial lipid and/or one or more fractions thereof selected from the triacylglycerol fraction, the diacylglycerol fraction, the sterol fraction, the sterol esters fraction, the free fatty acids fraction, the phospholipid fraction, and combinations thereof, comprises fatty acids with 5% or less, less than 5%, 4% or less, 3% or less, or 2% or less by weight of oleic acid (18:1 n-9), linoleic acid (18:2 n-6), linolenic acid (18:3 n-3), eicosenoic acid (20:1 n-9), erucic acid (22:1 n-9), stearidonic acid (18:4 n-3), or combinations thereof.

[0206] In some embodiments, an extracted microbial lipid comprises at least 20% by weight eicosapentaenoic acid and less than 5% by weight each of arachidonic acid, docosapentaenoic acid n-6, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, erucic acid, and stearidonic acid. In some embodiments, an extracted microbial lipid comprises a triacylglycerol fraction of at least 10% by weight, wherein at least 12% by weight of the fatty acids in the triacylglycerol fraction is eicosapentaenoic acid, wherein at least 25% by weight of the fatty acids in the triacylglycerol fraction is docosahexaenoic acid, and wherein less than 5% by weight of the fatty acids in the triacylglycerol fraction is arachidonic acid. In some embodiments, an extracted microbial lipid having any of the above fatty acid profiles has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, 2 or less, or 1 or less, and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less. In some embodiments, an extracted microbial lipid having any of the above fatty acid profiles is extracted from an isolated thraustochytrid microorganism having the characteristics of the thraustochytrid species deposited under ATCC Accession No. PTA-10208, PTA-10209, PTA-10210, PTA-10211, PTA-10212, PTA-10213, PTA-10214, or PTA-10215. In some embodiments, an extracted microbial lipid having any of the above fatty acid profiles is a crude lipid. In some embodiments, the crude lipid has less than 5% by weight or volume of an organic solvent. In some embodiments a microbial lipid extracted according to the processes of the present invention result in a lower anisidine value, and/or lower peroxide value, and/or lower phosphorus content than if extraction was

performed using a typical hexane extraction or a FRIOLEX® process (Westfalia Separator AG, Germany).

[0207] In some embodiments, a lipid obtained by any of the processes of the present invention comprises at least 20% by weight eicosapentaenoic acid and less than 5% by weight each of arachidonic acid, docosapentaenoic acid n-6, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, erucic acid, and stearidonic acid. In some embodiments, a lipid obtained by any of the processes of the present invention comprises a triacylglycerol fraction of at least 10% by weight, wherein at least 12% by weight of the fatty acids in the triacylglycerol fraction is eicosapentaenoic acid, wherein at least 25% by weight of the fatty acids in the triacylglycerol fraction is docosahexaenoic acid, and wherein less than 5% by weight of the fatty acids in the triacylglycerol fraction is arachidonic acid. In some embodiments, a lipid obtained by any of the processes of the present invention having any of the above fatty acid profiles has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, or 2 or less, or 1 or less and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less, and/or a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less. In some embodiments, a lipid obtained by any of the processes of the present invention having any of the above fatty acid profiles is extracted from an isolated thraustochytrid microorganism having the characteristics of the thraustochytrid species deposited under ATCC Accession No. PTA-10208, PTA-10209, PTA-10210, PTA-10211, PTA-10212, PTA-10213, PTA-10214, or PTA-10215. In some embodiments, a lipid obtained by any of the processes of the present invention having any of the above fatty acid profiles is a crude lipid. In some embodiments, the crude lipid has less than 5% by weight or volume of an organic solvent. In some embodiments a lipid extracted according to the processes of the present invention result in a lower anisidine value, lower peroxide value, lower phosphorus content and/or a higher extraction yield if extraction was performed using a solvent (e.g., atypical hexane extraction or a FRIOLEX® process (Westfalia Separator AG, Germany)).

Lipids Extracted From an Isolated Microorganism of the Species *Crypthecodinium cohnii*

[0208] In some embodiments, the present invention is further directed to a crude lipid extracted from a microorganism of the species *Crypthecodinium cohnii*. In some embodiments, the method comprises growing a microorganism of the species *Crypthecodinium cohnii* in a culture to produce a biomass and extracting a lipid comprising omega-3 fatty acids from the biomass. The lipid can be extracted from a freshly harvested biomass or can be extracted from a previously harvested biomass that has been stored under conditions that prevent spoilage. Known methods can be used to culture a microorganism of the species *Crypthecodinium cohnii*, and to isolate a biomass from the culture. *See*, e.g., U.S. Patent No. 7,163,811, incorporated by reference herein in its entirety. The lipid can be extracted according to the processes of the present invention.

[0209] In some embodiments, the crude lipid extracted from a microorganism of the species *Crypthecodinium cohnii* according to the extraction methods of the present invention can have a lower phosphorus content compared to using a typical hexane extraction method. In some embodiments, the crude lipid extracted from a microorganism of the species *Crypthecodinium cohnii* comprises a phosphorus content of 100 ppm or less, 95 ppm or less, 90 ppm or less, 85 ppm or less, 80 ppm or less, 75 ppm or less, 70 ppm or less, 65 ppm or less, 60 ppm or less, 55 ppm or less, 50 ppm or less, 45 ppm or less, 40 ppm or less, 35 ppm or less, 30 ppm or less, 25 ppm or less, 20 ppm or less, 15 ppm or less, 10 ppm or less, 5 ppm or less, 4 ppm or less, 3 ppm or less, 2 ppm or less, or 1 ppm or less. In some embodiments, the crude oil has an anisidine value of 26 or less, 25 or less, 20 or less, 15 or less, 10 or less, 5 or less, or 2 or less, or 1 or less and/or a peroxide value of 5 or less, 4.5 or less, 4 or less, 3.5 or less, 3 or less, 2.5 or less, 2 or less, 1.5 or less, 1 or less, 0.5 or less, 0.2 or less, or 0.1 or less. In some embodiments a crude microbial lipid extracted according to the processes of the present invention result in a lower anisidine value, lower peroxide value, lower phosphorus content and/or a higher extraction yield if extraction was performed using a solvent (e.g., atypical hexane extraction or a FRIOLEX® process (Westfalia Separator AG, Germany)).

[0210] Having generally described the invention, a further understanding can be obtained by reference to the examples provided herein. These examples are given for purposes of illustration only and are not intended to be limiting. The following examples are illustrative, but not limiting, of a process and a lipid prepared by a process of the present invention. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in extraction of a lipid from a cell, and which would become apparent to those skilled in the art, and are within the spirit and scope of the invention.

EXAMPLES

Example 1

[0211] A cell broth (20,000 kg) containing microbial cells (*Schizochytrium*) was heated to 60 °C. Enzymes (i.e., Alcalase 2.4 L FG 0.5%) were added to the cell biomass to lyse the cells and form an emulsified lysed cell composition. The emulsified lysed cell composition was first treated with a first base (NaOH, 250 kg of 50% w/w solution) until the pH of the lysed cell composition was from 10.4 to 10.6. Next, a salt (solid NaCl, in an amount of 2%, by weight, of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then heated to a temperature of 85° C to 102° C and held at that temperature level for 24 hours to 70 hours. A second base (NaOH, 50% w/w solution, 40 kg) was then added to the lysed cell composition until the pH was above 8. The lysed cell composition was then centrifuged to separate the lysed cell composition into three phases: a top phase containing a lipid layer, a middle phase containing an emulsion layer, and a bottom phase containing a solid layer. The lysed cell composition was then centrifuged at 40° C to 80° C using a Westfalia RSE110 Centrifuge (Westfalia Separator Industry GmbH, Germany), operating at 6,000 rpms at a feed rate of 30 kg/min to separate a lipid from the lysed cell composition. The centrifuging provided three phases: an upper phase containing a lipid, a middle phase containing an emulsion, and a bottom phase containing a solid/liquid emulsion. The pH of the lysed cell composition was maintained at 7.5 to 8.5 during the centrifuging. The total time to centrifuge the entire 20,000 kg batch was approximately 10 to 11 hours. The lipid layer was separated and had a moisture content of approximately 1% by weight.

Example 2

[0212] A cell broth (500 g) containing microbial cells (*Crypthecodinium cohnii*) was concentrated from approximately 7% biomass to 13.5% biomass, by weight of the broth. The broth was homogenized at a pressure of 10,000 psi (2 passes) to form a lysed cell composition. The lysed cell composition was treated with a base (i.e., NaOH, 10 g of a 50% w/w solution) until the pH of the lysed cell composition was 10.4 to 10.6. A salt (solid NaCl, in an amount of 2% by weight of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then heated to a temperature of 85° C to 92° C and held at that temperature range for 15 minutes to 2 hours. The lysed cell composition was then centrifuged at

a temperature of 70° C to 90° C using a Bench Top Sigma 6K15 Centrifuge (SIGMA Laborzentrifugen GmbH, Germany), operating at 5,400 rpm to separate the lysed cell composition into three phases: an upper phase containing a lipid, a middle phase containing an emulsion, and a bottom phase containing a solid/liquid emulsion. The pH of the lysed cell composition during centrifuging was maintained at 6.5 to 8.5. The total time to centrifuge was 5 minutes. The lipid layer was separated and had a moisture content of approximately 1% by weight.

Example 3

[0213] A cell broth (20,000 kg) containing microbial cells (*Schizochytrium*) was heated to 60° C. Enzymes (i.e., *Alcalase* 2.4 L FG 0.5%) were added to the cell biomass to lyse the cells and form a lysed cell composition. Next, a salt (solid Na_2SO_4, 2,000 kg, or 10%, by weight, of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then agitated for 24 hours to 48 hours at room temperature. The lysed cell composition was then centrifuged at 40° C to 75° C using a Westfalia RSE110 Centrifuge (Westfalia Separator Industry GmbH, Germany), operating at 6,000 rpm at a feed rate of 40 kg/min to separate a lipid from the lysed cell composition. The centrifuging provided three phases: an upper phase containing a lipid, a middle phase containing an emulsion, and a bottom phase containing a solid/liquid emulsion. The total time to centrifuge the entire 20,000 kg batch was approximately 8 to 9 hrs. The lipid layer was separated from the centrifuged lysed cell composition.

Example 4

[0214] A pasteurized cell broth (500 g) containing microbial cells (ATCC Accession No. PTA-10208) was provided. Enzymes (i.e., *Alcalase* 2.4 L FG 0.5%) were added to the cell biomass to lyse the cells and form an emulsified lysed cell composition. The emulsified lysed cell composition was treated with a first base (i.e., a 25% solution of NaOH) to adjust the pH of the lysed cell composition to 10.5. Next, a salt (solid NaCl, in an amount of 2%, by weight, of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then heated to a temperature of 95° C and held at that temperature level for 2 hours while agitating the lysed cell composition. A second base (i.e., a 25% solution of NaOH) was then added to the lysed cell composition until the pH was 8.3. The lysed cell composition was then

centrifuged at 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a lipid layer and a small emulsion layer.

Example 5

[0215] A cell broth (500 g) that was concentrated and pasteurized containing microbial cells (ATCC Accession No. PTA-9695) was provided. Enzymes (i.e., *Alcalase* 2.4 L FG 0.5%) were added to the cell biomass to lyse the cells and form an emulsified lysed cell composition. The emulsified lysed cell composition was treated with a base (i.e., a 25% solution of NaOH) to adjust the pH of the lysed cell composition to 10.5. Next, a salt (solid NaCl, in an amount of 2%, by weight, of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then heated to a temperature of 95° C and held at that temperature level for 1 hour while agitating the lysed cell composition and the pH dropped to 8.5. After an hour in the fermentation broth having a total of 10 ml, there was an about 1 ml layer of oil (lipid) and an about 6 ml layer of emulsion. The lysed cell composition was heated for a total of 220 minutes and the emulsion layer started to disappear. The lysed cell composition was then centrifuged at 5,100 rpm for 5 minutes to separate the lysed cell composition. The extraction yield of the lipid was 58.8 by weight%. The anisidine value (AV) of the crude oil was 11.3. The cell breakage yield was in a range of 93% to 95% by weight.

Example 6

[0216] A pasteurized cell broth (473 g) containing microbial cells of the isolated thraustochytrid deposited under ATCC Accession No. PTA-9695 was provided. Enzymes (i.e., *Alcalase* 2.4 L FG 0.5%) were added to the cell biomass to lyse the cells and form an emulsified lysed cell composition. The emulsified lysed cell composition was treated with a first base (i.e., a 25% solution of NaOH) to adjust the pH of the lysed cell composition to 10.62. Next, a salt (solid NaCl, in an amount of 2%, by weight, of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then heated to a temperature of 95° C and held at that temperature level for 3 hours while agitating the lysed cell composition. A second base (i.e., a 25% caustic solution of NaOH) was then added to the lysed cell composition until the pH was 8.13. The lysed cell composition was then centrifuged at 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a lipid layer and an emulsion layer in equal amounts. In order to determine if raising the pH increased the yield of the lipid layer, additional second base (i.e., a

25% solution of NaOH) was added to the separated lysed cell composition until the pH was 9.02 and the lysed cell composition was again centrifuged at 5,100 rpm for 5 minutes. This resulted in a similar yield of lipid layer. Additional second base was added again to the separated lysed cell composition until the pH was 10.12 and the lysed cell composition was again centrifuged at 5,100 rpm for 5 minutes. Again, this resulted in a similar yield of lipid layer.

Example 7

[0217] A pasteurized cell broth (470 g) containing microbial cells (ATCC Accession No. PTA-9695) was provided. The cell biomass was mechanically homogenized to lyse the cells and form an emulsified lysed cell composition. The emulsified lysed cell composition was treated with a first base (i.e., a 25% solution of NaOH) to adjust the pH of the lysed cell composition to 10.5. Next, a salt (solid NaCl, in an amount of 2%, by weight, of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then heated to a temperature of 95° C and held at that temperature level for 3 hours while agitating the lysed cell composition. A second base (i.e., a 25% solution of NaOH) was then added to the lysed cell composition until the pH was 8.07. The lysed cell composition was then centrifuged at 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a lipid layer and an emulsion layer wherein the emulsion layer was larger than the lipid layer. In order to determine if raising the pH increased the yield of the lipid layer, additional second base was added to the separated lysed cell composition until the pH was 9.11 and the lysed cell composition was again centrifuged at 5,100 rpm for 5 minutes. This resulted in a similar yield of lipid layer. Additional second base was added again to the separated lysed cell composition until the pH was 10.09 and the lysed cell composition was again centrifuged at 5,100 rpm for 5 minutes. Again, the resulted in a similar yield of lipid layer.

Example 8

[0218] A cell broth containing microbial cells (*Crypthecodinium cohnii*) was used in a decreased biotin trial fermentor. 20,000 kg of washed, concentrated, and pasteurized broth was harvested. This was pulled out at the startup of pasteurization. It was held for approximately 1 day before being transferred and homogenized. The material was homogenized at 813 bar/one pass and collected back into a treatment tank. Through microscopic inspection, it was estimated that approximately 80% of the cells were lysed.

[0219] The broth was heated to about 40° C before treatment began. The pH was adjusted to 10.5 and 2% NaCl was added and heated to 66° C. At this point a significant oil layer had already formed and the pH had dropped to 9.5 after 1–2 hours. The broth was held at 66° C overnight.

[0220] The next day, the broth was centrifuged on the Westfalia RSE-110 with a 155 mm ring dam installed. The viscosity was about 180 cP at 40° C. The centrifuge was fed at 48 kg/min, with 5 – 10 psi backpressure on the light phase and 30 psi backpressure on the heavy phase. The feed temperature was maintained at 70° C. No oil was present in the waste phase and only a few drops were visible after isopropyl alcohol was added.

[0221] Table 1 shows the results of analyses performed on the crude oil obtained from this procedure.

Table 1. Specifications of crude oil obtained using process of Example 8.

% Oil	87.79
DHA (mg/g)	531.02
% DHA	60.49
PV	1.95 (0.6*)
AV	15
%FFA	0.18
Phosphorus (ppm)	8.65
Copper (ppm)	0.22
Iron (ppm)	0.7
Lead (ppm)	0.63

*PV of the centrifuged oil.

[0222] Of the 20,000 kg of broth provided, 10.5% by weight (2,100 kg) was biomass. Of the biomass, 50% by weight was oil (1,050 kg). Of the oil, 58.9% by weight was DHA (618 kg). After running the process described above, there was 592.5 kg of material in the lipid layer, of which about 87.8% by weight (520.2 kg) was oil. Thus, the extraction yield of oil from the biomass was 49.5%. Of that oil, 60.5% by weight (314.6 kg) was DHA, thereby resulting in an extraction yield of 51% by weight DHA from the biomass.

Example 9

[0223] A cell broth (about 500g) that was washed, concentrated, and pasteurized containing microbial cells (*Schizochytrium*) was provided. The broth was chemically treated with a base (i.e., a 25% solution of NaOH) without a prior cell lysis step. The addition of the base raised the

pH of the broth from 5.8 to 11.2. The addition of the base and the rise in the pH lysed the cells to form a lysed cell composition. Next, a salt (solid Na_2SO_4, in an amount of 5%, by weight, of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then heated to a temperature in a range of 90° C to 95° C and held at that temperature level for 90 minutes and the pH of the lysed cell composition dropped to 9.7. After the 90 minutes there was an about 2.5 ml of oil layer per 45 ml of fermentation broth and there was no moisture loss. After 3 hours, the pH had dropped to 9.2. The solution was then centrifuged at about 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a lipid layer. The extraction yield of the lipid was 81% by weight. The anisidine value (AV) of the crude oil was 20.1. The cell breakage yield was in a range of 92% to 98% by weight.

Example 10

[0224] A cell broth (about 500g) that was washed, concentrated, and pasteurized containing microbial cells (*Schizochytrium*) was provided. The broth was chemically treated with a base (i.e., a 25% solution of NaOH) without a prior cell lysis step. The addition of the base raised the pH of the broth from 4.8 to 11. The addition of the base and the rise in the pH lysed the cells to form a lysed cell composition. Next, a salt (solid NaCl, in an amount of 2%, by weight, of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then heated to a temperature in a range of 90° C to 95° C and held at that temperature level for 3.5 hours and the pH of the lysed cell composition dropped to 8.7 and there was no moisture loss. The solution was then centrifuged at about 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a lipid layer. The extraction yield of the lipid after 3.5 hours was 92% by weight.

[0225] A portion of the lysed cell composition was held for 6 hours and the pH of the lysed cell composition dropped to 8.6. The solution was then centrifuged at about 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a lipid layer. The extraction yield of the lipid after 6 hours was 89% by weight. The anisidine value (AV) of the crude oil was 14.4. The cell breakage yield was 95% by weight.

Example 11

[0226] A cell broth (about 500g) that was washed, concentrated, and pasteurized containing microbial cells (*Schizochytrium*) was provided. The broth was chemically treated with a base

(i.e., a 50% solution of NaOH) without a prior cell lysis step. The addition of the base raised the pH of the broth from 5.8 to 11.2. The addition of the base and the rise in the pH lysed the cells to form a lysed cell composition. The lysed cell composition was then heated to 70° C under vacuum to reduce the water content from 88.7% to 85.5%. During evaporation, the pH dropped to 10.36. The solution was then centrifuged at about 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a lipid layer. The extraction yield of the lipid was 83.9% by weight. The anisidine value (AV) of the crude oil was 10.5. The cell breakage yield was 93.17% by weight.

[0227] The process was repeated except the lysed cell composition was heated to 70° C under vacuum to reduce the water content from 88.7% to 79.2%. The extraction yield of the lipid was 87.5% by weight when the water content was reduced to 79.2% and the cell breakage yield was 92.3% by weight. The process was also repeated to reduce the water content from 88.7% to 80.8%. The extraction yield of the lipid was 90% by weight when the water content was reduced to 80.8% and the cell breakage yield was 95.9% by weight.

Example 12

[0228] A cell broth (about 500g) that was washed, concentrated, and pasteurized containing microbial cells (*Schizochytrium*) was provided. The broth was chemically treated with a base (i.e., a 50% solution of NaOH) without a prior cell lysis step. The addition of the base raised the pH of the broth from 5.6 to 11.1. The addition of the base and the rise in the pH lysed the cells to form a lysed cell composition. The lysed cell composition was then heated to 90° C in a closed system for 40 minutes. After the 40 minutes, there was an about 1 ml layer of oil (lipid) per 40 ml of fermentation broth. The solution was then centrifuged at about 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a lipid layer. The extraction yield of the lipid was 85.1% by weight. The anisidine value (AV) of the crude oil was 16.3. The cell breakage yield was 97.6% by weight.

Example 13

[0229] A cell broth (about 500g) that was washed, concentrated, and pasteurized containing microbial cells (*Schizochytrium*) was provided. The broth was chemically treated with a base (i.e., a 50% solution of NaOH) without a prior cell lysis step. The addition of the base raised the pH of the broth from 4.9 to 11.2. The addition of the base and the rise in the pH lysed the cells to

form a lysed cell composition. The lysed cell composition was then mixed at room temperature for 4 hours. The solution was then centrifuged at about 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a small lipid layer.

[0230] A portion of the lysed cell composition was mixed at room temperature for about 96 hours. The solution was then centrifuged at about 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a larger lipid layer. The extraction yield of lipid was 61.4% by weight. The anisidine value (AV) of the crude oil was 22.6.

Example 14

[0231] A cell broth (about 500g) that was washed, concentrated, and pasteurized containing microbial cells (ATCC Accession No. PTA-9695) was provided. The broth was chemically treated with a base (i.e., a 50% solution of NaOH) without a prior cell lysis step. The addition of the base raised the pH of the broth from 5.6 to 11.1. The addition of the base and the rise in the pH lysed the cells to form a lysed cell composition. The lysed cell composition was then heated in a range of 70° C to 75° C for 3 hours. The solution was then centrifuged at about 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a lipid layer. The extraction yield of the lipid was 84.4% by weight.

[0232] A portion of the lysed cell composition was heated for a total of 5 hours. The solution was then centrifuged at about 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a similar lipid layer. The extraction yield of lipid was 87.3% by weight. The cell breakage yield was 89.1% by weight.

Example 15

[0233] A cell broth (about 500g) that was washed, concentrated, and pasteurized containing microbial cells (*Schizochytrium*) was provided. The broth was chemically treated with a base (i.e., a 50% solution of NaOH) without a prior cell lysis step. The addition of the base raised the pH of the broth from 7.3 to 11. The addition of the base and the rise in the pH lysed the cells to form a lysed cell composition. Next, a salt (solid Na_2SO_4, in an amount of 5%, by weight, of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then heated to a temperature of 90° C and held at that temperature level for 2 hours. While maintaining a temperature of 90° C for additional 2 to 4 hours, the vessel containing the lysed cell composition was opened to allow evaporation of water. The solution was then centrifuged at

about 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a lipid layer. Extraction yield of the lipid was greater than 70% by weight. The anisidine value (AV) of the crude oil was 11.6.

Example 16

[0234] A cell broth (9,925 kg) containing microbial cells (ATCC Accession No. PTA-9695) was provided. The cell broth was diluted with water in a 1:1 ratio by weight to form a diluted broth of 20,000 kg. The solid content of the broth prior to dilution was 16.13% by weight and after dilution was 8.25% by weight. The diluted broth was mixed and centrifuged with a desludging centrifuge at 6,400 rpm to remove extracellular water-soluble or water-dispersible compounds. The concentrate (10,250 kg) from the centrifuge was collected and had a solids content of 10.5% by weight. The collected concentrate was heated to 62° C to 64° C to pasteurize the concentrate. Enzymes (i.e., *Alcalase* 2.4 L FG 0.5%) were added to the pasteurized concentrate to lyse the cells and form an emulsified lysed cell composition. The emulsified lysed cell composition was treated with a base (i.e., a 25% solution of NaOH) to adjust the pH of the lysed cell composition to 11. Next, a salt (solid Na_2SO_4, in an amount of 5%, by weight, of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then heated to a temperature of 95° C and held at that temperature level for 10 hours to 12 hours while agitating the lysed cell composition. After agitation, the pH of the lysed cell composition was 8.6 and there was a very small emulsion layer. The agitation tank was allowed to cool to 60° C and the pH of the lysed cell composition increased to 9.6 while cooling. The pH of the lysed cell composition was lowered to 8.2 by adding phosphoric acid. The addition of the phosphoric acid did not harm the separation of the lipid layer and the very small emulsion layer. The lysed cell composition was then centrifuged at 5,100 rpm at a feed rate of 48 kg/min for 5 minutes at 60° C to 63° C to separate the lysed cell composition and yield a lipid layer having a moisture content of 1.7% to 2.3% by weight.

Example 17

[0235] A cell broth (500 g) that was washed, concentrated, and pasteurized containing microbial cells (*Crypthecodinium cohnii*) was provided. The broth was homogenized at a pressure of 8,000 to 12,000 psi (2 passes) to form a lysed cell composition. The lysed cell composition was treated with a base (i.e., a 12.5% solution of NaOH) until the lysed cell composition reached a pH of 7.8

to 8.2. A salt (solid Na_2SO_4, in an amount of 5% by weight of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then heated to a temperature of 60° C and held at that temperature. The pH of the lysed cell composition was maintained at the 7.8 to 8.2 level by the addition of base (i.e., a 12.5% solution of NaOH) for 10 to 15 hours in a closed system with little to no moisture loss. The lysed cell composition was then centrifuged at about 5,100 rpm for 5 minutes to separate the lysed cell composition and yield an oil layer. This resulted in an oil layer of about 2 ml in a sample of 40 ml. The extraction yield of the oil was 73% by weight. The anisidine value (AV) of the crude oil was 13.5. The cell breakage yield was 82% to 86% by weight.

Example 18

[0236] A pasteurized cell broth (1,000 g) containing microbial cells (*Schizochytrium*) was provided. Enzymes (i.e., *Alcalase* 2.4 L FG 0.5%) were added to the cell biomass to lyse the cells and form an emulsified lysed cell composition. The emulsified lysed cell composition was treated with a base (i.e., a 12.5% solution of NaOH) to adjust the pH of the lysed cell composition from 7.21 to 10.52. Next, a salt (solid NaCl, in an amount of 2%, by weight, of the lysed cell composition) was added to the lysed cell composition. The broth was then separated into 4 portions with each portion being held at 4 different temperatures and times: 1) Trial #1 was held at 90°C for 22 hours; 2) Trial #2 was held at 90°C for 2 hours and then held at 25°C for 20 hours; 3) Trial #3 was held at 60°C for 22 hours; and 4) Trial #4 was held at 25°C for 22 hours. The individual trials were then centrifuged without further pH adjustment. For Trials #1, #2, and #3, the broth was centrifuged at approximately 6,600 rpm (a g-force of 4,800) for 5 minutes to separate the lysed cell composition. Because the separation for Trial #4 was not good (<20%) at a g-force of 4,800, the g-force was increased to 15,000 and the broth was spun at a g-force of 15,000 for 5 minutes. The extraction yield of the lipid as a weight percent and the anisidine value (AV) are listed in the table below.

Table 2. Conditions and Results When Varying Temperature and Heating Time of the Lysed Cell Composition.

Trial #	Treatment Time and Temp.	Centrifugation Conditions	AV	Oil Yield (%)
1	90°C for 22 hours	pH = 6.22 g-force = 4,800	58.7	51.4

2	90°C for 2 hours, 25°C for 20 hours	pH = 8.19 g-force = 4,800	109.2	82.2
3	60°C for 22 hours	pH = 8.38 g-force = 4,800	91.2	27.2
4	25°C for 22 hours	pH = 10.03 g-force = 15,000	105.2	55.7

[0237] The anisidine values in Table 2 were higher than expected. One difference between previous examples and this example was that the lysed cell composition was allowed to sit for a long period of time before the lipids were extracted. It is hypothesized that the long period of time before extraction leads to the oxidation of the dissolved oxygen present in the lysed cell composition. The increased oxidation then leads to an increase in the anisidine value. The fact that the trial heated at the highest temperature for the longest time (Trial #1) had the lowest anisidine value supports this hypothesis because the dissolved oxygen content of a lysed cell composition generally decreases as the temperature is increased. The increased anisidine values are therefore believed to be an anomaly that was a result of the delay in extracting the lipids from the lysed cell composition. In production, there would be no delay time in extracting the lipids from the lysed cell composition and the anisidine values would be consistent with previous results of anisidine values of 26 or less.

Example 19

[0238] A pasteurized cell broth (1,000 g) containing microbial cells (*Schizochytrium*) was provided. The broth was then split into 3 portions and diluted as follows: 1) Trial #1 was not diluted at all and served as the control portion; 2) Trial #2 was diluted 25% with water; and 3) Trial #3 was diluted 50% with water. Enzymes (i.e., *Alcalase* 2.4 L FG 0.5%) were added to the cell biomass to lyse the cells and form an emulsified lysed cell composition. The emulsified lysed cell composition was treated with a base (i.e., a 12.5% solution of NaOH) to adjust the pH of the lysed cell composition from 6.8 to 10.6. Next, a salt (solid NaCl, in an amount of 2%, by weight, of the lysed cell composition) was added to the lysed cell composition. The broth was then heated to 90°C and held for 20 hours. After the hold time, the broth for each trial was separated into two with one half being centrifuged as is and the other half having its pH adjusted to approximately 8.5 before centrifugation. Both portions were then centrifuged at

approximately 8,545 rpm (a g-force of 8,000) for 5 minutes. The extraction yield of the lipid as a weight percent and the anisidine value (AV) are listed in the table below.

Table 3. Conditions and Results When Varying Dilution of the Pasteurized Broth.

Trial #	Diluted?	Centrifugation Conditions	AV	Oil Yield (%)
1	No dilution	pH = 6.0 g-force = 8,000	51.8	81.2
1a	No dilution	pH = 8.4 g-force = 8,000	44.3	78.1
2	25% dilution with water	pH = 5.5 g-force = 8,000	76.1	88.9
2a	25% dilution with water	pH = 8.4 g-force = 8,000	85.3	82.1
3	50% dilution with water	pH = 5.7 g-force = 8,000	68.5	85.0
3a	50% dilution with water	pH = 8.5 g-force = 8,000	79.6	84.0

[0239] The anisidine values in Table 3 were higher than expected. One difference between previous examples (excluding Example 18) and this example was that the lysed cell composition was allowed to sit for a long period of time before the lipids were extracted. It is hypothesized that the long period of time before extraction leads to the oxidation of the dissolved oxygen present in the lysed cell composition. The increased oxidation then leads to an increase in the anisidine value. The increased anisidine values are therefore believed to be an anomaly that was a result of the delay in extracting the lipids from the lysed cell composition. In production, there would be no delay time in extracting the lipids from the lysed cell composition and the anisidine values would be consistent with previous results of anisidine values of 26 or less.

Example 20

[0240] Cell broths obtained from various fermentation lots were treated using the process described in Example 2 except the timing of adding the salt (e.g., before and after homogenization) and the amount of salt were varied. The resulting separated lipids were analyzed and the analyses are provided in Table 4.

Table 4. Specifications of lipids obtained using processes of the present invention varying the timing of salt addition and the amount of salt added.

Fermentation Lot	P2137	P2137	P4167	P4167	P2137	P2137
Addition of NaCl*	Before	Before	After	After	After	After
% NaCl by weight	2	5	2	5	5	5
% Lipid*	27	37	51	72	59	15
% Starting Solids by weight	13	12.9	19.2	19.2	13.3	7.5+
% Solids by weight before Centrifugation	19.7	21.7	19.7	20.1	20.2	8.6

* Addition of NaCl was before or after homogenization; % lipid refers to the percentage of lipids in triglyceride form

\+ This run was diluted to have a low percentage of starting solids.

[0241] The data provided in Table 4 demonstrates that adding the salt after homogenization results in higher % lipid values than adding the salt before homogenization. The data provided in Table 4 also demonstrates that diluting the sample resulted in lower a % lipid value.

Example 21

[0242] A sample of Alcalase enzyme treated lysed cell composition obtained from microbial cells (*Schizochytrium*) was used. The sample had a pH of approximately 5.5. The sample was divided into 4 smaller samples and the pH of three of the samples was adjusted to approximately 7.4, approximately 10.5, and approximately 12, respectively, by adding sodium hydroxide. The samples were diluted in a 1:1 ratio with deionized water. POBN (α-(4-Pyridyl 1-oxide)-N-tert-butylnitrone, 1.25 M; 50 µL) was added as a spin trap chemical to 0.5 g of each of the diluted samples. The samples were measured with a Bruker BioSpin e-scan EPR (Electron Paramagnetic Resonance) spectrometer (system number SC0274) (Bruker BioSpin, Billerica, MA) to measure the amount of free radicals present from lipid oxidation. The samples were incubated at room temperature (20° C) and 50 µL of each of the POBN containing samples was tested at hourly intervals for four hours after adjusting the pHs using the following spectrometer parameters: modulation frequency of 86 Hz, modulation amplitude of 2.11 gauss, microwave power of 5.19 mW, time constant of 20.48 seconds, sweep time of 10.49 seconds, sweep width of 100 gauss, and a number of scans of 8. The results of the EPR spectrometer readings are provided in **FIG. 5**.

[0243] The data in **FIG. 5** demonstrates that initially the level of free radicals was highest for the sample at pH 5.5 and lowest for the samples at pH 10.5 and 12. The data also demonstrates that over the 4 hour period the rate of radical formation was slowest for the sample at pH 10.5 and

highest for the sample at pH 5.5. The data also demonstrates that the addition of a base to the lysed cell composition inhibits lipid oxidation, and therefore leads to a low AV in the crude lipid and refined oil.

Example 22

[0244] Oilseeds are extracted from a rapeseed plant and are then passed through a grinding mill to crack and break the outer hull of the oilseeds. The oilseeds are then dehulled through known means, such as through aspiration, to remove the meat (interior) of the seeds from the hull of the oilseeds. The dehulled oilseeds are then homogenized or expelled by passing them through a press to grind the dehulled oilseeds into a cake in order to lyse the cells of the oilseeds. Water is added to form an emulsified lysed cell composition. The emulsified lysed cell composition is filtered to remove any excess hull fragments from the lysed cell composition. The emulsified lysed cell composition is treated with a base (i.e., a 25% solution of NaOH) to adjust the pH of the lysed cell composition to 11. Next a salt (solid NaCl, in an amount 2% by weight of the lysed cell composition) is added to the lysed cell composition. The lysed cell composition is then heated to a temperature of 90° C and held at that level for 6 hours to 48 hours while agitating the lysed cell composition. The lysed cell composition is then centrifuged at 5,100 rpm for 5 minutes to separate the lysed cell composition and yield a lipid layer and an emulsion layer.

Example 23

Comparative Analysis of Crude Lipids Obtained by Hexane Extraction

[0245] The crude lipids obtained from a lot using the process described in Example 2 was analyzed to determine various specifications. Additional crude lipids were obtained using a typical hexane extraction process on the same microbial cell utilized in Example 2. The hexane extraction process included spray drying a fermentation broth, adding hexane to the spray dried biomass to obtain a solution of 15% to 20% solids by weight. The solution was then homogenized to lyse the cells to form a lysed cell composition. The lysed cell composition was centrifuged and a layer containing lipid and hexane was removed. The hexane was then removed from the lipid. The results of the analyses are provided in Table 5.

Table 5. Specifications of lipids obtained using processes of the present invention or a hexane extraction process.

Fermentation Lot	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Extraction Method	Ex. 2	Hexane	Hexane	Hexane	Hexane	Hexane	Hexane
AV*	5.9	ND	ND	ND	14.7	17.18	6.7
PV*	1.21	0.65	1.56	0.46	ND	0.85	0.3
% Lipid*	89.61	86.94	84.31	86.75	85.53	86.05	86.54
DHA (mg/g)	537.47	508.15	459.32	465.31	510.49	495.82	506.33
% DHA*	59.98	58.39	54.49	53.65	59.71	57.68	58.51

* AV = Anisidine Value; PV = peroxide value; % lipid refers to the percentage of lipids in triglyceride form; % DHA refers to the percentage of DHA in the lipid

[0246] The data provided in Table 5 demonstrates that the crude lipids obtained by the processes of the present invention exhibit superior anisidine values, percentage of lipid, amount of DHA and percentage of DHA compared to lipids prepared by typical hexane extraction processes.

Comparative Analysis of Crude Lipids Obtained by the FRIOLEX® Processes

Example 24

[0247] The crude lipids obtained from various fermentation lots using the processes described in Examples 1 and 3 were analyzed to determine various specifications. Additional crude lipids were obtained using a FRIOLEX® process (Westfalia Separator AG, Germany), which is a process of extracting lipids with a water-soluble organic solvent as described in U.S. Patent No. 5,928,696 and International Pub. Nos. WO 01/76385 and WO 01/76715. The results of the analyses are provided in Table 6.

Table 6. Specifications of lipids obtained using processes of the present invention or a FRIOLEX® process.

Fermentation Lot	A	B	B	B	B	C	C	C
Extraction Method	Ex. 1	Ex. 1	Ex. 3	Ex. 1[a]	Ex. 1[b]	Ex. 1	Ex. 1	FRIOLEX®
AV*	3.1	1.6	3.9	300	7.1	3.5	4	36
PV*	1.8	0.17	0.14	6.16	0.34	0	0	0.35
% Lipid*	96.27	93.67	92.55	87.20	94.42	95.14	94.31	93.92
DHA (mg/g)	452.4	458.16	455.17	414.66	471.55	416.41	416.05	415.38
Extraction Yield (%)	93.5	87	ND	96	ND	94	94	94

* AV = Anisidine Value; PV = peroxide value; % lipid refers to the percentage of lipids in triglyceride form

a: The lysed cell composition was not heated.

b: The lysed cell composition was allowed to stand for 3 weeks prior to extraction.

[0248] The data provided in Table 6 demonstrates that the crude lipids obtained by the processes of the present invention exhibit superior anisidine values (with the exception of the lipid obtained when the lysed cell composition was not heated) compared to lipids prepared by a FRIOLEX® process. The lipids prepared by a process of the present invention exhibit anisidine values that are from 4.4% to 19.7% of the anisidine values of a lipid prepared using the FRIOLEX® process.

[0249] It is believed that a lipid prepared by a process of the present invention has increased stability. For example, as shown in Table 6, a process of the present invention was used to extract a lipid from a lysed cell composition, wherein the lysed cell composition was allowed to stand for 3 weeks prior to the extraction process. It is believed that the anisidine value of a lipid in a lysed cell composition increases with time, and thus, it would be expected that a lipid extracted from a 3 week old lysed cell composition have increased anisidine values. However, as shown in Table 6, the lipid obtained from the 3 week old lysed cell composition using a process of the present invention had an anisidine value which was 19.7% of the anisidine value of a lipid prepared by the FRIOLEX® process.

Example 25

[0250] The crude lipids obtained from a broth of microbial cells (ATCC Accession No. PTA-9695) using the process described in Example 16 were analyzed to determine various specifications. Additional crude lipids were obtained from a broth of microbial cells (ATCC Accession No. PTA-9695) using a FRIOLEX® process (Westfalia Separator AG, Germany), which is a process of extracting lipids with a water-soluble organic solvent (e.g., isopropyl alcohol) as

described in U.S. Patent No. 5,928,696 and International Pub. Nos. WO 01/76385 and WO 01/76715. The results of the analyses are provided in Table 7.

Table 7. Crude Lipid Comparison

	Example 16	**FRIOLEX®**
% Oil	9.19	93.73
DHA (mg/g)	570.68	574.33
% DHA	62.1	61.27
% FFA	1.13	0.22
PV	0	0.74
AV	10	73.9
Iron (ppm)	0.11	0
Copper (ppm)	0.67	0.3
Lead (ppm)	0.21	0
Phosphorus (ppm)	5.22	7.20
Extraction yield (%)	61.4	45.3

[0251] The data provided in Table 7 demonstrates that the crude lipids obtained by the processes of the present invention exhibit superior anisidine values (AV) and peroxide values (PV) compared to lipids prepared by a FRIOLEX® process. The data also demonstrates that the extraction yield of lipid obtained by the processes of the present invention are superior compared to the extraction yield of lipid obtained by a FRIOLEX® process.

Example 26

Refining of Crude Lipids

[0252] Crude lipids were obtained using the processes outlined in Example 1 and a FRIOLEX® process. The crude lipids were further processed by sequentially: 1) degumming and caustic refining; 2) bleaching; 3) chilled filtering; and 4) deodorizing with antioxidants. The data for the crude lipids, caustic refined lipids, bleached lipids, and deodorized lipids are presented in Tables 8a and 8b. A comparison of the refined oils is presented in Table 9.

Table 8a. Lipids obtained from the FRIOLEX® process (Example 24)

Processing Step	FFA %	PV	AV	DHA (mg/g)	Yield % (Lipid)	Yield % (DHA)
Crude Lipid	0.28	0.37	33.6	413.6	N/A	N/A
Caustic Refined Lipid	<0.1	0.29	--	416.4	85.7	86.3
Bleached Lipid	<0.1	0.16	13.9	413.1	71.5	70.9
Filtered Lipid	<0.1	0.19	13.3	424.86	70.3	76.0
Deodorized Lipid w/AOX*	0.06	<0.1	14.3	401.5	96.6	89.4

*AOX refers to antioxidants

Table 8b. Lipids obtained from an extraction process using NaCl (Example 1)

Processing Step	FFA %	PV	AV	DHA (mg/g)	Yield % (Lipid)	Yield % (DHA)
Crude Lipid	1.36	0	3.5	406.7	N/A	N/A
Caustic Refined Lipid	<0.1	0.27	2.3	410.8	85.9	86.7
Bleached Lipid	<0.1	0.16	0.8	404.3	97.0	95.5
Filtered Lipid	<0.1	0.37	1.0	414.1	59.3	60.7
Deodorized Lipid w/AOX	0.06	<0.1	1.8	379.9*	94.9	87.1

*Note: Increased dilution with high oleic sunflower oil (HOSO) was the reason for the decrease in DHA (mg/g).

Table 9. Refined Oil Comparison

	FRIOLEX® (Example 24)	Example 1
DHA (mg/g)	401.5	379.9*
% DHA	42.69	40.18
FFA%	0.06	0.06
PV	<0.1	<0.1
AV	14.3	1.8
Iron (ppm)	0.05	<0.02
Copper (ppm)	<0.02	<0.02
Lead (ppm)	<0.1	<0.1
Arsenic (ppm)	<0.1	<0.1
Mercury (ppm)	<0.01	<0.01
% Moisture and volatiles	<0.01	<0.01
Unsaponifiables (%)	1.17	1.33
Trans-fatty acid by IR (%)	<1	<1

*Note: Increased dilution with HOSO was the reason for the decrease in DHA (mg/g).

[0253] The data provided in Table 8a, Table 8b, and Table 9 demonstrate that a refined oil prepared by a process of the present invention exhibits lower anisidine values compared to a refined oil prepared by the FRIOLEX® process.

Example 27

Sensory Profile Comparison

[0254] The refined oils obtained in Example 26 were analyzed by a panel of 8 to 12 sensory analysts. The sensory analysts rated various lipid specifications based on aroma, aromatics, and aftertaste to provide an "overall aroma intensity" for each lipid. The Universal Spectrum descriptive analysis method was used to assess the aroma and aromatic characteristics of samples. This method uses an intensity scale of 0 - 15, where 0 = none detected and 15 = very high intensity, to measure the aroma and aromatic attributes of the oils. The results of the sensory data are provided in Table 10.

Table 10. Sensory specifications of a lipid prepared by the FRIOLEX® process (Example 24) and a lipid prepared by a process of the present invention (Example 1)

	Specifications						
	Aroma						
	Overall Intensity	Fishy Marine Complex	Green Complex	Herbaceous	Nutty Roasted	Painty	Other
FRIOLEX®	3	1	1	1	0	0	0
Ex. 1	2	0	1	1	0	0	0
	Aromatics						
	Overall Intensity	Fishy Marine Complex	Green Complex	Herbaceous	Nutty Roasted	Painty	Other
FRIOLEX®	4	1	1	2	0	0	0
Ex. 1	3	0	1	2	0	0	0
	Aftertaste						
FRIOLEX®	Herbal/slightly fishy						
Ex. 1	Herbal						

[0255] The data provided in Table 10 demonstrates that a refined oil prepared by a process of the present invention exhibits superior sensory data compared to a refined oil prepared by the FRIOLEX® process. As shown above, the lipids provided by the present invention had an overall aroma intensity of 3 and 2, whereas, the FRIOLEX® lipids provided an overall aroma intensity of 4 and 3, respectively.

Example 28

Comparative Example

[0256] An extraction process for obtaining lipids from microorganisms without the use of an organic solvent is disclosed in U.S. Patent No. 6,750,048. A comparison of a refined oil obtained from a crude lipid prepared by a process of the present invention and a refined oil obtained from a crude lipid prepared by the extraction process disclosed in U.S. Patent No. 6,750,048 is provided in Table 11.

Table 11. Comparative data for a lipid prepared by a process of the present invention (Example 1) and a lipid prepared by a process disclosed in U.S. Patent No. 6,750,048.

	Example 1	**U.S.P.N. 6,750,048**
DHA (mg/g)	379.9	346
% DHA	40.18	37.3
FFA%	0.06	ND
PV	<0.1	0.46
AV	1.8	ND
Iron (ppm)	<0.02	0.26
Copper (ppm)	<0.02	<0.05
Lead (ppm)	<0.1	<0.20
Arsenic (ppm)	<0.1	<0.20
Mercury (ppm)	<0.01	<0.20
% Moisture and volatiles	<0.01	0.02
Unsaponifiables (%)	1.33	ND
Trans-fatty acid by IR (%)	<1	ND

[0257] The data provided in Table 11 demonstrates that a refined oil obtained from a crude lipid prepared by a process of the present invention exhibits superior properties compared to a refined oil obtained from a crude lipid prepared by the extraction process disclosed in U.S. Patent No. 6,750,048.

EXAMPLE 29

[0258] The isolated thraustochytrid (ATCC Accession No. PTA-9695) was characterized for taxonomic classification.

[0259] Samples were collected from intertidal habitats during low tide. Water, sediment, living plant material and decaying plant/animal debris were placed into sterile 50 ml tubes. Portions of each sample along with the water were spread onto solid agar plates of isolation media. Isolation

media consisted of: 500 ml of artificial seawater, 500 ml of distilled water, 1 g of glucose, 1 g of glycerol, 13 g of agar, 1 g of glutamate, 0.5 g of yeast extract, 0.5 g casein hydrolysate, 1 ml of a vitamin solution (100 mg/L thiamine, 0.5 mg/L biotin, 0.5 mg B_{12}), 1 ml of a trace mineral solution (PII metals, containing per liter: 6.0 g $FeCl_36H_2O$, 6.84 g H_3BO_3, 0.86 g $MnCl_24H_2O$, 0.06 g $ZnCl_2$, 0.026 $CoCl_26H_20$, 0.052 g $NiSO_4H_2O$, 0.002 g $CuSO_45H_2O$ and 0.005 g $Na_2MoO_42H_2O$), and 500 mg each of penicillin G and streptomycin sulfate. The agar plates were incubated in the dark at 20-25°C. After 2-4 days the agar plates were examined under magnification, and colonies of cells were picked with a sterile toothpick and restreaked onto a fresh plate of media. Cells were repeatedly streaked onto fresh media until contaminated organisms were removed.

[0260] Colonies from agar plates were transferred to petri dishes with half-strength seawater and (1 ml) of a suspension of autoclaved newly hatched brine shrimp larvae. The brine shrimp larvae became heavily overgrown with clusters of sporangia after 2-3 days. Released zoospores were biflagellate at discharge, swimming actively away from the mature sporangium, wall remnants of which are clearly visible (in phase contrast) after spore release. Sporangia measured 12.5 μm to 25 μm in diameter, and zoospores were 2.5 μm to 2.8 μm x 4.5 μm to 4.8 μm in size. There were 8 to 24 spores per individual sporangium. Settled zoospores enlarged and rapidly underwent binary divisions leading to tetrads, octads, and finally to clusters of sporangia. Tetrad formation commenced at a very early stage prior to maturity of the sporangia. These characteristics are in agreement with the genus *Schizochytrium*.

[0261] The isolated thraustochytrid (ATCC Accession No. PTA-9695) was further characterized based on the similarity of its 18s rRNA gene to that of known species. Total genomic DNA from the thraustochytrid (ATCC Accession No. PTA-9695) was prepared by standard procedures (Sambrook J. and Russell D. 2001. Molecular cloning: A laboratory manual, 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York) and used for PCR amplification of the 18s RNA gene. The PCR amplification of the 18s rRNA gene was carried out with primers previously described (Honda *et. al., J. Eukaryot. Microbiol.* 46(6) 1999). The PCR conditions with chromosomal DNA template were as follows: 0.2 μM dNTPs, 0.1 uM each primer, 8% DMSO, 200 ng chromosomal DNA, 2.5 U PfuUltra® II fusion HS DNA polymerase (Stratagene), and 1X PfuUltra® buffer (Stratagene) in a 50 μL total volume. The PCR Protocol included the following steps: (1) 95°C for 2 minutes; (2) 95°C for 45 seconds; (3) 55°C for 30 seconds; (4) 72°C for 2 minutes; (5) repeat steps 2-4 for 40 cycles; (6) 72°C for 5 minutes; and (7) hold at 6°C.

[0262] PCR amplification yielded a distinct DNA product with the expected size using chromosomal template described above. The PCR product was cloned into the vector pJET1.2/blunt (Fermentas) according to the manufacturer's instructions, and the insert sequence was determined using supplied standard primers.

[0263] Table 12 shows a comparison of the 18s rRNA sequence from the thraustochytrid (ATCC Accession No. PTA-9695) to DNA sequences in the National Center for Biotechnology Information (NCBI) electronic database. Briefly, "% Identity" was determined by the scoring matrix "swgapdnamt" within the "AlignX" program of the VectorNTI program (Invitrogen), a standard for DNA alignment. The "% Coverage" was taken from the results of a Basic Local Alignment Search Tool (BLAST) calculation from the NCBI electronic database and is the percent of the query length that is included in the aligned segments.

Table 12: Comparison of 18s rRNA Sequences

Thraustochytrids	**% Identity Calculation #1**	**% Coverage Calculation #2**
Thraustochytrium aggregatum (p)	98	90
Thraustochutriidae sp. HU1	84	86
Thraustochutriidae sp. 8-7	84	91
Thraustochytrium multirudimentale	81	88
Thraustochutriidae sp. PW19	81	85
Schizochytrium sp. ATCC 20888	81	95

(p): indicates partial sequence

[0264] As shown in Table 12, it was found that, in terms of % identity, the 18s rRNA gene sequence from the thraustochytrid (ATCC Accession No. PTA-9695) is closely related, though not identical, to the 18s rRNA gene sequence of T. aggregatum provided in Honda, D. et al., J. Euk. Micro. 46(6): 637-647 (1999). The 18s rRNA sequence published for Thraustochytrium aggregatum is a partial sequence, with an approximately 71 DNA nucleotide gap in the middle of the sequence. In terms of percent coverage, the 18s rRNA gene sequence of the isolate of the invention is more closely related to Schizochytrium sp. ATCC 20888 than to T. aggregatum.

[0265] Highly conserved proteins such as actin and beta-tubulin have been widely used, along with 18s rRNA gene, as markers for assessing phylogenetic relationships between organisms (Baldauf, S. M. Am. Nat. 154, S178 (1999)). Total genomic DNA from the thraustochytrid

(ATCC Accession No. PTA-9695) was also used as a template for PCR amplification of both the actin and beta-tubulin genes. The PCR amplification was carried out with primers designed to conserved regions from the actin and beta-tubulin DNA sequences from T. aggregatum.

[0266] The PCR conditions with chromosomal DNA template were as follows: 0.2 μM dNTPs, 0.1 uM each primer, 8% DMSO, 200 ng chromosomal DNA, 2.5 U Herculase® II fusion DNA polymerase (Stratagene), and 1X Herculase® buffer (Stratagene) in a 50 μL total volume. The PCR Protocol included the following steps: (1) 95°C for 2 minutes; (2) 95°C for 30 seconds; (3) 55°C for 30 seconds; (4) 72°C for 2 minutes; (5) repeat steps 2-4 for 40 cycles; (6) 72°C for 5 minutes; and (7) hold at 6°C.

[0267] PCR amplification yielded distinct DNA products with the expected sizes using chromosomal template described above. The respective PCR products were cloned into the vector pJET1.2/blunt (Fermentas) according to the manufacturer's instructions, and the insert sequence of each were determined using supplied standard primers.

[0268] Table 13 shows identities for the actin amino acid sequence from the thraustochytrid (ATCC Accession No. PTA-9695) as compared to actin sequences available in the public database. Identities were determined through use of the scoring matrix "blosum62mt2" within the "AlignX" program of the VectorNTI program, a standard for protein alignment.

Table 13: Comparison of Actin Protein Sequence % Identities

Thraustochytrids	**% Identity**
Thraustochytriidae sp. RT49	98
Schizochytrium sp. ATCC 20888	96
Thraustochytrium striatum	96
Thraustochytrium aggregatum	96
Japonochytrium marinum	95
Thraustochytrium aureum	95

[0269] Table 14 shows identities for the beta-tubulin amino acid sequence from the thraustochytrid (ATCC Accession No. PTA-9695) as compared to beta-tubulin sequences available in the public database. Identities were determined through use of the scoring matrix

"blosum62mt2" within the "AlignX" program of the VectorNTI program, a standard for protein alignment.

Table 14: Comparison of Beta-Tubulin Protein Sequence % Identities

Thraustochytrids	**% Identity**
Aplanochytrium kerguelense	100
Aplanochytrium stocchinoi	100
Japonochytrium marinum	100
Labyrinthula sp. N8	100
Thraustochytriidae sp. RT49	100
Thraustochytrium aggregatum	100
Thraustochytriidae sp. HU1	100
Thraustochytrium aureum	100
Thraustochytrium kinnei	100
Thraustochytriidae sp. #32	100
Thraustochytriidae sp. PW19	100
Schizochytrium aggregatum	100
Schizochytrium sp. ATCC 20888	100

[0270] Based on the above characterizations, the isolated thraustochytrid (ATCC Accession No. PTA-9695) is believed to represent a new *Schizochytrium* species and is therefore also designated as *Schizochytrium sp.* ATCC PTA-9695.

EXAMPLE 30

[0271] The isolated thraustochytrid (ATCC Accession No. PTA-9695) produced high levels of cell growth under varying culture conditions, as described below. Typical media and cultivation conditions are shown in Table 15. Also, high levels of fatty acids and DHA were observed (i.e., greater than 50% by weight of the dry cell weight were fatty acids and greater than 50% by weight of the fatty acid methyl esters was DHA).

Table 15: Vessel Media

Ingredient	concentration		ranges
NaCl	g/L	12.5	0-25, 5-20, or 10-15
KCl	g/L	1.0	0-5, 0.25-3, or 0.5-2
$MgSO_4 \cdot 7H_2O$	g/L	5.0	0-10, 2-8, or 3-6
$(NH_4)_2SO_4$	g/L	0.6	0-10, 0.25-5, or 0.5-3
$CaCl_2$	g/L	0.29	0.1-5, 0.15-3, or 0.2-1
T 154 (yeast extract)	g/L	6.0	0-20, 1-15, or 5-10
KH_2PO_4	g/L	1.2	0.1-10, 0.5-5, or 1-3
Post autoclave (Metals)			
Citric acid	mg/L	3.5	0.1-100, 1-50, or 2-25
$FeSO_4 \cdot 7H_2O$	mg/L	10.30	0.1-100, 1-50, or 5-25
$MnCl_2 \cdot 4H_2O$	mg/L	3.10	0.1-100, 1-50, or 2-25
$ZnSO_4 \cdot 7H_2O$	mg/L	3.10	0.1-100, 1-50, or 2-25
$CoCl_2 \cdot 6H_2O$	mg/L	0.04	0.001-1, 0.005-0.5, or 0.01-0.1
$Na_2MoO_4 \cdot 2H_2O$	mg/L	0.04	0.001-1, 0.005-0.5, or 0.01-0.1
$CuSO_4 \cdot 5H_2O$	mg/L	2.07	0.1-100, 0.5-50, or 1-25
$NiSO_4 \cdot 6H_2O$	mg/L	2.07	0.1-100, 0.5-50, or 1-25
Post autoclave (Vitamins)			
Thiamine	mg/L	9.75	0.1-100, 1-50, or 5-25
Vitamin B12	mg/L	0.16	0.1-100, 0.1-10, or 0.1-1
Ca½-pantothenate	mg/L	3.33	0.1-100, 0.1-50, or 1-10
Post autoclave (Carbon)			
Glucose	g/L	30.0	5-150, 10-100, or 20-50

Nitrogen Feed:

Ingredient	Concentration		
NH_4OH	mL/L	21.6	0-150, 10-100, or 15-50

Typical cultivation conditions would include the following:

pH	about 6.5 – about 8.5, about 6.5 – about 8.0, or about 7.0 - about 7.5
temperature:	about 17 – about 30 degrees Celsius, about 20 – about 25 degrees Celsius, or about 22 to about 23 degrees Celsius
dissolved oxygen:	about 5 – about 100% saturation, about 10 – about 80% saturation, or about 20 – about 50% saturation
glucose controlled @:	about 5 - about 50 g/L, about 10 – about 40 g/L, or about 20 – about 35 g/L.

[0272] In carbon and nitrogen-fed cultures with 8200 ppm Cl^- at 22.5 °C with 20% dissolved oxygen at pH 7.0, the isolate produced a dry cell weight of 140 g/L after 7 days of culture, with a fatty acid content of 70% by weight. Closed loop ammonia feed was used and the pH was maintained at 7.0. The omega-3 productivity was 8.92 g/(L*day) under these conditions, with 4.7 g/L EPA (5% by weight of fatty acids) and 56.3 g/L DHA (57% by weight of fatty acids) in 7 days.

[0273] In carbon and nitrogen-fed cultures with 3640 ppm Cl^- at 22.5 °C with 20% dissolved oxygen at pH 7.0, the isolate produced a dry cell weight of 82 g/L after 7 days of culture, with a fatty acid content of 58% by weight. The omega-3 productivity was 4.5 g/(L*day) under these conditions, with 2.1 g/L EPA (4.3% by weight of fatty acids) and 28.5 g/L DHA (58.7% by weight of fatty acids) in 7 days.

[0274] In carbon and nitrogen-fed cultures with 980 ppm Cl^- at 22.5 °C with 20% dissolved oxygen at pH 7.0, the isolate produced a dry cell weight of 60 g/L after 7 days of culture, with a fatty acid content of 53% by weight. The omega-3 productivity was 2.8 g/(L*day) under these conditions, with 1.1 g/L EPA (3.4% by weight of fatty acids) and 18.4 g/L DHA (56.8% by weight of fatty acids) in 7 days.

EXAMPLE 31

[0275] Oils were extracted from a biomass sample (Sample A) of the isolated thraustochytrid (ATCC Accession No. PTA-9695). The biomass sample was produced in a carbon and nitrogen-fed culture with 980 ppm Cl^- at 22.5 °C with 20% dissolved oxygen at pH 7.0. Oils were extracted from biomass Sample A by the hexane extraction process to yield microbial oil Sample A1. Briefly, dried biomass was ground with hexane using stainless steel tubes and stainless steel ball bearings for approximately 2 hours. The slurry was vacuum filtered and the filtrate was collected. The hexane was removed using a rotary evaporator. Oils were also extracted from biomass Sample A using the FRIOLEX® process (GEA Westfalia Separator UK Ltd., Milton Keynes, England) to yield microbial oil Sample A2. Individual lipid classes were isolated from microbial oil Samples A1 and A2 using low pressure flash chromatography, and the weight percent of each class was determined. The fatty acid profile of each class was determined using gas chromatography with flame ionization detection (GC-FID) as fatty acid methyl esters (FAME).

[0276] *Flash Chromatography* – Flash chromatography was used to separate the lipid classes present in the crude oils, and to determine the weight percent of each class present in the oils. The chromatography system utilized Silica Gel 60 (EMD Chemical, Gibbstown, NJ) with mobile phase composed of Petroleum Ether and Ethyl Acetate at 3 mL/min. A step gradient was used to selectively elute each lipid class from the column. The mobile phase gradient started from 100% petroleum ether and finished with 50% ethyl acetate (followed by a 100% methanol wash). Fractions were collected in 10 mL test tubes using a Gilson FC 204 large-bed fraction collector (Gilson, Inc., Middleton, WI). Each tube was analyzed by thin layer chromatography (TLC) and the tubes containing individual lipid classes (as judged by single spots on TLC plate with expected retention factor (Rf)) were pooled, concentrated to dryness, and weighed. The total fraction content was then determined gravimetrically.

[0277] *TLC Analysis* – Thin layer chromatography was conducted on silica gel plates. The plates were eluted using a solvent system consisting of petroleum ether : ethyl ether : acetic acid (80:20:1) and were visualized using iodine vapor. The Rf values of each spot were then compared with reported literature values for each lipid class.

[0278] *Fatty Acid Analysis* – The samples of biomass and isolated lipid classes were analyzed for fatty acid composition as FAMEs. Samples were weighed directly into screw cap test tubes, and 1 mL of C19:0 internal standard (NuCheck, Elysian, MN) in toluene and 2 mL of 1.5 N HCl in methanol was added to each tube. The tubes were vortexed briefly and placed in a heating block for 2 hours at 100° C. The tubes were removed from the heating block, allowed to cool, and 1 mL of saturated NaCl in water was added. The tubes were vortexed again, centrifuged, and a portion of the top (organic) layer was placed in a GC vial and analyzed by GC-FID. FAME's were quantified using a 3-point internal standard calibration curve generated using Nu-Chek-Prep GLC reference standard (Nu-Chek Prep, Inc., Elysian, MN) and tentatively identified based on retention time. Fatty acids present were expressed as mg/g and % of total FAME.

[0279] Sample A1 was prepared by dissolving the crude oil in hexane and applying to the head of the column. After fractionation of the sample using flash chromatography, the sterol ester fraction accounted for 1.2% by weight, the triacylglycerol (TAG) fraction accounted for 82.7% by weight, the free fatty acid (FFA) fraction accounted for 0.9% by weight, and the diacylglycerol (DAG) fraction accounted for 2.9% by weight of the crude oil. The total fatty acid profiles of the Sample A1 crude oil and isolated fractions are shown below in Table 16 and Table 17 calculated as mg/g and %FAME, respectively.

Table 16: Sample A1 Fatty Acid Profiles Calculated as Milligrams per Gram FAME

	Biomass	Crude Oil	Sterol Esters	TAG	FFA	DAG
Wt. %	NA	38%	1.2%	82.7%	0.9	2.9%
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0*	0.6	0.0	1.9	3.2	1.7	0.0
C14:0*	5.7	13.6	12.8	20.2	13.0	17.6
C14:1*	0.0	0.0	0.0	0.0	0.0	0.0
C15:0	1.3	3.4	3.1	3.1	2.1	2.6
C16:0*	105.5	239.5	222.2	274.3	183.3	225.1
C16:1*	0.0	0.0	0.8	0.0	0.8	0.0
C18:0*	6.4	16.4	43.1	16.8	9.8	14.0
C18:1 N9*	0.0	3.8	1.9	3.3	1.0	3.5
C18:1 N7	0.0	0.0	0.0	0.0	0.0	0.0
C18:2 N6*	0.0	0.0	0.0	0.0	0.0	0.0
C20:0*	1.8	5.5	13.0	4.7	2.0	2.9
C18:3 N3*	0.0	0.0	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.0	0.6	0.0
C20:2 N6*	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.0	0.0	0.0	0.0
C22:0*	0.0	0.8	7.3	0.8	0.0	1.2
C20:4 N7	0.0	0.0	0.8	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0	0.0	0.0
C20:4N6*	1.0	3.4	0.0	2.6	2.0	1.9
C22:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N3	1.5	4.1	1.5	3.5	2.1	2.1
C20:5 N3*	18.2	39.5	3.5	38.4	30.6	42.8
C24:0*	0.0	0.0	6.3	0.0	0.0	0.0
C22:4 N9	0.0	0.0	0.0	0.0	0.0	0.0
C24:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C22:5 N6*	11.9	29.5	8.9	26.9	14.8	18.7
C22:5 N3*	1.1	4.7	0.9	3.6	3.4	2.7
C22:6 N3*	253.5	569.7	107.3	556.5	352.8	451.4
Sum of all FAME's	408.6	934.0	435.4	958.0	620.1	786.4

Table 17: Sample A1 Fatty Acid Profiles as a Percent of Total FAME

	Biomass	Crude Oil	Sterol Esters	TAG	FFA	DAG
Fatty Acid	% FAME	% FAME	% FAME	% FAME	% FAME	% FAME
C12:0*	0.1	0.0	0.4	0.3	0.3	0.0
C14:0*	1.4	1.5	2.9	2.1	2.1	2.2
C14:1*	0.0	0.0	0.0	0.0	0.0	0.0
C15:0	0.3	0.4	0.7	0.3	0.3	0.3
C16:0*	25.8	25.6	51.0	28.6	29.6	28.6
C16:1*	0.0	0.0	0.2	0.0	0.1	0.0
C18:0*	1.6	1.8	9.9	1.8	1.6	1.8
C18:1 N9*	0.0	0.4	0.4	0.3	0.2	0.4
C18:1 N7	0.0	0.0	0.0	0.0	0.0	0.0
C18:2 N6*	0.0	0.0	0.0	0.0	0.0	0.0
C20:0*	0.4	0.6	3.0	0.5	0.3	0.4
C18:3 N3*	0.0	0.0	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.0	0.1	0.0
C20:2 N6*	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.0	0.0	0.0	0.0
C22:0*	0.0	0.1	1.7	0.1	0.0	0.1
C20:4 N7	0.0	0.0	0.2	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0	0.0	0.0
C20:4N6*	0.3	0.4	0.0	0.3	0.3	0.2
C22:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N3	0.4	0.4	0.4	0.4	0.3	0.3
C20:5 N3*	4.5	4.2	0.8	4.0	4.9	5.4
C24:0*	0.0	0.0	1.4	0.0	0.0	0.0
C22:4 N9	0.0	0.0	0.0	0.0	0.0	0.0
C24:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C22:5 N6*	2.9	3.2	2.1	2.8	2.4	2.4
C22:5 N3*	0.3	0.5	0.2	0.4	0.5	0.3
C22:6 N3*	62.0	61.0	24.6	58.1	56.9	57.4
Sum of FAME %	100.0	100.0	100.0	100.0	100.0	100.0

[0280] Sample A2 was prepared by dissolving the crude oil in hexane and applying to the head of the column. After fractionation of the sample using flash chromatography, the sterol ester fraction accounted for 0.8% by weight, the triacylglycerol (TAG) fraction accounted for 83.4% by weight, the free fatty acid (FFA) fraction accounted for 1.8% by weight, and the diacylglycerol (DAG) fraction accounted for 5.6% by weight of the crude oil. The total fatty acid

profiles of the Sample A2 crude oil and isolated fractions are shown below in Table 18 and Table 19 calculated as mg/g and %FAME, respectively.

Table 18: Sample A2 Fatty Acid Profiles Calculated as Milligrams per Gram FAME

	Biomass	Crude Oil	Sterol Esters	TAG	FFA	DAG
Wt. %	NA	NA	0.8%	83.4%	1.8%	5.6%
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0*	0.6	0.0	0.0	1.5	0.0	1.0
C14:0*	5.7	13.2	8.9	14.1	9.5	5.4
C14:1*	0.0	0.0	0.0	0.0	0.0	0.0
C15:0	1.3	3.3	2.8	3.4	2.1	2.2
C16:0*	105.5	233.7	183.8	246.1	159.7	137.3
C16:1*	0.0	0.0	0.0	0.8	0.0	0.0
C18:0*	6.4	16.6	23.6	16.9	11.3	5.6
C18:1 N9*	0.0	7.6	5.0	4.3	2.4	2.6
C18:1 N7	0.0	0.0	0.0	0.0	0.0	0.0
C18:2 N6*	0.0	2.2	0.7	1.6	0.8	5.1
C20:0*	1.8	5.2	12.1	5.5	2.6	1.1
C18:3 N3*	0.0	0.0	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.8	1.0	0.0
C20:2 N6*	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.0	0.3	0.0	0.0
C22:0*	0.0	0.7	6.0	1.3	0.8	0.0
C20:4 N7	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N6*	1.0	3.0	0.0	3.1	2.3	1.2
C22:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N3	1.5	4.1	1.4	4.3	2.7	1.0
C20:5 N3*	18.2	38.6	2.7	38.6	39.5	45.5
C24:0*	0.0	0.0	4.7	0.6	0.0	0.3
C22:4 N9	0.0	0.0	0.0	0.0	0.0	0.0
C24:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C22:5 N6*	11.9	28.2	8.6	29.6	18.0	14.7
C22:5 N3*	1.1	3.4	0.0	3.5	2.5	2.2
C22:6 N3*	253.5	566.7	102.2	575.0	475.3	447.2
Sum of all FAME's	408.6	926.5	362.3	951.3	730.4	672.5

Table 19: Sample A2 Fatty Acid Profiles as a Percent of Total FAME

	Biomass	Crude Oil	Sterol Esters	TAG	FFA	DAG
Fatty Acid	% FAME	% FAME	% FAME	% FAME	% FAME	% FAME
C12:0*	0.1	0.0	0.0	0.2	0.0	0.2
C14:0*	1.4	1.4	2.4	1.5	1.3	0.8
C14:1*	0.0	0.0	0.0	0.0	0.0	0.0
C15:0	0.3	0.4	0.8	0.4	0.3	0.3
C16:0*	25.8	25.2	50.7	25.9	21.9	20.4
C16:1*	0.0	0.0	0.0	0.1	0.0	0.0
C18:0*	1.6	1.8	6.5	1.8	1.5	0.8
C18:1 N9*	0.0	0.8	1.4	0.5	0.3	0.4
C18:1 N7	0.0	0.0	0.0	0.0	0.0	0.0
C18:2 N6*	0.0	0.2	0.2	0.2	0.1	0.8
C20:0*	0.4	0.6	3.3	0.6	0.4	0.2
C18:3 N3*	0.0	0.0	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.1	0.1	0.0
C20:2 N6*	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.0	0.0	0.0	0.0
C22:0*	0.0	0.1	1.7	0.1	0.1	0.0
C20:4 N7	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N6*	0.3	0.3	0.0	0.3	0.3	0.2
C22:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N3	0.4	0.4	0.4	0.4	0.4	0.2
C20:5 N3*	4.5	4.2	0.7	4.1	5.4	6.8
C24:0*	0.0	0.0	1.3	0.1	0.0	0.0
C22:4 N9	0.0	0.0	0.0	0.0	0.0	0.0
C24:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C22:5 N6*	2.9	3.0	2.4	3.1	2.5	2.2
C22:5 N3*	0.3	0.4	0.0	0.4	0.3	0.3
C22:6 N3*	62.0	61.2	28.2	60.4	65.1	66.5
Sum of FAME %	100.0	100.0	100.0	100.0	100.0	100.0

[0281] It is noted that Samples A1 and A2 were extracted using a typical hexane extraction and a FRIOLEX® process, respectively. The fatty acid profiles of Tables 16-19 are expected to be the substantially the same if the samples were extracted using the processes of the present invention.

EXAMPLE 32

[0282] After oil was extracted from the fermentation broth using the Friolex process, as described in Example 31, the crude oil was further processed via refining, bleaching, and deodorizing steps to obtain a final oil. The final oil was diluted with high oleic sunflower oil to obtain finished commercial oil with a DHA content of approximately 400 mg/g. Individual lipid classes were isolated and the fatty acid profiles of each class was determined using gas chromatography with flame ionization detection (GC-FID) as fatty acid methyl esters (FAME).

[0283] *Flash Chromatography* – Flash chromatography was used to separate the lipid classes present in the final oil, and to determine the weight percent of each class present in the oil. The chromatography system utilized Silica Gel 60 (EMD Chemical, Gibbstown, NJ) with mobile phase composed of Petroleum Ether and Ethyl Acetate at 3 mL/min. A step gradient was used to selectively elute each lipid class from the column. The mobile phase gradient started from 100% petroleum ether and finished with 50% ethyl acetate (followed by a 100% methanol wash). Fractions were collected in 10 mL test tubes using a Gilson FC 204 large-bed fraction collector (Gilson, Inc., Middleton, WI). Each tube was analyzed by thin layer chromatography (TLC) and the tubes containing individual lipid classes (as judged by single spots on TLC plate with expected retention factor (Rf)) were pooled, concentrated to dryness, and weighed. The total fraction content was then determined gravimetrically.

[0284] *TLC Analysis* – Thin layer chromatography was conducted on silica gel plates. The plates were eluted using a solvent system consisting of petroleum ether : ethyl ether : acetic acid (80:20:1) and were visualized using iodine vapor. The Rf values of each spot were then compared with reported literature values for each lipid class.

[0285] *Fatty Acid Analysis* – The final oil sample and isolated lipid classes were analyzed for fatty acid composition as FAMEs. Samples were weighed directly into screw cap test tubes, and 1 mL of C19:0 internal standard (NuCheck, Elysian, MN) in toluene and 2 mL of 1.5 N HCl in methanol was added to each tube. The tubes were vortexed briefly and placed in a heating block for 2 hours at 100° C. The tubes were removed from the heating block, allowed to cool, and 1 mL of saturated NaCl in water was added. The tubes were vortexed again, centrifuged, and a portion of the top (organic) layer was placed in a GC vial and analyzed by GC-FID. FAME's were quantified using a 3-point internal standard calibration curve generated using Nu-Chek-Prep GLC reference standard (Nu-Chek Prep, Inc., Elysian, MN) and tentatively identified based on retention time. Fatty acids present were expressed as mg/g and % of total FAME.

[0286] The sample was prepared by dissolving 250 mg of final oil in 600 µL of hexane and applying to the head of the column. After fractionation of the sample using flash chromatography, the sterol ester fraction accounted for 1.2% by weight, the triacylglyceride (TAG) fraction accounted for 92.1% by weight, the free fatty acid (FFA) fraction accounted for 2.1% by weight, the sterol fraction accounted for 1.1%, the diacylglyceride (DAG) fraction accounted for 2.8% by weight of the final oil.

[0287] The TLC analysis of the pooled fractions showed that the FFA and sterol fractions were mixed with TAG and DAG respectively. The total fatty acid profiles of the FRIOLEX® final oil and isolated fractions are shown below in Table 20 and Table 21 calculated as mg/g and %FAME, respectively.

Table 20: Fatty Acid Profile Calculated as Milligrams per Gram of FAME

	Final Oil	Sterol Esters	TAG	FFA	Sterol	DAG
Wt. %	NA	1.2	92.1	2.1	1.1	2.8
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0*	0.0	0.0	1.0	0.0	1.2	0.6
C14:0*	11.5	5.1	11.3	6.0	9.6	5.7
C14:1*	0.0	0.0	0.0	0.0	0.0	0.0
C15:0	2.3	0.0	2.3	1.2	2.0	1.9
C16:0*	183.3	80.0	180.8	99.9	149.3	132.2
C16:1*	0.0	0.0	0.9	0.0	0.8	0.6
C18:0*	19.6	17.5	19.6	7.5	16.2	6.7
C18:1 N9*	243.3	242.8	249.6	62.9	190.5	84.0
C18:1 N7	1.9	1.7	2.0	0.8	1.9	0.9
C18:2 N6*	13.8	5.6	13.8	6.2	14.3	9.1
C20:0*	4.3	6.6	4.5	1.5	3.6	1.4
C18:3 N3*	0.0	0.0	0.3	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.8	0.0	0.8	0.0
C18:4 N3	0.0	0.0	0.7	1.3	0.9	0.4
C20:2 N6*	0.0	0.0	0.6	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.3	0.0	0.0	0.0
C22:0*	3.3	61.0	3.2	1.1	3.0	1.2
C20:4 N7	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0	0.0	0.0
C20:4N6*	1.7	0.0	2.3	1.4	1.9	1.3
C22:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N3	2.4	4.5	3.0	2.2	2.6	1.3

	Final Oil	Sterol Esters	TAG	FFA	Sterol	DAG
C20:5 N3*	28.1	3.0	27.7	38.6	25.6	43.2
C24:0*	1.4	64.3	1.4	0.0	2.0	1.0
C22:4 N9	0.0	0.0	0.0	0.0	0.0	0.0
C24:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C22:5 N6*	20.0	7.6	21.0	10.1	17.2	14.4
C22:5 N3*	2.8	0.0	3.1	3.7	3.4	2.9
C22:6 N3*	407.1	72.5	417.4	443.6	350.5	428.5
Sum of all FAME's	936.1	572.1	967.6	688.0	797.3	737.3

Table 21: Fatty Acid Profiles as a Percent of Total FAME

	Final Oil	Sterol Esters	TAG	FFA	Sterol	DAG
Fatty Acid	% FAME	% FAME	% FAME	% FAME	% FAME	% FAME
C12:0*	0.0	0.0	0.1	0.0	0.2	0.1
C14:0*	1.2	0.9	1.2	0.9	1.2	0.8
C14:1*	0.0	0.0	0.0	0.0	0.0	0.0
C15:0	0.2	0.0	0.2	0.2	0.2	0.3
C16:0*	19.6	14.0	18.7	14.5	18.7	17.9
C16:1*	0.0	0.0	0.1	0.0	0.1	0.1
C18:0*	2.1	3.1	2.0	1.1	2.0	0.9
C18:1 N9*	26.0	42.4	25.8	9.1	23.9	11.4
C18:1 N7	0.2	0.3	0.2	0.1	0.2	0.1
C18:2 N6*	1.5	1.0	1.4	0.9	1.8	1.2
C20:0*	0.5	1.1	0.5	0.2	0.5	0.2
C18:3 N3*	0.0	0.0	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.1	0.0	0.1	0.0
C18:4 N3	0.0	0.0	0.1	0.2	0.1	0.1
C20:2 N6*	0.0	0.0	0.1	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.0	0.0	0.0	0.0
C22:0*	0.4	10.7	0.3	0.2	0.4	0.2
C20:4 N7	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0	0.0	0.0
C20:4N6*	0.2	0.0	0.2	0.2	0.2	0.2
C22:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N3	0.3	0.8	0.3	0.3	0.3	0.2
C20:5 N3*	3.0	0.5	2.9	5.6	3.2	5.9
C24:0*	0.2	11.2	0.1	0.0	0.2	0.1
C22:4 N9	0.0	0.0	0.0	0.0	0.0	0.0

	Final Oil	Sterol Esters	TAG	FFA	Sterol	DAG
C24:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C22:5 N6*	2.1	1.3	2.2	1.5	2.2	1.9
C22:5 N3*	0.3	0.0	0.3	0.5	0.4	0.4
C22:6 N3*	43.6	12.7	43.1	64.5	44.0	58.1
Sum of FAME %	100	100	100	100	100	100

[0288] It is noted that fatty acid profiles of Tables 20 and 21 were obtained from samples extracted using a FRIOLEX® process. The fatty acid profiles of Tables 20 and 21 are expected to be the substantially the same if the samples were extracted using the processes of the present invention.

EXAMPLE 33

[0289] A two-day old inoculum flask of the isolated thraustochytrid (ATCC Accession No. PTA-9695) was prepared in a carbon and nitrogen-fed culture with 980 ppm Cl^- (thraustochytrid media).

[0290] Mutagenesis was carried out according to following procedure:

[0291] A sterile T=2 day old flask, approximately 50 ml, was poured into a sterile 40 ml glass homogenizer. The culture received 50 plunges in the homogenizer. The culture was pipeted out and filtered through a sterile 50 micron mesh filter, which was placed in a 50 ml sterile tube (the mesh was used as a means of retaining the larger clumps of colonies while letting the smaller clusters and single cells pass through the 50 micron mesh.). The entire concentrated macerate was collected in a sterile 50 ml tube. The macerated culture was vortexed and dilutions at levels up to 1:100 fold were made in tubes containing thraustochytrid media. The diluted macerate samples were vortexed prior to adding 200 µl of inoculum to a thraustochytrid media agar petri dish, 100 x 15 mm, containing 4-5 glass beads (3 mm glass beads). Each plate was gently agitated in an effort to have the beads spread the inoculum evenly around the plate. Beads were dumped off of plates and plates were left to sit with covers on for approximately 5 minutes to dry. Lights in both the sterile hood and adjoining areas were turned off as the procedure was performed in dim light. There was minimal light available to be able to run the procedure but only indirect and dim.

[0292] Five replicate plates were placed on the floor of the XL crosslinker (Spectronics Corporation, New York) with the lids off while the samples were irradiated. The crosslinker

delivered power in terms of microjoules and a level was sought that achieved a 90%-95% Kill. Five replicate control plates were inoculated with un-mutagenized cells using the same protocol. These cell counts were used to calculate the % Kill. Once the irradiation was finished the plates were taken out, the lids were replaced, and the plates were wrapped in parafilm followed by a wrap in aluminum foil. It was imperative that the plates grew for the first week in the dark so that they were not able to repair the damaged genes.

[0293] Plates were placed in a 22.5°C room for about 10 days prior to counting the colonies. When final counts were made, individual colonies were picked with a sterile inoculating loop and re-streaked on new thraustochytrid media plates. Each colony was plated on an individual plate. As plates grew dense a sample was taken, using a inoculating loop, and inoculated into a sterile 250 ml shake flask containing 50 ml of thraustochytrid media. This flask was placed on a shaker at 200 rpm in a 22.5°C room. On T=7 days the shake flask culture was harvested into a 50 ml sterile tube. The pH was taken and the sample was spun down to collect the biomass pellet. Each sample was rinsed and re-suspended in a 50:50 mixture of isopropyl alcohol and distilled water prior to being re-spun. The collected pellet was freeze dried, weighed, and a FAME analysis was performed. The data in Tables 22-28 represents mutants produced with the above process.

Table 22: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA-9695	Mutant 1	Mutant 2	Mutant 3	Mutant 4	Mutant 5	Mutant 8	Mutant 9	Mutant 10
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.10	0.10	0.08	0.08	0.13	0.07	0.11	0.08	0.08
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.11	0.11	0.17	0.13	0.12	0.18	0.11	0.15	0.14
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 14:0	1.79	1.85	1.49	1.37	2.36	1.29	1.85	1.72	1.57
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 15:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:0	30.98	28.75	29.96	29.97	30.33	29.86	30.97	30.11	29.20
% 16:1	0.27	0.20	0.31	0.14	0.25	0.27	0.16	0.27	0.24
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.12	0.15	0.13	0.17	0.27	0.12	0.16	0.13	0.13
% 18:0	1.29	1.22	1.38	1.47	1.22	1.57	1.25	1.34	1.34
% 18:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.03	0.00	0.00	0.07	0.00	0.03	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.39	0.36	0.42	0.45	0.34	0.46	0.37	0.40	0.40
% 20:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.37	0.38	0.32	0.42	0.44	0.32	0.41	0.33	0.36
% 20:4 ARA	0.55	0.55	0.94	0.57	0.80	0.89	0.60	0.73	0.75

Table 22 continued

Fatty Acids	control ATCC PTA-9695	Mutant 1	Mutant 2	Mutant 3	Mutant 4	Mutant 5	Mutant 8	Mutant 9	Mutant 10
% 20:5 n-3 EPA	2.62	2.94	3.01	2.40	3.64	2.83	2.54	2.81	2.81
% 22:0	0.08	0.08	0.09	0.09	0.07	0.10	0.07	0.09	0.09
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:5 n-6	3.19	3.19	2.94	3.43	3.35	2.87	3.34	3.01	3.15
% 22:5 n-3	0.18	0.18	0.21	0.23	0.20	0.18	0.20	0.17	0.18
% 22:6 n-3 DHA	56.88	58.63	57.56	57.85	54.87	57.98	56.62	57.53	58.52
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.09	0.00
% Fat	46.83	46.10	31.23	47.39	49.78	30.62	54.71	37.72	37.87
% Unknown	0.85	0.46	0.35	0.51	0.51	0.36	0.50	0.38	0.39

Table 23: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA-9695	Mutant 11	Mutant 13	Mutant 14	Mutant 15	Mutant 16	Mutant 20	Mutant 21	Mutant 22
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.10	0.10	0.08	0.09	0.11	0.11	0.09	0.09	0.10
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.11	0.15	0.16	0.14	0.13	0.12	0.17	0.16	0.13
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 14:0	1.79	1.89	1.43	1.75	1.83	1.98	1.76	1.77	1.81
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 15:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:0	30.98	31.08	30.27	29.92	31.79	30.18	28.84	30.05	30.81
% 16:1	0.27	0.32	0.26	0.28	0.21	0.24	0.23	0.23	0.33
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.12	0.24	0.15	0.13	0.15	0.12	0.14	0.16	0.14
% 18:0	1.29	1.36	1.44	1.31	1.36	1.21	1.28	1.34	1.33
% 18:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.05	0.00	0.00	0.00	0.03	0.00	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.39	0.38	0.42	0.39	0.40	0.37	0.37	0.38	0.38
% 20:1 n-9	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.37	0.43	0.36	0.33	0.36	0.37	0.33	0.35	0.34
% 20:4 ARA	0.55	0.79	0.72	0.80	0.64	0.62	0.83	0.73	0.69
% 20:5 n-3 EPA	2.62	3.17	2.72	2.97	2.52	2.66	3.03	2.90	2.87
% 22:0	0.08	0.08	0.09	0.08	0.08	0.08	0.08	0.08	0.08

Table 23 continued

Fatty Acids	control ATCC PTA-9695	Mutant 11	Mutant 13	Mutant 14	Mutant 15	Mutant 16	Mutant 20	Mutant 21	Mutant 22
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:5 n-6	3.19	3.25	3.06	2.97	3.07	3.16	2.98	3.01	3.02
% 22:5 n-3	0.18	0.20	0.19	0.17	0.19	0.16	0.17	0.18	0.18
% 22:6 n-3 DHA	56.88	55.17	57.52	57.63	56.02	57.38	58.58	57.45	56.65
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.08
% Fat	46.83	46.19	37.00	38.41	48.46	47.32	37.71	40.23	43.55
% Unknown	0.85	0.47	0.39	0.36	0.47	0.44	0.37	0.39	0.38

Table 24: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA-9695	Mutant 24	Mutant 26	Mutant 27	Mutant 29	Mutant 30	Mutant 33	Mutant 34	Mutant 35
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.10	0.11	0.09	0.09	0.08	0.08	0.10	0.11	0.09
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.11	0.12	0.13	0.14	0.16	0.14	0.12	0.12	0.10
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 14:0	1.79	1.98	1.71	1.69	1.63	1.66	1.93	2.01	1.59
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 15:1	0.00	0.00	0.00	0.00	0.00	0.00	0.70	0.54	0.39
% 16:0	30.98	30.61	30.32	30.21	29.70	29.50	30.26	32.28	30.78
% 16:1	0.27	0.19	0.22	0.22	0.26	0.26	0.29	0.26	0.16
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.12	0.15	0.18	0.16	0.13	0.13	0.26	0.16	0.12
% 18:0	1.29	1.24	1.31	1.31	1.32	1.30	1.32	1.37	1.34
% 18:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.11	0.09
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.39	0.37	0.39	0.40	0.40	0.39	0.37	0.40	0.40
% 20:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.14
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.37	0.38	0.37	0.35	0.35	0.35	0.00	0.00	0.00
% 20:4 ARA	0.55	0.61	0.59	0.69	0.68	0.32	0.34	0.24	0.28
% 20:5 n-3 EPA	2.62	2.62	2.70	2.85	2.90	2.91	3.28	2.51	2.59
% 22:0	0.08	0.08	0.08	0.08	0.09	0.08	0.08	0.08	0.08

Table 24 continued

Fatty Acids	control ATCC PTA-9695	Mutant 24	Mutant 26	Mutant 27	Mutant 29	Mutant 30	Mutant 33	Mutant 34	Mutant 35
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:5 n-6	3.19	3.10	3.11	3.05	3.10	3.11	3.43	3.26	3.56
% 22:5 n-3	0.18	0.16	0.18	0.19	0.18	0.18	0.18	0.15	0.24
% 22:6 n-3 DHA	56.88	57.03	57.46	57.46	57.96	58.52	55.92	54.96	56.73
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.08	0.00	0.00	0.00	0.00	0.07	0.07	0.07
% Fat	46.83	47.80	43.50	38.86	38.60	38.16	46.95	46.43	51.55
% Unknown	0.85	0.45	0.42	0.39	0.37	0.82	1.25	1.23	1.25

Table 25: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA-9695	Mutant 36	Mutant 37	Mutant 38	Mutant 39	Mutant 40	Mutant 42	Mutant 43	Mutant 44
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.10	0.00	0.11	0.00	0.11	0.09	0.08	0.12	0.09
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.11	0.44	0.09	0.24	0.12	0.11	0.12	0.08	0.15
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 14:0	1.79	1.25	1.99	1.48	1.96	1.76	1.43	2.17	1.75
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 15:1	0.00	2.12	0.48	0.71	0.54	0.55	0.36	0.62	0.50
% 16:0	30.98	26.95	28.04	32.28	30.84	30.25	25.77	43.37	30.18
% 16:1	0.27	0.00	0.26	0.23	0.22	0.21	0.10	1.05	0.22
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.12	0.95	0.13	0.28	0.16	0.16	0.10	0.26	0.13
% 18:0	1.29	1.58	1.11	1.79	1.30	1.29	1.25	2.21	1.34
% 18:1 n-9	0.00	0.37	0.08	0.25	0.09	0.09	0.12	0.09	0.10
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.39	0.34	0.31	0.43	0.38	0.39	0.36	0.61	0.40
% 20:1 n-9	0.00	0.00	0.00	0.43	0.00	0.14	0.15	0.15	0.49
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:4 ARA	0.55	0.41	0.31	0.24	0.27	0.24	0.30	0.35	0.23
% 20:5 n-3 EPA	2.62	5.36	2.77	4.00	2.72	2.80	3.21	3.47	2.80
% 22:0	0.08	0.00	0.07	0.14	0.07	0.08	0.07	0.14	0.08

Table 25 continued

Fatty Acids	control ATCC PTA-9695	Mutant 36	Mutant 37	Mutant 38	Mutant 39	Mutant 40	Mutant 42	Mutant 43	Mutant 44
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
% 22:5 n-6	3.19	2.40	3.94	2.57	3.48	3.29	3.89	2.37	3.33
% 22:5 n-3	0.18	0.00	0.19	0.00	0.17	0.17	0.30	0.33	0.17
% 22:6 n-3 DHA	56.88	57.52	58.57	54.20	56.24	57.09	60.99	41.61	56.76
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.00	0.08	0.00	0.08	0.09	0.08	0.06	0.09
% Fat	46.83	12.73	54.86	18.08	45.74	42.59	42.48	56.44	41.20
% Unknown	0.85	0.29	1.36	0.73	1.28	1.20	1.31	0.90	1.20

Table 26: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA-9695	Mutant 45	Mutant 46	Mutant 47	Mutant 48	Mutant 49	Mutant 50	Mutant 51	Mutant 52
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.10	0.10	0.13	0.11	0.07	0.09	0.09	0.09	0.11
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.11	0.11	0.10	0.09	0.13	0.09	0.13	0.10	0.09
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 14:0	1.79	1.79	2.07	1.86	1.52	1.62	1.78	1.78	1.85
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 15:1	0.00	0.41	0.76	0.57	0.46	0.48	0.55	0.53	0.53
% 16:0	30.98	28.79	24.90	30.07	29.07	31.21	30.46	30.79	32.53
% 16:1	0.27	0.19	0.24	0.18	0.17	0.17	0.18	0.21	0.22
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.12	0.11	0.24	0.16	0.12	0.14	0.17	0.18	0.15
% 18:0	1.29	1.24	1.07	1.28	1.41	1.43	1.36	1.48	1.35
% 18:1 n-9	0.00	0.08	0.07	0.09	0.09	0.08	0.10	0.09	0.06
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.00	0.12	0.05	0.00	0.00	0.00	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.39	0.36	0.29	0.37	0.42	0.42	0.39	0.40	0.41
% 20:1 n-9	0.00	0.15	0.13	0.11	0.24	0.13	0.19	0.16	0.19
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.37	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00
% 20:4 ARA	0.55	0.29	0.65	0.26	0.18	0.21	0.22	0.24	0.24
% 20:5 n-3 EPA	2.62	3.05	4.28	2.66	2.93	2.46	2.71	2.94	2.44

Table 26 continued

Fatty Acids	control ATCC PTA-9695	Mutant 45	Mutant 46	Mutant 47	Mutant 48	Mutant 49	Mutant 50	Mutant 51	Mutant 52
% 22:0	0.08	0.07	0.06	0.07	0.09	0.09	0.08	0.08	0.08
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.06	0.07	0.05	0.00	0.00	0.00	0.00	0.00
% 22:5 n-6	3.19	3.59	4.28	3.46	3.07	3.32	3.17	3.18	3.24
% 22:5 n-3	0.18	0.25	0.27	0.18	0.17	0.17	0.16	0.17	0.17
% 22:6 n-3 DHA	56.88	57.74	58.32	56.70	58.65	56.45	56.83	56.19	55.06
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.07	0.15	0.10	0.10	0.11	0.10	0.10	0.07
% Fat	46.83	48.91	58.95	54.80	35.41	48.60	44.93	43.01	51.93
% Unknown	0.85	1.55	1.63	1.57	1.09	1.35	1.31	1.28	1.19

Table 27: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA-9695	Mutant 53	Mutant 54	Mutant 55	Mutant 56	Mutant 57	Mutant 58	Mutant 60	Mutant 61	Mutant 65
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.10	0.09	0.08	0.12	0.08	0.08	0.08	0.08	0.10	0.08
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.11	0.11	0.12	0.08	0.09	0.13	0.16	0.14	0.09	0.14
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 14:0	1.79	1.74	1.63	2.13	1.67	1.59	1.59	1.59	1.85	1.58
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 15:1	0.00	0.53	0.52	0.48	0.51	0.52	0.45	0.50	0.51	0.48
% 16:0	30.98	30.13	29.54	33.01	31.08	29.37	30.65	29.39	31.15	30.03
% 16:1	0.27	0.21	0.23	0.26	0.26	0.14	0.25	0.22	0.26	0.25
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.12	0.15	0.14	0.14	0.14	0.16	0.12	0.13	0.14	0.13
% 18:0	1.29	1.30	1.30	1.37	1.38	1.37	1.46	1.30	1.30	1.35
% 18:1 n-9	0.00	0.08	0.08	0.00	0.06	0.11	0.09	0.10	0.07	0.07
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.39	0.38	0.39	0.40	0.42	0.38	0.43	0.39	0.39	0.41
% 20:1 n-9	0.00	0.19	0.16	0.13	0.19	0.20	0.17	0.14	0.13	0.21
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:4 ARA	0.55	0.25	0.21	0.26	0.22	0.25	0.51	0.20	0.24	0.19
% 20:5 n-3 EPA	2.62	2.75	2.78	2.81	2.67	2.78	5.76	2.72	2.59	2.82
% 22:0	0.08	0.08	0.08	0.08	0.09	0.08	0.09	0.08	0.08	0.09

Table 27 continued

Fatty Acids	control ATCC PTA-9695	Mutant 53	Mutant 54	Mutant 55	Mutant 56	Mutant 57	Mutant 58	Mutant 60	Mutant 61	Mutant 65
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00
% 22:5 n-6	3.19	3.47	3.20	3.25	3.19	3.43	2.62	3.30	3.42	3.18
% 22:5 n-3	0.18	0.18	0.18	0.17	0.17	0.20	0.59	0.17	0.17	0.17
% 22:6 n-3 DHA	56.88	56.99	58.07	54.04	56.38	57.76	54.09	58.21	55.91	57.56
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.09	0.09	0.07	0.10	0.09	0.11	0.10	0.07	0.08
% Fat	46.83	45.83	39.59	48.81	41.92	43.97	33.96	36.97	50.40	36.21
% Unknown	0.85	1.28	1.19	1.19	1.29	1.35	0.77	1.24	1.48	1.17

Table 28: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA-9695	Mutant 66	Mutant 67	Mutant 68 ATCC PTA-9696	Mutant 69	Mutant 70 ATCC PTA-9697	Mutant 71	Mutant 72 ATCC PTA-9698	Mutant 73	Mutant 74
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00
% 14:0	2.42	2.29	2.07	2.09	2.11	2.21	2.27	2.29	1.97	2.05
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00
% 15:1	0.55	0.47	0.48	0.47	0.47	0.44	0.46	0.40	0.50	0.47
% 16:0	39.19	31.02	26.20	25.84	27.79	28.14	28.89	33.49	24.50	23.95
% 16:1	0.43	0.19	0.00	0.00	0.00	0.00	0.19	0.21	0.00	0.00
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00
% 18:0	1.67	1.68	1.22	1.22	1.44	1.49	1.51	2.24	1.11	1.02
% 18:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-3	0.00	0.18	0.20	0.21	0.19	0.17	0.22	0.16	0.22	0.22
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.49	0.41	0.32	0.31	0.35	0.37	0.44	0.52	0.29	0.27
% 20:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 28 continued

Fatty Acids	control ATCC PTA-9695	Mutant 66	Mutant 67	Mutant 68 ATCC PTA-9696	Mutant 69	Mutant 70 ATCC PTA-9697	Mutant 71	Mutant 72 ATCC PTA-9698	Mutant 73	Mutant 74
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:4 ARA	0.18	0.16	0.33	0.27	0.24	0.37	0.30	0.27	0.38	0.39
% 20:5 n-3 EPA	1.76	2.30	3.86	3.97	3.32	4.12	3.09	2.74	4.43	4.53
% 22:0	0.33	0.46	0.35	0.44	0.48	0.38	0.43	0.12	0.35	0.34
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:5 n-6	2.62	2.83	3.17	2.66	2.72	2.95	3.46	2.79	3.17	3.19
% 22:5 n-3	0.18	0.18	0.46	0.42	0.34	0.61	0.25	0.27	0.48	0.57
% 22:6 n-3 DHA	49.52	57.01	60.60	61.42	59.74	58.03	55.62	53.06	61.83	62.23
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% Fat	52.70	49.32	48.51	49.49	48.80	53.65	40.38	63.40	48.27	46.63
% Unknown	0.35	0.82	0.73	0.66	0.67	0.73	2.46	1.18	0.78	0.76

EXAMPLE 34

Isolation of Microorganisms

[0294] Samples were collected from intertidal habitats during low tide, including bays and estuaries along the West Coast of North America (California, Oregon, and Washington) and Hawaii. Water, sediment, living plant material, and decaying plant/animal debris were placed into sterile 50 ml tubes. Portions of each sample along with the water were spread onto solid agar plates of isolation media. Isolation media consisted of: 500 ml of artificial seawater, 500 ml of distilled water, 1 g of glucose, 1 g of glycerol, 13 g of agar, 1 g of glutamate, 0.5 g of yeast extract, 0.5 g casein hydrolysate, 1 ml of a vitamin solution (100 mg/L thiamine, 0.5 mg/L biotin, 0.5 mg B_{12}), 1 ml of a trace mineral solution (PII metals, containing per liter: 6.0 g $FeCl_36H_2O$, 6.84 g H_3BO_3, 0.86 g $MnCl_24H_2O$, 0.06 g $ZnCl_2$, 0.026 $CoCl_26H_20$, 0.052 g $NiSO_4H_2O$, 0.002 g $CuSO_45H_2O$ and 0.005 g $Na_2MoO_42H_2O$), and 500 mg each of penicillin G and streptomycin sulfate. The agar plates were incubated in the dark at 20-25°C. After 2-4 days the agar plates were examined under magnification, and colonies of cells were picked with a sterile toothpick and restreaked onto a fresh plate of media. Cells were repeatedly streaked onto fresh media until contaminated organisms were removed. Two of the isolated microorganisms were deposited under ATCC Accession Nos. PTA-10212 and PTA-10208.

Taxonomic Characteristics of the Isolated Microorganism Deposited Under ATCC Accession No. PTA-10212

[0295] Cultures of the isolated microorganism deposited under ATCC Accession No. PTA-10212 ("PTA-10212") appeared as white, wet, smeared colonies without visible isolated sori.

[0296] PTA-10212 was grown on solid and liquid FFM, solid KMV, KMV slush (1%), KMV broth, and MH broth to further examine growth characteristics. PTA-10212 was observed to grow rapidly on KMV and MH. *See, e.g.,* Porter D., 1989. *Phylum Labyrinthulomycota.* In Margulis, L., Corliss, J.O., Melkonian, M., Chapman, D.J. (Eds.) Handbook of Protoctista, Jones and Bartlett, Boston, pp. 388-398 (KMV); Honda *et al.*, *Mycol. Res. 102*:439-448 (1998) (MH); and U.S. Patent No. 5,130,242 (FFM).

[0297] The following observations were made after growth of PTA-10212 over several days on solid FFM media, after 72 hours growth in KMV medias, and MH broth. Sporangia were not clumped in/on any media and were very small (5-10 μm). PTA-10212 did not demonstrate the

copious tetrads characteristic of *Schizochytrium* cleavage patterns. Amoeboid cells appeared about 24 hours after transfer to fresh solid media. These amoeboid cells were gone after a few days and were not evident in liquid or slush media. Unlike *Aurantiochytrium*, described by Yokoyama, R. *et al.*, *Mycoscience 48*(6): 329-341 (2007), as having the appearance of "small sandgrains on the bottom of the flask" when grown in liquid media, PTA-10212 did not settle at the bottom of the flask but was suspended in both KMV and MH liquid media. The sporangia were not as dense as typical of *Schizochytrium* or *Oligochytrium*, which also have robust ectoplasmic networks that were absent from PTA-10212. While most species undergo vegetative cleavage of small sporangia or assimilative cells by the division of a larger sporangium over several hours, PTA-10212 formed dumbbell-shaped elongated assimilative cells, which then formed a thin isthmus that pulled apart as the ends of the dumbbell separated. The resulting cells appeared to be small assimilative cells. Direct transformation of an amoeboid cell into the dumbbell shaped assimilative cell was not observed. Typical biflagellate zoospores were observed swimming but were relatively rare. PTA-10212 was non-prolific, dividing by vegetative cleavage. Direct release of zoospores was not observed, although zoospores were observed swimming. Vegetative cells were very small (2 μm to 5 μm).

[0298] PTA-10212 was further examined using the flow-through technique, in which microscopic slides were prepared by suspending a small portion of an agar-grown colony in a drop of half-strength sea water. With this technique, primary sporangia were observed to be globose and approximately 10 μm in diameter. Walls were very thin and remnants were not observed when binary division of the protoplast was initiated. Repeated binary division produced 8-16 smaller (4-5 μm in diameter) secondary sporangia. The secondary sporangia remained quiescent for several hours before again releasing an amorphous protoplast. The amorphous protoplast divided by pinching and pulling, initially producing typical dumbbell-shaped intermediate stages and finally resulting in 4-8 small globose bodies 2.5-2.8 μm in diameter. The latter rested for several minutes up to 1-2 hours, then changed shape (elongated) and turned into biflagellate zoospores, 2.3-2.5 x 3.7-3.9 μm. Zoospores were abundant and could be precisely measured when they came to rest. Zoospores then rounded off and started a new cycle of development. The zoospores were larger than *Sicyoidochytrium minutum* and smaller than *Ulkenia visurgensis*.

[0299] PTA-10212 was further characterized based on the similarity of its 18s rRNA gene to that of known species. Genomic DNA was prepared from PTA-10212 by standard procedures. *See*,

for example, Sambrook J. and Russell D. 2001. Molecular cloning: A laboratory manual, 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. Briefly: (1) 500 µL of cells were centrifuged from mid-log culture. The cells were re-centrifuged, and all traces of liquid were removed from the cell pellet with a small-bore tip; (2) pellets were resuspended with 200 µL lysis buffer (20 mM Tris pH 8.0, 125 µg/mL Proteinase K, 50 mM NaCl, 10 mM EDTA pH 8.0, 0.5% SDS); (3) cells were lysed at 50°C for 1 hour; (4) the lysis mixture was pipetted into phase-lock gel (PLG-Eppendorf) 2 mL tubes; (5) equal volume of P:C:I was added and allowed to mix for 1.5 hours; (6) the tubes were centrifuged at 12,000 x g for 5 minutes; (7) the aqueous phase was removed from above the gel within the PLG tube and an equal volume of chloroform was added to the aqueous phase, and mixed for 30 minutes; (8) the tubes were centrifuged at 14,000 x g for approximately 5 minutes; (9) the top layer (aqueous phase) was pipetted away from the chloroform, and placed in a new tube; (10) 0.1 volume of 3 M NaOAC was added and mixed (inverted several times); (11) 2 volumes of 100% EtOH were added and mixed (inverted several times) with genomic DNA precipitant forming at this stage; (12) the tubes were centrifuged at 4°C in a microcentrifuge at 14,000 x g for approximately 15 minutes; (13) the liquid was gently poured off with genomic DNA remaining at the bottom of the tube; (14) the pellet was washed with 0.5 mL 70% EtOH; (15) the tubes were centrifuged at 4°C in a microcentrifuge at 14,000 x g for approximately 5 minutes; (16) the EtOH was gently poured off and the genomic DNA pellet was dried; and (17) a suitable volume of H_2O and RNase was added directly to the genomic DNA pellet. The PCR amplification of the 18s rRNA gene was carried out with primers previously described (Honda *et. al., J. Euk. Micro. 46*(6): 637-647 (1999). The PCR conditions with chromosomal DNA template were as follows: 0.2 µM dNTPs, 0.1 µM each primer, 8% DMSO, 200 ng chromosomal DNA, 2.5 U Herculase® II Fusion DNA Polymerase (Stratagene), and Herculase® buffer (Stratagene) in a 50 µL total volume. The PCR Protocol included the following steps: (1) 95°C for 2 minutes; (2) 95°C for 35 seconds; (3) 55°C for 35 seconds; (4) 72°C for 1 minute and 30 seconds; (5) repeat steps 2-4 for 30 cycles; (6) 72°C for 5 minutes; and (7) hold at 4°C.

[0300] PCR amplification yielded a distinct DNA product with the expected size using chromosomal template described above. The PCR product was cloned into the vector pJET1.2/blunt (Fermentas) according to the manufacturer's instructions, and the insert sequence was determined using supplied standard primers.

[0301] Phylogenetic analysis places PTA-10212 within the lineage that includes *Thraustochytrium pachydermum* and *Thraustochytrium aggregatum* with moderate support. The sporangia of *T. pachydermum* have very thick cell walls. *T. aggregatum* forms clearly visible clumps of sori that are opaque. PTA-10212 shows neither of these characteristics. The presence of many amoeboid cells has been described in other taxa, such as *Ulkenia*, *T. gaertnerium*, *A. limiacinum*, and *S. mangrovei*; however, the descriptions associated with those taxa differ from the observed characteristics of the isolate. Moreover, PTA-10212 did not show phylogenetic affinity towards any of these taxa.

[0302] Table 29 shows a comparison of the 18s rRNA sequence from the microorganism deposited under ATCC Accession No. PTA-10212 to DNA sequences in the National Center for Biotechnology Information (NCBI) electronic database. The percent identity was determined using two different calculations. "Calculation #1" takes into consideration any "gaps" that occurs in the sequences, either from non-homologous regions or partial sequence (AlignX-VectorNTI default settings). "Calculation #2" does not include calculated penalties for gaps (AlignX-VectorNTI "IDENTITY" matrix setting).

Table 29: Comparison of 18s rRNA Sequences

Thraustochytrids	**% Identity Calculation #1**	**% Identity Calculation #2**
Thraustochytrium pachydermum	85%	93%
Thraustochytrium aggregatum (p)	83%	92%
Thraustochytrium gaertnerium	82%	92%
Ulkenia visurgensis	82%	92%
Schizochytrium sp. PTA-9695	80%	92%
Schizochytrium mangrovei	80%	91%
Schizochytrium sp. ATCC 20888	80%	90%
Aurantiochytrium limiacinum	79%	90%

(p): indicates partial sequence

[0303] As shown in Table 29, it was found that, in terms of % identity, the 18s rRNA gene sequence from the microorganism deposited under ATCC Accession No. PTA-10212 is related, though not identical, to 18s rRNA gene sequences available in the NCBI database. It is generally recognized that organisms can have closely related 18s rRNA gene sequences while belonging to a different genus or species.

[0304] Based on the above characterization, the isolated microorganism (ATCC Accession No. PTA-10212) is believed to represent a new *Thraustochytrium* species and is therefore also designated as *Thraustochytrium sp.* ATCC PTA-10212.

Taxonomic Characteristics of the Isolated Microorganism Deposited Under ATCC Accession No. PTA-10208

[0305] The microorganism deposited under ATCC Accession No. PTA-10208 ("PTA-10208") was identified as a sub-isolate (an individual cell isolated from a culture and maintained as a new separate and distinct culture) of the microorganism deposited under ATCC Accession No. PTA-9695 ("PTA-9695"), described in U.S. Pub. No. 2010/0239533 and Int'l. Pub. No. WO 2010/107415.

[0306] PTA-10208 shares taxonomic characteristics with PTA-9695. PTA-9695 was found to have biflagellate zoospores at discharge that swim actively away from the mature sporangium, wall remnants of which were clearly visible (in phase contrast) after spore release. PTA-9695 sporangia measured 12.5 μm to 25 μm in diameter, and zoospores were 2.5 μm to 2.8 μm x 4.5 μm to 4.8 μm in size. There were 8 to 24 spores per individual PTA-9695 sporangium. Settled PTA-9695 zoospores enlarged and rapidly underwent binary divisions leading to tetrads, octads, and finally to clusters of sporangia. Tetrad formation commenced at a very early stage prior to maturity of the sporangia. These characteristics are in agreement with the genus *Schizochytrium*. In terms of % identity, the PTA-9695 18s rRNA gene sequence, which is shared by PTA-10208, was found to be closely related, though not identical, to the 18s rRNA gene sequence of *T. aggregatum* provided in Honda, D. *et al., J. Euk. Micro. 46*(6): 637-647 (1999). The 18s rRNA sequence published for *Thraustochytrium aggregatum* is a partial sequence, with an approximately 71 DNA nucleotide gap in the middle of the sequence. PTA-9695 is believed to represent a new *Schizochytrium* species. As such, the sub-isolate PTA-10208 is also designated as *Schizochytrium sp.* ATCC PTA-10208.

EXAMPLE 35

Growth Characteristics of the Isolated Microorganism Deposited Under ATCC Accession No. PTA-10212

[0307] The isolated microorganism (ATCC Accession No. PTA-10212) was examined for growth characteristics in individual fermentation runs, as described below. Typical media and cultivation conditions are shown in Table 30.

Table 30: PTA-10212 Vessel Media

Ingredient	concentration		ranges
Na_2SO_4	g/L	31.0	0-50, 15-45, or 25-35
NaCl	g/L	0.625	0-25, 0.1-10, or 0.5-5
KCl	g/L	1.0	0-5, 0.25-3, or 0.5-2
$MgSO_4 \cdot 7H_2O$	g/L	5.0	0-10, 2-8, or 3-6
$(NH_4)_2SO_4$	g/L	0.44	0-10, 0.25-5, or 0.05-3
$MSG \cdot 1H_2O$	g/L	6.0	0-10, 4-8, or 5-7
$CaCl_2$	g/L	0.29	0.1-5, 0.15-3, or 0.2-1
T 154 (yeast extract)	g/L	6.0	0-20, 0.1-10, or 1-7
KH_2PO_4	g/L	0.8	0.1-10, 0.5-5, or 0.6-1.8
Post autoclave (Metals)			
Citric acid	mg/L	3.5	0.1-5000, 10-3000, or 3-2500
$FeSO_4 \cdot 7H_2O$	mg/L	10.30	0.1-100, 1-50, or 5-25
$MnCl_2 \cdot 4H_2O$	mg/L	3.10	0.1-100, 1-50, or 2-25
$ZnSO_4 \cdot 7H_2O$	mg/L	3.10	0.01-100, 1-50, or 2-25
$CoCl_2 \cdot 6H_2O$	mg/L	0.04	0-1, 0.001-0.1, or 0.01-0.1
$Na_2MoO_4 \cdot 2H_2O$	mg/L	0.04	0.001-1, 0.005-0.5, or 0.01-0.1
$CuSO_4 \cdot 5H_2O$	mg/L	2.07	0.1-100, 0.5-50, or 1-25
$NiSO_4 \cdot 6H_2O$	mg/L	2.07	0.1-100, 0.5-50, or 1-25
Post autoclave (Vitamins)			
Thiamine	mg/L	9.75	0.1-100, 1-50, or 5-25
Vitamin B12	mg/L	0.16	0.01-100, 0.05-5, or 0.1-1
Ca½-pantothenate	mg/L	2.06	0.1-100, 0.1-50, or 1-10
Biotin	mg/L	3.21	0.1-100, 0.1-50, or 1-10
Post autoclave (Carbon)			
Glycerol	g/L	30.0	5-150, 10-100, or 20-50

Nitrogen Feed:

Ingredient	Concentration		
$MSG{\cdot}1H_2O$	g/L	17	0-150, 10-100, or 15-50

Typical cultivation conditions would include the following:

pH	about 6.5 – about 9.5, about 6.5 – about 8.0, or about 6.8 – about 7.8;
temperature:	about 15 – about 30 degrees Celsius, about 18 – about 28 degrees Celsius, or about 21 to about 23 degrees Celsius;
dissolved oxygen:	about 0.1 – about 100% saturation, about 5 – about 50% saturation, or about 10 – about 30% saturation; and/or
glycerol controlled @:	about 5 – about 50 g/L, about 10 – about 40 g/L, or about 15 – about 35 g/L.

[0308] In carbon (glycerol) and nitrogen-fed cultures with 1000 ppm Cl^- at 22.5°C with 20% dissolved oxygen at pH 7.3, PTA-10212 produced a dry cell weight of 26.2 g/L after 138 hours of culture in a 10 L fermentor volume. The lipid yield was 7.9 g/L; the omega-3 yield was 5.3 g/L; the EPA yield was 3.3 g/L; and the DHA yield was 1.8 g/L. The fatty acid content was 30.3% by weight; the EPA content was 41.4% of fatty acid methyl esters (FAME); and the DHA content was 26.2% of FAME. The lipid productivity was 1.38 g/L/day, and the omega-3 productivity was 0.92 g/L/day under these conditions, with 0.57 g/L/day EPA productivity and 0.31 g/L/day DHA productivity.

[0309] In carbon (glycerol) and nitrogen-fed cultures with 1000 ppm Cl^- at 22.5°C with 20% dissolved oxygen at pH 7.3, PTA-10212 produced a dry cell weight of 38.4 g/L after 189 hours of culture in a 10 L fermentor volume. The lipid yield was 18 g/L; the omega-3 yield was 12 g/L; the EPA yield was 5 g/L; and the DHA yield was 6.8 g/L. The fatty acid content was 45% by weight; the EPA content was 27.8% of FAME; and the DHA content was 37.9% of FAME. The lipid productivity was 2.3 g/L/day, and the omega-3 productivity was 1.5 g/L/day under these conditions, with 0.63 g/L/day EPA productivity and 0.86 g/L/day DHA productivity.

[0310] In carbon (glycerol) and nitrogen-fed cultures with 1000 ppm Cl^- at 22.5°C with 20% dissolved oxygen at pH 6.8-7.7, PTA-10212 produced a dry cell weight of 13 g/L after 189 hours of culture in a 10 L fermentor volume. The lipid yield was 5.6 g/L; the omega-3 yield was 3.5 g/L; the EPA yield was 1.55 g/L; and the DHA yield was 1.9 g/L. The fatty acid content was 38% by weight; the EPA content was 29.5% of FAME; and the DHA content was 36% of

FAME. The lipid productivity was 0.67 g/L/day, and the omega-3 productivity was 0.4 g/L/day under these conditions, with 0.20 g/L/day EPA productivity and 0.24 g/L/day DHA productivity.

[0311] In carbon (glycerol) and nitrogen-fed cultures with 1000 ppm Cl^- at 22.5-28.5°C with 20% dissolved oxygen at pH 6.6-7.2, PTA-10212 produced a dry cell weight of 36.7 g/L – 48.7 g/L after 191 hours of culture in a 10 L fermentor volume. The lipid yield was 15.2 g/L – 25.3 g/L; the omega-3 yield was 9.3 g/L – 13.8 g/L; the EPA yield was 2.5 g/L – 3.3 g/L; and the DHA yield was 5.8 g/L – 11 g/L. The fatty acid content was 42.4% - 53% by weight; the EPA content was 9.8% - 22% of FAME; and the DHA content was 38.1% - 43.6% of FAME. The lipid productivity was 1.9 g/L/day – 3.2 g/L/day, and the omega-3 productivity was 1.2 g/L/day – 1.7 g/L/day under these conditions, with 0.31 g/L/day – 0.41 g/L/day EPA productivity and 0.72 g/L/day – 1.4 g/L/day DHA productivity.

Growth Characteristics of the Isolated Microorganism Deposited Under ATCC Accession No. PTA-10208

[0312] The isolated microorganism (ATCC Accession No. PTA-10208) was examined for growth characteristics in individual fermentation runs, as described below. Typical media and cultivation conditions are shown in Table 31.

Table 31: PTA-10208 Vessel Media

Ingredient	concentration		ranges
Na_2SO_4	g/L	8.8	0-25, 2-20, or 3-10
NaCl	g/L	0.625	0-25, 0.1-10, or 0.5-5
KCl	g/L	1.0	0-5, 0.25-3, or 0.5-2
$MgSO_4 \cdot 7H_2O$	g/L	5.0	0-10, 2-8, or 3-6
$(NH_4)_2SO_4$	g/L	0.42	0-10, 0.25-5, or 0.05-3
$CaCl_2$	g/L	0.29	0.1-5, 0.15-3, or 0.2-1
T 154 (yeast extract)	g/L	1.0	0-20, 0.1-10, or 0.5-5
KH_2PO_4	g/L	1.765	0.1-10, 0.5-5, or 1-3
Post autoclave (Metals)			
Citric acid	mg/L	46.82	0.1-5000, 10-3000, or 40-2500
$FeSO_4 \cdot 7H_2O$	mg/L	10.30	0.1-100, 1-50, or 5-25
$MnCl_2 \cdot 4H_2O$	mg/L	3.10	0.1-100, 1-50, or 2-25
$ZnSO_4 \cdot 7H_2O$	mg/L	9.3	0.01-100, 1-50, or 2-25
$CoCl_2 \cdot 6H_2O$	mg/L	0.04	0-1, 0.001-0.1, or 0.01-0.1
$Na_2MoO_4 \cdot 2H_2O$	mg/L	0.04	0.001-1, 0.005-0.5, or 0.01-0.1
$CuSO_4 \cdot 5H_2O$	mg/L	2.07	0.1-100, 0.5-50, or 1-25
$NiSO_4 \cdot 6H_2O$	mg/L	2.07	0.1-100, 0.5-50, or 1-25

Post autoclave (Vitamins)			
Thiamine	mg/L	9.75	0.1-100, 1-50, or 5-25
Ca½-pantothenate	mg/L	3.33	0.1-100, 0.1-50, or 1-10
Biotin	mg/L	3.58	0.1-100, 0.1-50, or 1-10
Post autoclave (Carbon)			
Glucose	g/L	30.0	5-150, 10-100, or 20-50

Nitrogen Feed:

Ingredient	Concentration		
NH_4OH	mL/L	23.6	0-150, 10-100, or 15-50

Typical cultivation conditions would include the following:

pH	about 6.5 – about 8.5, about 6.5 – about 8.0, or about 7.0 – about 8.0;
temperature:	about 17 – about 30 degrees Celsius, about 20 – about 28 degrees Celsius, or about 22 to about 24 degrees Celsius;
dissolved oxygen:	about 2 – about 100% saturation, about 5 – about 50% saturation, or about 7 – about 20% saturation; and/or
glucose controlled @: 20	about 5 – about 50 g/L, about 10 – about 40 g/L, or about – about 35 g/L.

[0313] In carbon (glucose) and nitrogen-fed cultures with 1000 ppm Cl^- at 22.5°C at pH 7.0 with 20% dissolved oxygen during the nitrogen feed and 10% dissolved oxygen thereafter, PTA-10208 produced a dry cell weight of 95 g/L after 200 hours of culture in a 10 L fermentor volume. The lipid yield was 53.7 g/L; the omega-3 yield was 37 g/L; the EPA yield was 14.3 g/L; and the DHA yield was 21 g/L. The fatty acid content was 57% by weight; the EPA content was 27.7% of FAME; and the DHA content was 39.1% of FAME. The lipid productivity was 6.4 g/L/day, and the omega-3 productivity was 4.4 g/L/day under these conditions, with 1.7 g/L/day EPA productivity and 2.5 g/L/day DHA productivity.

[0314] In carbon (glucose) and nitrogen-fed cultures with 1000 ppm Cl^- at 22.5°C at pH 7.5 with 20% dissolved oxygen during the nitrogen feed and 10% dissolved oxygen thereafter, PTA-10208 produced a dry cell weight of 56 g/L after 139 hours of culture in a 10 L fermentor volume. The lipid yield was 53 g/L; the omega-3 yield was 34 g/L; the EPA yield was 11.5 g/L; and the DHA yield was 22 g/L. The fatty acid content was 58% by weight; the EPA content was 21.7% of FAME; and the DHA content was 41.7% of FAME. The lipid productivity was 9.2

g/L/day, and the omega-3 productivity was 5.9 g/L/day under these conditions, with 2 g/L/day EPA productivity and 3.8 g/L/day DHA productivity.

[0315] In carbon (glucose) and nitrogen-fed cultures with 1000 ppm Cl^- at 22.5°C at pH 7.0 with 20% dissolved oxygen during the nitrogen feed and 10% dissolved oxygen thereafter, PTA-10208 produced a dry cell weight of 93.8 g/L after 167 hours of culture in a 2000 L fermentor volume. The lipid yield was 47.2 g/L; the omega-3 yield was 33.1 g/L; the EPA yield was 10.5 g/L; and the DHA yield was 20.4 g/L. The fatty acid content was 50.6% by weight; the EPA content was 23% of FAME; and the DHA content was 42.6% of FAME. The lipid productivity was 6.8 g/L/day, and the omega-3 productivity was 4.7 g/L/day under these conditions, with 1.5 g/L/day EPA productivity and 2.9 g/L/day DHA productivity.

[0316] In carbon (glucose) and nitrogen-fed cultures with 1000 ppm Cl^- at 22.5°C at pH 7.0 with 20% dissolved oxygen during the nitrogen feed and 10% dissolved oxygen thereafter, PTA-10208 produced a dry cell weight of 105 g/L after 168 hours of culture in a 2000 L fermentor volume. The lipid yield was 46.4g/L; the omega-3 yield was 33 g/L; the EPA yield was 10.7 g/L; and the DHA yield was 20.3 g/L. The fatty acid content was 43.9% by weight; the EPA content was 24% of FAME; and the DHA content was 43.7% of FAME. The lipid productivity was 6.6 g/L/day, and the omega-3 productivity was 4.7 g/L/day under these conditions, with 1.5 g/L/day EPA productivity and 2.9 g/L/day DHA productivity.

[0317] In carbon (glucose) and nitrogen-fed cultures with 1000 ppm Cl^- at 22.5°C at pH 7.0 with 20% dissolved oxygen during the nitrogen feed and 10% dissolved oxygen thereafter, PTA-10208 produced a dry cell weight of 64.8 g/L after 168 hours of culture in a 2000 L fermentor volume. The lipid yield was 38.7 g/L; the omega-3 yield was 29.9 g/L; the EPA yield was 8.5 g/L; and the DHA yield was 16.7 g/L. The fatty acid content was 59.6% by weight; the EPA content was 23% of FAME; and the DHA content was 42.3% of FAME. The lipid productivity was 5.53 g/L/day, and the omega-3 productivity was 3.8 g/L/day under these conditions, with 1.2 g/L/day EPA productivity and 2.3 g/L/day DHA productivity.

EXAMPLE 36

Fatty Acid Profiles of Microorganism Strains ATCC PTA-10208 and PTA-10212

[0318] Four samples of biomass (PTA-10208 Sample #1; PTA-10208 Sample #2; PTA-10212 Sample #1; and PTA-10212 Sample #2) were analyzed for total crude oil content by solvent extraction, lipid classes were determined by high performance liquid chromatography/evaporative light scattering detection (HPLC/ELSD), triacylglycerol (TAG) were analyzed by HPLC/mass spectrometry (HPLC/MS), and fatty acid (FA) profiles were determined by gas chromatography with flame ionization detection (GC-FID). The crude lipid content of each freeze dried biomass was determined using solvent grinding with hexane and compared to the sum of FAME (mg/g) generated by direct transesterification, and the resultant fatty acid methyl esters (FAME) were quantified by GC/FID analysis. Fatty acids in the extracted crude lipid were also quantified by transesterification and quantified using GC/FID analysis of the resultant FAME. The weight percent of all neutral lipids (NL) and free fatty acids (FFA) were determined in the extracted crude lipid using normal phase HPLC with ELSD and atmospheric pressure chemical ionization-MS (APCI-MS) identification. The method separates and quantifies sterol esters (SE), TAG, free fatty acids (FFA), 1,3-diacylglycerols (1,3-DAG), sterols, 1,2-diacylglycerols (1,2-DAG), and monoacylglycerols (MAG). Results are shown in Tables 32 and 33, below. It is noted that fatty acid profiles of Tables 32 and 33 were obtained from samples extracted using a solvent. The fatty acid profiles of Tables 32 and 33 are expected to be the substantially the same if the samples were extracted using the processes of the present invention.

[0319] TAG and phospholipids (PL) were isolated from the crude oils extracted from the four samples of biomass (PTA-10208 Sample #1; PTA-10208 Sample #2; PTA-10212 Sample #1; and PTA-10212 Sample #2). TAG was isolated using low pressure flash chromatography and PL was isolated using solid phase extraction (SPE). The identity of each isolated fraction was confirmed by thin layer chromatography (TLC). The fatty acid profiles of the isolated TAG and PL fractions were determined following direct transesterification using GC-FID as FAME. Results are shown in Tables 34 and 35, below.

[0320] The total crude oil content and fatty acid profiles of isolated lipid classes were also determined for two additional samples of biomass from microorganism strain ATCC PTA-10212 (PTA-10212 Sample #3 and PTA-10212 Sample #4). Crude oil was obtained from each sample

by hexane extraction, and individual lipid classes were isolated using low pressure flash chromatography. The fatty acid profiles of the biomass, crude oil, and isolated fractions were determined using GC-FID as FAME. Results are shown in Tables 36-39, below. It is noted that fatty acid profiles of Tables 36-39 were obtained from samples extracted using a typical hexane extraction. The fatty acid profiles of Tables 36-39 are expected to be the substantially the same if the samples were extracted using the processes of the present invention.

[0321] Individual lipid classes were isolated from a sample of crude oil from microorganism strain ATCC PTA-10212 (PTA-10212 Sample #5) previously extracted using the FRIOLEX® process, and the fatty acid profiles of each class were determined using GC-FID as FAME. Results are shown in Tables 40 and 41, below. It is noted that fatty acid profiles of Tables 40 and 41 were obtained from samples extracted using a FRIOLEX® process. The fatty acid profiles of Tables 40 and 41 are expected to be the substantially the same if the samples were extracted using the processes of the present invention.

[0322] Individual lipid classes were isolated from a sample of crude oil from microorganism strain ATCC PTA-10208 (PTA-10208 Sample #3) using normal HPLC with ELSD and APCI-MS identification.

Experimental Procedures

[0323] *Crude Oil Extraction* – Crude oil was extracted from samples of freeze-dried biomass using solvent grinding. For example, approximately 3 grams of biomass was weighed into a Swedish tube. Three ball bearings and 30 mL of hexane were added to the Swedish tube, which was sealed with a neoprene stopper and placed in a shaker for 2 hours. The resultant slurry was filtered using a Buchner funnel and Whatman filter paper. The filtered liquid was collected, the solvent removed under vacuum, and the amount of remaining crude lipid determined gravimetrically.

[0324] *Fatty Acid Analysis* – The samples of biomass, extracted crude lipid, and isolated lipid classes were analyzed for fatty acid composition as FAME. Briefly, freeze-dried biomass and isolated lipid classes were weighed directly into a screw cap test tubes, while samples of the crude oil were dissolved in hexane to give a concentration of approximately 2 mg/mL. Toluene, containing internal standard, and 1.5 N HCl in methanol was added to each tube. The tubes were vortexed, then capped and heated to 100° C for 2 hours. The tubes were allowed to cool, and saturated NaCl in water was added. The tubes were vortexed again and centrifuged to allow the

layers to separate. A portion of the organic layer was then placed in a GC vial and analyzed by GC-FID. FAME was quantified using a 3-point calibration curve generated using Nu-Check-Prep GLC Reference Standard (NuCheck, Elysian, MN). Fatty acids present in the extract were expressed as mg/g and as a weight percent. Fat content in the samples was estimated assuming equal response to the internal standard when analyzed by GC-FID.

[0325] *HPLC/ELSD/MS Method* –

Instrument	Agilent 1100 HPLC, Alltech 3300 ELSD, Agilent 1100 MSD
Column	Phenomenex Luna Silica, 250 x 4.6 mm, 5 μm particle size w/ Guard Column
Mobile Phase	A – 99.5% Hexanes (Omnisolv) 0.4% Isopropyl alcohol (Omnisolv) 0.1% Acetic Acid B – 99.9% Ethanol (Omnisolv, 95:5 Ethanol:IPA) 0.1% Acetic Acid

Gradient

Time, min.	% A	% B
0	100	0
5	100	0
15	85	10
20	0	100
25	0	100
26	100	0
35	100	0

Column Temp.	30°C
Flow Rate	1.5 mL/min
Injection Volume	5 μL
ELSD Detection	Temperature 35°C, Gas flow 1.2 L/min
MSD	Mass Range 200 – 1200, Fragmentor 225 V Drying Gas Temperature 350°C Vaporizer Temperature 325°C Capillary Voltage 3500 V Corona Current 10 μA

[0326] *Solid Phase Extraction* –PL fractions were separated from the crude lipid by solid phase extraction (SPE) using 2 g aminopropyl cartridges (Biotage, Uppsala, Sweden) placed in a Vac Elut apparatus (Varian Inc, Palo Alto, USA). The cartridge was conditioned with 15 mL of hexane, and ~60 mg of each sample was dissolved in 1 mL $CHCl_3$ and applied to the cartridge.

The column was washed with 15 mL of 2:1 $CHCl_3$:isopropyl alcohol to elute all the neutral lipids, which was discarded. The fatty acids were then eluted with 15 mL of 2% acetic acid (HOAc) in ether, which was discarded. The PL portion was eluted with 15 mL of 6:1 Methanol:Chloroform, which was collected, dried under nitrogen, and weighed.

[0327] *Flash Chromatography* – Flash chromatography was used to separate the lipid classes present in the crude oil. Approximately 200 mg of crude oil dissolved in hexane was injected onto the head of the column. The chromatography system utilized Silica Gel 60 (EMD Chemical, Gibbstown, NJ) with mobile phase composed of Petroleum Ether and Ethyl Acetate at 5 mL/min (Tables 6-7) or 3 mL/min (Tables 8-13). A step gradient was used to selectively elute each lipid class from the column. The mobile phase gradient started from 100% petroleum ether and finished with 50% ethyl acetate. Fractions were collected in 10 mL test tubes using a Gilson FC 204 large-bed fraction collector (Gilson, Inc., Middleton, WI). Each tube was analyzed by thin layer chromatography (TLC) and the tubes containing individual lipid classes (as judged by single spots on TLC plate with expected retention factor (Rf)) were pooled, concentrated to dryness, and weighed. The total fraction content was then determined gravimetrically.

[0328] *TLC Analysis* – Thin layer chromatography was conducted on silica gel plates. The plates were eluted using a solvent system consisting of petroleum ether : ethyl ether : acetic acid (80:20:1) and were visualized using iodine vapor. The Rf values of each spot were then compared with reported literature values for each lipid class.

[0329] *Analysis of TAG and PL fractions* – The isolated TAG and PL fractions were analyzed for fatty acid composition as fatty acid methyl esters (FAME). The TAG fractions were dissolved in hexane to give a concentration of approximately 1-2 mg/mL. 1 mL aliquots of the solutions were concentrated to dryness under nitrogen. Toluene, containing internal standard, and 1.5 N HCl in methanol was added to each tube. The tubes were vortexed, then capped and heated to 100° C for 2 hours. Internal standard and HCl methanol were added directly to the tubes containing the PL fraction and heated. The tubes were allowed to cool, and saturated NaCl in water was added. The tubes were vortexed again and centrifuged to allow the layers to separate. A portion of the organic layer was then placed in a GC vial and analyzed by GC-FID. FAMEs were quantified using a 3-point calibration curve generated using Nu-Check-Prep GLC 502B Reference Standard (NuCheck, Elysian, MN). Fatty acids present in the extract were expressed as mg/g and as a % of FAME.

Results

PTA-10208 Sample #1

[0330] The fatty acid profile of the biomass and extracted crude lipid for PTA-10208 Sample #1 was determined using GC/FID. Fatty acids in the biomass were transesterified *in situ* by weighing 28.6 mg of biomass directly into a FAME tube, while a sample of the extracted crude lipid was prepared by weighing 55.0 mg of crude lipid into a 50 mL volumetric flask and transferring 1 ml to a separate FAME tube. The estimated crude lipid content of the biomass was determined to be 53.2% (as SUM of FAME) using GC with FID detection, while 52.0% (wt/wt) lipid was extracted from the dry biomass, giving a 97.8% recovery of total lipid. The crude lipid was determined to be 91.9% fatty acids (as SUM of FAME) using GC/FID. The major fatty acids contained in the crude lipid were C16:0 (182.5 mg/g), C20:5 n-3 (186.8 mg/g), and C22:6 n-3 (423.1 mg/g).

[0331] The lipid class profile of the extracted crude lipid was determined by weighing 55.0 mg of crude lipid into a 50 mL volumetric flask and transferring an aliquot into an HPLC vial for HPLC/ELSD/MS analysis. According to the HPLC/ELSD/MS analysis, the crude lipid contained 0.2% sterol esters (SE), 95.1% TAG, 0.4% sterols, and 0.5% 1,2-diacylglycerol (DAG). 5% of the TAG fraction included a peak that eluted directly after the TAG peak, but did not give a recognizable mass spectrum.

[0332] Isolated TAG from this sample as determined by flash chromatography made up approximately 92.4% of the crude oil. PL was not detected by weight or TLC after SPE isolation. The major fatty acids (>50 mg/g) contained in the TAG were C16:0 (189 mg/g), C20:5 n-3 (197 mg/g), and C22:6 n-3 (441 mg/g).

PTA-10208 Sample #2

[0333] The fatty acid profile of the biomass and extracted crude lipid for PTA-10208 Sample #2 was determined using GC/FID. Fatty acids in the biomass were transesterified *in situ* by weighing 32.0 mg of biomass directly into a FAME tube, while a sample of the extracted crude lipid was prepared by weighing 60.1 mg of crude lipid into a 50 mL volumetric flask and transferring 1 ml to a separate FAME tube. The estimated crude lipid content of the biomass was determined to be 52.4% (as SUM of FAME) using GC with FID detection, while 48.0% (wt/wt) lipid was extracted from the dry biomass, giving a 91.7% recovery of total lipid. The crude lipid was determined to be 95.3% fatty acids (as SUM of FAME) using GC/FID. The major fatty

acids contained in the crude lipid were C16:0 (217.5 mg/g), C20:5 n-3 (169.3 mg/g), and C22:6 n-3 (444.1 mg/g).

[0334] The lipid class profile of the extracted crude lipid was determined by weighing 60.1 mg of crude lipid into a 50 mL volumetric flask and transferring an aliquot into an HPLC vial for HPLC/ELSD/MS analysis. According to the HPLC/ELSD/MS analysis, the crude lipid contained 0.2% SE, 95.7% TAG, 0.3% sterols, and 0.7% 1,2-DAG. 5.1% of the TAG fraction included a peak that eluted directly after the TAG peak, but did not give a recognizable mass spectrum.

[0335] Isolated TAG from this sample made up approximately 93.9% of the crude oil. PL was not detected by weight or TLC after SPE isolation. The major fatty acids (>50mg/g) contained in the TAG were C16:0 (218 mg/g), C20:5 n-3 (167 mg/g) and C22:6 n-3 (430 mg/g).

PTA-10208 Sample #3

[0336] A sample of crude oil from the microorganism deposited under ATCC Accession No. PTA-10208 (Sample PTA-10208 #3) was analyzed using HPLC/ELSD/MS. A total of 98.38% of lipids were recovered, with the sterol ester (SE) fraction accounting for 0.32%, the TAG fraction accounting for 96.13%, the 1,3-diacylglycerol (DAG) fraction accounting for 0.22%, the 1,2-DAG fraction accounting for 0.78%, and the sterol fraction accounting for 0.93%.

PTA-10212 Sample #1

[0337] The fatty acid profile of the biomass and extracted crude lipid for PTA-10212 Sample #1 was determined using GC/FID. Fatty acids in the biomass were transesterified *in situ* by weighing 27.0 mg of biomass directly into a FAME tube, while a sample of the extracted crude lipid was prepared by weighing 52.5 mg of crude lipid into a 50 mL volumetric flask and transferring 1 ml to a separate FAME tube. The estimated crude lipid content of the biomass was determined to be 38.3% (as SUM of FAME) using GC with FID detection, while 36.3% (wt/wt) lipid was extracted from the dry biomass, giving a 94.6% recovery of total lipid. The crude lipid was determined to be 83.2% fatty acids (as SUM of FAME) using GC/FID. The major fatty acids contained in the crude lipid were C16:0 (328.5 mg/g), C20:5 n-3 (90.08 mg/g), and C22:6 n-3 (289.3 mg/g).

[0338] The lipid class profile of the extracted crude lipid was determined by weighing 52.5 mg of crude lipid into a 50 mL volumetric flask and transferring an aliquot into an HPLC vial for HPLC/ELSD/MS analysis. According to the HPLC/ELSD/MS analysis, the crude lipid contained 0.2% SE, 64.2% TAG, 1.9% FFA, 2.8% 1,3-DAG, 1.4% sterols, 18.8% 1,2-DAG, and 0.5%

MAG. 3.4% of the TAG fraction included a peak that eluted directly after the TAG peak, but did not give a recognizable mass spectrum.

[0339] Isolated TAG from this sample made up approximately 49.8% of the crude oil. Isolated PL made up approximately 8.1% of the crude oil. The major fatty acids (>50mg/g) contained in the TAG fraction are C16:0 (400 mg/g), C20:5 n-3 (91 mg/g), and C22:6 n-3 (273 mg/g). The major fatty acids (>50mg/g) contained in the PL fraction are C16:0 (98 mg/g), C20:5 n-3 (33 mg/g), and C22:6 n-3 (227 mg/g).

PTA-10212 Sample #2

[0340] The fatty acid profile of the biomass and extracted crude lipid PTA-10212 Sample #2 was determined using GC/FID. Fatty acids in the biomass were transesterified *in situ* by weighing 29.5 mg of biomass directly into a FAME tube, while a sample of the extracted crude lipid was prepared by weighing 56.5 mg of crude lipid into a 50 mL volumetric flask and transferring 1 ml to a separate FAME tube. The estimated crude lipid content of the biomass was determined to be 40.0% (as SUM of FAME) using GC with FID detection, while 41.3% (wt/wt) lipid was extracted from the dry biomass, giving a 106.1% recovery of total lipid. The crude lipid was determined to be 82.8% fatty acids (as SUM of FAME) using GC/FID. The major fatty acids contained in the crude lipid were C16:0 (327.3 mg/g), C20:5 n-3 (92.5 mg/g), and C22:6 n-3 (277.6 mg/g).

[0341] The lipid class profile of the extracted crude lipid was determined by weighing 56.5 mg of crude lipid into a 50 mL volumetric flask and transferring an aliquot into an HPLC vial for HPLC/ELSD/MS analysis. According to the HPLC/ELSD/MS analysis, the crude lipid contained 0.2% SE, 58.2% TAG, 2.3% FFA, 3.4% 1,3-DAG, 1.7% sterols, 23.4% 1,2-DAG, and 0.6% MAG. 3.3% of the TAG fraction included a peak that eluted directly after the TAG peak, but did not give a recognizable mass spectrum.

[0342] Isolated TAG from this sample made up approximately 51.9% of the crude oil. Isolated PL made up approximately 8.8% of the crude oil. The major fatty acids (>50mg/g) contained in the TAG fraction are C16:0 (402 mg/g), C20:5 n-3 (92 mg/g), and C22:6 n-3 (245 mg/g). The major fatty acids (>50mg/g) contained in the PL fraction are C16:0 (121 mg/g), C20:5 n-3 (48 mg/g), and C22:6 n-3 (246 mg/g).

Table 32: Fatty Acid Profiles of PTA-10208 and PTA-10212 Biomasses and Extracted Crude Lipids (mg/g)

	PTA-10208 Sample #1 Biomass	PTA-10208 Sample #1 Crude Lipid	PTA-10208 Sample #2 Biomass	PTA-10208 Sample #2 Crude Lipid	PTA-10212 Sample #1 Biomass	PTA-10212 Sample #1 Crude Lipid	PTA-10212 Sample #2 Biomass	PTA-10212 Sample #2 Crude Lipid
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0	1.47	2.43	1.80	3.14	0.99	1.90	0.87	1.91
C14:0	11.62	20.12	16.72	31.03	5.51	12.91	5.97	13.69
C14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C15:0	2.43	3.75	3.60	6.22	9.13	20.42	9.39	20.81
C16:0	105.04	182.47	117.72	217.49	145.87	328.45	147.87	327.27
C16:1	0.00	0.00	0.06	0.01	6.26	14.53	7.46	16.89
C18:0	5.37	8.96	4.77	8.37	6.77	15.39	6.77	15.15
C18:1 n-9	0.00	3.26	0.00	3.09	0.03	4.04	0.08	5.87
C18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18:2 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:0	1.48	1.79	1.40	1.85	1.60	3.09	1.67	3.20
C18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18:4 n-3	0.91	1.61	1.10	2.00	2.28	2.56	2.21	2.64
C20:2 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C22:0	0.10	0.00	0.08	0.00	0.30	0.12	0.35	0.24
C20:4 n-7	0.81	0.45	0.67	0.41	0.00	0.00	0.00	0.00
C20:4 n-6	7.22	12.23	6.84	12.18	1.19	2.26	1.31	2.32
C22:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:4 n-5	0.63	0.52	0.00	0.46	0.00	0.00	0.00	0.00
C20:4 n-3	3.45	5.45	3.33	5.58	0.00	2.40	0.00	2.34
C20:3 n-3	0.09	0.00	0.11	0.00	0.00	0.00	0.00	0.00
C20:5 n-3	107.31	186.83	92.99	169.32	40.32	90.08	43.15	92.54
C22:4 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24:0	0.60	0.00	0.52	0.00	2.81	6.83	2.74	6.53
C24:1 n-9	1.55	3.26	0.85	2.04	0.43	1.34	0.42	1.24
C22:5 n-6	9.66	15.84	10.27	17.98	2.42	4.68	2.32	4.21

	PTA-10208 Sample #1 Biomass	PTA-10208 Sample #1 Crude Lipid	PTA-10208 Sample #2 Biomass	PTA-10208 Sample #2 Crude Lipid	PTA-10212 Sample #1 Biomass	PTA-10212 Sample #1 Crude Lipid	PTA-10212 Sample #2 Biomass	PTA-10212 Sample #2 Crude Lipid
C22:5 n-3	20.44	35.13	9.92	17.50	2.41	4.94	2.69	5.23
C22:6 n-3	246.98	423.10	245.96	444.08	139.58	289.34	137.35	277.57
Sum of FAME	527.15	907.18	518.71	942.75	367.89	805.29	372.63	799.68

Table 33: Fatty Acid Profiles of PTA-10208 and PTA-10212 Biomasses and Extracted Crude Lipids (%)

	PTA-10208 Sample #1 Biomass	PTA-10208 Sample #1 Crude Lipid	PTA-10208 Sample #2 Biomass	PTA-10208 Sample #2 Crude Lipid	PTA-10212 Sample #1 Biomass	PTA-10212 Sample #1 Crude Lipid	PTA-10212 Sample #2 Biomass	PTA-10212 Sample #2 Crude Lipid
Fatty Acid	% FAME	% FAME	% FAME	% FAME	% FAME	% FAME	% FAME	% FAME
C12:0	0.28	0.27	0.35	0.33	0.27	0.24	0.23	0.24
C14:0	2.20	2.22	3.22	3.29	1.50	1.60	1.60	1.71
C14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C15:0	0.46	0.41	0.69	0.66	2.48	2.54	2.52	2.60
C16:0	19.93	20.11	22.70	23.07	39.65	40.79	39.68	40.93
C16:1	0.00	0.00	0.01	0.00	1.70	1.80	2.00	2.11
C18:0	1.02	0.99	0.92	0.89	1.84	1.91	1.82	1.89
C18:1 n-9	0.00	0.36	0.00	0.33	0.01	0.50	0.02	0.73
C18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18:2 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:0	0.28	0.20	0.27	0.20	0.43	0.38	0.45	0.40
C18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18:4 n-3	0.17	0.18	0.21	0.21	0.62	0.32	0.59	0.33
C20:2 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C22:0	0.02	0.00	0.01	0.00	0.08	0.02	0.09	0.03
C20:4 n-7	0.15	0.05	0.13	0.04	0.00	0.00	0.00	0.00
C20:4 n-6	1.37	1.35	1.32	1.29	0.32	0.28	0.35	0.29
C22:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

	PTA-10208 Sample #1 Biomass	PTA-10208 Sample #1 Crude Lipid	PTA-10208 Sample #2 Biomass	PTA-10208 Sample #2 Crude Lipid	PTA-10212 Sample #1 Biomass	PTA-10212 Sample #1 Crude Lipid	PTA-10212 Sample #2 Biomass	PTA-10212 Sample #2 Crude Lipid
C20:4 n-5	0.12	0.06	0.00	0.05	0.00	0.00	0.00	0.00
C20:4 n-3	0.65	0.60	0.64	0.59	0.00	0.30	0.00	0.29
C20:3 n-3	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00
C20:5 n-3	20.36	20.59	17.93	17.96	10.96	11.19	11.58	11.57
C22:4 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24:0	0.11	0.00	0.10	0.00	0.76	0.85	0.74	0.82
C24:1 n-9	0.29	0.36	0.16	0.22	0.12	0.17	0.11	0.16
C22:5 n-6	1.83	1.75	1.98	1.91	0.66	0.58	0.62	0.53
C22:5 n-3	3.88	3.87	1.91	1.86	0.65	0.61	0.72	0.65
C22:6 n-3	46.85	46.64	47.42	47.10	37.94	35.93	36.86	34.71
Sum of FAME %	100	100	100	100	100	100	100	100

Table 34: Fatty Acid Profiles of PTA-10208 and PTA-10212 Isolated TAG

	PTA-10208 Sample #1	PTA-10208 Sample #1	PTA-10208 Sample #2	PTA-10208 Sample #2	PTA-10212 Sample #1	PTA-10212 Sample #1	PTA-10212 Sample #2	PTA-10212 Sample #2
Fatty Acid	FAME (mg/g)	% FAME	FAME (mg/g)	% FAME	FAME (mg/g)	% FAME	FAME (mg/g)	% FAME
C12:0	2.57	0.27	3.35	0.36	0.00	0.00	0.00	0.00
C14:0	21.07	2.23	31.37	3.41	14.05	1.61	14.45	1.69
C14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C15:0	3.89	0.41	6.17	0.67	23.27	2.66	23.14	2.71
C16:0	189.28	20.07	218.78	23.75	399.51	45.75	402.43	47.07
C16:1	0.00	0.00	0.00	0.00	15.23	1.74	17.62	2.06
C18:0	9.21	0.98	8.07	0.88	22.70	2.60	23.10	2.70
C18:1 n-9	3.35	0.36	3.64	0.40	6.12	0.70	7.48	0.87
C18:1 n-7	0.00	0.00	0.00	0.00	<0.1	<0.1	<0.1	<0.1
C18:2 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:0	1.86	0.20	1.55	0.17	4.76	0.55	5.32	0.62
C18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

	PTA-10208 Sample #1	PTA-10208 Sample #1	PTA-10208 Sample #2	PTA-10208 Sample #2	PTA-10212 Sample #1	PTA-10212 Sample #1	PTA-10212 Sample #2	PTA-10212 Sample #2
C18:4 n-3	1.64	0.17	2.00	0.22	2.25	0.26	2.24	0.26
C20:2 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C22:0	0.00	0.00	0.00	0.00	0.55	0.06	0.89	0.10
Un-known	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:4 n-7	0.39	0.04	0.05	0.01	0.00	0.00	0.00	0.00
C20:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:4 n-6	12.79	1.36	11.82	1.28	2.33	0.27	2.25	0.26
C22:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:4 n-5	0.39	0.04	0.07	0.01	0.00	0.00	0.00	0.00
C20:4 n-3	5.52	0.59	5.09	0.55	2.87	0.33	2.98	0.35
C20:5 n-3	197.14	20.90	166.68	18.10	91.17	10.44	91.78	10.74
C24:0	0.00	0.00	0.00	0.00	6.93	0.79	7.36	0.86
C22:4 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24:1 n-9	1.08	0.11	<0.1	<0.1	0.00	0.00	0.00	0.00
C22:5 n-6	15.88	1.68	16.57	1.80	4.01	0.46	3.39	0.40
C22:5 n-3	36.05	3.82	16.00	1.74	4.53	0.52	5.07	0.59
C22:6 n-3	440.99	46.76	429.83	46.67	273.02	31.26	245.38	28.70
Sum of FAME	943.11	-	921.03	-	873.31	-	854.89	-
Total % FAME	-	100.00	-	100.00	-	100.00	-	100.00

Table 35: Fatty Acid Profiles of PTA-10212 Isolated PL

	PTA-10212 Sample #1	PTA-10212 Sample #1	PTA-10212 Sample #2	PTA-10212 Sample #2
Fatty Acid	FAME (mg/g)	% FAME	FAME (mg/g)	% FAME
C12:0	0.00	0.00	0.00	0.00
C14:0	0.93	0.22	1.89	0.39
C14:1	0.00	0.00	0.00	0.00
C15:0	3.44	0.82	5.07	1.05
C16:0	98.29	23.50	120.98	25.00
C16:1	1.15	0.27	3.07	0.63
C18:0	3.25	0.78	3.72	0.77
C18:1 n-9	1.12	0.27	0.95	0.20
C18:1 n-7	<0.1	<0.1	0.02	0.003
C18:2 n-6	0.00	0.00	0.00	0.00
C20:0	<0.1	<0.1	<0.1	<0.1
C18:3 n-3	0.00	0.00	0.00	0.00
C20:1 n-9	0.00	0.00	0.00	0.00
C18:4 n-3	3.71	0.89	3.24	0.67
C20:2 n-6	0.00	0.00	0.00	0.00
C20:3 n-6	0.00	0.00	0.00	0.00
C22:0	0.00	0.00	0.00	0.00
Unknown	42.33	10.12	44.71	9.24
C20:4 n-7	0.00	0.00	0.00	0.00
C20:3 n-3	0.00	0.00	0.00	0.00
C20:4 n-6	0.84	0.20	1.54	0.32
C22:1 n-9	0.00	0.00	0.00	0.00
C20:4 n-5	0.00	0.00	0.00	0.00
C20:4 n-3	<0.1	<0.1	0.27	0.06
C20:5 n-3	33.39	7.98	47.91	9.90
C24:0	<0.1	<0.1	0.01	0.001
C22:4 n-9	0.00	0.00	0.00	0.00
C24:1 n-9	0.00	0.00	0.00	0.00
C22:5 n-6	3.08	0.74	3.82	0.79
C22:5 n-3	<0.1	<0.1	0.66	0.14
C22:6 n-3	226.68	54.20	246.09	50.85
Sum of FAME	418.21	-	483.94	-
Total % FAME	-	100	-	100

PTA-10212 Sample #3

[0343] The lipid content of the biomass for PTA-10212 Sample #3 was estimated to be 34% as the sum of FAME, and the amount of crude oil obtained after solvent extraction was 37% by weight, giving a 109% recovery of fat present in the biomass. After fractionation using flash chromatography, approximately 46% of the crude oil was isolated as TAG, 28% was isolated as DAG. The crude oil contained 309 mg/g DHA and 264 mg/g EPA. The isolated TAG contained 341 mg/g DHA and 274 mg/g EPA. The isolated DAG fraction contained 262 mg/g DHA and 237 mg/g EPA. The total fatty acid profiles of the biomass, extracted crude oil, and isolated fractions are shown below in Table 36 and Table 37 calculated as mg/g and % FAME, respectively.

Table 36: Fatty Acid Profiles of PTA-10212 Sample #3 Biomass and Extracted Crude Lipid (mg/g)

	Biomass	Crude Oil	TAG	DAG
Wt. %	NA	37.2%	46.0%	27.9%
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0	0.0	0.0	0.0	0.0
C14:0	3.6	10.3	11.5	9.4
C14:1	0.0	0.0	0.0	0.0
C15:0	4.1	10.6	9.8	6.6
C16:0	70.5	181.8	231.7	111.3
C16:1	6.7	19.1	18.7	17.1
C18:0	2.4	10.2	14.2	0.0
C18:1 n-9	0.0	6.7	0.0	0.0
C18:1 n-7	0.0	1.2	0.0	0.0
C18:2 n-6	0.0	1.8	0.0	0.0
C20:0	0.0	2.4	0.0	0.0
C18:3 n-3	0.0	0.0	0.0	0.0
C20:1 n-9	0.0	0.3	0.0	1.7
C18:4 n-3	1.9	3.4	3.2	4.4
C20:2 n-6	0.0	0.0	0.0	0.0
C20:3 n-6	0.0	0.0	0.0	0.0
C22:0	3.3	0.0	0.0	0.0
C20:4 n-7	0.0	2.1	1.5	0.0
C20:3 n-3	0.0	0.0	0.0	0.0
C20:4 n-6	6.8	17.9	21.4	13.8
C22:1 n-9	0.0	0.0	0.0	0.0
C20:4 n-5	0.0	1.3	1.3	0.0

	Biomass	Crude Oil	TAG	DAG
C20:4 n-3	3.0	8.5	10.9	6.4
C20:5 n-3	102.0	263.6	274.2	237.4
C24:0	0.0	1.7	3.9	0.0
C22:4 n-9	0.0	0.0	0.0	0.0
C24:1 n-9	0.0	0.0	4.2	0.0
C22:5 n-6	3.2	8.3	10.7	6.1
C22:5 n-3	3.8	10.4	15.1	6.6
C22:6 n-3	131.2	309.4	341.1	261.9
Sum of FAME	342.4	871.1	973.2	682.6

Table 37: Fatty Acid Profiles of PTA-10212 Sample #3 Biomass and Extracted Crude Lipid (%)

	Biomass	Crude Oil	TAG	DAG
Wt. %	NA	37.2%	46.0%	27.9%
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0	0.0	0.0	0.0	0.0
C14:0	1.1	1.2	1.2	1.4
C14:1	0.0	0.0	0.0	0.0
C15:0	1.2	1.2	1.0	1.0
C16:0	20.6	20.9	23.8	16.3
C16:1	2.0	2.2	1.9	2.5
C18:0	0.7	1.2	1.5	0.0
C18:1 n-9	0.0	0.8	0.0	0.0
C18:1 n-7	0.0	0.1	0.0	0.0
C18:2 n-6	0.0	0.2	0.0	0.0
C20:0	0.0	0.3	0.0	0.0
C18:3 n-3	0.0	0.0	0.0	0.0
C20:1 n-9	0.0	0.0	0.0	0.2
C18:4 n-3	0.6	0.4	0.3	0.6
C20:2 n-6	0.0	0.0	0.0	0.0
C20:3 n-6	0.0	0.0	0.0	0.0
C22:0	1.0	0.0	0.0	0.0
C20:4 n-7	0.0	0.2	0.2	0.0
C20:3 n-3	0.0	0.0	0.0	0.0
C20:4 n-6	2.0	2.1	2.2	2.0
C22:1 n-9	0.0	0.0	0.0	0.0
C20:4 n-5	0.0	0.1	0.1	0.0
C20:4 n-3	0.9	1.0	1.1	0.9
C20:5 n-3	29.8	30.3	28.2	34.8
C24:0	0.0	0.2	0.4	0.0
C22:4 n-9	0.0	0.0	0.0	0.0

	Biomass	Crude Oil	TAG	DAG
C24:1 n-9	0.0	0.0	0.4	0.0
C22:5 n-6	0.9	1.0	1.1	0.9
C22:5 n-3	1.1	1.2	1.6	1.0
C22:6 n-3	38.3	35.5	35.1	38.4
Total % FAME	100.0	100.0	100.0	100.0

PTA-10212 Sample #4

[0344] PTA-10212 Sample #4 contained approximately 23% lipid determined as the sum of FAME, of which 107% was recovered using hexane extraction. After fractionation using flash chromatography, approximately 42% of the crude oil was isolated as TAG, 18% was isolated as DAG. The crude oil contained 275 mg/g DHA and 209 mg/g EPA. The isolated TAG contained 296 mg/g DHA and 205 mg/g EPA. The isolated DAG fraction contained 245 mg/g DHA and 219 mg/g EPA. The total fatty acid profiles of the biomass, extracted crude oil, and isolated fractions are shown below in Table 38 (mg/g) and Table 39 (%FAME).

Table 38: Fatty Acid Profiles of PTA-10212 Sample #4 Biomass and Extracted Crude Lipid (mg/g)

	Biomass	Crude Oil	TAG	DAG
Wt. %	NA	24.7%	42.2%	18.4%
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0	0.0	0.0	2.1	2.4
C14:0	2.0	8.3	9.8	9.6
C14:1	0.0	0.0	0.0	0.0
C15:0	4.8	16.8	0.4	0.9
C16:0	63.3	210.6	285.7	138.0
C16:1	1.6	6.7	7.4	7.5
C18:0	2.8	12.2	19.9	4.6
C18:1 n-9	0.0	3.7	0.7	1.1
C18:1 n-7	0.0	0.0	0.3	1.2
C18:2 n-6	0.0	0.0	0.0	0.0
C20:0	0.0	3.3	6.0	1.5
C18:3 n-3	0.0	0.0	0.0	0.0
C20:1 n-9	0.0	0.0	0.7	1.2
C18:4 n-3	1.4	3.8	3.6	5.0
C20:2 n-6	0.0	0.0	0.0	0.0
C20:3 n-6	0.0	0.0	0.4	0.0
C22:0	1.5	0.0	1.9	0.0
C20:4 n-7	0.0	0.0	0.9	0.6
C20:3 n-3	0.0	0.0	0.0	0.0

	Biomass	Crude Oil	TAG	DAG
C20:4 n-6	2.5	10.1	13.0	10.3
C22:1 n-9	0.0	0.0	0.0	0.0
C20:4 n-5	0.0	0.0	0.8	0.7
C20:4 n-3	1.4	6.3	8.6	6.0
C20:5 n-3	57.6	209.1	205.4	219.0
C24:0	0.0	2.6	0.8	0.0
C22:4 n-9	0.1	0.0	0.0	0.0
C24:1 n-9	0.0	0.0	1.1	0.5
C22:5 n-6	1.4	6.1	7.9	4.5
C22:5 n-3	4.0	15.8	20.8	12.9
C22:6 n-3	87.7	275.0	296.4	244.8
Sum of FAME	232.2	790.1	894.8	672.4

Table 39: Fatty Acid Profiles of PTA-10212 Sample #4 Biomass and Extracted Crude Lipid (%)

	Biomass	Crude Oil	TAG	DAG
Wt. %	NA	24.7%	42.2%	18.4%
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0	0.0	0.0	0.2	0.4
C14:0	0.9	1.0	1.1	1.4
C14:1	0.0	0.0	0.0	0.0
C15:0	2.1	2.1	0.0	0.1
C16:0	27.3	26.7	31.9	20.5
C16:1	0.7	0.8	0.8	1.1
C18:0	1.2	1.5	2.2	0.7
C18:1 n-9	0.0	0.5	0.1	0.2
C18:1 n-7	0.0	0.0	0.0	0.2
C18:2 n-6	0.0	0.0	0.0	0.0
C20:0	0.0	0.4	0.7	0.2
C18:3 n-3	0.0	0.0	0.0	0.0
C20:1 n-9	0.0	0.0	0.1	0.2
C18:4 n-3	0.6	0.5	0.4	0.7
C20:2 n-6	0.0	0.0	0.0	0.0
C20:3 n-6	0.0	0.0	0.0	0.0
C22:0	0.6	0.0	0.2	0.0
C20:4 n-7	0.0	0.0	0.1	0.1
C20:3 n-3	0.0	0.0	0.0	0.0
C20:4 n-6	1.1	1.3	1.5	1.5
C22:1 n-9	0.0	0.0	0.0	0.0
C20:4 n-5	0.0	0.0	0.1	0.1
C20:4 n-3	0.6	0.8	1.0	0.9

	Biomass	Crude Oil	TAG	DAG
C20:5 n-3	24.8	26.5	23.0	32.6
C24:0	0.0	0.3	0.1	0.0
C22:4 n-9	0.0	0.0	0.0	0.0
C24:1 n-9	0.0	0.0	0.1	0.1
C22:5 n-6	0.6	0.8	0.9	0.7
C22:5 n-3	1.7	2.0	2.3	1.9
C22:6 n-3	37.8	34.8	33.1	36.4
Total % FAME	100.0	100.0	100.0	100.0

PTA-10212 Sample #5

[0345] A sample of crude oil was extracted from a biomass of PTA-10212 using the FRIOLEX® process (GEA Westfalia Separator UK Ltd., Milton Keynes, England) to yield microbial oil PTA-10212 Sample #5. Individual lipid classes were isolated from PTA-10212 Sample #5 using low pressure flash chromatography, and the weight percent of each class was determined. The fatty acid profile of each class was determined using GC-FID.

[0346] Briefly, the sample was prepared by dissolving 240 mg of crude oil in 600 µL of hexane and applying to the head of the column. After fractionation of the sample using flash chromatography, the combined weights of all the fractions was 240 mg giving a 100% recovery. The sterol ester fraction accounted for 0.9%, the TAG fraction accounted for 42.6%, the free fatty acid (FFA) fraction accounted for 1.3%, the sterol fraction accounted for 2.2%, and the DAG fraction accounted for 41.6%. The total fatty acid profiles of the FRIOLEX® crude oil and isolated fractions are shown below in Table 40 and Table 41 calculated as mg/g and % FAME, respectively.

Table 40: Fatty Acid Profiles of PTA-10212 Sample #5 Crude Oil (mg/g)

	Crude Oil	TAG	DAG
Wt. %	NA	42.6%	41.6%
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0	0	0.7	1.0
C14:0	7.7	7.7	8.5
C14:1	0	0.0	0.0
C15:0	10.3	11.7	9.3
C16:0	179.3	217.7	134.6
C16:1	18.1	16.3	25.9
C18:0	8.1	13.2	2.3
C18:1 n-9	4.7	8.4	0.7
C18:1 n-7	0	1.8	1.0
C18:2 n-6	1.8	3.3	0.7
C20:0	1.9	3.6	0.2
C18:3 n-3	0	0.0	0.0
C20:1 n-9	0	0.7	1.0
C18:4 n-3	3.1	2.8	3.8
C20:2 n-6	0	0.0	0.0
C20:3 n-6	0	0.6	0.4
C22:0	0	1.5	0.0
C20:4 n-7	0	1.0	0.7
C20:3 n-3	0	0.0	0.0
C20:4 n-6	12.7	16.1	13.6
C22:1 n-9	0	0.0	0.0
C20:4 n-5	0	1.5	0.8
C20:4 n-3	6.5	9.3	6.4
C20:5 n-3	213.3	223.7	252.8
C24:0	2.3	4.4	0.6
C22:4 n-9	0	1.9	0.9
C24:1 n-9	0	0.0	0.0
C22:5 n-6	7.9	9.5	8.3
C22:5 n-3	13	20.6	9.7
C22:6 n-3	305.6	327.4	353.8
Sum of FAME	796.6	905.3	837.4

Table 41: Fatty Acid Profiles of PTA-10212 Sample #5 Crude Oil (%)

	Crude Oil	TAG	DAG
Fatty Acid	% FAME	% FAME	% FAME
C12:0	0	0.1	0.1
C14:0	1	0.9	1.0
C14:1	0	0.0	0.0
C15:0	1.3	1.3	1.1
C16:0	22.5	24.0	16.1
C16:1	2.3	1.8	3.1
C18:0	1	1.5	0.3
C18:1 n-9	0.6	0.9	0.1
C18:1 n-7	0	0.2	0.1
C18:2 n-6	0.2	0.4	0.1
C20:0	0.2	0.4	0.0
C18:3 n-3	0	0.0	0.0
C20:1 n-9	0	0.1	0.1
C18:4 n-3	0.4	0.3	0.5
C20:2 n-6	0	0.0	0.0
C20:3 n-6	0	0.1	0.0
C22:0	0	0.2	0.0
C20:4 n-7	0	0.1	0.1
C20:3 n-3	0	0.0	0.0
C20:4 n-6	1.6	1.8	1.6
C22:1 n-9	0	0.0	0.0
C20:4 n-5	0	0.2	0.1
C20:4 n-3	0.8	1.0	0.8
C20:5 n-3	26.8	24.7	30.2
C24:0	0.3	0.5	0.1
C22:4 n-9	0	0.2	0.1
C24:1 n-9	0	0.0	0.0
C22:5 n-6	1	1.1	1.0
C22:5 n-3	1.6	2.3	1.2
C22:6 n-3	38.4	36.2	42.3
Total % FAME	100	100	100

EXAMPLE 37

[0347] Crude oils were further processed via refining, bleaching, and deodorizing to obtain refined oils. The refined oils were diluted with high oleic sunflower oil to obtain final oils with a DHA content of approximately 400 mg/g. Individual lipid classes were isolated and the fatty acid profiles of each class were determined using GC-FID as FAME.

PTA-10208 Final Oils

[0348] The fatty acid profiles for PTA-10208 Final Oils #1-5 are summarized in Tables 42-43, including profiles associated within the isolated TAG fraction (Tables 44-45) and the isolated sterols/DAG fraction (Tables 46-47).

[0349] Individual lipid classes in the final oils were also determined using flash chromatography (Table 48) and normal HPLC with ELSD and APCI-MS confirmation (Table 49).

Table 42: Fatty Acid Profiles of PTA-10208 Final Oils (mg/g)

	PTA-10208 Final Oil #1	PTA-10208 Final Oil #2	PTA-10208 Final Oil #3	PTA-10208 Final Oil #4	PTA-10208 Final Oil #5
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0	2.5	2.4	2.8	2.7	2.7
C14:0	16.1	14.9	21.0	18.4	17.5
C14:1	0.0	0.0	0.0	0.0	0.0
C15:0	3.8	3.6	4.4	3.9	3.9
C16:0	192.1	179.1	193.1	184.3	194.6
C16:1	0.4	0.5	0.5	0.5	0.5
C17:0	0.6	0.5	0.9	0.8	2.1
C18:0	12.8	13.9	11.5	12.3	12.9
C18:1 n-9	23.5	82.0	25.7	26.0	29.5
C18:1 n-7	0.2	0.7	0.1	0.1	0.1
C18:2 n-6	3.7	8.1	4.0	4.1	4.3
C20:0	4.3	4.1	3.7	4.0	4.0
C18:3 n-3	0.0	0.0	0.0	0.0	0.0
C20:1 n-9	<0.1	0.1	<0.1	<0.1	<0.1
C18:4 n-3	2.4	2.5	2.8	2.7	2.8
C20:2 n-6	0.0	0.0	0.0	0.0	0.0
C20:3 n-6	0.2	0.1	0.1	0.1	0.1
C22:0	1.2	1.8	1.0	1.1	1.1
C20:4 n-7	1.7	1.6	1.7	1.8	1.6
C20:3 n-3	0.0	0.0	0.0	0.0	0.0
C20:4 n-6	12.9	12.1	13.5	13.5	13.3
C22:1 n-9	0.0	0.0	0.0	0.0	0.0
C20:4 n-5	1.6	1.4	1.5	1.7	1.5
C20:4 n-3	6.0	5.7	6.0	6.0	6.1
C20:5 n-3	173.8	163.3	196.4	209.6	197.9
C24:0	1.4	1.6	1.3	1.3	1.0
C22:4 \n-9	0.0	0.0	0.0	0.0	0.0
C24:1 n-9	3.4	3.2	2.3	2.6	2.3
C22:5 n-6	14.9	14.0	14.4	13.0	12.9
C22:5 n-3	43.9	41.3	32.8	40.3	36.9
C22:6 n-3	394.8	373.7	373.2	374.3	364.2
Sum of FAME	918.1	932.2	914.7	925.1	914.1

Table 43: Fatty Acid Profiles of PTA-10208 Final Oils (%)

	PTA-10208 Final Oil #1	PTA-10208 Final Oil #2	PTA-10208 Final Oil #3	PTA-10208 Final Oil #4	PTA-10208 Final Oil #5
Fatty Acid	% FAME	% FAME	% FAME	% FAME	% FAME
C12:0	0.3	0.3	0.3	0.3	0.3
C14:0	1.8	1.6	2.3	2.0	1.9
C14:1	0.0	0.0	0.0	0.0	0.0
C15:0	0.4	0.4	0.5	0.4	0.4
C16:0	20.9	19.2	21.1	19.9	21.3
C16:1	<0.1	<0.1	<0.1	<0.1	0.1
C17:0	0.1	0.1	0.1	0.1	0.2
C18:0	1.4	1.5	1.3	1.3	1.4
C18:1 n-9	2.6	8.8	2.8	2.8	3.2
C18:1 n-7	<0.1	0.1	<0.1	<0.1	<0.1
C18:2 n-6	0.4	0.9	0.4	0.4	0.5
C20:0	0.5	0.4	0.4	0.4	0.4
C18:3 n-3	0.0	0.0	0.0	0.0	0.0
C20:1 n-9	<0.1	<0.1	<0.1	<0.1	<0.1
C18:4 n-3	0.3	0.3	0.3	0.3	0.3
C20:2 n-6	0.0	0.0	0.0	0.0	0.0
C20:3 n-6	<0.1	<0.1	<0.1	<0.1	<0.1
C22:0	0.1	0.2	0.1	0.1	0.1
C20:4 n-7	0.2	0.2	0.2	0.2	0.2
C20:3 n-3	0.0	0.0	0.0	0.0	0.0
C20:4 n-6	1.4	1.3	1.5	1.5	1.5
C22:1 n-9	0.0	0.0	0.0	0.0	0.0
C20:4 \n-5	0.2	0.2	0.2	0.2	0.2
C20:4 \n-3	0.7	0.6	0.7	0.7	0.7
C20:5 n-3	18.9	17.5	21.5	22.7	21.6
C24:0	0.1	0.2	0.1	0.1	0.1
C22:4 n-9	0.0	0.0	0.0	0.0	0.0
C24:1 n-9	0.4	0.3	0.2	0.3	0.2
C22:5 n-6	1.6	1.5	1.6	1.4	1.4
C22:5 n-3	4.8	4.4	3.6	4.4	4.0
C22:6 n-3	43.0	40.1	40.8	40.5	39.9

Table 44: Isolated TAG Fatty Acid Profiles: PTA-10208 Final Oils (mg/g)

	PTA-10208 Final Oil #1	PTA-10208 Final Oil #2	PTA-10208 Final Oil #3	PTA-10208 Final Oil #4	PTA-10208 Final Oil #5
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0	2.5	2.3	2.7	2.5	2.6
C14:0	16.3	15.1	21.3	18.6	18.1
C14:1	0.0	0.0	0.0	0.0	0.0
C15:0	3.9	3.6	4.4	4.0	4.0
C16:0	194.2	181.9	196.1	186.1	199.8
C16:1	0.4	0.4	0.6	0.5	0.7
C17:0	0.6	0.5	0.9	0.8	0.8
C18:0	12.9	14.2	11.7	12.5	13.2
C18:1 n-9	24.3	84.0	26.8	26.1	34.0
C18:1 n-7	0.1	0.7	0.1	0.1	0.3
C18:2 n-6	3.2	7.7	3.4	3.5	4.0
C20:0	4.4	4.2	3.8	4.0	4.2
C18:3 n-3	0.0	0.0	0.0	0.0	0.0
C20:1 n-9	<0.1	0.2	<0.1	<0.1	0.1
C18:4 n-3	2.5	2.4	2.8	2.6	2.7
C20:2 n-6	0.0	0.0	0.0	0.0	0.0
C20:3 n-6	0.2	0.2	0.1	0.1	0.1
C22:0	1.2	1.9	1.0	1.1	1.1
C20:4 n-7	1.7	1.6	1.8	1.8	1.7
C20:3 n-3	0.0	0.0	0.0	0.0	0.0
C20:4 n-6	13.2	12.3	13.8	13.7	13.8
C22:1 n-9	0.0	0.0	0.0	0.0	0.0
C20:4 n-5	1.6	1.5	1.6	1.7	1.5
C20:4 n-3	6.1	5.7	6.1	5.9	6.2
C20:5 n-3	176.0	166.1	199.0	211.2	204.2
C24:0	1.2	1.3	1.0	1.1	1.2
C22:4 n-9	0.0	0.0	0.0	0.0	0.0
C24:1 n-9	3.3	3.2	2.2	2.5	2.4
C22:5 n-6	15.0	14.2	14.7	13.2	13.5
C22:5 n-3	44.4	42.0	33.3	40.5	38.3
C22:6 n-3	397.9	378.4	376.4	375.5	375.5
Sum of FAME	926.9	945.7	925.5	929.6	944.1

Table 45: Isolated TAG Fatty Acid Profiles: PTA-10208 Final Oils (%)

	PTA-10208 Final Oil #1	PTA-10208 Final Oil #2	PTA-10208 Final Oil #3	PTA-10208 Final Oil #4	PTA-10208 Final Oil #5
Fatty Acid	% FAME	% FAME	% FAME	% FAME	% FAME
C12:0	0.3	0.2	0.3	0.3	0.3
C14:0	1.8	1.6	0.3	0.3	0.3
C14:1	0.0	0.0	0.0	0.0	0.0
C15:0	0.4	0.4	0.5	0.4	0.4
C16:0	20.9	19.2	21.2	20.0	21.2
C16:1	<0.1	<0.1	0.1	0.1	0.1
C17:0	0.1	0.1	0.1	0.1	0.1
C18:0	1.4	1.5	1.3	1.3	1.4
C18:1 n-9	2.6	8.9	2.9	2.8	3.6
C18:1 n-7	<0.1	0.1	<0.1	<0.1	<0.1
C18:2 n-6	0.3	0.8	0.4	0.4	0.4
C20:0	0.5	0.4	0.4	0.4	0.4
C18:3 n-3	0.0	0.0	0.0	0.0	0.0
C20:1 n-9	<0.1	<0.1	<0.1	<0.1	<0.1
C18:4 n-3	0.3	0.3	0.3	0.3	0.3
C20:2 n-6	0.0	0.0	0.0	0.0	0.0
C20:3 n-6	<0.1	<0.1	<0.1	<0.1	<0.1
C22:0	0.1	0.2	0.1	0.1	0.1
C20:4 n-7	0.2	0.2	0.2	0.2	0.2
C20:3 n-3	0.0	0.0	0.0	0.0	0.0
C20:4 n-6	1.4	1.3	1.5	1.5	1.5
C22:1 n-9	0.0	0.0	0.0	0.0	0.0
C20:4 n-5	0.2	0.2	0.2	0.2	0.2
C20:4 n-3	0.7	0.6	0.7	0.6	0.7
C20:5 n-3	19.0	17.6	21.5	22.7	21.6
C24:0	0.1	0.1	0.1	0.1	0.1
C22:4 n-9	0.0	0.0	0.0	0.0	0.0
C24:1 n-9	0.4	0.3	0.2	0.3	0.3
C22:5 n-6	1.6	1.5	1.6	1.4	1.4
C22:5 n-3	4.8	4.4	3.6	4.4	4.1
C22:6 n-3	42.9	40.0	40.7	40.4	39.8

Table 46: Isolated Sterols/DAG Fatty Acid Profiles: PTA-10208 Final Oils (mg/g)

	PTA-10208 Final Oil #1	PTA-10208 Final Oil #2	PTA-10208 Final Oil #3	PTA-10208 Final Oil #4	PTA-10208 Final Oil #5
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0	1.9	2.1	2.9	2.1	1.9
C14:0	9.9	9.5	9.7	10.3	8.0
C14:1	0.0	0.0	0.0	0.0	0.0
C15:0	2.4	2.3	2.2	2.3	2.0
C16:0	132.6	128.6	110.1	116.8	106.4
C16:1	0.2	0.3	<0.1	0.3	0.4
C17:0	0.3	0.2	0.3	0.3	0.3
C18:0	7.3	8.1	6.4	6.8	6.1
C18:1 n-9	15.0	55.1	47.4	19.0	30.1
C18:1 n-7	0.4	0.7	0.1	<0.1	0.2
C18:2 n-6	13.1	16.7	21.6	13.5	18.4
C20:0	2.0	2.1	1.2	1.8	1.4
C18:3 n-3	0.0	0.0	0.0	0.0	0.0
C20:1 n-9	<0.1	<0.1	<0.1	<0.1	<0.1
C18:4 n-3	2.3	2.4	2.4	2.4	2.0
C20:2 n-6	0.0	0.0	0.0	0.0	0.0
C20:3 n-6	<0.1	<0.1	<0.1	<0.1	<0.1
C22:0	0.6	1.0	0.5	0.6	0.5
C20:4 n-7	0.8	0.9	2.1	0.9	0.7
C20:3 n-3	0.0	0.0	0.0	0.0	0.0
C20:4 n-6	5.7	5.8	4.8	6.1	4.5
C22:1 n-9	0.0	0.0	0.0	0.0	0.0
C20:4 n-5	<0.1	<0.1	<0.1	0.6	<0.1
C20:4 n-3	2.7	2.7	2.1	2.7	2.0
C20:5 n-3	92.9	94.5	91.9	111.6	84.8
C24:0	1.2	1.3	1.1	1.1	1.3
C22:4 n-9	0.0	0.0	0.0	0.0	0.0
C24:1 n-9	1.9	2.0	1.2	1.5	1.2
C22:5 n-6	7.8	8.0	6.7	7.0	5.5
C22:5 n-3	22.2	22.9	13.9	20.7	14.2
C22:6 n-3	246.3	252.7	223.5	240.3	196.3
Sum of FAME	569.3	619.8	552.1	568.7	488.2

Table 47: Isolated Sterols/DAG Fatty Acid Profiles: PTA-10208 Final Oils (%)

	PTA-10208 Final Oil #1	PTA-10208 Final Oil #2	PTA-10208 Final Oil #3	PTA-10208 Final Oil #4	PTA-10208 Final Oil #5
Fatty Acid	% FAME	% FAME	% FAME	% FAME	% FAME
C12:0	0.3	0.3	0.5	0.4	0.4
C14:0	1.7	1.5	1.8	1.8	1.6
C14:1	0.0	0.0	0.0	0.0	0.0
C15:0	0.4	0.4	0.4	0.4	0.4
C16:0	23.3	20.8	19.9	20.5	21.8
C16:1	<0.1	<0.1	<0.1	<0.1	0.1
C17:0	0.0	0.0	0.1	0.1	0.1
C18:0	1.3	1.3	1.2	1.2	1.2
C18:1 n-9	2.6	8.9	8.6	3.3	6.2
C18:1 n-7	0.1	0.1	<0.1	<0.1	<0.1
C18:2 n-6	2.3	2.7	3.9	2.4	3.8
C20:0	0.4	0.3	0.2	0.3	0.3
C18:3 n-3	0.0	0.0	0.0	0.0	0.0
C20:1 n-9	<0.1	<0.1	<0.1	<0.1	<0.1
C18:4 n-3	0.4	0.4	0.4	0.4	0.4
C20:2 n-6	0.0	0.0	0.0	0.0	0.0
C20:3 n-6	<0.1	<0.1	<0.1	<0.1	<0.1
C22:0	0.1	0.2	0.1	0.1	0.1
C20:4 n-7	0.1	0.1	0.4	0.2	0.1
C20:3 n-3	0.0	0.0	0.0	0.0	0.0
C20:4 n-6	1.0	0.9	0.9	1.1	0.9
C22:1 n-9	0.0	0.0	0.0	0.0	0.0
C20:4 n-5	<0.1	<0.1	<0.1	0.1	<0.1
C20:4 n-3	0.5	0.4	0.4	0.5	0.4
C20:5 n-3	16.3	15.2	16.6	19.6	17.4
C24:0	0.2	0.2	0.2	0.2	0.3
C22:4 n-9	0.0	0.0	0.0	0.0	0.0
C24:1 n-9	0.3	0.3	0.2	0.3	0.2
C22:5 n-6	1.4	1.3	1.2	1.2	1.1
C22:5 n-3	3.9	3.7	2.5	3.6	2.9
C22:6 n-3	43.3	40.8	40.5	42.3	40.2

Table 48: Lipid class separation by flash chromatography (wt %)

Lipid Class Separation	PTA-10208 Final Oil #1	PTA-10208 Final Oil #2	PTA-10208 Final Oil #3	PTA-10208 Final Oil #4	PTA-10208 Final Oil #5
TAG	93.4	95.4	94.0	95.7	95.1
Sterols/DAG	3.1	2.9	2.6	3.0	2.9
Recovery (%)	96.5	98.3	96.6	98.7	98.0

Table 49: Lipid class separation by HPLC-ELSD (wt %)

	Sterol Esters	TAG	FFA	Sterols	1,3-DAG	1,2-DAG	MAG	Total
PTA-10208 Final Oil #1	0.4	90.8	ND	0.8	0.5	0.5	N.D.	93.0
PTA-10208 Final Oil #2	0.4	88.5	ND	0.6	0.6	0.6	N.D.	90.7
PTA-10208 Final Oil #3	0.3	89.4	ND	0.8	0.6	0.5	N.D.	91.6
PTA-10208 Final Oil #4	0.3	88.0	ND	0.8	0.5	0.5	N.D.	90.1
PTA-10208 Final Oil #5	0.3	86.3	ND	0.7	0.8	0.5	N.D.	88.6
PTA-10208 Final Oil #6	0.36	100.76	ND	0.84	0.54	0.61	N.D.	103.11

ND= Not Detected

PTA-10212 Final Oil

[0350] DHA was present in a PTA-10212 Final Oil at 41.63% and 366.9 mg/g, while EPA was present at 16.52%. Individual fatty acid profiles were determined and are summarized in Table 50.

Table 50: Fatty Acid Profiles of PTA-10212 Final Oil (% FAME)

Fatty Acid	% FAME
C6:0	ND
C7:0	ND
C8:0	ND
C9:0	ND
C10:0	ND
C11:0	ND
C12:0	ND
C13:0	ND
C14:0	0.84
C14:1	ND
C15:0	1.33
C16:0	27.09
C16:1	1.03
C17:0	0.34
C17:1	ND
C18:0	1.26
C18:1 n-9	2.14
C18:1 n-7	0.18
C19:0	ND
C18:2 n-6	0.58
C20:0	0.32
C18:3 n-3	ND
C20:1 n-9	ND
C18:3 n-6	ND
C20:2 n-6	0.26
C20:3 n-6	ND
C22:0	0.14
C20:3 n-3	ND
C20:4 n-6	1.34
C22:1 n-9	ND
C23:0	ND
C20:5 n-3	16.53
C24:0	0.53
C24:1 n-9	ND
C22:5 n-6	1.50
C22:5 n-3	1.30
C22:6 n-3	41.63
Unknown	0.87

ND= Not Detected

EXAMPLE 38

[0351] A two-day old inoculum flask of the isolated microorganisms deposited under ATCC Accession Nos. PTA-10208 and 10212 was prepared as a carbon and nitrogen-fed culture in media according to Tables 30 and 31.

[0352] Mutagenesis was carried out according to following procedure:

[0353] A sterile T=2 day old flask, approximately 50 ml, was poured into a sterile 40 ml glass homogenizer. The culture received 50 plunges in the homogenizer. The culture was pipeted out and filtered through a sterile 50 micron mesh filter, which was placed in a 50 ml sterile tube (the mesh was used as a means of retaining the larger clumps of colonies while letting the smaller clusters and single cells pass through the 50 micron mesh.). The entire concentrated macerate was collected in a sterile 50 ml tube. The macerated culture was vortexed and dilutions at levels up to 1:100 fold were made. The diluted macerate samples were vortexed prior to adding 200 µl of inoculum to a media agar plate, 100 x 15 mm, containing 4-5 glass beads (3 mm glass beads). Each plate was gently agitated in an effort to have the beads spread the inoculum evenly around the plate. Beads were dumped off of plates and plates were left to sit with covers on for approximately 5 minutes to dry. Lights in both the sterile hood and adjoining areas were turned off as the procedure was performed in dim light. There was minimal light available to be able to run the procedure but only indirect and dim.

[0354] Five replicate plates were placed on the floor of the XL crosslinker (Spectronics Corporation, New York) with the lids off while the samples were irradiated. The crosslinker delivered power in terms of microjoules and a level was sought that achieved a 90%-95% Kill. Five replicate control plates were inoculated with un-mutagenized cells using the same protocol. These cell counts were used to calculate the % Kill. Once the irradiation was finished the plates were taken out, the lids were replaced, and the plates were wrapped in parafilm followed by a wrap in aluminum foil. It was imperative that the plates grew for the first week in the dark so that they were not able to repair the damaged genes.

[0355] Plates were placed in a 22.5°C room for about 10 days prior to counting the colonies. When final counts were made, individual colonies were picked with a sterile inoculating loop and re-streaked on new media plates. Each colony was plated on an individual plate. As plates grew dense a sample was taken, using a inoculating loop, and inoculated into a sterile 250 ml shake flask containing 50 ml of media. This flask was placed on a shaker at 200 rpm in a 22.5°C room. On T=7 days the shake flask culture was harvested into a 50 ml sterile tube. The pH was taken

and the sample was centrifuged to collect the biomass pellet. Each sample was rinsed and re-suspended in a 50:50 mixture of isopropyl alcohol and distilled water prior to being re-centrifuged. The collected pellet was freeze dried, weighed, and a FAME analysis was performed. The data in Tables 51 and 52 represents mutants produced with the above process from strains PTA-10208 and PTA-10212, respectively.

Table 51: PTA-10208 Mutants

Fatty Acids	control PTA-10208	Mutant 1 PTA-10209	Mutant 2 PTA-10210	Mutant 3 PTA-10211
% 08:0	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00
% 12:0	0.11	0.10	0.22	0.19
% 12:1	0.00	0.00	0.00	0.00
% 13:0	0.19	0.19	0.15	0.16
% 13:1	0.00	0.00	0.00	0.00
% 14:0	1.94	1.82	2.98	2.59
% 14:1	0.00	0.00	0.00	0.00
% 15:1	2.66	2.22	1.76	1.66
% 16:0	24.87	24.97	23.71	25.01
% 16:1	0.20	0.25	0.07	0.07
% 16:2	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00
% 17:0	1.49	1.21	0.62	0.66
% 18:0	1.13	1.14	0.91	1.01
% 18:1 n-9	0.07	0.07	0.06	0.06
% 18:1 n-7	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.00	0.05	0.04
% 18:3 n-3	0.09	0.08	0.17	0.14
% 18:4 n-3	0.00	0.00	0.00	0.00
% 20:0	0.31	0.33	0.24	0.30
% 20:1 n-9	0.00	0.04	0.00	0.00
% 20:2	0.00	0.00	0.05	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00
% 20:3 n-6	0.12	0.13	0.08	0.04
% 20:3 n-3	0.42	0.42	0.08	0.06
% 20:4 ARA	0.68	0.67	1.44	1.11
% 20:5 n-3				

Fatty Acids	control PTA-10208	Mutant 1 PTA-10209	Mutant 2 PTA-10210	Mutant 3 PTA-10211
EPA	6.56	6.47	11.99	9.87
% 22:0	0.07	0.07	0.06	0.07
% 22:1	0.00	0.00	0.00	0.00
% 22:2	0.11	0.09	0.10	0.08
% 22:3	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.00	0.00
% 22:5 n-6	2.32	2.36	2.36	2.36
% 22:5 n-3	0.48	0.66	0.66	0.52
% 22:6 n-3 DHA	51.58	52.27	48.17	49.35
% 24:0	0.00	0.00	0.00	0.00
% 24:1	0.00	0.00	0.00	0.00
% Fat	47.87	49.41	66.00	63.12
% Unknown	4.61	4.45	4.07	4.64

Table 52: PTA-10212 Mutants

Fatty Acids	Control PTA-10212	Mutant 1 PTA-10213	Mutant 2 PTA-10214	Mutant 3 PTA-10215
% 08:0	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00
% 12:0	0.00	0.00	0.00	0.00
% 12:1	0.00	0.00	0.00	0.00
% 13:0	0.00	0.00	0.21	0.20
% 13:1	0.00	0.00	0.00	0.00
% 14:0	0.68	0.77	0.62	0.97
% 14:1	0.00	0.00	0.00	0.00
% 15:1	0.00	0.00	0.00	0.00
% 16:0	17.36	19.94	15.27	23.61
% 16:1	1.45	2.33	1.40	2.57
% 16:2	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00
% 17:0	0.20	0.21	0.18	0.27
% 18:0	0.78	0.82	0.79	0.81
% 18:1 n-9	0.00	0.00	0.00	0.00
% 18:1 n-7	0.18	0.27	0.20	0.19
% 18:2	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.00	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00

Fatty Acids	Control PTA-10212	Mutant 1 PTA-10213	Mutant 2 PTA-10214	Mutant 3 PTA-10215
% 20:0	0.00	0.00	0.00	0.00
% 20:1 n-9	0.00	0.00	0.00	0.00
% 20:2	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00
% 20:3 n-3	0.90	0.77	0.99	0.66
% 20:4 ARA	1.43	1.32	1.65	0.72
% 20:5 n-3 EPA	13.33	14.93	14.14	8.54
% 22:0	0.00	0.00	0.00	0.00
% 22:1	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.00	0.00
% 22:5 n-6	2.39	1.95	2.59	2.18
% 22:5 n-3	0.73	0.79	0.80	0.68
% 22:6 n-3 DHA	59.18	54.31	59.89	56.39
% 24:0	0.00	0.00	0.00	0.00
% 24:1	0.00	0.00	0.00	0.00
% Fat	45.69	38.08	42.88	48.48
% Unknown	1.38	1.58	1.27	2.19

Example 39

[0356] Two cell broths (approximately 13.3 kg) containing microbial cells (*Schizochytrium*) were heated to 60 °C in a 20 liter fermentor. The fermentor had two 6-blade Rushton impellers having a diameter of 15 cm. The top impeller was positioned at the 12 liter mark and the bottom impeller was positioned 10 cm below the top impeller. The first cell broth was continuously agitated at 307 centimeters/second. The second cell broth was continuously agitated at 464 centimeters/second. Enzymes (i.e., Alcalase 2.4 L FG 0.5%) were added to the cell biomass to lyse the cells and form an emulsified lysed cell composition. The emulsified lysed cell composition was first treated with a base (NaOH, 250 kg of 50% w/w solution) until the pH of the lysed cell composition was from 10.4 to 10.6. Next, a salt (solid NaCl, in an amount of 2%, by weight, of the lysed cell composition) was added to the lysed cell composition. The lysed cell composition was then heated to a temperature of 90° C and held at that temperature level for 20 hours. A sample of each cell broth was taken and the pH was adjusted to 8.0 and placed in 50 ml

test tubes. The test tubes were centrifuged and the oil extraction data was measured. The oil extraction data is provided in Table 53.

Table 53. Results from extraction testing in 50 mL tubes at pH 8.0.

307 centimeters/second			
Wet broth tested for Extraction (g)	Mass of Oil Recovered (g)	% yield (oil/broth)	%yield (oil/solids)*
49.990	3.881	7.76	27.81
50.814	2.747	5.41	19.36
50.772	2.418	4.76	17.05
464 centimeters/second			
Wet broth tested for Extraction (g)	Mass of Oil Recovered (g)	% yield (oil/broth)	%yield (oil/solids)*
51.154	7.067	13.81	49.13
51.092	7.055	13.81	49.11
50.132	6.606	13.18	46.86

*based on solids content of untreated pasteurized broth

[0357] The data provided in Table 53 demonstrates that the higher agitation speed resulted in a greater mass of oil recovered, a greater % yield of oil from the broth, and a greater % yield of oil from the solids content of the untreated pasteurized broth.

CONCLUSION

[0358] All of the various embodiments or options described herein can be combined in any and all variations. While the invention has been particularly shown and described with reference to some embodiments thereof, it will be understood by those skilled in the art that they have been presented by way of example only, and not limitation, and various changes in form and details can be made therein without departing from the spirit and scope of the invention. Thus, the breadth and scope of the present invention should not be limited by any of the above described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents.

[0359] All documents cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued or foreign patents, or any other documents, are each entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited documents.

WHAT IS CLAIMED IS:

1. A process for obtaining a lipid from a microbial cell composition, comprising:

raising the pH of the cell composition to 8 or above; and

separating a lipid from the cell composition,

wherein the lipid contains less than 5% by weight of an organic solvent.

2. The process of claim 1, wherein the raising the pH lyses the cell composition.

3. The process of claim 1, wherein the raising the pH demulsifies the cell composition.

4. The process of any of claims 1-3, further comprising adding a salt to the cell composition to demulsify the cell composition.

5. The process of claim 4, wherein the adding a salt is performed after the raising the pH.

6. The process of any of claims 1-5, further comprising heating the cell composition to demulsify the cell composition.

7. The process of claim 6, wherein the heating is performed after the raising the pH.

8. The process of any of claims 1-7, further comprising raising the pH of the cell composition a second time to demulsify the cell composition.

9. The process of claim 8, wherein the raising the pH a second time is performed after the adding a salt or the heating.

10. A process for obtaining a lipid from a cell, comprising:

lysing a cell to form a lysed cell composition;

raising the pH of the lysed cell composition to 8 or above to demulsify the cell composition;

adding a salt to the lysed cell composition to demulsify the cell composition; and

separating a lipid from the demulsified cell composition,

wherein the lipid contains less than 5% by weight of an organic solvent.

11. A process for obtaining a lipid from a cell composition, comprising:

raising the pH of a cell composition to 8 or above to lyse the cell composition and demulsify the cell composition;

adding a salt to the cell composition; and

separating a lipid from the demulsified cell composition,

wherein the lipid contains less than 5% by weight of an organic solvent.

12. The process of claim 10 or 11, further comprising heating the lysed cell composition to demulsify the cell composition.

13. The process of claim 12, wherein the heating is performed after the adding a salt.

14. The process of any of claims 1-13, further comprising agitating the lysed cell composition to demulsify the cell composition.

15. The process of any of claims 10-14, further comprising raising a pH of the lysed cell composition to demulsify the cell composition.

16. The process of claim 15, wherein the raising the pH of the lysed cell composition is performed after the adding a salt or the heating.

17. The process of any of claims 1-16, wherein raising the pH comprises adding a base.

18. The process of any of claims 1-17, wherein the base has a pK_b of 1 to 12.

19. The process of any of claims 14-18, wherein the process comprises agitating the lysed cell composition by stirring, mixing, blending, shaking, vibrating, or a combination thereof.

20. The process of any of claims 1-19, wherein the lysing comprises mechanical treatment, physical treatment, chemical treatment, enzymatic treatment, or a combination thereof.

21. The process of claim 20, wherein the mechanical treatment is homogenization.

22. The process of any of claims 4-21, wherein the salt is added in an amount of 0.1% to 20% by weight of the lysed cell composition.

23. The process of any of claims 4-22, wherein the salt is selected from the group consisting of: alkali metal salts, alkali earth metal salts, sulfate salts, and combinations thereof.

24. The process of any of claims 1-23, wherein the separating comprises centrifuging.

25. The process of any of claims 1-24, wherein the process provides a lipid comprising at least 50% by weight triglyceride.

26. The process of any of claims 1-25, wherein the process provides a lipid having an anisidine value of 26 or less.

27. The process of any of claims 10-26, wherein the cell is a microbial cell.

28. The process of any of claims 1-9 and 27, further comprising concentrating a fermentation broth comprising the microbial cell.

29. The process of any of claims 10-26, wherein the cell is an oilseed.

30. The process of claim 29, wherein the oilseed is selected from the group consisting of sunflower seeds, canola seeds, rapeseeds, linseeds, castor oil seeds, coriander seeds, calendula seeds, and genetically modified variants thereof.

31. The process of any of claims 1-30, further comprising washing the cell or cell composition.

32. The process of any of claims 1-31, further comprising pasteurizing the cell or cell composition.

33. The process of any of claims 1-32, further comprising concentrating the lysed cell composition.

34. The process of any of claims 1-33, further comprising refining the lipid.

35. The process of claim 34, wherein the refining is selected from the group consisting of: refining, degumming, acid treatment, alkali treatment, cooling, heating, bleaching, deodorizing, deacidification, and combinations thereof.

36. The process of any of claims 1-35, further comprising harvesting the lipid, wherein the harvesting comprises pumping the lipid without agitation.

37. A process for obtaining a lipid from a cell, comprising:

lysing a cell to form a lysed cell composition;

agitating the cell composition to demulsify the cell composition; and

separating a lipid from the demulsified cell composition,

wherein the lipid contains less than 5% by weight of an organic solvent.

38. A process of claim 37, wherein the agitating comprises agitating the cell composition with an impeller having a tip speed of 350 centimeters per second to 900 centimeters per second.

39. A lipid obtained by the process of any of claims 1-38.

40. The lipid of claim 39, wherein the lipid comprises one or more polyunsaturated fatty acids.

41. The lipid of any of claims 39-40, wherein the lipid comprises at least 35% by weight docosahexaenoic acid.

42. The lipid of any of claims 39-41, wherein the lipid has an overall aroma intensity of 3 or less.

43. The lipid of any of claims 39-41, wherein the lipid has an overall aromatic intensity of 2 or less.

44. The lipid of any of claims 39-43, wherein the lipid has an anisidine value of 26 or less.

45. The lipid of any of claims 39-44, wherein the lipid has a phosphorus content of 100 ppm or less.

46. The lipid of any of claims 39-45, wherein the lipid has a peroxide value of 5 or less.

47. The lipid of claim 39, comprising at least 20% by weight eicosapentaenoic acid and less than 5% by weight each of arachidonic acid, docosapentaenoic acid n-6, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, erucic acid, and stearidonic acid.

48. The lipid of claim 39, comprising a triacylglycerol fraction of at least 10% by weight, wherein at least 12% by weight of the fatty acids in the triacylglycerol fraction is eicosapentaenoic acid, wherein at least 25% by weight of the fatty acids in the triacylglycerol fraction is docosahexaenoic acid, and wherein less than 5% by weight of the fatty acids in the triacylglycerol fraction is arachidonic acid.

49. The lipid of claim 47 or 48, wherein the lipid has a peroxide value of 5 or less.

50. The lipid of any of claims 39-49, wherein the lipid is a crude oil.

51. A crude microbial lipid having an anisidine value of 26 or less, a peroxide value of 5 or less, a phosphorus content of 100 ppm or less, and less than 5% by weight of an organic solvent.

52. An extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, and wherein the oil has an anisidine value of 26 or less.

53. An extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1, and wherein the oil has an anisidine value of 26 or less.

54. An extracted microbial lipid comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight and wherein the oil has an anisidine value of 26 or less.

55. The extracted microbial lipid of any of claims 52-54, wherein the oil has a peroxide value of 5 or less.

56. The extracted microbial lipid of any of claims 52-55, wherein the oil has a phosphorus content of 100 ppm or less.

57. The extracted microbial lipid of any of claims 52-56, wherein the lipid is a crude oil.

58. A process for obtaining a lipid, the process comprising refining a crude lipid of any of claims 50, 51, or 57.

59. The process of claim 58, wherein the refining is selected from the group consisting of: caustic refining, degumming, acid treatment, alkali treatment, bleaching, deodorizing, deacidification, and combinations thereof.

ABSTRACT

The present invention relates to processes for obtaining a lipid from a cell by lysing the cell, contacting the cell with a base and/or salt, and separating the lipid. The present invention is also directed to a lipid prepared by the processes of the present invention. The present invention is also directed to microbial lipids having a particular anisidine value, peroxide value, and/or phosphorus content.

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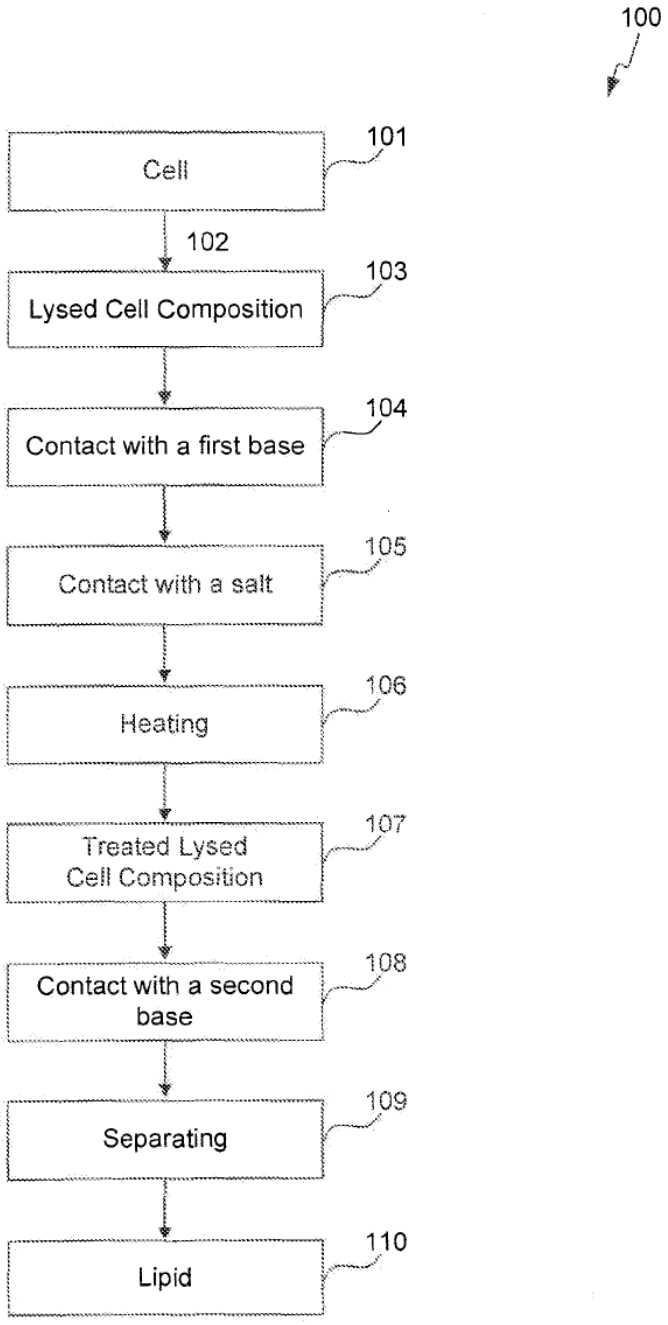

100
Cell
101
102
Lysed Cell Composition
103
Contact with a first base
104
Contact with a salt
105
Heating
106
Treated Lysed Cell Composition
107
Contact with a second base
108
Separating
109
Lipid
110

FIG. 1

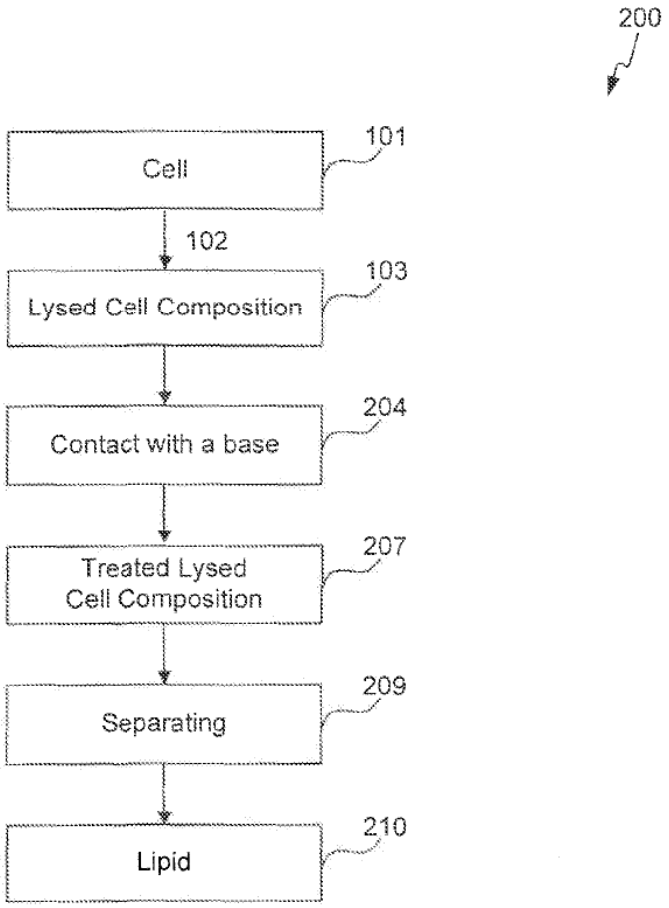

200
Cell
101
102
Lysed Cell Composition
103
Contact with a base
204
Treated Lysed Cell Composition
207
Separating
209
Lipid
210

FIG. 2

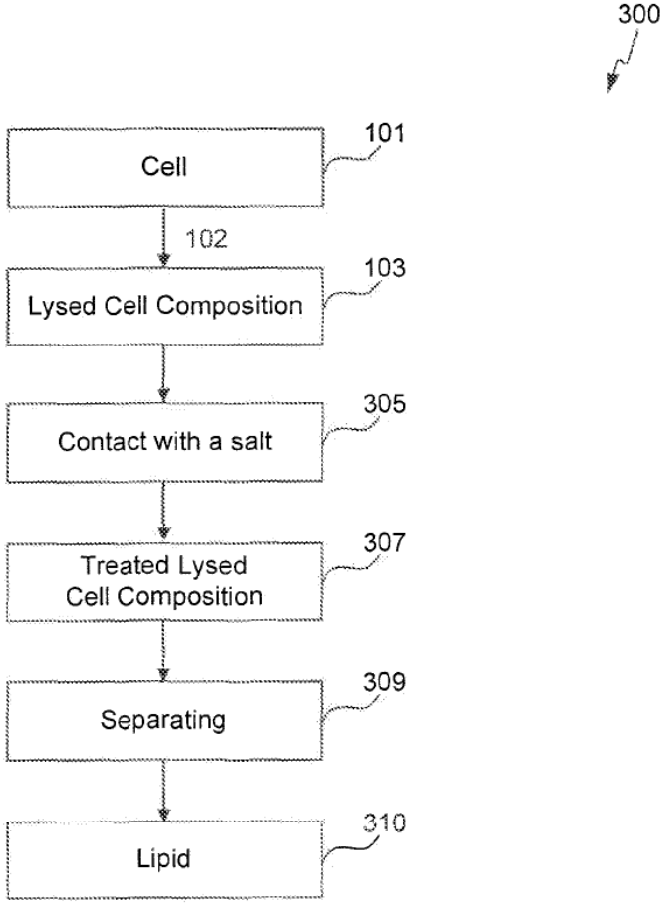

300
Cell
101
102
Lysed Cell Composition
103
Contact with a salt
305
Treated Lysed
Cell Composition
307
Separating
309
Lipid
310

FIG. 3

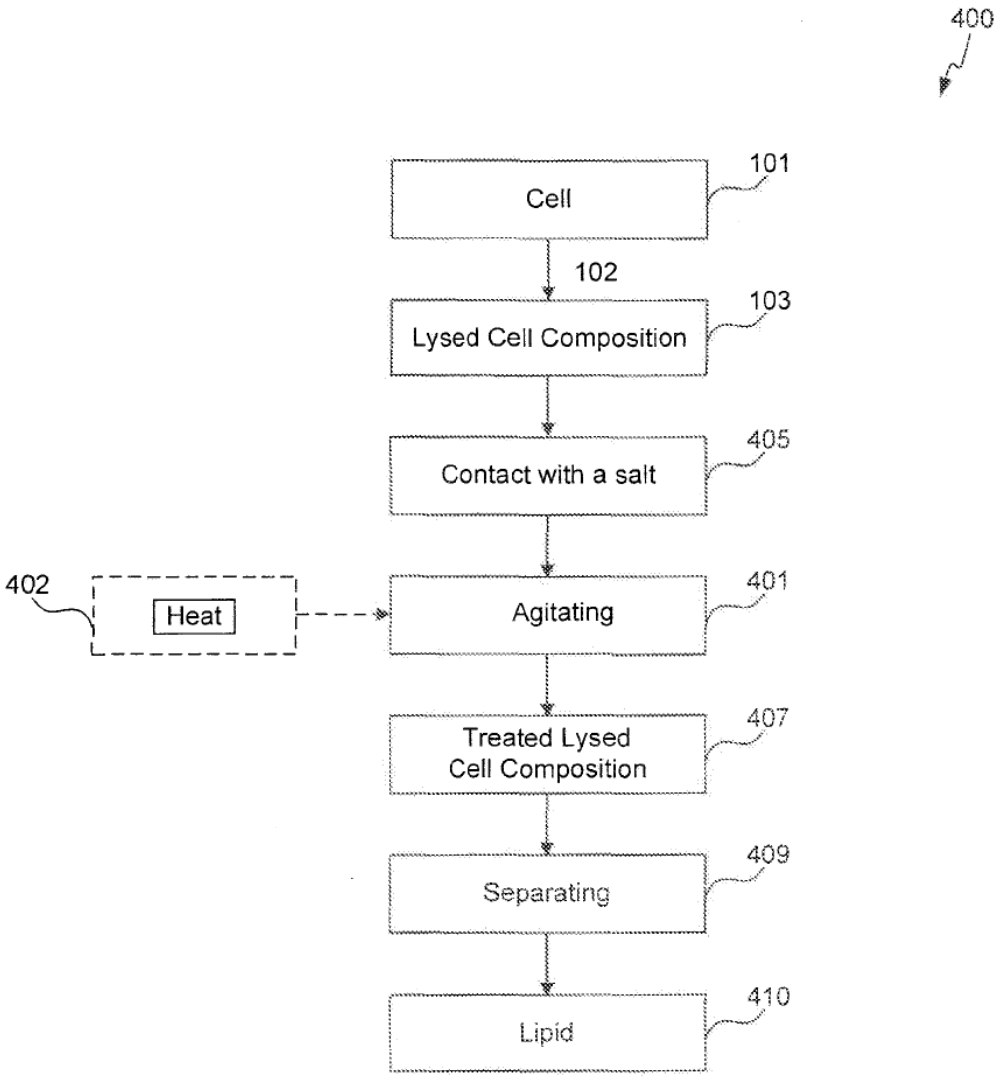
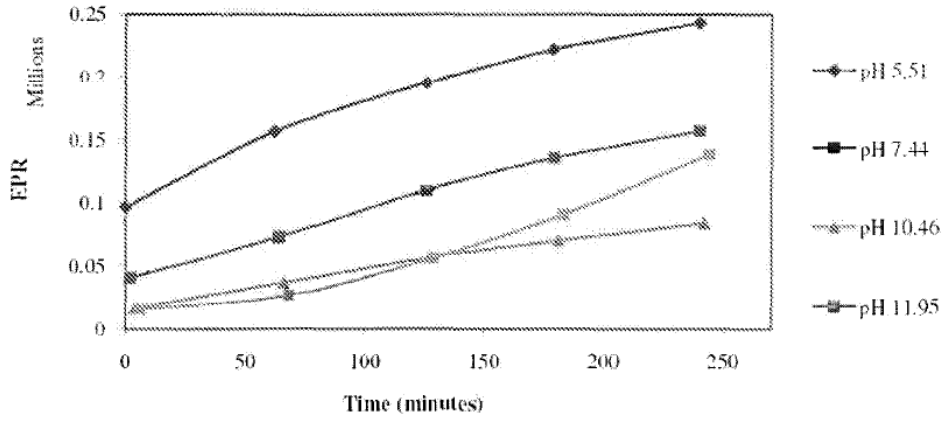

400
Cell
101
102
Lysed Cell Composition
103
Contact with a salt
405
402
Heat
Agitating
401
Treated Lysed
Cell Composition
407
Separating
409
Lipid
410

FIG. 4

FIG. 5

Effect of Alkaline Treatment Using EPR Spectroscopy



Electronic Acknowledgement Receipt	
EFS ID:	10209814
Application Number:	13151014
International Application Number:	
Confirmation Number:	4148
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom
First Named Inventor/Applicant Name:	Stephen Robert CHERINKO
Customer Number:	91335
Filer:	John Thomas Haran/Hilda Morgan Beale
Filer Authorized By:	John Thomas Haran
Attorney Docket Number:	2715.1350001/JUK/JHH
Receipt Date:	01-JUN-2011
Filing Date:	
Time Stamp:	18:05:13
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		27151350003_tl_trans_auth_ads_spec_drwgsxx.pdf	23634534 6b477b10186f24c6b776ea888145ac1d9e7de9ef	yes	170

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Miscellaneous Incoming Letter	1	2
	Transmittal of New Application	3	3
	Authorization for Extension of Time all replies	4	4
	Application Data Sheet	5	10
	Specification	11	158
	Claims	159	164
	Abstract	165	165
	Drawings-only black and white line drawings	166	170
Warnings:			
Information:			
	Total Files Size (in bytes):	23634534	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

JOHN T. HARAN
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Sterne Kessler Goldstein Fox
ATTORNEYS AT LAW

June 1, 2011

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Re: U.S. Non-Provisional Utility Patent Application under 37 C.F.R. § 1.53(b)
Appl. No. To be assigned; Filed: Herewith
For: **Extraction of Lipid From Cells and Products Therefrom**
Inventors: CHERINKO *et al.*
Our Ref: 2715.1350003/JUK/JHH

Sir:

The following documents are transmitted herewith for appropriate action by the U.S. Patent and Trademark Office:

1. Utility Patent Application Transmittal Form (PTO/SB/05);

2. Authorization to Treat a Reply As Incorporating An Extension of Time Under 37 C.F.R. § 1.136(a)(3);

3. U.S. Utility Patent Application entitled:

   Extraction of Lipid From Cells and Products Therefrom

   and naming as inventors:

   Stephen Robert CHERINKO, Robert Cody KERTIS, Kirk E. APT, Paul Warren BEHRENS, Jon Milton HANSEN, Joseph W. PFEIFER III, Tracey Lynn STAHL and Ross ZIRKLE

   the application comprising:

   a. an Application Data Sheet (37 C.F.R. § 1.76);

   b. specification containing:

      i. 148 pages of description prior to the claims;

      ii. 6 pages of claims (59 claims);

      iii. a one (1) page abstract; and

c. 5 sheets of drawings: (Figures 1-5).

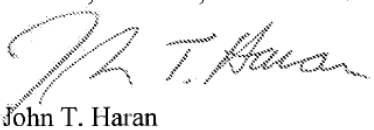
The above-listed documents are filed electronically through EFS-Web.

Correspondence should be sent to Customer Number 91335.

This patent application is being submitted under 37 C.F.R. § 1.53(b) without Declaration and without filing fee.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



John T. Haran
Attorney for Applicants
Registration No. 58,010

JUK/JHH/shr
Enclosures

SKGF_1367859.1

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/151,014	06/01/2011	1633	0.00	2715.1350003/JUK/JHH	59	8

CONFIRMATION NO. 4148

91335
Sterne, Kessler, Goldstein & Fox P.L.L.C
1100 New York Avenue, N.W.
Washington, DC 20005

FILING RECEIPT

OC000000048197884

Date Mailed: 06/15/2011

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

Stephen Robert Cherinko, Georgetown, SC;
Robert Cody Kertis, Timmonsville, SC;
Kirk E. Apt, Ellicott City, MD;
Paul Warren Behrens, Ellicott City, MD;
Jon Milton Hansen, Ellicott City, MD;
Joseph W. Pfeifer III, Westminster, MD;
Tracey Lynn Stahl, Pasadena, MD;
Ross Zirkle, Mt. Airy, MD;

Power of Attorney: None

Domestic Priority data as claimed by applicant

This appln claims benefit of 61/350,363 06/01/2010
and claims benefit of 61/378,923 08/31/2010
and claims benefit of 61/452,721 03/15/2011

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)

If Required, Foreign Filing License Granted: 06/14/2011

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/151,014**

Projected Publication Date: To Be Determined - pending completion of Missing Parts

Non-Publication Request: No

Early Publication Request: No

Title

Extraction of Lipid From Cells and Products Therefrom

Preliminary Class

424

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as

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This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

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NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH

CONFIRMATION NO. 4148

91335
Sterne, Kessler, Goldstein & Fox P.L.L.C
1100 New York Avenue, N.W.
Washington, DC 20005

FORMALITIES LETTER

OC000000048197885

Date Mailed: 06/15/2011

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The statutory basic filing fee is missing.
  *Applicant must submit $**330** to complete the basic filing fee for a non-small entity. If appropriate, applicant may make a written assertion of entitlement to small entity status and pay the small entity filing fee (37 CFR 1.27).*
- The oath or declaration is missing.
  A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
  Note: If a petition under 37 CFR 1.47 is being filed, an oath or declaration in compliance with 37 CFR 1.63 signed by all available joint inventors, or if no inventor is available by a party with sufficient proprietary interest, is required.

The applicant needs to satisfy supplemental fees problems indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- Additional claim fees of $**3934** as a non-small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
- A surcharge (for late submission of filing fee, search fee, examination fee or oath or declaration) as set forth in 37 CFR 1.16(f) of $**130** for a non-small entity, must be submitted.

SUMMARY OF FEES DUE:

Total fee(s) required within **TWO MONTHS** from the date of this Notice is $**5424** for a non-small entity
- $**330** Statutory basic filing fee.
- $**130** Surcharge.
- The application search fee has not been paid. Applicant must submit $**540** to complete the search fee.

- The application examination fee has not been paid. Applicant must submit $**220** to complete the examination fee for a non-small entity.
- The specification and drawings submitted electronically contain the equivalent of more than 100 pages. Applicant owes $**270** for **20** pages in excess of **100** pages for a non-small entity.
- Total additional claim fee(s) for this application is $**3934**
  - $**1100** for **5** independent claims over 3.
  - $**2444** for **47** total claims over 20.
  - $**390** for multiple dependent claim surcharge.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

/fhadera/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/151,014

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	330
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	540
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	220
TOTAL CLAIMS (37 CFR 1.16(i))	67 minus 20 =	* 47			OR	x 52 =	2444
INDEPENDENT CLAIMS (37 CFR 1.16(h))	8 minus 3 =	* 5				x 220 =	1100
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						270
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							390
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	5294

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET Substitute for Form PTO-1360 (For use with Form PTO/SB/06)	Application Number 13151014	Filing Date
	Applicant(s) Stephen Cherinko	

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
1	1					
2		1				
3		1				
4		3				
5		3				
6		(1)				
7		(1)				
8		(1)				
9		(1)				
10	1					
11	1					
12		2				
13		2				
14		(1)				
15		(1)				
16		(1)				
17		(1)				
18		(1)				
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47		(1)				
48		(1)				
49		(1)				
50		(1)				
Total Indep	8		0		0	
Total Depend	59	↵	0	↵	0	↵
Total Claims	67		0		0	

	*		*		*	
	Indep	Depend	Indep	Depend	Indep	Depend
51	1					
52	1					
53	1					
54	1					
55		3				
56		(1)				
57		(1)				
58		(1)				
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 4148
CHERINKO *et al.*	Art Unit: 1633
Appl. No.: 13/151,014	Examiner: To Be Assigned
Filed: June 1, 2011	Atty. Docket: 2715.1350003/JUK/JHH
For: **Extraction of Lipid From Cells and Products Therefrom**	

Preliminary Amendment Under 37 C.F.R. § 1.115

Mail Stop Amendment

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

In advance of prosecution, Applicants submit the following amendments and remarks.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks and Arguments begin on page 7 of this paper.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Original) A process for obtaining a lipid from a microbial cell composition, comprising:

   raising the pH of the cell composition to 8 or above; and

   separating a lipid from the cell composition,

   wherein the lipid contains less than 5% by weight of an organic solvent.

2. (Original) The process of claim 1, wherein the raising the pH lyses the cell composition.

3. (Original) The process of claim 1, wherein the raising the pH demulsifies the cell composition.

4. (Currently amended) The process of claim 1, ~~any of claims 1-3,~~ further comprising adding a salt to the cell composition to demulsify the cell composition.

5. (Cancelled)

6. (Currently amended) The process of claim 1, ~~any of claims 1-5,~~ further comprising heating the cell composition to demulsify the cell composition.

7. (Cancelled)

8. (Currently amended) The process of claim 1, ~~any of claims 1-7,~~ further comprising raising the pH of the cell composition a second time to demulsify the cell composition.

9. (Cancelled)

10. (Original) A process for obtaining a lipid from a cell, comprising:

lysing a cell to form a lysed cell composition;

raising the pH of the lysed cell composition to 8 or above to demulsify the cell composition;

adding a salt to the lysed cell composition to demulsify the cell composition; and

separating a lipid from the demulsified cell composition,

wherein the lipid contains less than 5% by weight of an organic solvent.

11. (Original) A process for obtaining a lipid from a cell composition, comprising:

raising the pH of a cell composition to 8 or above to lyse the cell composition and demulsify the cell composition;

adding a salt to the cell composition; and

separating a lipid from the demulsified cell composition,

wherein the lipid contains less than 5% by weight of an organic solvent.

12. (Currently amended) The process of claim 10 ~~or 11~~, further comprising heating the lysed cell composition to demulsify the cell composition.

13. (Cancelled)

14. (Currently amended) The process of claim 1, ~~any of claims 1-13,~~ further comprising agitating the lysed cell composition to demulsify the cell composition.

15. (Currently amended) The process of claim 10, ~~any of claims 10-14,~~ further comprising raising a pH of the lysed cell composition to demulsify the cell composition.

16-30. (Cancelled)

31. (Currently amended) The process of claim 1, ~~any of claims 1-30,~~ further comprising washing the cell or cell composition.

32. (Currently amended) The process of claim 1, ~~any of claims 1-31,~~ further comprising pasteurizing the cell or cell composition.

33. (Currently amended) The process of claim 1, ~~any of claims 1-32,~~ further comprising concentrating the lysed cell composition.

34-35. (Cancelled)

36. (Currently amended) The process of claim 1, ~~any of claims 1-35,~~ further comprising harvesting the lipid, wherein the harvesting comprises pumping the lipid without agitation.

37. (Original) A process for obtaining a lipid from a cell, comprising:

lysing a cell to form a lysed cell composition;

agitating the cell composition to demulsify the cell composition; and

separating a lipid from the demulsified cell composition,

wherein the lipid contains less than 5% by weight of an organic solvent.

38. (Cancelled)

39. (Currently amended) A lipid obtained by the process of claim 1. ~~any of claims 1-38.~~

40. (Cancelled)

41. (Currently amended) The lipid of claim 39, ~~any of claims 39-40,~~ wherein the lipid comprises at least 35% by weight docosahexaenoic acid.

42. (Currently amended) The lipid of claim 39, ~~any of claims 39-41,~~ wherein the lipid has an overall aroma intensity of 3 or less.

43. (Cancelled)

44. (Currently amended) The lipid of claim 39, ~~any of claims 39-43,~~ wherein the lipid has an anisidine value of 26 or less.

45. (Currently amended) The lipid of claim 39, ~~any of claims 39-44,~~ wherein the lipid has a phosphorus content of 100 ppm or less.

46. (Currently amended) The lipid of claim 39, ~~any of claims 39-45,~~ wherein the lipid has a peroxide value of 5 or less.

47. (Original) The lipid of claim 39, comprising at least 20% by weight eicosapentaenoic acid and less than 5% by weight each of arachidonic acid, docosapentaenoic acid n-6, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, erucic acid, and stearidonic acid.

48. (Cancelled)

49. (Currently amended) The lipid of claim 47 ~~or 48~~, wherein the lipid has a peroxide value of 5 or less.

50. (Currently amended) The lipid of claim 39 ~~any of claims 39-49,~~ wherein the lipid is a crude oil.

51. (Original) A crude microbial lipid having an anisidine value of 26 or less, a peroxide value of 5 or less, a phosphorus content of 100 ppm or less, and less than 5% by weight of an organic solvent.

52. (Original) An extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight, wherein the docosapentaenoic acid

n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, and wherein the oil has an anisidine value of 26 or less.

53. (Original) An extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1, and wherein the oil has an anisidine value of 26 or less.

54. (Original) An extracted microbial lipid comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight and wherein the oil has an anisidine value of 26 or less.

55. (Currently amended) The extracted microbial lipid of claim 52, ~~any of claims 52-54,~~ wherein the oil has a peroxide value of 5 or less.

56. (Currently amended) The extracted microbial lipid of claim 52, ~~any of claims 52-55,~~ wherein the oil has a phosphorus content of 100 ppm or less.

57. (Currently amended) The extracted microbial lipid of claim 52, ~~any of claims 52-56,~~ wherein the lipid is a crude oil.

58. (Currently amended) A process for obtaining a lipid, the process comprising refining a crude lipid of claim 50. ~~any of claims 50, 51, or 57.~~

59. (Cancelled)

60. (New) A lipid obtained by the process of claim 10.

61. (New) A lipid obtained by the process of claim 11.

62. (New) A lipid obtained by the process of claim 37.

Remarks

Upon entry of the foregoing amendment, claims 1-4, 6, 8, 10-12, 14, 15, 31-33, 36, 37, 39, 41, 42, 44-47, 49-58, and 60-62 are pending in the application, with claims 1, 10, 11, 37, 51, 52, 53, and 54 being the independent claims. Claims 5, 7, 9, 13, 16-30, 38, 40, 43, 48, and 59 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. New claims 60-62 are sought to be added.

The claims are amended to remove multiple dependencies.

Support for new claims 60-62 is found, *inter alia*, in original claim 39.

These changes are believed to introduce no new matter, and their entry is respectfully requested.

Conclusion

Prompt and favorable consideration of this Preliminary Amendment is respectfully requested. Applicants believe the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Judith U. Kim Reg. No. 49,679

for John T. Haran
Attorney for Applicants
Registration No. 58,010

Date: August 15, 2011

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600\
1393835_2.DOCX

Electronic Patent Application Fee Transmittal	
Application Number:	13151014
Filing Date:	01-Jun-2011
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom
First Named Inventor/Applicant Name:	Stephen Robert Cherinko
Filer:	John Thomas Haran/Jake Koster
Attorney Docket Number:	2715.1350003/JUK/JHH
Filed as Large Entity	

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	330	330
Utility Search Fee	1111	1	540	540
Utility Examination Fee	1311	1	220	220
Pages:				
Utility Appl Size fee per 50 sheets >100	1081	1	270	270
Claims:				
Claims in excess of 20	1202	16	52	832
Independent claims in excess of 3	1201	5	220	1100

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous-Filing:				
Late filing fee for oath or declaration	1051	1	130	130
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**3422**

Electronic Acknowledgement Receipt	
EFS ID:	10733009
Application Number:	13151014
International Application Number:	
Confirmation Number:	4148
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom
First Named Inventor/Applicant Name:	Stephen Robert Cherinko
Customer Number:	91335
Filer:	John Thomas Haran/Jake Koster
Filer Authorized By:	John Thomas Haran
Attorney Docket Number:	2715.1350003/JUK/JHH
Receipt Date:	15-AUG-2011
Filing Date:	01-JUN-2011
Time Stamp:	12:17:12
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$3422
RAM confirmation Number	9065
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1		27151350003_Filing_RNFMP.pdf	4081379 1666864c0565602420122870 51fc19068cf8 6bb3	yes	36

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Miscellaneous Incoming Letter	1	2
	Oath or Declaration filed	3	8
	Oath or Declaration filed	9	14
	Oath or Declaration filed	15	20
	Application Data Sheet	21	28
	Preliminary Amendment	29	36

Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	41043 d88d681f1485f28438bfacd5810e8057457e f1a5	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	4122422		

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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Sterne Kessler Goldstein Fox
ATTORNEYS AT LAW

August 15, 2011

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Confirmation No. 4148
Mail Stop Missing Parts

Re: U.S. Patent Application
Appl. No. 13/151,014; Filed: June 1, 2011
For: **Extraction of Lipid From Cells and Products Therefrom**
Inventors: CHERINKO *et al.*
Our Ref: 2715.1350003/JUK/JHH

Sir:

In reply to the "Notice to File Missing Parts of Non-Provisional Application," dated June 15, 2011, Applicants transmit the following documents for appropriate action by the U.S. Patent and Trademark Office:

1. Online Credit Card Payment Authorization for $3,422.00 to cover:

   $ 1,090.00 Patent Application fee (including basic filing, search and examination fees)
   $ 1,932.00 Excess claims fee
   $ 270.00 Application size fee
   $ 130.00 Surcharge for late filing fee or late Oath or Declaration

2. Original Declaration, executed by the inventors;

3. Supplemental Application Data Sheet (adding 3 additional inventors); and

4. Preliminary Amendment Under 37 C.F.R. § 1.115.

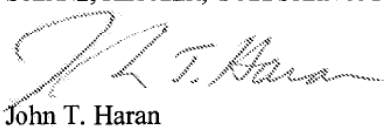
The above-listed documents are filed electronically through EFS-Web.

Fee payment is provided through online credit card payment. The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036. If extensions of time under 37 C.F.R. § 1.136 other than those otherwise provided for herewith are required to prevent abandonment of the present patent

application, then such extensions of time are hereby petitioned, and any fees therefor are hereby authorized to be charged to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



John T. Haran
Attorney for Applicants
Registration No. 58,010

JHH/hmb
Enclosures

1393842_1.DOCX

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/151,014	Filing Date 06/01/2011	☐ To be Mailed

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)	SMALL ENTITY ☐ RATE ($)	SMALL ENTITY ☐ FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
FOR	NUMBER FILED	NUMBER EXTRA					
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	08/15/2011	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 36	Minus	** 67	= 0	X $ =		OR	X $52=	0
	Independent (37 CFR 1.16(h))	* 8	Minus	***8	= 0	X $ =		OR	X $220=	0
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)	OR	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/SHAREILL COLES/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/151,014	06/01/2011	1633	3422	2715.1350003/JUK/JHH	36	8

CONFIRMATION NO. 4148

91335
Sterne, Kessler, Goldstein & Fox P.L.L.C
1100 New York Avenue, N.W.
Washington, DC 20005

UPDATED FILING RECEIPT

OC000000049401366

Date Mailed: 08/19/2011

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

Stephen Robert Cherinko, Georgetown, SC;
Robert Cody Kertis, Timmonsville, SC;
Kirk E. Apt, Ellicott City, MD;
Paul Warren Behrens, Ellicott City, MD;
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Nasrin Tabayeh Burke, Lexington, KY;
Krishna Raman, Wilmington, DE;
Neil Francis Leininger, Winchester, KY;

Power of Attorney: None

Domestic Priority data as claimed by applicant

This appln claims benefit of 61/350,363 06/01/2010
and claims benefit of 61/378,923 08/31/2010
and claims benefit of 61/452,721 03/15/2011

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)

Permission to Access - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 06/14/2011

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/151,014**

Projected Publication Date: 12/01/2011

Non-Publication Request: No

Early Publication Request: No
Title

Extraction of Lipid From Cells and Products Therefrom

Preliminary Class

424

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/151,014

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	330
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	540
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	220
TOTAL CLAIMS (37 CFR 1.16(i))	36 minus 20 =	* 16			OR	x 52 =	832
INDEPENDENT CLAIMS (37 CFR 1.16(h))	8 minus 3 =	* 5				x 220 =	1100
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						270
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	3292

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH

CONFIRMATION NO. 4148

91335
Sterne, Kessler, Goldstein & Fox P.L.L.C
1100 New York Avenue, N.W.
Washington, DC 20005

PUBLICATION NOTICE

OC00000051241541

Title: Extraction of Lipid From Cells and Products Therefrom

Publication No.US-2011-0295028-A1
Publication Date:12/01/2011

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

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In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH	4148

91335 7590 11/27/2013
Sterne, Kessler, Goldstein & Fox P.L.L.C
1100 New York Avenue, N.W.
Washington, DC 20005

EXAMINER	
CARR, DEBORAH D	
ART UNIT	PAPER NUMBER
1621	

MAIL DATE	DELIVERY MODE
11/27/2013	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 13/151,014	Applicant(s) CHERINKO ET AL.	
	Examiner DEBORAH D. CARR	Art Unit 1621	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *1* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-63* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *1-63* are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-8, 14, 31-33, 36, 39, 41-42, 44-47, 49-58, drawn to a process for obtaining a lipid from a microbial cell composition, classified in 435.

II. Claims 10-12, 15, 37, 60-63, drawn to a method for obtaining a lipid from a cell, classified in 554.

The inventions are distinct, each from the other because of the following reasons:

2. Inventions I and II are directed to related distinct processes. The related inventions are distinct if: (1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.05(j). In the instant case, the inventions as claimed can have a materially different design in that the staring source for the process are materially different. Group I utilizes microbial cell composition which are materially different that the cells in which the lipids are obtained from. As shown in sections [0102] and [0103], the source of te lipids are obtaining from a materially different design and are not obvious variants.

Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants.

3. Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a

serious search and/or examination burden if restriction were not required because one or more of the following reasons apply:

(a) the inventions have acquired a separate status in the art in view of their different classification;

(b) the inventions have acquired a separate status in the art due to their recognized divergent subject matter;

(c) the inventions require a different field of search (for example, searching different classes/subclasses or electronic resources, or employing different search queries);

(d) the prior art applicable to one invention would not likely be applicable to another invention;

(e) the inventions are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of an invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected invention**.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103 or pre-AIA 35 U.S.C. 103(a) of the other invention.

4. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be corrected in compliance with 37 CFR 1.48(a) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. A request to correct inventorship under 37 CFR 1.48(a) must be accompanied by an application data sheet in accordance with 37 CFR 1.76 that identifies each inventor by his or her legal name and by the processing fee required under 37 CFR 1.17(i).

5. Any inquiry concerning this communication or earlier communications from the examiner should be directed to DEBORAH D. CARR whose telephone number is (571)272-0637. The examiner can normally be reached on Monday-Friday 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Wu-Cheng Winston Shen can be reached on 571-272-3157. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like

assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/DEBORAH D CARR/
Primary Examiner, Art Unit 1621

Ddc

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1621

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	11/25/2013								
	1	÷								
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Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
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	Examiner	Art Unit
	DEBORAH D CARR	1621

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	11/25/2013								
	37	÷								
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 4148
Cherinko *et al.*	Art Unit: 1672
Appl. No.: 13/151,014	Examiner: Carr, Deborah D
Filing Date: June 1, 2011	Atty. Docket: 28612-US-NP

For: EXTRACTION OF LIPID FROM CELLS AND PRODUCTS THEREFROM

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

REPLY TO RESTRICTION REQUIREMENT MAILED NOVEMBER 27, 2013

Dear Commissioner:

Applicants herein respond to the Restriction Requirement mailed November 27, 2013. A petition for a one-month extension of time accompanies this response. Please amend the application as follows and consider the following remarks:

Amendments to the claims begin on page 2 of this paper.

Remarks/Arguments begin on page 5 of this paper.

Amendments to the claim:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A process for obtaining a lipid from a ~~microbial~~ cell composition, comprising:
(a) lysing a cell of said cell composition to form a lysed cell composition;
(b) demulsifying said lysed cell composition to form a demulsified cell composition; ~~raising the pH of the cell composition to 8 or above; and~~
(c) separating ~~a~~ said lipid from the demulsified cell composition~~,~~; and
(d) harvesting said lipid;
wherein (b) comprises heating said lysed cell composition to a temperature of at least 70° C~~the lipid contains less than 5% by weight of an organic solvent~~.

2. (Currently Amended) The process of claim 1, wherein (b) further comprises contacting said lysed cell composition with a first base to raise ~~the raising~~ the pH of ~~lyses~~ the lysed cell composition to 8 or above.

3. (Currently Amended) The process of claim 2 ~~claim 1~~, wherein (b) further comprises contacting said ~~the raising the pH demulsifies the~~ lysed cell composition with a second base to raise the pH of the lysed cell composition to 7 or above.

4. (Currently Amended) The process of claim 1, wherein (b) further ~~comprising~~ comprises contacting said lysed cell composition with ~~adding~~ a salt ~~to the cell composition to demulsify the cell composition~~.

5. (Cancelled)

6. (Currently Amended) The process of ~~claim 1~~claim 4, wherein the salt is added in an amount of 0.1% to 20% by weight of ~~further comprising heating the cell composition to demulsify~~ the lysed cell composition.

7. (Cancelled).

8. (Currently Amended) The process of claim 4~~claim 1~~, wherein ~~further~~ ~~comprising raising~~ the salt is selected from: alkali metal salts, alkali earth metal salts, sulfate salts,

and combinations thereof ~~pH of the cell composition a second time to demulsify the cell composition~~.

9-30. (Cancelled)

31. (Previously Presented) The process of claim 1, further comprising washing the cell or cell composition.

32. (Previously Presented) The process of claim 1, further comprising pasteurizing the cell or cell composition.

33. (Previously Presented) The process of claim 1, further comprising concentrating the lysed cell composition.

34-38. (Cancelled)

39. (Previously Presented) A lipid obtained by the process of claim 1.

40. (Cancelled)

41. (Previously Presented) The lipid of claim 39, wherein the lipid comprises at least 35% by weight docosahexaenoic acid.

42. (Previously Presented) The lipid of claim 39, wherein the lipid has an overall aroma intensity of 3 or less.

43. (Cancelled)

44. (Previously Presented) The lipid of claim 39, wherein the lipid has an anisidine value of 26 or less.

45. (Previously Presented) The lipid of claim 39, wherein the lipid has a phosphorus content of 100 ppm or less.

46. (Previously Presented) The lipid of claim 39, wherein the lipid has a peroxide value of 5 or less.

47. (Original) The lipid of claim 39, comprising at least 20% by weight eicosapentaenoic acid and less than 5% by weight each of arachidonic acid, docosapentaenoic acid n-6, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, erucic acid, and stearidonic acid.

48. (Cancelled)

49. (Previously Presented) The lipid of claim 47, wherein the lipid has a peroxide value of 5 or less.

50. (Previously Presented) The lipid of claim 39, wherein the lipid is a crude oil.

51. (Original) A crude microbial lipid having an anisidine value of 26 or less, a peroxide value of 5 or less, a phosphorus content of 100 ppm or less, and less than 5% by weight of an organic solvent.

52. (Original) An extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, and wherein the oil has an anisidine value of 26 or less.

53. (Original) An extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1, and wherein the oil has an anisidine value of 26 or less.

54. (Original) An extracted microbial lipid comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight and wherein the oil has an anisidine value of 26 or less.

55. (Previously Presented) The extracted microbial lipid of claim 52, wherein the oil has a peroxide value of 5 or less.

56. (Previously Presented) The extracted microbial lipid of claim 52, wherein the oil has a phosphorus content of 100 ppm or less.

57. (Previously Presented) The extracted microbial lipid of claim 52, wherein the lipid is a crude oil.

58. (Previously Presented) A process for obtaining a lipid, the process comprising refining a crude lipid of claim 50.

59-62. (Cancelled)

63. (New) The process of claim 1, wherein (b) comprises heating said lysed cell composition to a temperature of from 70° C to 100° C.

64. (New) The process of claim 1, wherein (b) further comprises agitating the lysed cell composition.

65. (New) The process of claim 1, wherein (a) further comprises adding a salt to said cell composition.

66. (New) The process of claim 1, wherein (a) comprises raising the pH of said cell composition to 8 or above.

67. (New) The process of claim 1, wherein the lysed cell composition is in the form of an oil-in-water emulsion comprising a mixture of a continuous aqueous phase and a dispersed lipid phase.

68. (New) The process of claim 1, wherein (c) comprises centrifuging the demulsified cell composition.

69. (New) The process of claim 1, wherein the lipid is a crude lipid.

70. (New) The process of claim 69, further comprising (e) refining said crude lipid to obtain a refined lipid.

71. (New) The process of claim 1, wherein said lipid has an anisidine value of 26 or less.

72. (New) The process of claim 1, wherein said lipid has a peroxide value of 5 or less.

73. (New) The process of claim 1, wherein said cell is a microbial cell.

74. (New) The process of claim 73, wherein said microbial cell is of the genus *Thraustochytrium*, *Schizochytrium*, or mixtures thereof.

75. (New) The process of claim 1, wherein said lipid comprises omega 3 and/or omega-6 polyunsaturated fatty acids.

76. (New) The process of claim 1, wherein said lipid comprises at least one of DHA, DPA(n-3), DPA(n-6), EPA, and ARA.

77. (New) The process of claim 1, wherein an organic solvent is not added to said cell composition, said lysed cell composition, or said lipid in an amount or concentration sufficient to extract said lipid from said cell.

78. (New) The process of claim 1, wherein the lipid contains less than 5% by weight of an organic solvent.

Remarks

On August 15, 2011, Applicants submitted a Preliminary Amendment wherein claims 4, 6, 8, 14, 15, 31-33, 36, 39, 41, 42, 44-46, 49-50, and 55-58 were amended; claims 5, 7, 9, 13, 16-30, 34-35, 38, 40, 43, 48, and 59 were cancelled; and claims 60-62 were added such that claims 1-4, 6, 8, 10-12, 14, 15, 31-33, 36, 37, 39, 41-42, 44-47, 49-58, and 60-62 were pending. Applicants herein respond to the Restriction Requirement on the basis of the claims that were pending pursuant to the August 15, 2011 Preliminary Amendment. Accordingly, Applicants herein elect without traverse to prosecute the invention of Group I represented by claims 1-4, 6, 8, 14, 31-33, 36, 39, 41-42, 44-47, and 49-58. This election is made without prejudice to or disclaimer of the other claims or inventions disclosed in the above-referenced patent application.

In conjunction with making this election, Applicants herein amend claims 1-4, 6, and 8; cancel claims 10-12, 14, 15, 36-37, and 60-62; and submit new claims 63-78 such that claims 1-4, 6, 8, 31-33, 39, 41-42, 44-47, 49-58, and 63-78 are pending.

Support for claim 1 can be found, for example, in original claim 6; at paragraphs 113, 124, 125, 128, 139, 140, 156 and 160; and at Examples 11-12 and 14.

Support for claim 2 can be found, for example, at paragraphs 128, 129, and 132.

Support for claim 3 can be found, for example, at paragraphs 129 and 158

Support for claims 4, 6, and 8 can be found, for example, in original claims 4 and 22-23 and at paragraphs 128 and 135.

Support for claim 63 can be found at, for example, paragraph 139 and Examples 11-12 and 14.

Support for claim 64 can be found at, for example, original claim 14 and at paragraph 146.

Support for claim 65 can be found at, for example, paragraphs 135, 157, and Example 20.

Support for claim 66 can be found at, for example, paragraphs 113, 119-120, and 129.

Support for claim 67 can be found at, for example, paragraph 122.

Support for claim 68 can be found at, for example, original claim 24 and paragraphs 157 and 161.

Support for claims 69-70 can be found at, for example, original claims 50 and 58 and paragraph 167.

Support for claims 71-72 can be found at, for example, original claims 44 and 46 and paragraphs 134, 178-179, and 207.

Support for claims 73-74 can be found at, for example, paragraphs 102-103, and 106.

Support for claims 75-76 can be found at, for example, paragraph 111.

Support for claims 77-78 can be found at, for example, paragraph 91.

No new matter has been added.

Summary

In view of the foregoing amendments and remarks, Applicants respectfully submit this application is in condition for allowance and respectfully solicit same. In order to expedite disposition of this case, the Office is invited to contact Applicants' representative at the telephone number listed below to resolve any remaining issues. Although Applicants believe no fees beyond those accompanying this response are due, the Commissioner is hereby authorized to charge any deficiency in fees or credit any over payment to deposit account No. 50-5958, referencing attorney docket No. 28612-US-NP.

Respectfully submitted,

/Jacqueline Cohen/

Jacqueline Cohen
Attorney for Applicants
Registration No. 51,574

Date: January 27, 2014

DSM Nutritional Products
6480 Dobbin Road
Columbia, Maryland 21045
(240) 512-2704

Electronic Patent Application Fee Transmittal	
Application Number:	13151014
Filing Date:	01-Jun-2011
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom
First Named Inventor/Applicant Name:	Stephen Robert Cherinko
Filer:	Jacqueline Marie Cohen/Kimberly Sauter-Bray
Attorney Docket Number:	2715.1350003/JUK/JHH
Filed as Large Entity	

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 1 month with $0 paid	1251	1	200	200

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**200**

Electronic Acknowledgement Receipt	
EFS ID:	18040580
Application Number:	13151014
International Application Number:	
Confirmation Number:	4148
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom
First Named Inventor/Applicant Name:	Stephen Robert Cherinko
Customer Number:	91335
Filer:	Jacqueline Marie Cohen/Kimberly Sauter-Bray
Filer Authorized By:	Jacqueline Marie Cohen
Attorney Docket Number:	2715.1350003/JUK/JHH
Receipt Date:	27-JAN-2014
Filing Date:	01-JUN-2011
Time Stamp:	23:23:09
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$200
RAM confirmation Number	8721
Deposit Account	505958
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Extension of Time	20140127_1MthEOT_28612-US-NP.pdf	163190 8135aad03dc3bfc784abd030bd548068081 1afaa	no	2
Warnings:					
Information:					
2	Response to Election / Restriction Filed	20140127_Response_to_Restriction_Req_28612-US-NP.pdf	119300 01cdef88a4fed35f5a5089529a7c45761dbd 5109	no	8
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30199 24306e5d62af4a2410269b00a25230da0b1 09507	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	312689		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/22 (03-13)
Approved for use through 7/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) 28612-US-NP
Application Number 13/151014	Filed June 1, 2011
For EXTRACTION OF LIPID FROM CELLS AND PRODUCTS THEREFROM	
Art Unit 1621	Examiner CARR, DEBORAH D.

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

		Fee	Small Entity Fee	Micro Entity Fee	
☑	One month (37 CFR 1.17(a)(1))	$200	$100	$50	$ 200.00
☐	Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
☐	Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$
☐	Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
☐	Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☑ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-5958.

☑ Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant.

☐ attorney or agent of record. Registration number _____.

☑ attorney or agent acting under 37 CFR 1.34. Registration number 51574.

/Jacqueline Cohen/ — Signature

January 27, 2014 — Date

Jacqueline Cohen — Typed or printed name

240-512-2704 — Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☑ * Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.136(a). The information is required to obtain or retain a benefit by the public, which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 6 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/151,014	Filing Date 06/01/2011	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)		
FOR	NUMBER FILED	NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

		(Column 1)		(Column 2)	(Column 3)		
	01/27/2014	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
AMENDMENT	Total (37 CFR 1.16(i))	* 42	Minus	** 36	= 6	X $80 =	480
	Independent (37 CFR 1.16(h))	* 6	Minus	***8	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**480**

		(Column 1)		(Column 2)	(Column 3)		
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
AMENDMENT	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/KIM WATSON/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Document code: WFEE

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91335 e 2014-01-31

Sterne, Kessler, Goldstein & Fox P.L.L.C
1100 New York Avenue, N.W.
Washington, DC 20005

Paper No.

Application No.:	**13/151,014**	**Date Mailed:**	**2014-01-31**
First Named Inventor:	Cherinko, Stephen, Robert	Examiner:	CARR, DEBORAH D
Attorney Docket No.:	2715.1350003/JUK/JHH	Art Unit:	1672
Confirmation No.:	4148	Filing Date:	2011-06-01

Please find attached an Office communication concerning this application or proceeding.

Commissioner for Patents

PTO-90c (Rev.08-06)

Notice of Non-Compliant Amendment (37 CFR 1.121)	Application No. 13/151,014	Applicant(s) CHERINKO ET AL.
		Art Unit 2100

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

The amendment document filed on *27 January, 2014* is considered non-compliant because it has failed to meet the requirements of 37 CFR 1.121 or 1.4. In order for the amendment document to be compliant, correction of the following item(s) is required.

THE FOLLOWING MARKED (X) ITEM(S) CAUSE THE AMENDMENT DOCUMENT TO BE NON-COMPLIANT:

- ☐ 1. Amendments to the specification:
  - ☐ A. Amended paragraph(s) do not include markings.
  - ☐ B. New paragraph(s) should not be underlined.
  - ☐ C. Other _____.
- ☐ 2. Abstract:
  - ☐ A. Not presented on a separate sheet. 37 CFR 1.72.
  - ☐ B. Other _____.
- ☐ 3. Amendments to the drawings:
  - ☐ A. The drawings are not properly identified in the top margin as "Replacement Sheet," "New Sheet," or "Annotated Sheet" as required by 37 CFR 1.121(d).
  - ☐ B. The practice of submitting proposed drawing correction has been eliminated. Replacement drawings showing amended figures, without markings, in compliance with 37 CFR 1.84 are required.
  - ☐ C. Other _____.
- ☒ 4. Amendments to the claims:
  - ☐ A. A complete listing of all of the claims is not present.
  - ☐ B. The listing of claims does not include the text of all pending claims (including withdrawn claims)
  - ☐ C. Each claim has not been provided with the proper status identifier, and as such, the individual status of each claim cannot be identified. Note: the status of every claim must be indicated after its claim number by using one of the following status identifiers: (Original), (Currently amended), (Canceled), (Previously presented), (New), (Not entered), (Withdrawn) and (Withdrawn-currently amended).
  - ☐ D. The claims of this amendment paper have not been presented in ascending numerical order.
  - ☒ E. Other: *See Continuation Sheet*.
- ☐ 5. Other (e.g., the amendment is unsigned or not signed in accordance with 37 CFR 1.4): For further explanation of the amendment format required by 37 CFR 1.121, see MPEP § 714.

TIME PERIODS FOR FILING A REPLY TO THIS NOTICE:

1. Applicant is given **no new time period if the non-compliant amendment is an** after-final amendment or an amendment filed after allowance, or a drawing submission (only) If applicant wishes to resubmit the non-compliant after-final amendment with corrections, the **entire corrected amendment** must be resubmitted.

2. Applicant is given **two months** from the mail date of this notice to supply the correction, if the non-compliant amendment is one of the following: a preliminary amendment, a non-final amendment (including a submission for a request for continued examination (RCE) under 37 CFR 1.114), a supplemental amendment filed within a suspension period under 37 CFR 1.103(a) or (c), and an amendment filed in response to a Quayle action. If any of above boxes 1 to 4 are checked, the correction required is only the corrected section of the non-compliant amendment in compliance with 37 CFR 1.121.

Extensions of time are available under 37 CFR 1.136(a) only if the non-compliant amendment is a non-final amendment or an amendment filed in response to a *Quayle* action.

Failure to timely respond to this notice will result in:

Abandonment of the application if the non-compliant amendment is a non-final amendment or an amendment filed in response to a *Quayle* action; or

Non-entry of the amendment if the non-compliant amendment is a preliminary amendment or supplemental amendment.

Legal Instruments Examiner (LIE), if applicable /KIM WATSON/ Telephone No: (571)272-3616

Continuation of 4. Other: The text of (New) claims must not be underlined. See claims 63-78.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 4148
Cherinko *et al.*	Art Unit: 1672
Appl. No.: 13/151,014	Examiner: Carr, Deborah D
Filing Date: June 1, 2011	Atty. Docket: 28612-US-NP

For: EXTRACTION OF LIPID FROM CELLS AND PRODUCTS THEREFROM

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

REPLY TO NOTICE OF NON-COMPLIANT AMENDMENT MAILED JANUARY 31, 2014

Dear Commissioner:

Applicants herein respond to the Notice of Non-Compliant Amendment mailed January 31, 2014. Please amend the application as follows and consider the following remarks:

Amendments to the claims begin on page 2 of this paper.

Remarks/Arguments begin on page 7 of this paper.

Amendments to the claim:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A process for obtaining a lipid from a ~~microbial~~ cell composition, comprising:
(a) lysing a cell of said cell composition to form a lysed cell composition;
(b) demulsifying said lysed cell composition to form a demulsified cell composition; ~~raising the pH of the cell composition to 8 or above; and~~
(c) separating ~~a~~ said lipid from the demulsified cell composition~~,~~; and
(d) harvesting said lipid;
wherein (b) comprises heating said lysed cell composition to a temperature of at least 70° C~~the lipid contains less than 5% by weight of an organic solvent~~.

2. (Currently Amended) The process of claim 1, wherein (b) further comprises contacting said lysed cell composition with a first base to raise ~~the raising~~ the pH of ~~lyses~~ the lysed cell composition to 8 or above.

3. (Currently Amended) The process of claim 2 ~~claim 1~~, wherein (b) further comprises contacting said ~~the raising the pH demulsifies the~~ lysed cell composition with a second base to raise the pH of the lysed cell composition to 7 or above.

4. (Currently Amended) The process of claim 1, wherein (b) further ~~comprising~~ comprises contacting said lysed cell composition with ~~adding~~ a salt ~~to the cell composition to demulsify the cell composition~~.

5. (Cancelled)

6. (Currently Amended) The process of ~~claim 1~~claim 4, wherein the salt is added in an amount of 0.1% to 20% by weight of ~~further comprising heating the cell composition to demulsify~~ the lysed cell composition.

7. (Cancelled).

8. (Currently Amended) The process of claim 4~~claim 1~~, wherein ~~further~~ ~~comprising raising~~ the salt is selected from: alkali metal salts, alkali earth metal salts, sulfate salts,

and combinations thereof ~~pH of the cell composition a second time to demulsify the cell composition~~.

9-30. (Cancelled)

31. (Previously Presented) The process of claim 1, further comprising washing the cell or cell composition.

32. (Previously Presented) The process of claim 1, further comprising pasteurizing the cell or cell composition.

33. (Previously Presented) The process of claim 1, further comprising concentrating the lysed cell composition.

34-38. (Cancelled)

39. (Previously Presented) A lipid obtained by the process of claim 1.

40. (Cancelled)

41. (Previously Presented) The lipid of claim 39, wherein the lipid comprises at least 35% by weight docosahexaenoic acid.

42. (Previously Presented) The lipid of claim 39, wherein the lipid has an overall aroma intensity of 3 or less.

43. (Cancelled)

44. (Previously Presented) The lipid of claim 39, wherein the lipid has an anisidine value of 26 or less.

45. (Previously Presented) The lipid of claim 39, wherein the lipid has a phosphorus content of 100 ppm or less.

46. (Previously Presented) The lipid of claim 39, wherein the lipid has a peroxide value of 5 or less.

47. (Original) The lipid of claim 39, comprising at least 20% by weight eicosapentaenoic acid and less than 5% by weight each of arachidonic acid, docosapentaenoic acid n-6, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, erucic acid, and stearidonic acid.

48. (Cancelled)

49. (Previously Presented) The lipid of claim 47, wherein the lipid has a peroxide value of 5 or less.

50. (Previously Presented) The lipid of claim 39, wherein the lipid is a crude oil.

51. (Original) A crude microbial lipid having an anisidine value of 26 or less, a peroxide value of 5 or less, a phosphorus content of 100 ppm or less, and less than 5% by weight of an organic solvent.

52. (Original) An extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, and wherein the oil has an anisidine value of 26 or less.

53. (Original) An extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1, and wherein the oil has an anisidine value of 26 or less.

54. (Original) An extracted microbial lipid comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight and wherein the oil has an anisidine value of 26 or less.

55. (Previously Presented) The extracted microbial lipid of claim 52, wherein the oil has a peroxide value of 5 or less.

56. (Previously Presented) The extracted microbial lipid of claim 52, wherein the oil has a phosphorus content of 100 ppm or less.

57. (Previously Presented) The extracted microbial lipid of claim 52, wherein the lipid is a crude oil.

58. (Previously Presented) A process for obtaining a lipid, the process comprising refining a crude lipid of claim 50.

59-62. (Cancelled)

63. (New) The process of claim 1, wherein (b) comprises heating said lysed cell composition to a temperature of from 70° C to 100° C.

64. (New) The process of claim 1, wherein (b) further comprises agitating the lysed cell composition.

65. (New) The process of claim 1, wherein (a) further comprises adding a salt to said cell composition.

66. (New) The process of claim 1, wherein (a) comprises raising the pH of said cell composition to 8 or above.

67. (New) The process of claim 1, wherein the lysed cell composition is in the form of an oil-in-water emulsion comprising a mixture of a continuous aqueous phase and a dispersed lipid phase.

68. (New) The process of claim 1, wherein (c) comprises centrifuging the demulsified cell composition.

69. (New) The process of claim 1, wherein the lipid is a crude lipid.

70. (New) The process of claim 69, further comprising (e) refining said crude lipid to obtain a refined lipid.

71. (New) The process of claim 1, wherein said lipid has an anisidine value of 26 or less.

72. (New) The process of claim 1, wherein said lipid has a peroxide value of 5 or less.

73. (New) The process of claim 1, wherein said cell is a microbial cell.

74. (New) The process of claim 73, wherein said microbial cell is of the genus *Thraustochytrium*, *Schizochytrium*, or mixtures thereof.

75. (New) The process of claim 1, wherein said lipid comprises omega 3 and/or omega-6 polyunsaturated fatty acids.

76. (New) The process of claim 1, wherein said lipid comprises at least one of DHA, DPA(n-3), DPA(n-6), EPA, and ARA.

77. (New) The process of claim 1, wherein an organic solvent is not added to said cell composition, said lysed cell composition, or said lipid in an amount or concentration sufficient to extract said lipid from said cell.

78. (New) The process of claim 1, wherein the lipid contains less than 5% by weight of an organic solvent.

Remarks

Pursuant to the Notice of Non-compliance, which indicates "the correction required is only the corrected section of the non-compliant amendment in compliance with 37 CFR 1.121", Applicants herein submit a revised listing of claims (which reflects all of the amendments made via the amendment submitted January 27, 2014) in which the underlining of new claims 63-78 has been removed.

In the Reply to the Restriction Requirement dated January 27, 2014, Applicants amended claims 1-4, 6, and 8; cancelled claims 10-12, 14, 15, 36-37, and 60-62; and submitted new claims 63-78 such that claims 1-4, 6, 8, 31-33, 39, 41-42, 44-47, 49-58, and 63-78 were pending. Support for the amendments and the election made therein can be found in the Reply dated January 27, 2014.

Summary

In view of the foregoing amendments and remarks, Applicants respectfully submit this application is in condition for allowance and respectfully solicit same. In order to expedite disposition of this case, the Office is invited to contact Applicants' representative at the telephone number listed below to resolve any remaining issues. Although Applicants believe no fees beyond those accompanying this response are due, the Commissioner is hereby authorized to charge any deficiency in fees or credit any over payment to deposit account No. 50-5958, referencing attorney docket No. 28612-US-NP.

Respectfully submitted,

/Jacqueline Cohen/

Jacqueline Cohen
Attorney for Applicants
Registration No. 51,574

Date: March 31, 2014

DSM Nutritional Products
6480 Dobbin Road
Columbia, Maryland 21045
(240) 512-2704

Electronic Acknowledgement Receipt	
EFS ID:	18627429
Application Number:	13151014
International Application Number:	
Confirmation Number:	4148
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom
First Named Inventor/Applicant Name:	Stephen Robert Cherinko
Customer Number:	91335
Filer:	Jacqueline Marie Cohen/Kimberly Sauter-Bray
Filer Authorized By:	Jacqueline Marie Cohen
Attorney Docket Number:	2715.1350003/JUK/JHH
Receipt Date:	31-MAR-2014
Filing Date:	01-JUN-2011
Time Stamp:	14:56:22
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Arguments/Remarks Made in an Amendment	20140331_Response_to_NonCompliantAmendment_28612-US-NP.pdf	115274 2b94e3854acadf5140aaf4bddd790b7ecf0df191	no	7
Warnings:					
Information:					

Total Files Size (in bytes):	115274

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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NOTICE OF ALLOWANCE AND FEE(S) DUE

91335 7590 08/21/2014
Sterne, Kessler, Goldstein & Fox P.L.L.C
1100 New York Avenue, N.W.
Washington, DC 20005

EXAMINER	
CARR, DEBORAH D	
ART UNIT	PAPER NUMBER
1672	

DATE MAILED: 08/21/2014

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH	4148

TITLE OF INVENTION: Extraction of Lipid From Cells and Products Therefrom

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	11/21/2014

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

91335 7590 08/21/2014
Sterne, Kessler, Goldstein & Fox P.L.L.C
1100 New York Avenue, N.W.
Washington, DC 20005

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH	4148

TITLE OF INVENTION: Extraction of Lipid From Cells and Products Therefrom

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	11/21/2014

EXAMINER	ART UNIT	CLASS-SUBCLASS
CARR, DEBORAH D	1672	554-175000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee
❑ Publication Fee (No small entity discount permitted)
❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.
❑ Payment by credit card. Form PTO-2038 is attached.
❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH	4148

91335 7590 08/21/2014
Sterne, Kessler, Goldstein & Fox P.L.L.C
1100 New York Avenue, N.W.
Washington, DC 20005

EXAMINER	
CARR, DEBORAH D	

ART UNIT	PAPER NUMBER
1672	

DATE MAILED: 08/21/2014

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 13/151,014	Applicant(s) CHERINKO ET AL.	
	Examiner DEBORAH D. CARR	Art Unit 1672	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *31 March 2014*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1-4,6,8,31-33,39,41,42,44-47,49-58 and 63-78*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   Certified copies:
   a) ☐ All b) ☐ Some *c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☐ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/DEBORAH D CARR/
Primary Examiner, Art Unit 1672

Notice of References Cited	Application/Control No. 13/151,014	Applicant(s)/Patent Under Reexamination CHERINKO ET AL.	
	Examiner DEBORAH D. CARR	Art Unit 1672	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
554	8, 223, 224, 227	8/2014	ddc
435	134	8/2014	ddc

SEARCH NOTES		
Search Notes	Date	Examiner
see transcript	8/2014	ddc

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
435	134	8/2014	ddc
554	8, 223, 224, 227	8/2014	ddc

	/DEBORAH D CARR/ Primary Examiner.Art Unit 1672

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

CPC						
Symbol					Type	Version
C11B		3	/	02	I	2013-01-01
C11B		3	/	16	I	2013-01-01
C11B		1	/	10	F	2013-01-01
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CPC Combination Sets							
Symbol					Type	Set	Ranking	Version
			/					
			/					

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	42	
/DEBORAH D CARR/ Primary Examiner.Art Unit 1672	08/04/2014	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

U.S. Patent and Trademark Office — Part of Paper No. 20140804

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	**Art Unit**
	DEBORAH D CARR	1672

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS
554	8

CROSS REFERENCE(S)					
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				
435	134				
554	223	224	227		

INTERNATIONAL CLASSIFICATION						
CLAIMED					NON-CLAIMED	
C	1	2	P	7 / 64 (2006.0)		
C	1	1	B	1 / 00 (2006.0)		
A	0	1	H	5 / 10 (2006.0)		
A	2	3	D	9 / 00 (2006.01.01)		

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 42	
/DEBORAH D CARR/ Primary Examiner.Art Unit 1672 (Primary Examiner)	08/04/2014 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 1

U.S. Patent and Trademark Office

Part of Paper No. 20140804

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	**Art Unit**
	DEBORAH D CARR	1672

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
	1		17		33		49		65						
	2		18		34		50		66						
	3		19		35		51		67						
	4		20		36		52		68						
	5		21		37		53		69						
	6		22		38		54		70						
	7		23		39		55		71						
	8		24		40		56		72						
	9		25		41		57		73						
	10		26		42		58		74						
	11		27		43		59		75						
	12		28		44		60		76						
	13		29		45		61		77						
	14		30		46		62		78						
	15		31		47		63								
	16		32		48		64								

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	42	
/DEBORAH D CARR/ Primary Examiner.Art Unit 1672	08/04/2014	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

U.S. Patent and Trademark Office

Part of Paper No. 20140804

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S595	318043	"435"/$	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 15:22
S597	358079	dehydrat$	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:21
S599	18804191	preparing or method	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:21
S601	18241	lysing adj3 cell	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:23
S602	3707	demulsifying	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:23
S603	147906	harvesting	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:23
S604	797706	extracting	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:24
S605	338955	lipid	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:24
S606	3032	S604 with S605	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:24
S607	0	S606 and S601 and S602 and S595	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:24
S608	98	S606 and S601 and S595	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:24
S609	38	S606 and S601 and S603 and S595	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:25
S610	10	heating adj4 (lysed adj2 cell)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:39
S611	14053	heating adj4 cell	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:39
S612	0	S606 and S601 and S603 and S595 and S611	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:42
S613	1	S606 and S601 and S603 and S611	US-PGPUB; USPAT; USOCR; EPO; JPO;	ADJ	ON	2014/08/01 16:42

			DERWENT			
S614	12	S606 and S611	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:44
S615	1	S606 and S611 and S602	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:47
S616	71393	S599 with S605	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/08/01 16:47

EAST Search History (Interference)

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8/ 4/ 2014 1:13:55 PM
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BIB DATA SHEET

CONFIRMATION NO. 4148

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
13/151,014	06/01/2011 RULE	554	1672	2715.1350003/JUK/JHH

APPLICANTS

INVENTORS

Stephen Robert Cherinko, Georgetown, SC;
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Tracey Lynn Stahl, Pasadena, MD;
Ross Zirkle, Mt. Airy, MD;
Nasrin Tabayeh Burke, Lexington, KY;
Krishna Raman, Wilmington, DE;
Neil Francis Leininger, Winchester, KY;

**** CONTINUING DATA ***************************

This appln claims benefit of 61/350,363 06/01/2010
and claims benefit of 61/378,923 08/31/2010
and claims benefit of 61/452,721 03/15/2011

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

06/14/2011

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☑ No Verified and Acknowledged /DEBORAH D CARR/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		SC	5	36	8

ADDRESS

Sterne, Kessler, Goldstein & Fox P.L.L.C
1100 New York Avenue, N.W.
Washington, DC 20005
UNITED STATES

TITLE

Extraction of Lipid From Cells and Products Therefrom

FILING FEE RECEIVED		
3902	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

BIB (Rev. 05/07).

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	11/25/2013	08/04/2014							
	1	÷	=							
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CLAIM		DATE								
Final	Original	11/25/2013	08/04/2014							
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CLAIM		DATE								
Final	Original	11/25/2013	08/04/2014							
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PTO/SB/30EFS (07-09)
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Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	13151014	Filing Date	2011-06-01	Docket Number (if applicable)	28612-US-NP	Art Unit	1672
First Named Inventor	Stephen Robert Cherinko			Examiner Name	CARR, DEBORAH D.		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the orde in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____

☐ Other _____

☒ Enclosed

☐ Amendment/Reply

☒ Information Disclosure Statement (IDS)

☐ Affidavit(s)/ Declaration(s)

☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____ (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

☒ **The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.**
The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 505958

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

☐ Applicant Signature

PTO/SB/30EFS (07-09)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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Signature of Registered U.S. Patent Practitioner			
Signature	/Shannon McGarrah/	Date (YYYY-MM-DD)	2014-11-21
Name	Shannon McGarrah	Registration Number	55442

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is t
file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection
estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Tim
will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions fo
reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce,
P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the informatio solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record ma be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: DSM IP Assets B.V.

Application No./Patent No.: 13/151014 Filed/Issue Date: June 1, 2011

Titled: EXTRACTION OF LIPID FROM CELLS AND PRODUCTS THEREFROM

DSM IP Assets B.V. , a Dutch Corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.

states that it is:

1. ☒ the assignee of the entire right, title, and interest in;

2. ☐ an assignee of less than the entire right, title, and interest in (The extent (by percentage) of its ownership interest is _____ %); or

3. ☐ the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made)

the patent application/patent identified above, by virtue of either:

A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy therefore is attached.

OR

B. ☒ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Inventors To: Martek Biosciences Corporation

The document was recorded in the United States Patent and Trademark Office at
Reel 028774 , Frame 0825 , or for which a copy thereof is attached.

2. From: Martek Biosciences Corporation To: DSM IP Assets B.V.

The document was recorded in the United States Patent and Trademark Office at
Reel 028938 , Frame 0620 , or for which a copy thereof is attached.

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Shannon McGarrah/ 2014-11-21

Signature Date

Shannon McGarrah, 55442 Patent Attorney

Printed or Typed Name Title

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	13151014
Filing Date	2011-06-01
First Named Inventor	Stephen Robert Cherinko
Art Unit	1672
Examiner Name	CARR, DEBORAH D.
Attorney Docket Number	28612-US-NP

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	5130242		1992-07-14	Phycotech, Inc.	
	2	5340594		1994-08-23	OmegaTech, Inc.	
	3	5340742		1994-08-23	Omegatech, Inc.	
	4	5583019		1996-12-10	Omegatech, Inc.	
	5	5928696		1999-07-27	Dr. Frische GmbH	
	6	6750048		2004-06-15	Martek Biosciences Corporation	
	7	7163811		2007-01-16	Martek Biosciences Corporation	
	8	7431952		2008-10-07	DSM IP Assets B.V.	

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	Attorney Docket Number	28612-US-NP

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20050115897		2005-06-02	Dueppen, et al.	
	2	20060099693		2006-05-11	Kobzeff, et al.	
	3	20080107791		2008-05-08	Martek Biosciences Corporation	
	4	20080088489		2008-04-17	Samsung Electronics Co., Ltd.	
	5	20100239533		2010-09-23	Martek Biosciences Corporation	
	6	20110177031		2011-07-21	Apt, et al.	

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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	01/0076385	WO	A1	2001-10-18	West-Falia Separator Industry GmbH		☐
	2	01/0076715	WO	A2	2001-10-18	West-Falia Separator Industry GmbH		☐

EFS Web 2.1.17

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	Examiner Name	CARR, DEBORAH D.
	Attorney Docket Number	28612-US-NP

	3	2010/107415	WO	A1	2010-09-23	Martek Biosciences Corporation		☐
	4	2011/090493	WO	A1	2011-07-28	Martek Biosciences Corporation		☐
	5	2011/153246	WO	A2	2011-12-08	Martek Biosciences Corporation		☐

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NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	Baldauf, S.M. Am. Nat. 154, S178 (1999) (BOOK)	☐
	2	Edward B. Roche ed., Bioreversible Carriers in Drug Design: Theory and Application, American Pharmaceutical Association, Pergamon 1987, 13-94 (BOOK)	☐
	3	Higuchi, T. et al Pro-drugs as Noval Delivery Systems Vol 14, ACS Symposium Series, American Chemical Society, 1975 (BOOK)	☐
	4	Honda et al, Schizochytrium limacinum sp. Nov., a new thraustochytrid from a mangrove area in the west Pacific Ocean, Mycol. Res. 102:439-448 (1998)	☐
	5	Honda et al., Molecular phylogency of labyrinthulids and thraustochytrids based on the sequencing of 18S ribosomal RNA gene, J. of Eukaryotic Microbiology, 46(6) 637-647 1999	☐
	6	Margulis, L., Corliss, J.O., Melkonian, M. and Chapman D.J. (Eds) handbook of Protoctista, Boston MA, Jones and Bartlett pp. 388-398 KMV;	☐

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	Examiner Name	CARR, DEBORAH D.
	Attorney Docket Number	28612-US-NP

	7	McOmie, J.F., Protective Groups in Organic Chemistry, Plenum Press, New York 1973 (BOOK)	☐
	8	Sambrook J. and Russell D 2001 Molecular cloning: A laboratory manual, 3rd edition. (BOOK)	☐
	9	Yokoyama, R. et al., Taxonomic rearrangement of the genus Ulkenia sensu lato based on morphology, chemotaxonomical characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomcetes): emendation for Ulkenia and erection of Botryochytrium, Parietichytrium, and Sicyoidochytrium gen. nov., Mycoscience 48(6): 329-341 (2007)	☐
	10	ZHU, et al. Extraction of lipid from sea urchin (Strongylocentrotus nudus) gonad by enzyme-assisted aqueous and supercritical carbon dioxide methods. Eur Food Research Technol. 20 January 2010, Vol. 230, Pages 737-743, especially page 737, abstract; page 740, right col, second para.	☐

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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	Art Unit	1672
	Examiner Name	CARR, DEBORAH D.
	Attorney Docket Number	28612-US-NP

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Shannon McGarrah/	Date (YYYY-MM-DD)	2014-11-21
Name/Print	Shannon McGarrah, Patent Attorney	Registration Number	55442

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal				
Application Number:	13151014			
Filing Date:	01-Jun-2011			
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom			
First Named Inventor/Applicant Name:	Stephen Robert Cherinko			
Filer:	Shannon Elizabeth McGarrah/Kimberly Sauter-Bray			
Attorney Docket Number:	2715.1350003/JUK/JHH			
Filed as Large Entity				
Utility under 35 USC 111(a) Filing Fees				
Description	**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Late Filing Fee for Oath or Declaration	1051	1	140	140
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Request for Continued Examination	1801	1	1200	1200
		Total in USD ($)		**1340**

Electronic Acknowledgement Receipt	
EFS ID:	20770070
Application Number:	13151014
International Application Number:	
Confirmation Number:	4148
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom
First Named Inventor/Applicant Name:	Stephen Robert Cherinko
Customer Number:	91335
Filer:	Shannon Elizabeth McGarrah/Kimberly Sauter-Bray
Filer Authorized By:	Shannon Elizabeth McGarrah
Attorney Docket Number:	2715.1350003/JUK/JHH
Receipt Date:	21-NOV-2014
Filing Date:	01-JUN-2011
Time Stamp:	16:32:44
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1340
RAM confirmation Number	3342
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1	Request for Continued Examination (RCE)	20141121_28612-US-NP_01_RCETransmittal.pdf	797755 c477ce8b205b6d72fa21e9cdbf4045e31bcb0e34	no	3
Warnings:					
Information:					
2	Assignee showing of ownership per 37 CFR 3.73.	20141121_28612-US-NP_03_StatementUnder37CFR3_73b.pdf	426747 dcfb223fa6fa678b7b35bf6829b075ee72628a4d	no	2
Warnings:					
Information:					
3	Power of Attorney	20141121_28612-US-NP_04_POA.pdf	503738 4ec3a3707939ccb0dfa985093e483f4b947da562	no	1
Warnings:					
Information:					
4	Foreign Reference	WO0176385.pdf	780110 b27dcc3ae26b676ff2ba1caaca2678ae78f7f66a	no	19
Warnings:					
Information:					
5	Foreign Reference	WO0176715.pdf	1294251 82cf6f468d9e75ffd1d89c83e9efa589dfe2b7ee	no	26
Warnings:					
Information:					
6	Foreign Reference	WO2010107415.pdf	4005231 6cb80d2a29991bb1ef12bae051e9544022abed3b	no	83
Warnings:					
Information:					
7	Foreign Reference	WO2011090493.pdf	5819978 1c622c78105192febc91ca6d374405e368d8efb5	no	97
Warnings:					
Information:					
8	Foreign Reference	WO2011153246.pdf	10317911 fb145c8e658a03ac4cafd31281d11a9e3cdfc220	no	161
Warnings:					
Information:					
9	Information Disclosure Statement (IDS) Form (SB08)	20141121_28612-US-NP_02_IDS1.pdf	613491 373b09860b33003a58a2211f2d0a5b363fcb73e1	no	6
Warnings:					
Information:					

10	Non Patent Literature	Honda_etal_1998.pdf	5116214 6f39e635584f35ac3fe32addbdd26577364c0431	no	10
Warnings:					
Information:					
11	Non Patent Literature	Yokoyama_etal_2007.pdf	7313924 8abfa2973c01591eeff2f19d6076327b5212df76	no	13
Warnings:					
Information:					
12	Non Patent Literature	Zhu_etal_2010.pdf	3557375 8be1b7140192cf04e854ac8a47c621865446daa4	no	7
Warnings:					
Information:					
13	Non Patent Literature	Honda_etal_1999.pdf	5792378 b5891df49d9bbaeb7a85dc5af204711bfe0b851c	no	11
Warnings:					
Information:					
14	Fee Worksheet (SB06)	fee-info.pdf	32016 5edb7bf5e4c52e3f3e94bc34b1ecf16a7a54e33e	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	46371119		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/80 (07-12)
Approved for use through 11/30/2014. OMB 0651-0035
U.S. Patent and Trademark Office; U.S DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

■ Practitioners associated with Customer Number: 109143

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

■ The address associated with Customer Number: 109143

OR

☐	Firm or Individual Name			
	Address			
	City		State	Zip
	Country			
	Telephone		Email	

Assignee Name and Address: DSM IP Assets B.V.
Het Overloon 1
Heerlen, Netherlands TE 6411

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be Filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of The practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature	Shannon McGarrah	Date	October 15, 2014
Name	Shannon McGarrah	Telephone	443-542-2395
Title	Patent Attorney		

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH

CONFIRMATION NO. 4148

109143
DSM Services USA
6480 Dobbin Road
Columbia, MD 21045

POA ACCEPTANCE LETTER

OC000000072112350

Date Mailed: 12/02/2014

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 11/21/2014.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/ltaba/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH	4148

109143 7590 01/23/2015

DSM Services USA
6480 Dobbin Road
Columbia, MD 21045

EXAMINER
CARR, DEBORAH D

ART UNIT	PAPER NUMBER
1672	

NOTIFICATION DATE	DELIVERY MODE
01/23/2015	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

dsmna.ip@dsm.com

PTOL-90A (Rev. 04/07)

Office Action Summary	Application No. 13/151,014	Applicant(s) CHERINKO ET AL.	
	Examiner DEBORAH D. CARR	Art Unit 1672	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 November 2014*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-4,6,8,31-33,39,41,42,44-47,49-58 and 63-78* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☒ Claim(s) *1-4,6,8,31-33 and 63-78* is/are allowed.

7)☒ Claim(s) *39,41,42 and 44-58* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *11/14*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Notice of Pre-AIA or AIA Status

1. The present application is being examined under the pre-AIA first to invent provisions.

Claim Rejections - 35 USC § 103

2. The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. Claim 51 rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Fichtali et al. (US Pub. 2008/0107791).

US Pub'791 discloses a crude microbial lipid (para [0011], [0016]. (0092)) having an anisidine value of 26 or less (para [0046]), a peroxide value of 5 or less (para [0045]), a phosphorus content of 100 ppm or less (para [0044]).

US Pub'791 does not explicitly disclose wherein the lipid comprises less than 5% by weight of an organic solvent.

It would have been obvious to a person skilled in the art to modify the product such that the lipid has the claimed value for the organic solvent, since US Pub'791 discloses wherein the product can be refined without a solvent (para [0082], [0085]) or through desolventization (para [0038], [0074]) where the amount of organic solvent could have been routinely optimized to form a purer lipid product.

4. Claims 52, 54-57 rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Dueppen et al. (US Pub. 2005/0115897).

As to claim 52, US Pub'897 discloses an extracted microbial lipid (abstract: para [0011]) comprising a triglyceride fraction of at least 70% by weight (para [0035]). US Pub'897 discloses wherein the triglyceride fraction comprises docoshexaenoic acid (para [0011]), the docosapentaenoic acid n-6 content of the triglyceride fraction (para [0010], [0011]) is from at least 0.5% by weight to 6% by weight (para [0056]), and wherein the oil has an anisidine value of 26 or less (para [0061], Table 5). US Pub'897 does not specifically disclose wherein the DHA content is at least 50% by weight.

However, US Pub'897 discloses wherein the lipid are preferably at least 85% triglyceride (para [0035]), and wherein "preferred attributes of the process include the selective recovery of only LCPUFA-containing triglycerides at the expense of trisaturated glycerides" (para [0038]).

It would have been obvious to a one of ordinary skill in the art at the time the invention was made to obtain a DHA content of at least 50 wt.%, from a DHA-producing microbe, based upon the disclosure of US Pub'897.

As to claim 54, US Pub'897 discloses an extracted microbial lipid (abstract: para [0011]) comprising a triglyceride fraction of at least 70% by weight (para [0035]). US Pub'897 discloses wherein the docoshexaenoic acid content of the triglyceride fraction (para [0011]), wherein the oil has a peroxide value of 5 or less (para [0064], Table 2]), and wherein the oil has an anisidine value of 26 or less (para [0061], Table 5).

US Pub'897 does not disclose an embodiment where DHA content is at least 60% by weight.

However, US Pub'897 discloses wherein the lipid are preferably at least 85% triglyceride (para [0035]), and wherein "preferred attributes of the process include the selective recovery of only LCPUFA-containing triglycerides at the expense of trisaturated glycerides· (para [0038]).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to obtain a DHA content of at least 60 wt.%, from a DHA-containing microbe, based upon the disclosure of US Pub'897.

5. Claim 53 rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Dueppen et al. (US Pub. 2005/0115897) in view of Metz et al. (US Pub. 2008/0148433).

US Pub'897 discloses an extracted microbial lipid (abstract: para [0011]) comprising a triglyceride fraction of at least 70% by weight (para [0035]). US Pub'897 discloses wherein the docoshexaenoic acid content of the triglyceride fraction (para [0011]), wherein the oil has a peroxide value of 5 or less (para [0064], Table 2]), and wherein the oil has an anisidine value of 26 or less (para [0061], Table 5).

US Pub'897 discloses a ratio of DHA to DPA about 3:1 (para [0040]), but does not explicitly disclose wherein the ratio of docoshexaenoic add to docosapentaenoic acid n-6 is greater than 6:1. Metz discloses plants engineered to produce at least two target PUFAs (e.g., DHA and DPA n-6) at desired ratios ("In one aspect, the plant produces DHA and DPAn-6 (the products of a PUFA PKS system described herein), and the ratio of DHA to DPAn-6 is from about 1:10 to about 10:1, including any ratio in between. In a one embodiment, the ratio of DHA to DPA is from about 1: 1 to about 3:1, and in another embodiment about 2.5:1"; para [0220]).

It would have been obvious to one of ordinary skill in that art at the time the invention was made to use the specific PUFA-producing processes of US Pub'433 to adjust the DHA produced in the as taught by US Pub'897 in order to achieve a DHA:DPA ratio of 6:1 or greater, in order to produce a specifically DHA-rich product.

Claim Rejections - 35 USC § 102

6. The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

7. Claims 39, 44-46, 50, 58 rejected under pre-AIA 35 U.S.C. 102b as being anticipated by Fichtali et al. (US Pub. 2008/0107791).

Claim(s) 39, 44-46 are product-by-process claims. M.P.E.P. § 2113 reads, "Product-by-process claims are not limited to the manipulations of the recited steps, only the structure implied by the steps."

"Even though product-by-process claims are limited by and defined by the process, determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process." *In re Thorpe*, 777 F.2d 695, 698, 227 USPQ 964, 966 (Fed. Cir. 1985) (citations omitted).

The structure implied by the process steps should be considered when assessing the patentability of product-by-process claims over the prior art, especially where the product can only be defined by the process steps by which the product is made, or where the manufacturing process steps would be expected to impart distinctive structural characteristics to the final product. See, e.g., *In re Garnero*, 412 F.2d 276, 279, 162 USPQ 221, 223 (CCPA 1979)

The use of 35 U.S.C. §§ 102 and 103 rejections for product-by-process claims has been approved by the courts. "[T]he lack of physical description in a product-by-process claim makes

determination of the patentability of the claim more difficult, since in spite of the fact that the claim may recite only process limitations, it is the patentability of the product claimed and not of the recited process steps which must be established. We are therefore of the opinion that when the prior art discloses a product which reasonably appears to be either identical with or only slightly different than a product claimed in a product-by-process claim, a rejection based alternatively on either section 102 or section 103 of the statute is eminently fair and acceptable. As a practical matter, the Patent Office is not equipped to manufacture products by the myriad of processes put before it and then obtain prior art products and make physical comparisons therewith." *In re Brown*, 459 F.2d 531, 535, 173 USPQ 685, 688 (CCPA 1972).

US Pub'791 discloses a crude microbial lipid (para [0011], [0016]. (0092)) having an anisidine value of 26 or less (para [0046]), a peroxide value of 5 or less (para [0045]), a phosphorus content of 100 ppm or less (para [0044]).

8. Claims 39, 41-42, 44, 46 rejected under pre-AIA 35 U.S.C. 102b as being anticipated by Dueppen et al. (US Pub. 2005/0115897).

Claim(s) 39, 41-42, 44, 46 are product-by-process claims. M.P.E.P. § 2113 reads, "Product-by-process claims are not limited to the manipulations of the recited steps, only the structure implied by the steps."

"Even though product-by-process claims are limited by and defined by the process, determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process." *In re Thorpe*, 777 F.2d 695, 698, 227 USPQ 964, 966 (Fed. Cir. 1985) (citations omitted).

The structure implied by the process steps should be considered when assessing the patentability of product-by-process claims over the prior art, especially where the product can only be defined by the process steps by which the product is made, or where the manufacturing process steps would be expected to impart distinctive structural characteristics to the final product. See, e.g., *In re Garnero*, 412 F.2d 276, 279, 162 USPQ 221, 223 (CCPA 1979)

The use of 35 U.S.C. §§ 102 and 103 rejections for product-by-process claims has been approved by the courts. "[T]he lack of physical description in a product-by-process claim makes determination of the patentability of the claim more difficult, since in spite of the fact that the claim may recite only process limitations, it is the patentability of the product claimed and not of the recited process steps which must be established. We are therefore of the opinion that when the prior art discloses a product which reasonably appears to be either identical with or only slightly different than a product claimed in a product-by-process claim, a rejection based alternatively on either section 102 or section 103 of the statute is eminently fair and acceptable. As a practical matter, the Patent Office is not equipped to manufacture products by the myriad of processes put before it and then obtain prior art products and make physical comparisons therewith." *In re Brown*, 459 F.2d 531, 535, 173 USPQ 685, 688 (CCPA 1972).

US Pub'897 discloses an extracted microbial lipid (abstract: para [0011]) comprising a triglyceride fraction of at least 70% by weight (para [0035]). US Pub'897 discloses wherein the triglyceride fraction comprises docoshexaenoic acid (para [0011]), the docosapentaenoic acid n-6 content of the triglyceride fraction (para [0010], [0011]) is from at least 0.5% by weight to 6% by weight (para [0056]), and wherein the oil has an anisidine value of 26 or less (para [0061], Table 5).

Allowable Subject Matter

9. Claims 1-4, 6, 8, 31-33, 63-78 are allowed.

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to DEBORAH D. CARR whose telephone number is (571)272-0637. The examiner can normally be reached on Monday-Friday 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brandon J. Fetterolf can be reached on 571-272-2919. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/DEBORAH D CARR/
Primary Examiner, Art Unit 1672

Ddc

Notice of References Cited	Application/Control No. 13/151,014	Applicant(s)/Patent Under Reexamination CHERINKO ET AL.	
	Examiner DEBORAH D. CARR	Art Unit 1672	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-2005/0115897 A1	06-2005	Dueppen et al.	210/634
*	B	US-2008/0107791 A1	05-2008	Fichtali et al.	426/601
*	C	US-2008/0148433 A1	06-2008	Metz et al.	800/281
	D	US-			
	E	US-			
	F	US-			
	G	US-			
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	I	US-			
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	13151014
Filing Date	2011-06-01
First Named Inventor	Stephen Robert Cherinko
Art Unit	1672
Examiner Name	CARR, DEBORAH D.
Attorney Docket Number	28612-US-NP

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	5130242		1992-07-14	Phycotech, Inc.	
	2	5340594		1994-08-23	OmegaTech, Inc.	
	3	5340742		1994-08-23	Omegatech, Inc.	
	4	5583019		1996-12-10	Omegatech, Inc.	
	5	5928696		1999-07-27	Dr. Frische GmbH	
	6	6750048		2004-06-15	Martek Biosciences Corporation	
	7	7163811		2007-01-16	Martek Biosciences Corporation	
	8	7431952		2008-10-07	DSM IP Assets B.V.	

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	Application Number	13151014
	Filing Date	2011-06-01
	First Named Inventor	Stephen Robert Cherinko
	Art Unit	1672
	Examiner Name	CARR, DEBORAH D.
	Attorney Docket Number	28612-US-NP

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20050115897		2005-06-02	Dueppen, et al.	
	2	20060099693		2006-05-11	Kobzeff, et al.	
	3	20080107791		2008-05-08	Martek Biosciences Corporation	
	4	20080088489		2008-04-17	Samsung Electronics Co., Ltd.	
	5	20100239533		2010-09-23	Martek Biosciences Corporation	
	6	20110177031		2011-07-21	Apt, et al.	

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	1	01/0076385	WO	A1	2001-10-18	West-Falia Separator Industry GmbH		☐
	2	01/0076715	WO	A2	2001-10-18	West-Falia Separator Industry GmbH		☐

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	Application Number	13151014
	Filing Date	2011-06-01
	First Named Inventor	Stephen Robert Cherinko
	Art Unit	1672
	Examiner Name	CARR, DEBORAH D.
	Attorney Docket Number	28612-US-NP

	3	2010/107415	WO	A1	2010-09-23	Martek Biosciences Corporation		☐
	4	2011/090493	WO	A1	2011-07-28	Martek Biosciences Corporation		☐
	5	2011/153246	WO	A2	2011-12-08	Martek Biosciences Corporation		☐

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
	1	Baldauf, S.M. Am. Nat. 154, S178 (1999) (BOOK)	☐
	2	Edward B. Roche ed., Bioreversible Carriers in Drug Design: Theory and Application, American Pharmaceutical Association, Pergamon 1987, 13-94 (BOOK)	☐
	3	Higuchi, T. et al Pro-drugs as Noval Delivery Systems Vol 14, ACS Symposium Series, American Chemical Society, 1975 (BOOK)	☐
	4	Honda et al, Schizochytrium limacinum sp. Nov., a new thraustochytrid from a mangrove area in the west Pacific Ocean, Mycol. Res. 102:439-448 (1998)	☐
	5	Honda et al., Molecular phylogency of labyrinthulids and thraustochytrids based on the sequencing of 18S ribosomal RNA gene, J. of Eukaryotic Microbiology, 46(6) 637-647 1999	☐
	6	Margulis, L., Corliss, J.O., Melkonian, M. and Chapman D.J. (Eds) handbook of Protoctista, Boston MA, Jones and Bartlett pp. 388-398 KMV;	☐

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	First Named Inventor	Stephen Robert Cherinko
	Art Unit	1672
	Examiner Name	CARR, DEBORAH D.
	Attorney Docket Number	28612-US-NP

	7	McOmie, J.F., Protective Groups in Organic Chemistry, Plenum Press, New York 1973 (BOOK)	☐
	8	Sambrook J. and Russell D 2001 Molecular cloning: A laboratory manual, 3rd edition. (BOOK)	☐
	9	Yokoyama, R. et al., Taxonomic rearrangement of the genus Ulkenia sensu lato based on morphology, chemotaxonomical characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomcetes): emendation for Ulkenia and erection of Botryochytrium, Parietichytrium, and Sicyoidochytrium gen. nov., Mycoscience 48(6): 329-341 (2007)	☐
	10	ZHU, et al. Extraction of lipid from sea urchin (Strongylocentrotus nudus) gonad by enzyme-assisted aqueous and supercritical carbon dioxide methods. Eur Food Research Technol. 20 January 2010, Vol. 230, Pages 737-743, especially page 737, abstract; page 740, right col, second para.	☐

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EXAMINER SIGNATURE

Examiner Signature	/Deborah Carr/	Date Considered	01/16/2015

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
554	8, 223, 224, 227	8/2014	ddc
435	134	8/2014	ddc

SEARCH NOTES		
Search Notes	Date	Examiner
see transcript	8/2014	ddc
updated search	1/2015	ddc

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
435	134	8/2014	ddc
554	8, 223, 224, 227	8/2014	ddc

	/DEBORAH D CARR/ Primary Examiner.Art Unit 1672

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	**Art Unit**
	DEBORAH D CARR	1672

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
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Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

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Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	**Art Unit**
	DEBORAH D CARR	1672

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
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	74		=	=						
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	77		=	=						
	78		=	=						

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Attorney Docket No: 2715.1350003/JUK/JHH
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 4148
Cherinko et al.	Art Unit: 1672
Appl. No.: 13/151,014	Examiner: CARR, DEBORAH D.
Filed: June 1, 2011	Atty. Docket: 28612-US-NP

For: **EXTRACTION OF LIPID FROM CELLS AND PRODUCTS THEREFROM**

Reply and Amendment

Mail Stop Amendment

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the non-final Office Action dated January 23, 2015, Applicants submit the following amendments and remarks.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks begin on page 7 of this paper.

Listing of Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously Presented) A process for obtaining a lipid from a cell composition, comprising:
(a) lysing a cell of said cell composition to form a lysed cell composition;
(b) demulsifying said lysed cell composition to form a demulsified cell composition;
(c) separating said lipid from the demulsified cell composition; and
(d) harvesting said lipid;
wherein (b) comprises heating said lysed cell composition to a temperature of at least 70° C.

2. (Previously Presented) The process of claim 1, wherein (b) further comprises contacting said lysed cell composition with a first base to raise the pH of the lysed cell composition to 8 or above.

3. (Previously Presented) The process of claim 2, wherein (b) further comprises contacting said lysed cell composition with a second base to raise the pH of the lysed cell composition to 7 or above.

4. (Previously Presented) The process of claim 1, wherein (b) further comprises contacting said lysed cell composition with a salt.

5. (Cancelled)

6. (Previously Presented) The process of claim 4, wherein the salt is added in an amount of 0.1% to 20% by weight of the lysed cell composition.

7. (Cancelled).

8. (Previously Presented) The process of claim 4, wherein the salt is selected from: alkali metal salts, alkali earth metal salts, sulfate salts, and combinations thereof.

9-30. (Cancelled)

31. (Previously Presented) The process of claim 1, further comprising washing the cell or cell composition.

32. (Previously Presented) The process of claim 1, further comprising pasteurizing the cell or cell composition.

33. (Previously Presented) The process of claim 1, further comprising concentrating the lysed cell composition.

34-38. (Cancelled)

39. (Currently Amended) A lipid obtained by the process of claim 1, wherein the lipid has an overall aroma intensity of 3 or less.

40. (Cancelled)

41. (Previously Presented) The lipid of claim 39, wherein the lipid comprises at least 35% by weight docosahexaenoic acid.

42. (Cancelled)

43. (Cancelled)

44. (Previously Presented) The lipid of claim 39, wherein the lipid has an anisidine value of 26 or less.

45. (Previously Presented) The lipid of claim 39, wherein the lipid has a phosphorus content of 100 ppm or less.

46. (Previously Presented) The lipid of claim 39, wherein the lipid has a peroxide value of 5 or less.

47. (Original) The lipid of claim 39, comprising at least 20% by weight eicosapentaenoic acid and less than 5% by weight each of arachidonic acid, docosapentaenoic acid n-6, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, erucic acid, and stearidonic acid.

48. (Cancelled)

49. (Previously Presented) The lipid of claim 47, wherein the lipid has a peroxide value of 5 or less.

50. (Previously Presented) The lipid of claim 39, wherein the lipid is a crude oil.

51. (Cancelled)

52. (Currently Amended) An extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, and wherein the oil has an anisidine value of 26 or less, wherein the lipid has less than 5% by weight of an organic solvent.

53. (Currently Amended) An extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1, and wherein the oil has an anisidine value of 26 or less, wherein the lipid has less than 5% by weight of an organic solvent.

54. (Currently Amended) An extracted microbial lipid comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight and wherein the oil has an anisidine value of 26 or less, wherein the lipid has less than 5% by weigh of an organic solvent.

55. (Previously Presented) The extracted microbial lipid of claim 52, wherein the oil has a peroxide value of 5 or less.

56. (Previously Presented) The extracted microbial lipid of claim 52, wherein the oil has a phosphorus content of 100 ppm or less.

57. (Previously Presented) The extracted microbial lipid of claim 52, wherein the lipid is a crude oil.

58. (Previously Presented) A process for obtaining a lipid, the process comprising refining a crude lipid of claim 50.

59-62. (Cancelled)

63. (Previously Presented) The process of claim 1, wherein (b) comprises heating said lysed cell composition to a temperature of from 70° C to 100° C.

64. (Previously Presented) The process of claim 1, wherein (b) further comprises agitating the lysed cell composition.

65. (Previously Presented) The process of claim 1, wherein (a) further comprises adding a salt to said cell composition.

66. (Previously Presented) The process of claim 1, wherein (a) comprises raising the pH of said cell composition to 8 or above.

67. (Previously Presented) The process of claim 1, wherein the lysed cell composition is in the form of an oil-in-water emulsion comprising a mixture of a continuous aqueous phase and a dispersed lipid phase.

68. (Previously Presented) The process of claim 1, wherein (c) comprises centrifuging the demulsified cell composition.

69. (Previously Presented) The process of claim 1, wherein the lipid is a crude lipid.

70. (Previously Presented) The process of claim 69, further comprising (e) refining said crude lipid to obtain a refined lipid.

71. (Previously Presented) The process of claim 1, wherein said lipid has an anisidine value of 26 or less.

72. (Previously Presented) The process of claim 1, wherein said lipid has a peroxide value of 5 or less.

73. (Previously Presented) The process of claim 1, wherein said cell is a microbial cell.

74. (Previously Presented) The process of claim 73, wherein said microbial cell is of the genus *Thraustochytrium*, *Schizochytrium*, or mixtures thereof.

75. (Previously Presented) The process of claim 1, wherein said lipid comprises omega 3 and/or omega-6 polyunsaturated fatty acids.

76. (Previously Presented) The process of claim 1, wherein said lipid comprises at least one of DHA, DPA(n-3), DPA(n-6), EPA, and ARA.

77. (Previously Presented) The process of claim 1, wherein an organic solvent is not added to said cell composition, said lysed cell composition, or said lipid in an amount or concentration sufficient to extract said lipid from said cell.

78. (Previously Presented) The process of claim 1, wherein the lipid contains less than 5% by weight of an organic solvent.

Remarks

After entry of the above amendments, the claims pending in the subject application are 1-4, 6, 8, 31-33, 39, 41, 42, 44-47, 49-58 and 63-78. Claims 1-4, 6, 8, 31-33 and 63-78 are allowed. Claims 39, 41, 42 and 44-58 are rejected. Claims 5, 7, 9-30 34-38, 40, 42-43, 48, 51 and 59-62 have been cancelled. Claims 39 and 52-54 have been amended. No new matter has been added by these amendments.

Applicants would like to provide clarification for the Restriction Requirement dated November 27, 2013. In the Restriction Requirement, the Examiner stated the pending claims were 1-63 and asked for restriction of Group 1, claims 1-8, 14, 31-33, 36, 39, 41-42, 44-47, 49-58 and Group II, claims 10-12, 15, 37, 60-63, but on the date of the Restriction Requirement, the claims pending were only 1-62. The response to the Restriction Requirement included election of Group I and new claims 63-78 were added. As the current claim numbering is correct, Applicants submit no further changes need to be made and only included these statements for clarification.

Reconsideration of this application based on the Amendments and Remarks presented herein is respectfully requested.

Rejections under 35 U.S.C. §103(a)

A. Claim 51 is rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Publication No. 2008/0107791 to Fichtali et al. ("Fichtali").

Claim 51 has been cancelled, thereby rendering this rejection moot.

B. Claims 52 and 54-57 are rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Publication No. 2005/0115897 to Dueppen et al. ("Dueppen").

Claim 52, as amended, is directed to an extracted microbial lipid comprising a triglyceride (TG) fraction of at least 70% by weight, wherein the docosahexaenoic acid (DHA) content of the TG fraction is at least 50% by weight, wherein the docosapentaenoic acid n-6 (DPA n-6) content of the TG fraction is from at least 0.5% by weight to 6% by weight, and wherein the oil has an anisidine value of 26 or less, wherein the lipid has less than 5% organic solvent.

The Examiner states Dueppen discloses an extracted microbial lipid comprising a TG fraction of at least 70% by weight, wherein the TG fraction comprises DHA, DPA n-6 from at least 0.5% by weight to 6% by weight, and wherein the oil has an anisidine value of less than 26 but does not disclose wherein the DHA content is at least 50% by weight. Based on paragraph [0035] of Dueppen, the Examiner states it would have been obvious to one of ordinary skill in the art at the time the invention was made to obtain a DHA content of at least 50 wt.% from a DHA-producing microbe.

One of ordinary skill in the art would not look to Dueppen for preparation of a composition comprising a triglyceride fraction wherein the DHA is at least 50% by weight and the DPA n-6 is from at least 0.5-6% and having less than 5% organic solvent. The Examiner cites paragraph [0056] as disclosing DPA n-6 in an amount from at least 0.5% by weight to 6% by weight. In example 1, a sample of unwinterized DHA-rich liquid was pulled from frozen storage. It was subjected to "acetone winterization" (*see*, [0054]) in which a portion of the sample was put in a flask and cold acetone was added. The sample was filtered, and the solid fraction washed with cold acetone and dried. The "lipid/acetone" fraction resulting from the filtration was concentrated to produce an orange-colored liquid. Both the solid fraction and the lipid/acetone fraction were analyzed by FAME for their fatty acid profile. FAME analysis measures fatty acid profile and does not analyze lipid class profile, which would show the quantitative value of the lipid composition (including triglycerides). As disclosed in [0056], the solid fraction had very little DHA (2.4%) and DPA n-6 (0.9%). In contrast to Dueppen, the present invention comprises a triglyceride fraction wherein the DHA is at least 50% by weight and the DPA n-6 is from at least 0.5-6%.

Dueppen relates to production of a lipid composition. The solid fraction in Dueppen, although containing minor amounts of lipids, is not the lipid composition which is the targeted inventive concept of Dueppen. In fact, Dueppen explicitly refers to the portions of the sample in Example 1 after winterization as a "solid fraction" and as the "lipid/acetone fraction". The sample cited by the Examiner for the amount of DPA n-6 is not the lipid-containing fraction of Dueppen. The amount of DHA and DPA n-6 contained in the sample in Dueppen is 2.4% and 0.9%, respectively. One of skill in the art would not look to Dueppen to produce a lipid composition having at least 50% DHA and from at least 0.5-6% DPA n-6, as the solid fraction containing 2.4% DHA and 0.9% DPA n-6 is not the lipid composition. Even if one of ordinary skill in the art would look to the non-lipid-containing

fraction (which position Applications do not support), the DHA amount found in the solid fraction is at least 20 times greater in the present application than that in Dueppen. The amount of DHA and DPA n-6 in the lipid/acetone fraction is 41% and 16%, respectively. One of ordinary skill in the art would not look to Dueppen, particularly the lipid/acetone fraction, as the amount of DPA n-6 is much greater than that of the present invention.

Further, Dueppen is a process for purifying a lipid composition having predominantly neutral lipid components wherein the process employs contacting the lipid composition with a polar solvent. Example 1 explicitly discloses use of a polar solvent (acetone) for purification of a lipid composition.

Applicants therefore respectfully submit that as the solid fraction the Examiner cites in his rejection to show the DPA n-6 levels is not a lipid composition and as Dueppen discloses production of a lipid composition, in which the lipid-acetone fraction shows significantly higher levels of DPA n-6 than in the present application, and also discloses use of a polar solvent for purification of the lipid-containing fraction, Dueppen actually teaches away from the present invention and one of skill in the art would not look to Dueppen to produce a composition wherein the DHA is at least 50% by weight and the DPA n-6 is from at least 0.5-6% and has less than 5% organic solvent.

Claim 54, as amended, is directed to an extracted microbial lipid comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight and wherein the oil has an anisidine value of 26 or less, wherein the lipid has less than 5% by weigh of an organic solvent.

The Examiner states Dueppen discloses an extracted microbial lipid comprising a TG fraction of at least 70% by weight, wherein the DHA content of the TG fraction, wherein the oil has a peroxide value of 5 or less and wherein the oil has an anisidine value of 26 or less but does not disclose wherein the DHA content is at least 60% by weight. Based on paragraph [0035] of Dueppen, the Examiner states it would have been obvious to one of ordinary skill in the art at the time the invention was made to obtain a DHA content of at least 60 wt.% from a DHA-producing microbe.

One of ordinary skill in the art would not look to Dueppen for preparation of a composition comprising a triglyceride fraction wherein the DHA is at least 60% by weight and has less than 5% organic solvent. In example 1, a sample of unwinterized DHA-rich

liquid was pulled from frozen storage. It was subjected to "acetone winterization" (*see*, [0054]) in which a portion of the sample was put in a flask and cold acetone was added. The sample was filtered, and the solid fraction washed with cold acetone and dried. The "lipid/acetone" fraction resulting from the filtration was concentrated to produce an orange-colored liquid.

Dueppen is a process for purifying a lipid composition having predominantly neutral lipid components wherein the process employs contacting the lipid composition with a polar solvent. Example 1 explicitly discloses use of a polar solvent (acetone) for purification of a lipid composition.

Applicants therefore respectfully submit that as Dueppen discloses use of a polar solvent for purification of the lipid-containing fraction, Dueppen actually teaches away from the present invention and one of skill in the art would not look to Dueppen to produce a composition wherein the DHA is at least 60% by weight and has less than 5% organic solvent.

Applicants submit that independent claims 52 and 54 and dependent claims therefrom are not unpatentable over Dueppen and request that this rejection be withdrawn.

C. Claim 53 is rejected under 35 U.S.C. §103(a) as being unpatentable over Dueppen in view of U.S. Publication No. 2008/0148433 ("Metz").

Claim 53, as amended, is an extracted microbial lipid comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight, wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1, and wherein the oil has an anisidine value of 26 or less, wherein the lipid has less than 5% by weight of an organic solvent.

As discussed in the arguments above, one of ordinary skill in the art would not look to Dueppen to produce a composition having at least 50% DHA and from at least 0.5-6% DPA n-6 and having less than 5% of an organic solvent. Metz is cited for disclosure of the ratio of DHA and DPA n-6. Metz does not cure the deficiencies of Dueppen.

Applicants submit that independent claim 53s not unpatentable over Dueppen and request that this rejection be withdrawn. Applicants submit that independent claims 52-54

are not unpatentable over Dueppen Claims 52 and 54-57 are rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Publication No. 2005/0115897 to Dueppen et al. ("Dueppen").

Applicants submit that independent claim 53 is not unpatentable over Dueppen in view of Metz and request that this rejection be withdrawn.

Rejections under 35 U.S.C. §102(b)

A. Claims 39, 44-46, 50 and 58 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by U.S. Publication No. 2008/0107791 to Fichtali et al. ("Fichtali").

Claim 39 has been amended to incorporate dependent claim 42. Claim 42 was not included in this rejection. Further, Claim 39, as amended, is a lipid obtained by the process of claim 1, wherein the lipid has an overall aroma intensity of 3 or less. Fichtali does not disclose any aroma intensity values, let alone a total aroma intensity value of 3 or less.

Applicants submit that independent claim 39 is not anticipated under 35 U.S.C. §102(b) by Fichtali. As claims 44-46 and 50 are dependent upon claim 39, and as claim 58 is dependent upon claim 50, these claims are also not anticipated under 35 U.S.C. §102(b) by Fichtali.

B. Claims 39, 41-42, 44 and 46 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by U.S. Publication No. 2005/0115897 to Dueppen et al. ("Dueppen").

Claim 39, as amended, is a lipid obtained by the process of claim 1, wherein the lipid has an overall aroma intensity value of 3 or less. Applicants respectfully submit that Dueppen does not disclose a lipid having an overall aroma intensity value of 3 or less and, if this rejection is maintained, request the Examiner to cite the specific paragraph in which this is disclosed.

Applicants submit that independent claim 39 is not anticipated under 35 U.S.C. §102(b) by Dueppen. As claims 41-42, 44 and 46 are dependent upon claim 39, these claims are also not anticipated under 35 U.S.C. §102(b) by Dueppen.

Conclusion

Prompt and favorable consideration of this Reply and Amendment is respectfully requested. Applicants believe the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided. A three month extension of time is due with this response and the Commissioner is hereby authorized to charge any deficiency in fees or credit any over payment to deposit account No. 50-5958, referencing attorney docket No. 28612-US-NP.

Respectfully submitted,

/Shannon McGarrah/

Shannon McGarrah
Attorney for Applicants
Registration No. 55,442

Date: July 23, 2015

DSM Services USA, Inc.
6480 Dobbin Road
Columbia, Maryland 21045
443-542-2395

PTO/AIA/22 (03-13)
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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) 28612-US-NP
Application Number 13/151014	Filed June 1, 2011
For EXTRACTION OF LIPID FROM CELLS AND PRODUCTS THEREFROM	
Art Unit 1621	Examiner Carr, Deborah D.

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

		Fee	Small Entity Fee	Micro Entity Fee	
☐	One month (37 CFR 1.17(a)(1))	$200	$100	$50	$
☐	Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
☑	Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$ 1400
☐	Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
☐	Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☑ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-5958.

☑ Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant.

☑ attorney or agent of record. Registration number 55442.

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

/Shannon McGarrah/ — Signature

July 22, 2015 — Date

Shannon McGarrah — Typed or printed name

443-542-2395 — Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☑ * Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.136(a). The information is required to obtain or retain a benefit by the public, which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 6 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal					
Application Number:	13151014				
Filing Date:	01-Jun-2011				
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom				
First Named Inventor/Applicant Name:	Stephen Robert Cherinko				
Filer:	Shannon Elizabeth McGarrah/Laurie Tollas				
Attorney Docket Number:	2715.1350003/JUK/JHH				
Filed as Large Entity					
Filing Fees for **Utility under 35 USC 111(a)**					
Description		**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:					
Pages:					
Claims:					
Miscellaneous-Filing:					
Petition:					
Patent-Appeals-and-Interference:					
Post-Allowance-and-Post-Issuance:					
Extension-of-Time:					

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	1253	1	1400	1400
Miscellaneous:				
			Total in USD ($)	**1400**

Electronic Acknowledgement Receipt	
EFS ID:	23000530
Application Number:	13151014
International Application Number:	
Confirmation Number:	4148
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom
First Named Inventor/Applicant Name:	Stephen Robert Cherinko
Customer Number:	109143
Filer:	Shannon Elizabeth McGarrah/Laurie Tollas
Filer Authorized By:	Shannon Elizabeth McGarrah
Attorney Docket Number:	2715.1350003/JUK/JHH
Receipt Date:	23-JUL-2015
Filing Date:	01-JUN-2011
Time Stamp:	11:59:10
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1400
RAM confirmation Number	11681
Deposit Account	505958
Authorized User	MCGARRAH, SHANNON
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Arguments/Remarks Made in an Amendment	20150723_28612_US_NP_Response_to_OA_012315.pdf	165518 4e34ed7d9a706c717579a5806a236f0ba8a3bcef	no	13
Warnings:					
Information:					
2	Extension of Time	20150722_3MthEOT_28612-US-NP.pdf	163174 b8b42132ff1ffcaf48f2c9f4b486f2b269e5582a	no	2
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30451 20eb3f0fa71c492d60889dc1e1ea5f6d3a050fe2	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	359143		

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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New International Application Filed with the USPTO as a Receiving Office
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PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/151,014	Filing Date 06/01/2011	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)		
FOR	NUMBER FILED	NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	07/23/2015	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 40	Minus	** 42	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 4	Minus	***6	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**0**

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/DEBORAH POLLARD/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
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NOTICE OF ALLOWANCE AND FEE(S) DUE

109143 7590 10/09/2015

DSM Services USA
6480 Dobbin Road
Columbia, MD 21045

EXAMINER	
CARR, DEBORAH D	
ART UNIT	PAPER NUMBER
1672	

DATE MAILED: 10/09/2015

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH	4148

TITLE OF INVENTION: Extraction of Lipid From Cells and Products Therefrom

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	01/11/2016

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

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II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

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PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
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Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

109143 7590 10/09/2015
DSM Services USA
6480 Dobbin Road
Columbia, MD 21045

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH	4148

TITLE OF INVENTION: Extraction of Lipid From Cells and Products Therefrom

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	01/11/2016

EXAMINER	ART UNIT	CLASS-SUBCLASS
CARR, DEBORAH D	1672	554-008000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee
❑ Publication Fee (No small entity discount permitted)
❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.
❑ Payment by credit card. Form PTO-2038 is attached.
❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH	4148

109143 7590 10/09/2015

DSM Services USA
6480 Dobbin Road
Columbia, MD 21045

EXAMINER	
CARR, DEBORAH D	
ART UNIT	PAPER NUMBER
1672	

DATE MAILED: 10/09/2015

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 13/151,014	Applicant(s) CHERINKO ET AL.	
	Examiner DEBORAH D. CARR	Art Unit 1672	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *the amendment filed 23 July 2015*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1-4,6,8,31-33,39,41,44-47,49,50,52-58 and 63-78*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   Certified copies:
   a) ☐ All b) ☐ Some *c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☐ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/DEBORAH D CARR/
Primary Examiner
Art Unit: 1672

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

CPC- SEARCHED		
Symbol	Date	Examiner
C11B1/10	9/2015	DDC
C11B3/02	9/2015	DDC
C11B3/16	9/2015	DDC

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
554	8, 223, 224, 227	8/2014	ddc
435	134	8/2014	ddc

SEARCH NOTES		
Search Notes	Date	Examiner
see transcript	8/2014	ddc
updated search	1/2015	ddc
updated search	9/2015	ddc

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
435	134	8/2014	ddc
554	8, 223, 224, 227	8/2014	ddc
see areas searched		9/2015	ddc

	/DEBORAH D CARR/ Primary Examiner.Art Unit 1672

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	11/25/2013	08/04/2014	01/12/2015	09/29/2015					
	1	÷	=	=	=					
	2	÷	=	=	=					
	3	÷	=	=	=					
	4	÷	=	=	=					
	5	÷	-	-	-					
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	36	÷	-	-	-					

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
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	40	÷	-	-	-					
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	70		=	=	=					
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	72		=	=	=					

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	11/25/2013	08/04/2014	01/12/2015	09/29/2015					
	73		=	=	=					
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	76		=	=	=					
	77		=	=	=					
	78		=	=	=					

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	**Art Unit**
	DEBORAH D CARR	1672

CPC						
Symbol					**Type**	**Version**
C11B		1	/	10	F	2013-01-01
C11B		3	/	02	I	2013-01-01
C11B		3	/	16	I	2013-01-01
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CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	40	
/DEBORAH D CARR/ Primary Examiner.Art Unit 1672	09/29/2015	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

U.S. Patent and Trademark Office — Part of Paper No. 20150929

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	**Art Unit**
	DEBORAH D CARR	1672

US ORIGINAL CLASSIFICATION						INTERNATIONAL CLASSIFICATION										
CLASS	SUBCLASS					CLAIMED						NON-CLAIMED				
554	8					C	1	2	P	7 / 64 (2006.0)						
CROSS REFERENCE(S)						C	1	1	B	1 / 00 (2006.0)						
						A	0	1	H	5 / 10 (2006.0)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)					A	2	3	D	9 / 00 (2006.0)						
435	134															
554	223	224	227													

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	40	
/DEBORAH D CARR/ Primary Examiner.Art Unit 1672	09/29/2015	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
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	2		18		34		50		66						
	3		19		35		51		67						
	4		20		36		52		68						
	5		21		37		53		69						
	6		22		38		54		70						
	7		23		39		55		71						
	8		24		40		56		72						
	9		25		41		57		73						
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	13		29		45		61		77						
	14		30		46		62		78						
	15		31		47		63								
	16		32		48		64								

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	40	
/DEBORAH D CARR/ Primary Examiner.Art Unit 1672	09/29/2015	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

U.S. Patent and Trademark Office Part of Paper No. 20150929

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DSM Services USA
6480 Dobbin Road
Columbia, MD 21045

Courtesy Reminder for
Application Serial No: 13/151,014

Attorney Docket No: 2715.1350003/JUK/JHH
Customer Number: 109143
Date of Electronic Notification: 10/09/2015

This is a courtesy reminder that new correspondence is available for this application. If you have not done so already, please review the correspondence. The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

An email notification regarding the correspondence was sent to the following email address(es) associated with your customer number:
dsmna.ip@dsm.com

To view your correspondence online or update your email addresses, please visit us anytime at **https://sportal.uspto.gov/secure/myportal/privatepair.** If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov or call 1-866-217-9197.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	13151014
Filing Date	2011-06-01
First Named Inventor	Stephen Robert Cherinko
Art Unit	1672
Examiner Name	Carr, Deborah D.
Attorney Docket Number	28612-US-NP

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20070138094		2007-06-21	Bomberger et al.	

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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	101224022	CN	A	2008-07-23	Univ Tianjin Science & Tech	Abstract	
	2	101455240	CN	A	2009-06-17	Univ Northeast Agriculture	Abstract	
	3	101531690	CN	A	2009-09-16	Zhejiang Simsun Tea Science & Technology Co. Ltd.	Abstract	

EFS Web 2.1.17

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13151014
	Filing Date	2011-06-01
	First Named Inventor	Stephen Robert Cherinko
	Art Unit	1672
	Examiner Name	Carr, Deborah D.
	Attorney Docket Number	28612-US-NP

	4	1374383	CN	A	2002-10-16	Westsouth China Agriculture	Abstract	
	5	2008088489	WO	A2	2008-07-24	Danisco US Inc.		

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	Zhang, et al., 2007, 23(9): pgs 213-219	

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13151014
	Filing Date	2011-06-01
	First Named Inventor	Stephen Robert Cherinko
	Art Unit	1672
	Examiner Name	Carr, Deborah D.
	Attorney Docket Number	28612-US-NP

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Shannon McGarrah/	Date (YYYY-MM-DD)	2015-12-21
Name/Print	Shannon McGarrah	Registration Number	55442

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal				
Application Number:	13151014			
Filing Date:	01-Jun-2011			
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom			
First Named Inventor/Applicant Name:	Stephen Robert Cherinko			
Filer:	Shannon Elizabeth McGarrah/Kimberly Sauter-Bray			
Attorney Docket Number:	2715.1350003/JUK/JHH			
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt	
EFS ID:	24422144
Application Number:	13151014
International Application Number:	
Confirmation Number:	4148
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom
First Named Inventor/Applicant Name:	Stephen Robert Cherinko
Customer Number:	109143
Filer:	Shannon Elizabeth McGarrah/Kimberly Sauter-Bray
Filer Authorized By:	Shannon Elizabeth McGarrah
Attorney Docket Number:	2715.1350003/JUK/JHH
Receipt Date:	21-DEC-2015
Filing Date:	01-JUN-2011
Time Stamp:	11:09:56
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$180
RAM confirmation Number	10406
Deposit Account	505958
Authorized User	SAUTER-BRAY, KIMBERLY
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

Charge any Additional Fees required under 37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	20151221_IDS2_28612-US-NP.pdf	1034923 f3c9bb5e527523eb2e21b18d72e6a318127 6bf7f	no	4
Warnings:					
Information:					
2	Foreign Reference	CN1374383A.pdf	62445 bd53102a1b9e745efa41029d2cbf3fd643d d7266	no	1
Warnings:					
Information:					
3	Foreign Reference	CN101224022A.pdf	70221 508eb71986a445ddd756d2051e83ee39aa 2a5bfd	no	1
Warnings:					
Information:					
4	Foreign Reference	CN101455240A.pdf	64092 b51fe04c2c04bb1fb6327214f8a6dc847f4a 5403	no	1
Warnings:					
Information:					
5	Foreign Reference	CN101531690A.pdf	65141 7c6c657c1b017cf6bb4505edaff9d4df73c5f e80	no	1
Warnings:					
Information:					
6	Foreign Reference	WO2008088489.pdf	1544631 ea236eb0166894a41c4358eed20e4e32f19 e921d	no	33
Warnings:					
Information:					
7	Non Patent Literature	Zhang_2007_pgs213-219_EF.pdf	3166862 1d47061c7e1758dad08cf21cf4cdbf9b2b69 b61b	no	7
Warnings:					
Information:					

8	Fee Worksheet (SB06)	fee-info.pdf	30705 b7711734e0882eb8bd7413195805ed33eb48426a	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	6039020	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH	4148

7590 12/24/2015

DSM Services USA
6480 Dobbin Road
Columbia, MD 21045

EXAMINER	
CARR, DEBORAH D	

ART UNIT	PAPER NUMBER
1672	

NOTIFICATION DATE	DELIVERY MODE
12/24/2015	ELECTRONIC

NOTICE OF NON-COMPLIANT INFORMATION DISCLOSURE STATEMENT

An Information Disclosure Statement (IDS) filed 12/21/15 in the above-identified application fails to meet the requirements of 37 CFR 1.97(d) for the reason(s) specified below. Accordingly, the IDS will be placed in the file, but the information referred to therein has not been considered.

The IDS is not compliant with 37 CFR 1.97(d) because:

☒ The IDS lacks a statement as specified in 37 CFR 1.97(e).

☐ The IDS lacks the fee set forth in 37 CFR 1.17(p).

☐ The IDS was filed after the issue fee was paid. Applicant may wish to consider filing a petition to withdraw the application from issue under 37 CFR 1.313(c) to have the IDS considered. See MPEP 1308.

571-272-4200 or 1-888-786-0101
Application Assistance Unit
Office of Data Management

FORM PTOM327-B (Rev. 02/08)

Doc code: RCEX
Doc description: Request for Continued Examination (RCE)

PTO/SB/30EFS (07-14)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	13151014	Filing Date	2011-06-01	Docket Number (if applicable)	28612-US-NP	Art Unit	1672
First Named Inventor	Stephen Robert Cherinko			Examiner Name	Carr, Deborah D.		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8 1995, to any international application that does not comply with the requirements of 35 U.S.C. 371, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV.

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the orde in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____

☐ Other _____

☒ Enclosed

☐ Amendment/Reply

☒ Information Disclosure Statement (IDS)

☐ Affidavit(s)/ Declaration(s)

☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____ (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

☒ **The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.**
The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 505958

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

Applicant Signature

EFS - Web 2.1.15

PTO/SB/30EFS (07-14)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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Signature of Registered U.S. Patent Practitioner			
Signature	/Shannon McGarrah/	Date (YYYY-MM-DD)	2016-01-06
Name	Shannon McGarrah	Registration Number	55442

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file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection
estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Tim
will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions fo
reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce,
P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

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Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
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Application Number	13151014
Filing Date	2011-06-01
First Named Inventor	Stephen Robert Cherinko
Art Unit	1672
Examiner Name	Carr, Deborah D.
Attorney Docket Number	28612-US-NP

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

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U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20070138094		2007-06-21	Bomberger et al.	

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FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	101224022	CN	A	2008-07-23	Univ Tianjin Science & Tech	Abstract	
	2	101455240	CN	A	2009-06-17	Univ Northeast Agriculture	Abstract	
	3	101531690	CN	A	2009-09-16	Zhejiang Simsun Tea Science & Technology Co. Ltd.	Abstract	

EFS Web 2.1.17

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	Filing Date	2011-06-01
	First Named Inventor	Stephen Robert Cherinko
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	Examiner Name	Carr, Deborah D.
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	4	1374383	CN	A	2002-10-16	Westsouth China Agriculture	Abstract	
	5	2008088489	WO	A2	2008-07-24	Danisco US Inc.		

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	Zhang, et al., 2007, 23(9): pgs 213-219	

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

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[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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Signature	/Shannon McGarrah/	Date (YYYY-MM-DD)	2015-12-21
Name/Print	Shannon McGarrah	Registration Number	55442

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4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal					
Application Number:	13151014				
Filing Date:	01-Jun-2011				
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom				
First Named Inventor/Applicant Name:	Stephen Robert Cherinko				
Filer:	Shannon Elizabeth McGarrah/Kimberly Sauter-Bray				
Attorney Docket Number:	2715.1350003/JUK/JHH				
Filed as Large Entity					
Filing Fees for Utility under 35 USC 111(a)					
Description		**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:					
Pages:					
Claims:					
Miscellaneous-Filing:					
Petition:					
Patent-Appeals-and-Interference:					
Post-Allowance-and-Post-Issuance:					
Extension-of-Time:					

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
RCE- 2nd and Subsequent Request	1820	1	1700	1700
		Total in USD ($)		**1880**

Electronic Acknowledgement Receipt	
EFS ID:	24543270
Application Number:	13151014
International Application Number:	
Confirmation Number:	4148
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom
First Named Inventor/Applicant Name:	Stephen Robert Cherinko
Customer Number:	109143
Filer:	Shannon Elizabeth McGarrah
Filer Authorized By:	
Attorney Docket Number:	2715.1350003/JUK/JHH
Receipt Date:	06-JAN-2016
Filing Date:	01-JUN-2011
Time Stamp:	16:32:12
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1880
RAM confirmation Number	3350
Deposit Account	505958
Authorized User	SAUTER-BRAY, KIMBERLY
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

Charge any Additional Fees required under 37 CFR 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Continued Examination (RCE)	20160106_RCETransmittal_28612-US-NP.pdf	1363798 bf5762c4b05b010d7b87e2f46660166cc242353e	no	3
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	20160106_IDS2_28612-US-NP.pdf	1034923 f3c9bb5e527523eb2e21b18d72e6a318127 6caf7f	no	4
Warnings:					
Information:					
3	Foreign Reference	CN101224022A.pdf	70221 508eb71986a445ddd756d2051e83ee39aa 2a5bfd	no	1
Warnings:					
Information:					
4	Foreign Reference	CN101455240A.pdf	64092 b51fe04c2c04bb1fb6327214f8a6dc847f4b 5403	no	1
Warnings:					
Information:					
5	Foreign Reference	CN101531690A.pdf	65141 7c6c657c1b017cf6bb4505edaff9d4df73c5f e80	no	1
Warnings:					
Information:					
6	Foreign Reference	CN1374383A.pdf	62445 bd53102a1b9e745efa41029d2cbf3fd643d d7266	no	1
Warnings:					
Information:					
7	Foreign Reference	WO2008088489.pdf	1544631 ea236eb0166894a41c4358eed20e4e32f19 e921d	no	33
Warnings:					
Information:					

8	Non Patent Literature	Zhang_2007_pgs213-219_EF.pdf	3166862 1d47061c7e1758dad08cf21cf4cdbf9b2b69b61b	no	7
Warnings:					
Information:					
9	Fee Worksheet (SB06)	fee-info.pdf	32270 a34fd5f6e963b566bc5b746f45233d9cb49a5321	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	7404383		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

109143 7590 02/01/2016

DSM Services USA
6480 Dobbin Road
Columbia, MD 21045

EXAMINER	
CARR, DEBORAH D	
ART UNIT	PAPER NUMBER
1672	

DATE MAILED: 02/01/2016

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH	4148

TITLE OF INVENTION: Extraction of Lipid From Cells and Products Therefrom

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	05/02/2016

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

109143 7590 02/01/2016
DSM Services USA
6480 Dobbin Road
Columbia, MD 21045

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH	4148

TITLE OF INVENTION: Extraction of Lipid From Cells and Products Therefrom

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	05/02/2016

EXAMINER	ART UNIT	CLASS-SUBCLASS
CARR, DEBORAH D	1672	554-008000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee
❑ Publication Fee (No small entity discount permitted)
❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.
❑ Payment by credit card. Form PTO-2038 is attached.
❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/151,014	06/01/2011	Stephen Robert Cherinko	2715.1350003/JUK/JHH	4148

109143 7590 02/01/2016

DSM Services USA
6480 Dobbin Road
Columbia, MD 21045

EXAMINER	
CARR, DEBORAH D	
ART UNIT	PAPER NUMBER
1672	

DATE MAILED: 02/01/2016

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 13/151,014	Applicant(s) CHERINKO ET AL.	
	Examiner DEBORAH D. CARR	Art Unit 1672	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *theIDS filed 6 January 2016*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1-4,6,8,31-33,39,41,44-47,49,50,52-58 and 63-78*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   Certified copies:
   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 12/15, 1/16
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☐ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/DEBORAH D CARR/
Primary Examiner
Art Unit: 1672

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	11/25/2013	08/04/2014	01/12/2015	09/29/2015	01/26/2016				
	1	÷	=	=	=	=				
	2	÷	=	=	=	=				
	3	÷	=	=	=	=				
	4	÷	=	=	=	=				
	5	÷	-	-	-	-				
	6	÷	=	=	=	=				
	7	÷	-	-	-	-				
	8	÷	=	=	=	=				
	9	÷	-	-	-	-				
	10	÷	-	-	-	-				
	11	÷	-	-	-	-				
	12	÷	-	-	-	-				
	13	÷	-	-	-	-				
	14	÷	-	-	-	-				
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	32	÷	=	=	=	=				
	33	÷	=	-	=	=				
	34	÷	-	-	-	-				
	35	÷	-	-	-	-				
	36	÷	-	-	-	-				

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	11/25/2013	08/04/2014	01/12/2015	09/29/2015	01/26/2016				
	37	÷	-	-	-	-				
	38	÷	-	-	-	-				
	39	÷	=	✓	=	=				
	40	÷	-	-	-	-				
	41	÷	=	✓	=	=				
	42	÷	=	✓	-	-				
	43	÷	-	-	-	-				
	44	÷	=	✓	=	=				
	45	÷	=	✓	=	=				
	46	÷	=	✓	=	=				
	47	÷	=	✓	=	=				
	48	÷	-	-	-	-				
	49	÷	=	✓	=	=				
	50	÷	=	✓	=	=				
	51	÷	=	✓	-	-				
	52	÷	=	✓	=	=				
	53	÷	=	✓	=	=				
	54	÷	=	✓	=	=				
	55	÷	=	✓	=	=				
	56	÷	=	✓	=	=				
	57	÷	=	✓	=	=				
	58	÷	=	✓	=	=				
	59	÷	-	-	-	-				
	60	÷	-	-	-	-				
	61	÷	-	-	-	-				
	62	÷	-	-	-	-				
	63	÷	=	=	=	=				
	64		=	=	=	=				
	65		=	=	=	=				
	66		=	=	=	=				
	67		=	=	=	=				
	68		=	=	=	=				
	69		=	=	=	=				
	70		=	=	=	=				
	71		=	=	=	=				
	72		=	=	=	=				

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	**Art Unit**
	DEBORAH D CARR	1672

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

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	73		=	=	=	=				
	74		=	=	=	=				
	75		=	=	=	=				
	76		=	=	=	=				
	77		=	=	=	=				
	78		=	=	=	=				

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	Art Unit
	DEBORAH D CARR	1672

CPC- SEARCHED		
Symbol	Date	Examiner
C11B1/10	9/2015, 1/2016	DDC
C11B3/02	9/2015, 1/2016	DDC
C11B3/16	9/2015, 1/2016	DDC

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
554	8, 223, 224, 227	8/2014, 1/2016	ddc
435	134	8/2014, 1/2016	ddc

SEARCH NOTES		
Search Notes	Date	Examiner
see transcript	8/2014	ddc
updated search	1/2015	ddc
updated search	9/2015	ddc
updated search	1/2016	ddc

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
435	134	8/2014	ddc
554	8, 223, 224, 227	8/2014	ddc
see areas searched		9/2015, 1/2016	ddc

	/DEBORAH D CARR/ Primary Examiner.Art Unit 1672

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
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Application Number	13151014
Filing Date	2011-06-01
First Named Inventor	Stephen Robert Cherinko
Art Unit	1672
Examiner Name	Carr, Deborah D.
Attorney Docket Number	28612-US-NP

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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	1	20070138094		2007-06-21	Bomberger et al.	

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	1	101224022	CN	A	2008-07-23	Univ Tianjin Science & Tech	Abstract	
	2	101455240	CN	A	2009-06-17	Univ Northeast Agriculture	Abstract	
	3	101531690	CN	A	2009-09-16	Zhejiang Simsun Tea Science & Technology Co. Ltd.	Abstract	

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	First Named Inventor	Stephen Robert Cherinko
	Art Unit	1672
	Examiner Name	Carr, Deborah D.
	Attorney Docket Number	28612-US-NP

	4	1374383	CN	A	2002-10-16	Westsouth China Agriculture	Abstract	
	5	2008088489	WO	A2	2008-07-24	Danisco US Inc.		

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	Zhang, et al., 2007, 23(9): pgs 213-219	

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Examiner Signature	/DEBORAH D CARR/	Date Considered	01/26/2016

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[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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	Art Unit	1672
	Examiner Name	Carr, Deborah D.
	Attorney Docket Number	28612-US-NP

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

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OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Shannon McGarrah/	Date (YYYY-MM-DD)	2015-12-21
Name/Print	Shannon McGarrah	Registration Number	55442

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13151014	CHERINKO ET AL.
	Examiner	**Art Unit**
	DEBORAH D CARR	1672

CPC						
Symbol					**Type**	**Version**
C11B		1	/	10	F	2013-01-01
C11B		3	/	02	I	2013-01-01
C11B		3	/	16	I	2013-01-01
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CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	40	
/DEBORAH D CARR/ Primary Examiner.Art Unit 1672	01/26/2016	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

U.S. Patent and Trademark Office — Part of Paper No. 20160126

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	13151014	CHERINKO ET AL.
	Examiner	**Art Unit**
	DEBORAH D CARR	1672

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**
554	8

CROSS REFERENCE(S)					
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**				
435	134				
554	223	224	227		

INTERNATIONAL CLASSIFICATION																
CLAIMED						**NON-CLAIMED**										
C	1	2	P	7 / 64 (2006.0)												
C	1	1	B	1 / 00 (2006.0)												
A	0	1	H	5 / 10 (2006.0)												
A	2	3	D	9 / 00 (2006.0)												

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	40	
/DEBORAH D CARR/ Primary Examiner.Art Unit 1672	01/26/2016	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

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Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
	1		17		33		49		65						
	2		18		34		50		66						
	3		19		35		51		67						
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	12		28		44		60		76						
	13		29		45		61		77						
	14		30		46		62		78						
	15		31		47		63								
	16		32		48		64								

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	40	
/DEBORAH D CARR/ Primary Examiner.Art Unit 1672	01/26/2016	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

U.S. Patent and Trademark Office Part of Paper No. 20160126

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
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Art Unit	1672
Examiner Name	Carr, Deborah D.
Attorney Docket Number	28612-US-NP

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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	1	20070138094		2007-06-21	Bomberger et al.	

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NON-PATENT LITERATURE DOCUMENTS [Remove]

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	Zhang, et al., 2007, 23(9): pgs 213-219	

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EXAMINER SIGNATURE

Examiner Signature	/DEBORAH D CARR/	Date Considered	01/26/2016

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Signature	/Shannon McGarrah/	Date (YYYY-MM-DD)	2015-12-21
Name/Print	Shannon McGarrah	Registration Number	55442

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REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	13151014	Filing Date	2011-06-01	Docket Number (if applicable)	28612-US-NP	Art Unit	1672
First Named Inventor	Stephen Robert Cherinko			Examiner Name	Deborah D. Carr		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8 1995, to any international application that does not comply with the requirements of 35 U.S.C. 371, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV.

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the orde in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
- ☐ Other _____

☒ Enclosed

- ☐ Amendment/Reply
- ☒ Information Disclosure Statement (IDS)
- ☐ Affidavit(s)/ Declaration(s)
- ☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____ (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.

☒ The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 505958

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

Applicant Signature

PTO/SB/30EFS (07-14)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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Signature of Registered U.S. Patent Practitioner			
Signature	/Shannon McGarrah/	Date (YYYY-MM-DD)	2016-05-02
Name	Shannon McGarrah	Registration Number	55442

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is t
file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection
estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Tim
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reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce,
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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal					
Application Number:	13151014				
Filing Date:	01-Jun-2011				
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom				
First Named Inventor/Applicant Name:	Stephen Robert Cherinko				
Filer:	Shannon Elizabeth McGarrah/Kimberly Sauter-Bray				
Attorney Docket Number:	2715.1350003/JUK/JHH				
Filed as Large Entity					
Filing Fees for Utility under 35 USC 111(a)					
Description		**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:					
Pages:					
Claims:					
Miscellaneous-Filing:					
Petition:					
Patent-Appeals-and-Interference:					
Post-Allowance-and-Post-Issuance:					
Extension-of-Time:					

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
RCE- 2nd and Subsequent Request	1820	1	1700	1700
		Total in USD ($)		**1880**

Electronic Acknowledgement Receipt	
EFS ID:	25656661
Application Number:	13151014
International Application Number:	
Confirmation Number:	4148
Title of Invention:	Extraction of Lipid From Cells and Products Therefrom
First Named Inventor/Applicant Name:	Stephen Robert Cherinko
Customer Number:	109143
Filer:	Shannon Elizabeth McGarrah/Kimberly Sauter-Bray
Filer Authorized By:	Shannon Elizabeth McGarrah
Attorney Docket Number:	2715.1350003/JUK/JHH
Receipt Date:	02-MAY-2016
Filing Date:	01-JUN-2011
Time Stamp:	15:44:28
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1880
RAM confirmation Number	16327
Deposit Account	505958
Authorized User	SAUTER-BRAY, KIMBERLY
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

Charge any Additional Fees required under 37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Continued Examination (RCE)	20160502_RCE_28612-US-NP.pdf	1363785 25157f4605b7765885aef7f597cf914a22f13e86	no	3
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Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	20160502_IDS_3_28612_US_NP.pdf	633672 698306190ef9bf3c6607d6c53c0d5e7e987fe359	no	0
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Information:					
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49	Foreign Reference	WO2011133181.pdf	3059416 956da4f512f4ed5e8a476f7a81c138fcc3e31645	no	67
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50	Foreign Reference	WO12061647.pdf	13094195 78bf1173a540f9945665bcd5cced8cbeab0dd54e	no	199
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51	Non Patent Literature	Akoh_1998.pdf	416216 7efece91ef7dea9dd7512db57efa55140567b65f	no	9
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52	Non Patent Literature	AlternativeMethodsofExtraction_OilsandFatsInternationalIssues_1992.pdf	426696 9377e63ae79c65127acaf07dc5f06a5871f09ad6	no	3
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53	Non Patent Literature	Atkinson_etal_1991.pdf	457839 959f8f78c9c093e94e910545faaaec264e34a651	no	8
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54	Non Patent Literature	Ayres_etal_1998.pdf	41049 d4437ea6b4b35ef4aa7959afd39844a7bb9ba9cc	no	1
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58	Non Patent Literature	Cartens_etal_1996.pdf	161723 e8ad8c370d6738f39781f70828f26d466c4c8d30	no	7
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60	Fee Worksheet (SB06)	fee-info.pdf	32270 f7aeaff0bdc6a24ef9f1270082e7d6300b9bc7b3	no	2
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Information:					
		Total Files Size (in bytes):	112737597		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 November 2003 (13.11.2003)

PCT

(10) International Publication Number
WO 2003/092628 A3

(51) International Patent Classification[7]: A61K 45/00, 47/44, C12N 15/88

(21) International Application Number: PCT/US2003/014177

(22) International Filing Date: 5 May 2003 (05.05.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/377,550 3 May 2002 (03.05.2002) US

(71) Applicant *(for all designated States except US)*: **MARTEK BIOSCIENCES CORPORATION** [US/US]; 6480 Dobbin Road, Columbia, MD 21045 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KOBZEFF, Joseph, M.** [US/US]; 2050 Red Feather Point, Lafayette, CO 80026 (US). **WEAVER, Craig, A.** [US/US]; 4542 Starboard Drive, Boulder, CO 80301 (US).

(74) Agents: **CONNELL, Gary, J.** et al.; Sheridan Ross P.C., Suite 1200, 1560 Broadway, Denver, CO 80202-5141 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 1 April 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/092628 A3

(54) Title: HIGH-QUALITY LIPIDS AND METHODS FOR PRODUCING BY ENZYMATIC LIBERATION FROM BIOMASS

(57) Abstract: A high-quality lipid composition is disclosed having low oxidative deterioration such as measured by low anisidine values. Also disclosed are methods of preparing the same from a lipid-containing material that include enzymatic degradation of protein and/or carbohydrate components of the material. Lipid-containing materials include biomass, such as microorganisms. The invention further includes products containing the lipid compositions, such as dietary supplements, food products, pharmaceutical formulations, humanized animal milk, and infant formula.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/14177

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61k 45/00, 47/44; C12N 15/88
US CL : 424/283.1; 435/458
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/283.1; 435/458

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,969,169 A (FAN) 19 October 1999 (19.10.1999), see abstract and column 9, lines 8-65, column 10, lines 20-45, column 11, lines 15-25 and 66.	1-56 --- 1-56
X --- Y	US 6,270,828 B1 (DEBONTE et al) 07 August 2001 (07.08.2001), see abstract and column 14, lines 40-67.	1-56 --- 1-56
X --- Y	US 6,255,505 B1 (BIJL et al) 03 July 2001 (03.07.2001), see abstract and columns 1-2, all lines, columns 11-12, all lines, column 28, lines 35-50.	1-56 --- 1-56
X --- Y	US 6,204,401 B1 (PERRUT et al) 20 March 2001 (20.03.2001), see abstract and columns 7-8, all lines.	1-56 --- 1-56

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"&" document member of the same patent family

Date of the actual completion of the international search
11 August 2003 (11.08.2003)

Date of mailing of the international search report
03 NOV 2003

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
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Authorized officer
Deborah K. Ware
Valerie Bell-Harris for

Telephone No. 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

PCT/US03/14177

INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,201,145 B1 (FAN) 13 March 2001 (13.03.2001) see abstract and column 6, all lines, column 10, all lines.	1-56
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Y		1-56
X	US 6127,185 A (MELTON et al) 03 October 2000 (03.10.2000), see entire document.	1-56
---		---
Y		1-56

Form PCT/ISA/210 (second sheet) (July 1998)

Continuation of B. FIELDS SEARCHED Item 3:
WEST, CAS ONLINE:

search terms: lipid, anisidine, value, fatty acid

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

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4 May 2006 (04.05.2006)

PCT

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WO 2006/046943 A3

(51) International Patent Classification:
C12P 7/64 (2006.01)

(21) International Application Number:
PCT/US2004/034965

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(26) Publication Language: English

(71) Applicant *(for all designated States except US)*: **MARTEK BIOSCIENCES CORPORATION** [US/US]; 6480 Dobbin Road, Columbia, MD 21045 (US).

(72) Inventors; and

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
22 November 2007

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR PRODUCING LIPIDS BY LIBERATION FROM BIOMASS

(57) Abstract: Disclosed are methods of preparing high-quality polyunsaturated fatty acid-containing lipids from a lipid-containing material that include enzymatic treatment of components of the material and/or pressure disruption of the material. Lipid-containing materials include biomass, such as microorganisms. The invention further includes products containing the lipid compositions, such as dietary supplements, food products, pharmaceutical formulations, humanized animal milk, and infant formula.

WO 2006/046943 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/34965

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **C12P 7/64**(2006.01)

USPC: 424/93.4;435/134,135,183
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/93.4;435/134,135,183

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Caplus, Biosis, Medline, Agricola, WEST, wpids.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 1178118 (BIJL et al.) 06 February 2002 (06.02.2002). See entire document.	1-49

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
17 August 2007 (17.08.2007)

Date of mailing of the international search report
26 SEP 2007

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/34965

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-49

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(2)) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/34965

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-49, drawn to a method of obtaining a polyunsaturated fatty-acid containing lipid by contacting a biomass with an enzyme.

Group II, claim(s) 50-97, drawn to a method of obtaining a polyunsaturated fatty-acid containing lipid by contacting a biomass with an enzyme and by pressure disruption..

Group III, claim(s) 98-146, drawn to drawn to a method of obtaining a polyunsaturated fatty-acid containing lipid by disrupting cells in the biomass by a pressure differential of less than about 12,000 psi.

Group IV, claim(s) 147-49 (second occurrence of claim 49; should be "149"), drawn to a polyunsaturated fatty acid containing lipid.

The inventions listed as groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

First, the inventions do not match a permitted category as PCT Rule 13.2 does not provide for multiple methods or products in one category.

Second, the method of group I does not share a special technical feature with the methods of group II and III because the process steps used in each process are different, independent and distinct.

No common inventive concept is shared among groups I-IV, since a technical relationship is lacking among the claimed inventions involving one or more special technical features . For example, Barclay *et al.* (U.S. Patent No. 5,130,242) adequately demonstrates that methods of disruption such as enzymatic digestion of cell walls and pressure disruption are old and well known and have been used for obtaining lipids containing unsaturated fatty acids. See, e.g., col. 13, lines 41-49. In addition Barclay *et al.* demonstrate that lipids containing polyunsaturated fatty acid are known in the art. See, e.g., Table 3.

The requirement of unity of invention is not fulfilled because there is no technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features " means those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

PATENT COOPERATION TREATY

RECEIVED
MAY 1 3 2002
SHERIDAN, ROSS

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: GARY J. CONNELL
SHERIDAN ROSS P.C.
1560 BROADWAY, SUITE 1200
DENVER, CO 80202-5141

Reviewed:
Date: May 15 2002
Initial:
Please return to Janice

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	06 MAY 2002
Applicant's or agent's file reference: 2997-19-PCT	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US01/01806	19 JANUARY 2001	19 JANUARY 2000

Applicant: OMEGATECH, INC.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	CHRISTOPHER TATE Faye Bridges for
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)*

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2997-19-PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US01/01806	International filing date *(day/month/year)* 19 JANUARY 2001	Priority date *(day/month/year)* 19 JANUARY 2000
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant OMEGATECH, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 26 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☒ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02 JULY 2001	26 MARCH 2002
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Taylor Bridges for CHRISTOPHER TATE Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US01/01806

I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed

☒ the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages (See Attached) , as originally filed
pages _____ , as amended (together with any statement) under Article 19
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the drawings:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the sequence listing part of the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

Form PCT/IPEA/409 (Box I) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US01/01806

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- [ ] restricted the claims.
- [X] paid additional fees.
- [ ] paid additional fees under protest.
- [ ] neither restricted nor paid additional fees.

2. [ ] This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- [ ] complied with.
- [X] not complied with for the following reasons:

Please See Supplemental Sheet.

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- [X] all parts.
- [ ] the parts relating to claims Nos. .

Form PCT/IPEA/409 (Box IV) (July 1998)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US01/01806

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	2-5, 8-86	YES
	Claims	1, 6, 7	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-86	NO
Industrial Applicability (IA)	Claims	1-86	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1, 6, and 7 lack novelty under PCT Article 33(2) as being anticipated by Gudin et al. (US 5,179,012).

Gudin et al. teaches a method of obtaining lipid (liposoluble components) from microorganisms using the steps recited in claims 1, 6, and 7 (see, for example, column 2, line 37 - column 6, line 4).

Claims 1-86 lack an inventive step under PCT Article 33(3) as being obvious over Gudin et al. (US 5,179,012) in view of Tanaka et al. (WO 98/03671), Barclay (US 5,180,242), Best et al. (US 5,928,696), Wagner (US 4,720,456), and/or Kohn et al. (US 5,539,133).

Gudin et al. teach a method of obtaining lipid (liposoluble components) from microorganisms using the steps recited in claims 1, 6, and 7.

The secondary references disclose other conventional steps/procedures used to beneficially obtain lipid (for example, liposoluble components)from microorganisms/cells (see, for example, Tanaka et al: column page 2, line 32 - page 11, line 23; Barclay: column 4, line 52 - column 13, line 49; Best et al: entire document; Wagner et al: column 1, lines 8-28; Kohn et al: column 1, line 10 - column 5, line 20) including those claimed.

It would have been obvious to one of ordinary skill in the art to incorporate other conventional art-recognized steps, such as those beneficially disclosed by the secondary references, within the process taught by Gudin so as to more effectively obtained high yields of desired lipid (liposoluble components) from such microorganisms, as well as to remove undesired solvents therefrom. Claimed limitations not expressly disclosed by the above teachings, including particular properties of such lipids, would be intrinsic thereto and/or obvious based upon the cited teachings as a whole.

(Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C12P 7/64, 1/04, 1/02; C12N 1/12; C12M 1/33, 3/08 and US Cl.: 435/134, 170, 171, 306.1, 946

I. BASIS OF REPORT:

This report has been drawn on the basis of the description,
page(s) NONE, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
Pages 1-16, filed with the letter of 26 November 2001.

This report has been drawn on the basis of the claims,
page(s) NONE, as originally filed.
page(s) NONE, as amended under Article 19.
page(s) NONE, filed with the demand.
and additional amendments:
Pages 17-26, filed with the letter of 26 November 2001.

This report has been drawn on the basis of the drawings,
page(s) 1, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the sequence listing part of the description:
page(s) NONE, as originally filed.
pages(s) NONE, filed with the demand.
and additional amendments:
NONE

IV. LACK OF UNITY OF INVENTION:

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2, and 13.3 is not complied with for the following reasons:
As applicant was previously notified this International Preliminary Examining Authority has found plural inventions claimed in the International Application covered by the claims indicated below:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-46, drawn to a method of obtaining lipid from icroorganisms comprising a step of lysing the microbial cells.

Group II, claim(s) 47-82, drawn to a method of obtaining lipid from microorganisms which does not necessarily comprise a step of lysing.

Group III, claim(s) 83-84, drawn to a lipid derived from a microorganism which has less than 0.2 ppm residual nonpolar organic solvent.

Group IV, claim(s) 85-86, drawn to a lipid comprising greater than 15% cholesterol and/or other fatty acids.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 11

and it considers that the International Application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The Group I process involves a step of lysing the microbial cells to produce a lysed cell mixture whereas the Group II process does not necessarily involve such a step. The special technical feature of the Group IV lipid is that it comprises greater than 15% cholesterol and/or other fatty acid, which is not shared by the lipid of Group III. Further, the lipids of Groups III and IV do not require that they be obtained using the special technical feature steps of the Group I process or of the Group II process.

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

---------- NEW CITATIONS ----------
NONE

SOLVENTLESS EXTRACTION PROCESS

FIELD OF THE INVENTION

The present invention is directed to a process for extracting lipids from microorganisms without the use of any significant amount of a nonpolar organic solvent.

BACKGROUND OF THE INVENTION

A typical microorganism lipid manufacturing process, such as production of omega-3 highly unsaturated fatty acid, in particular, a lipid mixture rich in docosahexaenoic acid (DHA), involves growing microorganisms which are capable of producing the desired lipid in a fermentor, pond or bioreactor, isolating the microbial biomass, drying it, and extracting intracellular lipids with a nonpolar organic solvent, *e.g.*, hexane. Generally, intracellular lipids of microorganisms are extracted after rupturing (i.e., lysing) dried cells of the microorganisms. The extracted lipids are can be further refined to produce high purity and/or quality lipids. The microorganisms are generally isolated by first diluting the fermentation broth with water, and centrifuging the mixture to isolate microorganisms. The cells are then dried and if the lipids are not extracted immediately or soon thereafter, the cells are packaged, for example, in vacuum-sealed bags, to prevent degradation of lipids.

Unfortunately, the drying process exposes the microorganisms to heat, which can damage, *i.e.*, degrade the quality of, lipids if done incorrectly. The vacuum-sealed bags may develop leaks, which can further degrade the quality of the lipids due to exposure of the microorganisms to air. In addition, if the dried microorganisms are not treated with an antioxidant, lipids can be further degraded due to exposure to air and or light. For example, carotenoids, xanthophylls and long chain fatty acids like DHA may degrade due to oxidation by air and/or light. Furthermore, in some cases operators who are exposed to the dried microorganisms can develop an allergic reaction creating a safety and/or health hazard to operators.

Moreover, in an industrial scale production, the large amount of volatile and flammable nonpolar organic solvent used in lipid extraction can create hazardous operating conditions. The use of nonpolar organic solvent in the extraction process may necessitate using an explosion-proof oil recovery system, thereby adding to the cost of lipid recovery. Moreover, use of a nonpolar organic solvent in extracting lipids from

microorganisms generates a nonpolar organic solvent waste stream requiring a proper disposal method, which further increases the overall production cost of lipid extraction.

Therefore, there is a need for a process for extracting lipids from microorganisms which does not require the use of a nonpolar organic solvent. There is also a need for a lipid extraction process from microorganisms which does not require the expensive step of drying the microorganisms prior to extraction.

SUMMARY OF THE INVENTION

One embodiment of the present invention provides a process for obtaining lipid from microorganisms comprising:

(a) lysing cells of the microorganisms to produce a lysed cell mixture;

(b) treating the lysed cell mixture to produce a phase separated mixture comprising a heavy layer and a lipid-rich light layer;

(c) separating the heavy layer from the lipid-rich light layer; and

(d) obtaining the lipid and/or lipid fractions from the light layer.

Another embodiment of the present invention provides a process for obtaining lipids from microorganisms comprising:

(a) growing microorganisms in a culture medium;

(b) treating said culture medium and the microorganism cells to release intercellular lipids;

(c) subjecting the culture medium containing the released intercellular lipids to gravity separation to form a light lipid-containing phase and a heavy phase;

(d) separating said light phase from said heavy phase;

(e) treating said light phase to break an emulsion formed between said lipid and water; and

(f) recovering a crude lipid.

In accordance with another embodiment of the present invention, a process is provided for recovering lipids from microorganisms comprising the steps:

(a) growing said microorganisms in a culture medium;

(b) treating microorganism cells from said culture medium without drying said cells to release intercellular lipids;

(c) subjecting the culture medium containing the released intercellular lipids to gravity separation to form a light lipid-containing phase and a heavy phase;

(d) separating said light phase from said heavy phase;

(e) treating said light phase to break an emulsion formed between said lipid and water; and

(f) recovering a crude lipid.

Preferably, the microorganisms are cultured in a fermentation medium in a fermentor. Alternatively, the microorganisms can be cultured photosynthetically in a photobioreactor or pond. Preferably, the microorganisms are lipid-rich microorganisms, more preferably, the microorganisms are selected from the group consisting of algae, bacteria, fungi and protists, more preferably, the microorganisms are selected from the group consisting of golden algae, green algae, dinoflagellates, yeast, fungi of the genus *Mortierella*, and Stramenopiles. Preferably, the microorganisms comprise microorganisms of the genus Mortierella, genus Crypthecodinium, and order Thraustochytriales, and more preferably, microorganisms are selected from the genus *Thraustochytrium*, *Schizochytrium* or mixtures thereof, more preferably, the microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and ATCC number 20892, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

The treatment of the cells includes a treatment to release the lipids such as lysing, rupturing or permeabilizing. As used herein, the terms lyse, lysing, lysed, etc., will be used generically to refer to a treatment to release intercellular lipids, including breaking or permeabilizing the cells. Preferably, the treatment is selected from the group consisting of heating the cells, exposing the cells to basic conditions, exposing the cells to a chelating compound or combinations thereof. More preferably the lysing or rupturing of the cells comprises heating the cells to at least 50°C while exposing the cells to basic conditions, a chelating compound or mixtures thereof.

Preferably, the gravity separation comprises passing the fermentation broth containing the released intercellular lipids through a centrifuge, such as stacked-disc-, separator- or decanter-type centrifuges.

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The separated lysed cell mixture comprises a heavy layer which comprises an aqueous solution which contains the solid materials resulting from the lysed cells, and a light layer which contains lipids. The light and heavy layers can be separated by centrifugation. The lipids may be present in an emulsified state. The light layer can be further washed with an aqueous washing solution until the lipid becomes substantially non-emulsified. Preferably the treatment to break the emulsion comprises mixing the emulsion with water, alcohol, acetone or mixtures thereof and subjecting the mixture to gravity separation. Preferably, the process is performed without using nonpolar organic solvents such as hexane.

When the lipid extraction process of the present invention includes using microorganisms from a fermentation process, the extraction process can also include solubilizing at least part of proteinaceous compounds in a fermentation broth by adding a base selected from the group consisting of hydroxides, carbonates, bicarbonates, phosphates and mixtures thereof.

The process of the present invention can also include heating the microorganisms to temperature of at least about 50 °C. Preferably, a chemical compound, such as a base, is added to the culture medium to aid in the lysing of the cells.

As an alternative to heating, the cells can be lysed with the assistance of a chelating compound such as EDTA. In addition to assisting in lysing or rupturing the cells, the chelators assist in preventing oxidation of the lipids during processing by chelating (binding with) free radical-producing metal ions in the fermentation broth such as iron or copper. Preferred forms of chelators are those that are considered food grade or GRAS (Generally Recognized As Safe). Effective chelating compounds include EDTA, citric acid or citrate, lactic acid, trisodium phosphate, polyphosphate, hexametaphosphate, EGTA, DTPA, phytic acid, or CDTA and other salt forms of these compounds. In one embodiment, sodium EDTA is added to the cells to degrade the cell walls by chelating the divalent cations which assist in holding the cell walls together. The process can be performed at higher temperatures with less EDTA or lower temperatures with higher concentrations of EDTA. For example, we have found that DHA-rich cells of *Schizochytrium* sp. can be permeabilized and/or ruptured by addition of EDTA to the cultures at the end of the fermentation process. A concentration of 10,000 ppm is required to assist in rupturing the cells at 30°C, a concentration of 5,000 ppm at 50°C and at temperatures above 70°C concentrations of lower than 1000 ppm are effective. Chelators can be added to the fermentation broth to make the cells easier to break by

physical processes such as homogenization. In addition to a chelator, water can also be added to increase the internal osmotic pressure in order to lyse the cells.

Preferably, the microorganisms are capable of growth at salinity level of less than about 12 g/L of sodium chloride, more preferably less than about 5 g/L of sodium chloride and most preferably less than about 3 g/L of sodium chloride. Preferably, the microorganisms are capable of growth at salinity levels of less than about 7 g/L of sodium and less than about 250 mg/L of chloride. Preferably, the chloride is present in an amount from about 70 to about 150 mg/L.

Preferably, the microorganisms comprise at least about 20% by weight of lipid, more preferably at least about 30% by weight, and most preferably at least about 40%. Alternatively at least about 20% of the lipid is cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof, preferably at least about 30%, and more preferably at least about 40%.

In one particular aspect of the present invention the microorganisms are capable of producing at least about 0.1 grams per liter per hour of a mixture of lipids, preferably including cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof, more preferably at least about 0.2 g/L/h, still more preferably at least about 0.3 g/L/h, and most preferably at least about 0.4 g/L/h.

In another aspect of the present invention, the microorganism is selected from the group consisting of algae, fungi, bacteria and protists. Preferably, the microorganisms are of the order Thraustochytriales. More preferably the microorganisms are selected from the genus *Thraustochytrium*, *Schizochytrium* and mixtures thereof. And most preferably, the microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and ATCC number 20892, mutant strains derived from any

of the foregoing, and mixtures thereof. Preferably, the microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888 and ATCC number 20889, and more preferably ATCC number 20888, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a flow diagram of one embodiment of a solventless extraction process of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a process for extracting, recovering, isolating or obtaining lipids from microorganisms. The process of the present invention is applicable to extracting a variety of lipids from a variety of microorganisms, for example, extracting lipids containing cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof, more preferably, omega-3 highly unsaturated fatty acids, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and/or docosapentaenoic acid (DPA) (i.e., the omega-3 form of DPA), in particular lipids containing a relatively large amount of DHA, from microorganisms producing the same; and extracting lipids containing omega-6 highly unsaturated fatty acids, such as arachidonic acid and docosapentaenoic acid (DPA) (i.e., the omega-6 form of DPA)from microorganisms producing the same. Exemplary microorganisms which produce a relatively large amount of omega-3 highly unsaturated fatty acids are disclosed in commonly assigned U.S. Patent Nos. 5,340,594 and 5,340,742, both issued to Barclay, and exemplary microorganisms which produce a relatively large amount of arachidonic acid are disclosed in commonly assigned U.S. Patent No. 5,583,019, issued to Barclay. All of the above disclosed patents are incorporated herein by reference in their entirety.

For the sake of brevity, however, this detailed description of the invention is presented for purposes of convenience and illustration for the case of extracting lipids

comprising omega-3 highly unsaturated fatty acid from microorganisms producing the same, in particular extracting lipids from microorganisms that produce a relatively high amount of DHA. It is to be understood, however, that the invention as a whole is not intended to be so limited, and that one skilled in the art will recognize that the concept of the present invention will be applicable to other microorganisms producing a variety of lipid compositions in accordance with the techniques discussed herein. These microorganisms include microorganisms, such as fungi, protists, algae and bacteria, which produce a variety of lipids, such as phospholipids; free fatty acids; esters of fatty acids, including triglycerides of fatty acids; sterols; pigments (*e.g.*, carotenoids and oxycarotenoids) and other lipids, and lipid associated compounds such as phytosterols, ergothionine, lipoic acid and antioxidants including beta-carotene, tocotrienols, and tocopherol. Preferred lipids and lipid associated compounds include, but are not limited to, cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof. For the sake of brevity, unless otherwise stated, the term "lipid" refers to lipid and/or lipid associated compounds. Other lipids and microorganisms which may be suitable for use in the instant invention will be readily apparent to those skilled in the art.

Typical microbial lipid (in particular an oil containing an omega-3 highly unsaturated fatty acid such as DHA) manufacturing processes involve growing microorganisms which produce DHA in a fermentor, isolating the microorganisms, drying the microbial biomass and extracting the intracellular lipids with a non-polar organic solvent, *e.g.*, hexane. The extracted lipid is generally further refined to produce a high purity and/or quality lipid. The isolation of microorganisms involves diluting the fermentation broth with water and centrifuging the mixture to isolate microorganisms. When lipids are not extracted immediately or soon after isolating the microorganisms, the isolated microorganisms are typically dried, *for example*, on a drum dryer, and sealed in a package, *e.g.*, in vacuum-sealed bags, to prevent degradation of lipids. Unfortunately, the drying process exposes the microorganisms to heat, which can damage, *i.e.*, degrade the quality of, the lipid if done incorrectly. The package may develop leaks, which can further degrade the quality of the lipids. Furthermore, if the dried microorganisms are not

treated with an antioxidant, the exposure of microorganisms to air and/or light can further degrade the lipids.

Recovering the crude oil directly from the fermentation broth avoids these problems. Avoiding the non-polar organic solvent extraction step reduces manufacturing costs and also eliminates operator exposure to the dried microorganisms, which can cause an allergic response in some individuals.

The present invention provides a method for obtaining lipids from microorganisms using a substantially nonpolar organic solvent free extraction process, *i.e.*, a "solventless" extraction process. The term "solventless extraction process" refers to an extraction process which, when an aqueous or polar solvent is used, the aqueous or polar solvent includes less than about 5% of a nonpolar organic solvent, preferably less than about 4%, more preferably less than about 2%, and most preferably less than 1%. However, solvents can be employed in downstream steps such as in a refining process. The process of the present invention can include obtaining or isolating microorganisms, preferably from a fermentation process. In contrast to the prior art processes such as the extraction of oils from soybeans in which the soybeans must be dried, the process of the present invention does not require a drying step prior to the extraction process. Thus, processes of the present invention are applicable to extracting lipids from a microbial biomass containing at least about 10% by weight entrained water, preferably at least about 20%, more preferably at least about 30%, and most preferably at least about 50%. When the microorganisms are obtained from a fermentation process, the process of the present invention can include adding a base to the fermentation broth to dissolve any proteinaceous compound that may be present in the broth. "Bases" are substances which show alkali (basic) reactions in watery solutions, i.e. they attach protons and dissociate hydroxide ions. The base should be strong enough to hydrolyze or solubilize at least a portion of proteinaceous compounds that may be present in the broth. Bases which are useful for solubilizing proteins are well known to one of ordinary skill in the art of chemistry. Exemplary bases which are useful in the processes of the present invention include, but are not limited to, hydroxides, carbonates and bicarbonates of lithium, sodium, potassium, calcium, and magnesium carbonate. Other very basic compounds like basic phosphate salts, such as trisodium phosphate, can also be used.

The process of the present invention can also include rupturing or lysing the cells of microorganisms to release the lipids which are present within the cells. Cells can be lysed using any of the known methods including chemical; thermal; mechanical,

including, but not limited to, french press, mills, ultrasonication, homogenization, and steam explosion; and combinations thereof. In a thermal lysing of cells, the fermentation broth containing microorganisms are heated until cells, *i.e.*, cell walls, of microorganisms degrade or breakdown. Typically, the fermentation broth is heated to a temperature of at least about 50°C, preferably at least about 75°C, more preferably to at least about 100°C, and most preferably to at least about 130°C. An important aspect of the process is to maintain the temperature below that temperature at which the extracted lipids can be degraded. Thermally lysing the cell walls of microorganisms is particularly useful for microorganisms whose cell walls are composed of proteins. During this process the headspace of the fermentor can be filled with nitrogen or another inert gas to prevent oxidation of the lipids by oxygen.

Heating the broth also denatures proteins and helps solubilize organic materials, including proteins. Heating of the fermentation broth step can be achieved by any of the known methods, including the use of an in-line heat exchanger, and preferably by sparging steam into the fermentation broth and maintaining the broth at a desired temperature for less than about 90 minutes, preferably less than about 60 minutes, and more preferably less than about 30 minutes.

The solventless extraction process of the present invention can also include at least partially separating the spent fermentation broth from the lipids. Typically, this is achieved by centrifuging, *e.g.*, by passing the broth through a stacked-disc, separator or decantor centrifuge, and collecting lipids as an emulsion phase. Centrifuging the mixture results in a two phase mixture comprising a heavy layer and a light layer. Typically, the heavy layer is an aqueous phase, which contains the majority of cellular debris. The light layer which contains emulsified lipids is then diluted with water, again separated into two phase mixture and the light layer is again isolated. This dilution with water, separation and isolation processes (*i.e.*, washing process) can be achieved continuously by feeding water and removing the heavy layer throughout the process or it can be conducted in discreet steps. The washing process is generally repeated until a substantially non-emulsified lipid layer is obtained, although minor amounts of emulsion can remain. It is believed that the oil-water interface of the emulsion is stabilized by residual cellular debris which is removed by the washing process. During the washing process, the successive amount of water added is reduced to increase the lipid content. While reducing the amount of feed water too quickly can result in loss of lipids to the aqueous phase, reducing the amount of feed water too slowly results in an inefficient washing

process. One can readily determine an appropriate rate of feed water reduction by observing or analyzing the separated aqueous layer. Generally, the lipid layer, *i.e.*, the light layer, is colored; therefore, in many cases one can determine an appropriate rate of feed water reduction by simply analyzing or observing the color of the aqueous layer which is separated from the lipid layer.

Alternatively, and preferably, the emulsion can be broken, and the oil recovered using the deoiling process outlined in WO 96/05278 which is incorporated herein by reference in its entirety. In this process a water soluble compound, e.g., alcohol and/or acetone, is added to the oil/water emulsion to break the emulsion and the resulting mixture is separated by centrifugation. The isolated lipid can be further refined using a process similar to that used to refine standard vegetable oils. Briefly, the lipid refining process generally involves hydrating phospholipids by adding phosphoric acid to the lipid followed by adding sodium hydroxide to neutralize free fatty acids. These compounds are removed via centrifugation. This is then followed by a water wash step to further remove any remaining amounts of hydrated phospholipids ("gums") and neutralized fatty acids ("soapstock") in the lipid. The resulting lipid is bleached using Trysil™ and a standard bleaching clay. Citric acid is also added to remove divalent metal ions by chelation. The Trysil™ and bleaching clay are then removed via filtration to produce refined lipid. The bleached lipid can be cold filtered to remove high melting point compounds that may be present in the lipid; however, this step is generally seldom required.

The resulting lipid can be further refined by removing any low molecular weight components that may be present. Typically, these components are removed by sparging with steam at high temperatures, under high vacuum. This process also destroys any peroxide bonds which may be present and reduces or removes off- odors and helps improve the stability of the oil. An antioxidant may then be added to the resulting deodorized lipid to improve product stability.

During the refining process, the isolated lipid can be winterized to remove high melting compounds, such as saturated fatty acids. The winterization process generally involves dissolving the isolated lipid in an organic solvent, *e.g.*, hexane, cooling the resulting organic solution, and filtering the solution to remove the high melting point components of the lipid or stearine phase. The winterization process generally produces a clear lipid, especially when the isolated lipid is cloudy or opaque. As will be appreciated, the use of a solvent such as hexane is acceptable in processes such as the above described

"refining" process. Alternatively, the isolated lipid can be chilled and solidified impurities can be filtered out, without employing a solvent.

The refining, bleaching, deodorizing process outlined above would be used for triglyceride-rich lipid mixtures. Alternatively, or in addition to this process, other lipids, for example, pigments or carotenoids can be separated and purified, e.g., by partition into various solvents, chromatographic methods, etc.

While, the process of the present invention can include isolating microorganisms from a fermentation process, one of the advantages of the present invention is that it allows fermentation of microorganisms and isolation of lipids to be carried out in a single vessel. For example, after the fermentation, one can add base to the fermentation vessel and heat the mixture to lyse cells. After separating the phase into a heavy layer and a light layer, the light layer can be transferred to another vessel for further processing or the heavy layer can be removed from the fermentation vessel, for example, by draining through the bottom of the fermentation vessel, and the remaining light layer can be further processed within the same fermentation vessel.

If the concentration of lipids in the cells of the microbial culture is high (e.g., greater than about 20%) but the concentration of cells is low (e.g., less than about 40 g/L), as in cells grown in continuous fermentation systems, or cultures of difficult (e.g., fragile) to grow cells, or cultures produced in photosynthetically-based culture systems, the cells can be concentrated, e.g., by centrifugation, filtration, or settling, prior to employing the methods of the invention if necessary.

Additional objects, advantages, and novel features of this invention will become apparent to those skilled in the art upon examination of the following examples thereof, which are not intended to be limiting.

Examples

Process reproducibility was characterized by producing three samples of fully refined oil using crude oil from the new solventless extraction process. A hexane-extracted sample was also fully refined to serve as a control. The fermentation, extraction and oil isolation steps were performed at a large scale, while the oil refining studies were performed at a small scale.

The fully refined oil samples were analyzed to demonstrate process reproducibility.

Fermentation:

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An oil-rich microorganism *(Schizochytrium sp.)* was grown in a 1200 gallon fermentor to produce a fermentation broth for the following extraction processes. A single batch was used to generate the starting broth for the three solventless extraction processes. The fermentation was allowed to run for 94 hours, while controlling the glucose levels at 13 g/L, after which time the corn syrup feed was terminated. Residual glucose levels dropped to <5 g/L four hours later. This resulted in a final age of 98 hours. The final broth volume was 958 gallons. The final yield was 146 g/L dry weight of cells. Both in-process contamination checks and a thorough analysis of a final broth sample failed to show any signs of contamination.

Hexane-Extracted Control Sample:

A small aliquot of broth from the fermentation batch was drum-dried and extracted with hexane to serve as a control sample. The biomass intermediate was recovered using a double-drum dryer. Analysis of this lipid is shown in Table 1.

Table 1. Analysis of drum dried *Schizochytrium* sp. biomass.

Parameter	Value
DHA Content (FAME basis)	35.7%
Oil Content	62.7%
Peroxide Value (meq/kg)	2.6
Total Plate Count (cfu/g)	<50
DHA Content*	20.3%
FAME Content	56.9%

* cellular dry weight basis

Solventless Extraction Process:

Crude oil was obtained by treating three 400-gallon aliquots (approx.) of the fermentation broth. Each 400-gallon aliquot from the fermentor was processed separately, starting with the caustic/heat treatment steps. Each aliquot was treated with 20 grams of 45% KOH per liter and heated to 130°C for about 30 minutes by passing steam through the fermentation broth. The crude oil was recovered from the treated broth using a commercial-scale Westfalia HFA-100 stacked-disc centrifuge. Summary results for various process parameters are reported in Table 2, and the final crude oil analysis results are shown in Table 3.

Table 2. Process Data from the Solventless Extraction Process.

	SFE-1	SFE-2	SFE-3
Broth Treatment			
Volume of Broth Processed	288 gal	288 gal	258 gal
Final Treated pH	7.5	8.0	8.7
Final Volume After Heat Treatment	388 gal	398 gal	308 gal
Volume Increase From Condensate	34.7%	38.2%	19.4%
1st Pass Emulsion			
Total Volume (gal)	180	133	149
Est. Oil Concentration (w/w)	12.0%	24.5%	16.1%
Apparent Density (g/mL)	0.986	0.991	0.999
Oil Isolation			
Total Crude Oil Recovered (lb)	182	165	174
DHA Oil Lot Number Assigned	SF1A	SF2A	SF3A

Table 3. Analysis of Lots of DHA oil from the Solventless Extraction Process.

Parameter	SF1A	SF2A	SF3A
DHA Content (%FAME)	39.0%	38.6%	39.2%
Peroxide Value (meq/kg)	4.6	1.8	2.0
Acid Value (mg KOH/g)	N/D	N/D	N/D
Moisture Content	N/D	N/D	N/D

Refining:

A sample from each aliquot of crude oil was winterized, refined, bleached and deodorized at a small scale, as was a sample of the crude oil from the hexane-extracted control. Miscellaneous process data from these small scale experiments is shown in Table 4, including recovery efficiencies for the various processing steps. While it is difficult to read too much into recovery efficiencies for bench-scale processes, as losses tend to be disproportionately large, the values listed in Table 4 show that values for the solventless-extracted samples tend to bracket the values measured for the hexane-extracted control, with the one exception being the winterization step. While the recovery efficiency during the winterization step for the hexane control was lower than those observed for the other three samples, this difference is insignificant from a statistical perspective. The high losses during the winterization step caused the overall recovery efficiency for the hexane-control sample to be lower as well. The lower yield would not be expected to have a significant impact on the overall quality of the oil. All in all, differences in the processing of the various oil samples were minimal.

PCT/US 00/01806
IPEA/US 26 NOV 2001

Table 4. Miscellaneous Process Data from the Oil Refining Steps.

	HEX-1	SF1A	SF2A	SF3A
Processing Conditions				
Miscella Concentration	45.0%	52.9%	52.8%	45.0%
Steam Sparge Rate	3.4%	3.4%	2.5%	2.2%
Recovery Efficiencies				
Winterization	80.6%	92.3%	87.7%	85.5%
Refining	89.4%	84.8%	91.8%	95.0%
Water Wash	90.6%	94.5%	95.8%	81.2%
Bleaching	86.1%	89.2%	87.3%	84.1%
Deodorization	97.4%	96.1%	97.2%	97.5%
Packaging	88.2%	89.7%	89.3%	95.8%
Overall	**48.2%**	**56.9%**	**58.5%**	**51.8%**

Fully refined oil samples from the three solventless extraction runs, and the hexane-extracted control, were analyzed and the results are shown in Table 5. Also shown are the corresponding release specifications for each parameter.

A sample of the starting crude oil from the solventless extraction run was also analyzed for iron content. The iron content of this sample was 0.08 ppm. The concentrations of the other trace metals were all below their respective detection limits.

Table 5. QC Results for RBD DHA Oil from the Solventless Extraction Process compared to hexane extracted oil.

	Hexane	Solventless Extrac. Process		
Run ID #	HEX-1	SFA1	SFA2	SFA3
Peroxide Value (meq/kg)	0.28	0.69	0.35	0.34
Acid Value (mg KOH/g)	0.17	0.11	0.57	0.24
Moisture & Volatiles	0.00%	0.06%**	0.00%	0.00%
Trace Metals (ppm)				
Lead	<0.20	<0.20	<0.20	<0.20
Arsenic	<0.20	<0.20	<0.20	<0.20
Iron	0.22	0.21	0.56***	0.02
Copper	<0.05	<0.05	<0.05	<0.05
Mercury	<0.20	<0.20	<0.20	<0.20
DHA (%FAME)	36.9	37.3	37.0	37.7
DHA (mg/g oil)	342	345	343	351
Hexane (ppm)	<3	<3	<3	<3

* Value was reduced to 0.22 mg KOH/g after repeating the refining and bleaching steps

** Sample analyzed by the San Diego Fermentation Sciences Analytical Group.

*** Value was reduced to <0.02 ppm after repeating the refining and bleaching steps

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Shown in Table 6 is a more direct comparison of the average analysis results for the three samples from the solventless extraction process versus those for the hexane control.

Table 6. Comparison of Average Values.

	Hexane	Solventless Extraction			
Parameter	Control	Mean	Std Dev	CV	%Diff
Peroxide Value (meq/kg)	0.28	0.46	0.20	43.3%	64.3%
Acid Value (mg KOH/g)	0.17	0.19*	0.06	33.3%	11.2%
Moisture & Volatiles	0.00%	0.02%	0.03%	173%	ND
Trace Metals (ppm)					
Lead	<0.20	<0.20	N/A	N/A	0.0%
Arsenic	<0.20	<0.20	N/A	N/A	0.0%
Iron	0.22	0.26	0.27	104%	18.2%
Copper	<0.05	<0.05	N/A	N/A	0.0%
Mercury	<0.20	<0.20	N/A	N/A	0.0%
DHA Content (%FAME)	36.9%	37.3%	0.4%	0.9%	1.1%
DHA Content (mg/g)	342	346	4	1.2%	1.2%
Hexane (ppm)	<3	<3	N/A	N/A	0.0%

* Calculated using the acid value for the re-worked sample.

The results from this experiment clearly demonstrate that the solventless extraction process is both reproducible and lipids from solventless extraction are relatively indistinguishable from the lipids obtained from hexane extraction process in terms of process performance and product quality. The final product from the solventless extraction process is substantially equivalent to lipids from a current hexane-based extraction process, as determined by similarities between the fatty acid and sterol profiles of the product from these two processes.

The present invention, in various embodiments, includes components, methods, processes, systems and/or apparatus substantially as depicted and described herein, including various embodiments, subcombinations, and subsets thereof. Those of skill in the art will understand how to make and use the present invention after understanding the present disclosure. The present invention, in various embodiments, includes providing devices and processes in the absence of items not depicted and/or described herein or in various embodiments hereof, including in the absence of such items as may have been used in previous devices or processes, *e.g.*, for improving performance, achieving ease and/or reducing cost of implementation.

The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, *e.g.*, as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

WHAT IS CLAIMED IS:

1. A process for obtaining lipid from microorganisms comprising:

(a) lysing cells of the microorganisms to produce a lysed cell mixture;

(b) treating said lysed cell mixture to produce a phase separated mixture comprising a heavy layer and a light layer, wherein said heavy layer comprises an aqueous solution and said light layer comprises said lipid;

(c) separating said heavy layer from said light layer; and

(d) obtaining said lipid from said light layer.

2. The process of Claim 1, wherein said step (b) comprises centrifuging said lysed cell mixture.

3. The process of Claim 2, wherein said light layer comprises an emulsified lipid.

4. The process of Claim 3 further comprising:

(e) adding an aqueous extraction solution to said light layer of step (c); and

(f) repeating said steps (c), (d) and (e) until said lipid becomes substantially non-emulsified prior to said step (d).

5. The process of Claim 3, wherein said emulsified lipid comprises a suspension of said lipid in an aqueous solution.

6. The process of Claim 1, wherein said aqueous solution comprises solid cell materials.

7. The process of Claim 1, wherein said microorganisms are obtained from a fermentation process.

8. The process of Claim 7 further comprising adding a base to a fermentation broth.

9. The process of Claim 8, wherein said base is selected from the group consisting of hydroxides, carbonates, bicarbonates, phosphates and mixtures thereof.

10. The process of Claim 7 further comprising solubilizing at least part of proteinaceous compounds in a fermentation broth.

11. The process of Claim 1, wherein said step (a) comprises heating said microorganisms to temperature of at least about 50°C.

12. The process of Claim 1, wherein said microorganism is capable of growth at salinity level of less than about 12 g/L of sodium chloride.

13. The process of Claim 1, wherein said microorganism comprises at least about 20% by weight of lipid.

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14. The process of Claim 1, wherein said microorganism is selected from the group consisting of algae, fungi, bacteria and protists.

15. The process of Claim 14, wherein said microorganisms comprise microorganisms of the order Thraustochytriales.

16. The process of Claim 15, wherein said microorganisms are selected from the genus *Thraustochytrium*, *Schizochytrium* and mixtures thereof.

17. The process of Claim 16, wherein said microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and ATCC number 20892, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

18. The process of Claim 1, wherein said microorganisms are capable of producing at least about 0.1 grams per liter per hour of cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

19. The process of Claim 1, wherein at least about 20% of said lipid is cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

20. A process for obtaining lipids from microorganisms comprising:

(a) growing said microorganisms in a fermentation medium to produce a fermentation broth;

(b) solubilizing at least a part of any proteins present in said fermentation broth;

(c) lysing cells of said microorganisms to produce a lysed cell mixture;

(d) treating said lysed cell mixture to produce a phase separated mixture comprising a heavy layer and a light layer, wherein said heavy layer comprises an aqueous solution and said light layer comprises emulsified lipids;

(e) separating said heavy layer from said light layer; and

(f) obtaining said lipids from said light layer.

21. The process of Claim 20, wherein said step of dissolving proteins comprises contacting said fermentation broth with a base.

22. The process of Claim 21, wherein said base is selected from the group consisting of hydroxides, carbonate, bicarbonates, phosphates and mixtures thereof.

23. The process of Claim 20, wherein said step of lysing cells comprises heating said microorganisms to a temperature of at least about 50°C.

24. The process of Claim 20, wherein said step of producing the phase separated mixture comprises centrifuging said lysed cell mixture.

25. The process of Claim 20, wherein said step of obtaining said lipids from said light layer comprises:

(a) adding an aqueous washing solution to said light layer;

(b) separating said aqueous washing solution from said light layer; and

(c) repeating said steps (a) and (b) until said lipid becomes substantially non-emulsified.

26. The process of Claim 20, wherein said aqueous solution comprises solid cell materials.

27. The process of Claim 20, wherein said microorganism is capable of growth at salinity level of less than about 12 g/L of sodium chloride.

28. The process of Claim 20, wherein said microorganism comprises at least about 20% by weight of lipid.

29. The process of Claim 20, wherein said microorganism is selected from the group consisting of algae, fungi, bacteria and protists.

30. The process of Claim 29, wherein said microorganisms comprise microorganisms of the order Thraustochytriales.

31. The process of Claim 30, wherein said microorganisms are selected from the genus *Thraustochytrium*, *Schizochytrium* and mixtures thereof.

32. The process of Claim 31, wherein said microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and

AMENDED SHEET

ATCC number 20892, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

33. The process of Claim 20, wherein said microorganisms are capable of producing at least about 0.1 grams per liter per hour of cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

34. The process of Claim 20, wherein at least about 20% of said lipid is cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

35. A process for obtaining lipids from microorganisms comprising:

(a) growing said microorganisms in a fermentation medium to produce a fermentation broth;

(b) contacting said fermentation broth with a base to dissolve at least a part of any proteins present in said fermentation broth;

(c) increasing the temperature of said fermentation broth to at least about 50°C to lyse cells of said microorganisms to produce a lysed cell mixture;

(d) separating substances of different densities from said lysed cell mixture to produce a phase separated mixture comprising a heavy layer and a light layer, wherein said heavy layer comprises an aqueous solution and said light layer comprises emulsified lipids;

(e) removing said heavy layer from said phase separated mixture;

(f) adding an aqueous washing solution to said light layer;

(g) separating substances of different densities from said mixture of step (f);

(h) removing said heavy layer from said phase separated mixture; and

(i) repeating said steps (f)-(h) until said lipid becomes substantially non-emulsified.

36. The process of Claim 35, wherein said base is selected from the group consisting of hydroxides, carbonates, bicarbonates, and mixtures thereof.

37. The process of Claim 35, wherein said step of producing the phase separated mixture comprises centrifuging said lysed cell mixture.

38. The process of Claim 35, wherein said aqueous solution of step (d) comprises solid cell materials.

39. The process of Claim 35, wherein said microorganism is capable of growth at salinity level of less than about 12 g/L of sodium chloride..

40. The process of Claim 35, wherein said microorganism comprises at least about 20% by weight of lipid.

41. The process of Claim 35, wherein said microorganism is selected from the group consisting of algae, fungi, bacteria and protists.

42. The process of Claim 35, wherein said microorganisms comprise microorganisms from the group consisting of golden algae, green algae, dinoflagellates, yeast, fungi of the genus *Mortierella*, and Stramenopiles.

43. The process of Claim 35, wherein said microorganisms comprise microorganisms of the order Thraustochytriales.

44. The process of Claim 43, wherein said microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and ATCC number 20892, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

45. The process of Claim 35, wherein said microorganisms are capable of producing at least about 0.1 grams per liter per hour of cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

46. The process of Claim 35, wherein at least about 20% of said lipid is cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-

6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

47. A process for obtaining lipids from microorganisms comprising:

(a) growing microorganisms in a culture medium;

(b) treating said culture medium and the microorganism cells to release intercellular lipids;

(c) subjecting the culture medium containing the released intercellular lipids to gravity separation to form a light lipid-containing phase and a heavy phase;

(d) separating said light phase from said heavy phase;

(e) treating said light phase to break an emulsion formed between said lipid and water; and

(f) recovering a crude lipid.

48. The process of Claim 47 wherein said microorganisms are lipid-rich microorganisms.

49. The process of Claim 47 wherein said microorganisms are selected from the group consisting of algae, bacteria, fungi and protists.

50. The process of Claim 47 wherein said microorganisms are selected from the group consisting of golden algae, green algae, dinoflagellates, yeast, fungi of the genus *Mortierella*, and Stramenopiles.

51. The process of Claim 47, wherein said microorganisms comprise microorganisms of the order Thraustochytriales.

52. The process of Claim 51, wherein said microorganisms are selected from the genus *Thraustochytrium*, *Schizochytrium* and mixtures thereof.

53. The process of Claim 52, wherein said microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and ATCC number 20892, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

54. The process of Claim 47, wherein said microorganisms are capable of producing at least about 0.1 grams per liter per hour of cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty

acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

55. The process of Claim 54, wherein at least about 20% of said lipid is cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

56. The process of Claim 47, wherein said treatment includes a treatment selected from the group consisting of heating said cells, exposing said cells to a basic compound, exposing said cells to a chelating compound or combinations thereof.

57. The process of Claim 47, wherein said treatment comprises heating the cells to at least 50°C before, during or after exposing the cells to a basic compound, a chelating compound or mixtures thereof.

58. The process of Claim 47, wherein said gravity separation of step (c) comprises passing the culture medium containing the released intercellular lipids through a stacked-disc, separator or decanter centrifuge.

59. The process of Claim 47, wherein the treatment of step to break the emulsion comprises mixing the emulsion with water, alcohol and/or acetone and subjecting the mixture to gravity separation.

60. The process of Claim 59, wherein said gravity separation comprises centrifugation.

61. The process of Claim 60, wherein said centrifugation includes treatment in a stacked-disc-, separator- or decanter-type centrifuge.

62. The process of Claim 59, wherein said treatment is repeated at least 3 times to obtain said crude lipid.

63. The process of Claim 47, wherein said crude lipid is subjected to further refining or processing to obtain a refined lipid.

64. The process of Claim 63, wherein said crude lipid is bleached and deodorized.

65. A process for recovering lipids from microorganisms comprising the steps:

(a) growing said microorganisms in a culture medium;

(b) treating microorganism cells from said culture medium without drying said cells to release intercellular lipids;

(c) subjecting the culture medium containing the released intercellular lipids to gravity separation to form a light lipid-containing phase and a heavy phase;

(d) separating said light phase from said heavy phase;

(e) treating said light phase to break an emulsion formed between said lipid and water; and

(f) recovering a crude lipid.

66. The process of Claim 65 wherein said microorganisms are lipid-rich microorganisms.

67. The process of Claim 65 wherein said microorganisms are selected from the group consisting of algae, bacteria, fungi and protists.

68. The process of Claim 65 wherein said microorganisms are selected from the group consisting of golden algae, green algae, dinoflagellates, yeast, fungi of the genus *Mortierella*, and Stramenopiles.

69. The process of Claim 65, wherein said microorganisms comprise microorganisms of the order Thraustochytriales.

70. The process of Claim 69, wherein said microorganisms are selected from the genus *Thraustochytrium*, *Schizochytrium* and mixtures thereof.

71. The process of Claim 70, wherein said microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and ATCC number 20892, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

72. The process of Claim 65, wherein said microorganisms are capable of producing at least about 0.1 grams per liter per hour of cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

73. The process of Claim 72, wherein at least about 20% of said lipid is cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

74. The process of Claim 65, wherein said treatment of the cells includes a treatment selected from the group consisting of heating said cells, exposing said cells to basic conditions, exposing said cells to a chelating compound or combinations thereof.

75. The process of Claim 65, wherein said treatment of the cells comprises heating the cells to at least 50°C before, during or after exposing the cells to a basic condition, a chelating compound or mixtures thereof.

76. The process of Claim 65, wherein said gravity separation of step (c) comprises passing the culture medium containing the released intercellular lipids through a stacked-disc, separator or decanter centrifuge.

77. The process of Claim 65, wherein the treatment to break the emulsion comprises mixing the emulsion with water, alcohol and/or acetone and subjecting the mixture to gravity separation.

78. The process of Claim 77, wherein said gravity separation comprises centrifugation.

79. The process of Claim 78, wherein said centrifugation includes treatment in a stacked-disc-, separator- or decanter-type centrifuge.

80. The process of Claim 77, wherein said treatment is repeated at least 3 times to obtain said crude lipid.

81. The process of Claim 65, wherein said crude lipid is subjected to further refining or purification to obtain a refined lipid.

82. The process of Claim 81, wherein said crude lipid is bleached and deodorized.

83. A lipid made by the process of any one of claims 1-82.

84. A lipid derived from microorganisms with less than 0.2ppm residual nonpolar organic solvent.

85. A lipid comprising greater than 15% cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as

beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof with less than 0.2 ppm residual nonpolar organic solvent

86. A lipid comprising greater than 15% docosahexaenoic acid and less than 0.2 ppm residual nonpolar organic solvent.

RECEIVED
SHERIDAN, ROSS

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: GARY J. CONNELL
SHERIDAN ROSS P.C.
1560 BROADWAY, SUITE 1200
DENVER, CO 80202-5141

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	27 SEP 2002
Applicant's or agent's file reference: 2997-19-PCT	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US01/01806	19 JANUARY 2001	19 JANUARY 2000

Applicant
OMEGATECH, INC.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	CHRISTOPHER TATE
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)*

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2997-19-PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US01/01806	International filing date *(day/month/year)* 19 JANUARY 2001	Priority date *(day/month/year)* 19 JANUARY 2000
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant OMEGATECH, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

   [✓] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 26 sheets.

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [ ] Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV [X] Lack of unity of invention

   V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [ ] Certain defects in the international application

   VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02 JULY 2001	05 SEPTEMBER 2002
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Gayle Bridges for CHRISTOPHER TATE Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US01/01806

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages (See Attached) , as originally filed
pages _____ , as amended (together with any statement) under Article 19
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the drawings:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the sequence listing part of the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~Fig~~ NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

Form PCT/IPEA/409 (Box I) (July 1998) ★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US01/01806

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- [ ] restricted the claims.
- [X] paid additional fees.
- [ ] paid additional fees under protest.
- [ ] neither restricted nor paid additional fees.

2. [ ] This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- [ ] complied with.
- [X] not complied with for the following reasons:

Please See Supplemental Sheet.

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- [X] all parts.
- [ ] the parts relating to claims Nos. .

Form PCT/IPEA/409 (Box IV) (July 1998)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US01/01806

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	2-5, 8-86	YES
	Claims	1, 6, 7	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-86	NO
Industrial Applicability (IA)	Claims	1-86	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1, 6, and 7 lack novelty under PCT Article 33(2) as being anticipated by Gudin et al. (US 5,179,012).

Gudin et al. teaches a method of obtaining lipid (liposoluble components) from microorganisms using the steps recited in claims 1, 6, and 7 (see, for example, column 2, line 37 - column 6, line 4).

Claims 1-86 lack an inventive step under PCT Article 33(3) as being obvious over Gudin et al. (US 5,179,012) in view of Tanaka et al. (WO 98/03671), Barclay (US 5,130,242), Best et al. (US 5,928,696), Wagner (US 4,720,456), and/or Kohn et al. (US 5,539,133).

Gudin et al. teach a method of obtaining lipid (liposoluble components) from microorganisms using the steps recited in claims 1, 6, and 7.

The secondary references disclose other conventional steps/procedures used to beneficially obtain lipid (for example, liposoluble components)from microorganisms/cells (see, for example, Tanaka et al: column page 2, line 32 - page 11, line 23; Barclay: column 4, line 52 - column 13, line 49; Best et al: entire document; Wagner et al: column 1, lines 8-28; Kohn et al: column 1, line 10 - column 5, line 20) including those claimed.

It would have been obvious to one of ordinary skill in the art to incorporate other conventional art-recognized steps, such as those beneficially disclosed by the secondary references, within the process taught by Gudin so as to more effectively obtained high yields of desired lipid (liposoluble components) from such microorganisms, as well as to remove undesired solvents therefrom. Claimed limitations not expressly disclosed by the above teachings, including particular properties of such lipids, would be intrinsic thereto and/or obvious based upon the cited teachings as a whole.

---------- NEW CITATIONS ----------

NONE

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

and it considers that the International Application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The Group I process involves a step of lysing the microbial cells to produce a lysed cell mixture whereas the Group II process does not necessarily involve such a step. The special technical feature of the Group IV lipid is that it comprises greater than 15% cholesterol and/or other fatty acid, which is not shared by the lipid of Group III. Further, the lipids of Groups III and IV do not require that they be obtained using the special technical feature steps of the Group I process or of the Group II process.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C12P 7/64, 1/04, 1/02; C12N 1/12; C12M 1/33, 3/08 and US Cl.: 435/134, 170, 171, 306.1, 946

I. BASIS OF REPORT:

This report has been drawn on the basis of the description,
page(s) NONE, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
Pages 1 and 4-16, filed with the letter of 26 November 2001 and pages 2-3, filed with the letter of 06 May 2002.

This report has been drawn on the basis of the claims,
page(s) NONE, as originally filed.
page(s) NONE, as amended under Article 19.
page(s) NONE, filed with the demand.
and additional amendments:
Pages 17-21 and 26, filed with the letter of 26 November 2001 and pages 22-25, filed with the letter of 06 May 2002.

This report has been drawn on the basis of the drawings,
page(s) 1, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the sequence listing part of the description:
page(s) NONE, as originally filed.
pages(s) NONE, filed with the demand.
and additional amendments:
NONE

IV. LACK OF UNITY OF INVENTION:

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2, and 13.3 is not complied with for the following reasons:
As applicant was previously notified this International Preliminary Examining Authority has found plural inventions claimed in the International Application covered by the claims indicated below:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-46, drawn to a method of obtaining lipid from microorganisms comprising a step of lysing the microbial cells.

Group II, claim(s) 47-82, drawn to a method of obtaining lipid from microorganisms which does not necessarily comprise a step of lysing.

Group III, claim(s) 83-84, drawn to a lipid derived from a microorganism which has less than 0.2 ppm residual nonpolar organic solvent.

Group IV, claim(s) 85-86, drawn to a lipid comprising greater than 15% cholesterol and/or other fatty acids.

SOLVENTLESS EXTRACTION PROCESS

FIELD OF THE INVENTION

The present invention is directed to a process for extracting lipids from microorganisms without the use of any significant amount of a nonpolar organic solvent.

BACKGROUND OF THE INVENTION

A typical microorganism lipid manufacturing process, such as production of omega-3 highly unsaturated fatty acid, in particular, a lipid mixture rich in docosahexaenoic acid (DHA), involves growing microorganisms which are capable of producing the desired lipid in a fermentor, pond or bioreactor, isolating the microbial biomass, drying it, and extracting intracellular lipids with a nonpolar organic solvent, *e.g.*, hexane. Generally, intracellular lipids of microorganisms are extracted after rupturing (i.e., lysing) dried cells of the microorganisms. The extracted lipids are can be further refined to produce high purity and/or quality lipids. The microorganisms are generally isolated by first diluting the fermentation broth with water, and centrifuging the mixture to isolate microorganisms. The cells are then dried and if the lipids are not extracted immediately or soon thereafter, the cells are packaged, for example, in vacuum-sealed bags, to prevent degradation of lipids.

Unfortunately, the drying process exposes the microorganisms to heat, which can damage, *i.e.*, degrade the quality of, lipids if done incorrectly. The vacuum-sealed bags may develop leaks, which can further degrade the quality of the lipids due to exposure of the microorganisms to air. In addition, if the dried microorganisms are not treated with an antioxidant, lipids can be further degraded due to exposure to air and or light. For example, carotenoids, xanthophylls and long chain fatty acids like DHA may degrade due to oxidation by air and/or light. Furthermore, in some cases operators who are exposed to the dried microorganisms can develop an allergic reaction creating a safety and/or health hazard to operators.

Moreover, in an industrial scale production, the large amount of volatile and flammable nonpolar organic solvent used in lipid extraction can create hazardous operating conditions. The use of nonpolar organic solvent in the extraction process may necessitate using an explosion-proof oil recovery system, thereby adding to the cost of lipid recovery. Moreover, use of a nonpolar organic solvent in extracting lipids from

microorganisms generates a nonpolar organic solvent waste stream requiring a proper disposal method, which further increases the overall production cost of lipid extraction.

Therefore, there is a need for a process for extracting lipids from microorganisms which does not require the use of a nonpolar organic solvent. There is also a need for a lipid extraction process from microorganisms which does not require the expensive step of drying the microorganisms prior to extraction.

SUMMARY OF THE INVENTION

One embodiment of the present invention provides a process for obtaining lipid from microorganisms comprising:

(a) lysing cells of the microorganisms to produce a lysed cell mixture;

(b) treating the lysed cell mixture to produce a phase separated mixture comprising a heavy layer and a lipid-rich light layer;

(c) separating the heavy layer from the lipid-rich light layer; and

(d) obtaining the lipid and/or lipid fractions from the light layer.

Another embodiment of the present invention provides a process for obtaining lipids from microorganisms comprising:

(a) growing microorganisms in a culture medium;

(b) treating said culture medium and the microorganism cells to release intracellular lipids;

(c) subjecting the culture medium containing the released intracellular lipids to gravity separation to form a light lipid-containing phase and a heavy phase;

(d) separating said light phase from said heavy phase;

(e) treating said light phase to break an emulsion formed between said lipid and water; and

(f) recovering a crude lipid.

In accordance with another embodiment of the present invention, a process is provided for recovering lipids from microorganisms comprising the steps:

(a) growing said microorganisms in a culture medium;

(b) treating microorganism cells from said culture medium without drying said cells to release intracellular lipids;

AMENDED SHEET

PCT/US 01/01806
PEA/US 06 MAY 2002

(c) subjecting the culture medium containing the released intracellular lipids to gravity separation to form a light lipid-containing phase and a heavy phase;

(d) separating said light phase from said heavy phase;

(e) treating said light phase to break an emulsion formed between said lipid and water; and

(f) recovering a crude lipid.

Preferably, the microorganisms are cultured in a fermentation medium in a fermentor. Alternatively, the microorganisms can be cultured photosynthetically in a photobioreactor or pond. Preferably, the microorganisms are lipid-rich microorganisms, more preferably, the microorganisms are selected from the group consisting of algae, bacteria, fungi and protists, more preferably, the microorganisms are selected from the group consisting of golden algae, green algae, dinoflagellates, yeast, fungi of the genus *Mortierella*, and Stramenopiles. Preferably, the microorganisms comprise microorganisms of the genus Mortierella, genus Crypthecodinium, and order Thraustochytriales, and more preferably, microorganisms are selected from the genus *Thraustochytrium*, *Schizochytrium* or mixtures thereof, more preferably, the microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and ATCC number 20892, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

The treatment of the cells includes a treatment to release the lipids such as lysing, rupturing or permeabilizing. As used herein, the terms lyse, lysing, lysed, etc., will be used generically to refer to a treatment to release intracellular lipids, including breaking or permeabilizing the cells. Preferably, the treatment is selected from the group consisting of heating the cells, exposing the cells to basic conditions, exposing the cells to a chelating compound or combinations thereof. More preferably the lysing or rupturing of the cells comprises heating the cells to at least 50°C while exposing the cells to basic conditions, a chelating compound or mixtures thereof.

Preferably, the gravity separation comprises passing the fermentation broth containing the released intracellular lipids through a centrifuge, such as stacked-disc-, separator- or decanter-type centrifuges.

AMENDED SHEET

The separated lysed cell mixture comprises a heavy layer which comprises an aqueous solution which contains the solid materials resulting from the lysed cells, and a light layer which contains lipids. The light and heavy layers can be separated by centrifugation. The lipids may be present in an emulsified state. The light layer can be further washed with an aqueous washing solution until the lipid becomes substantially non-emulsified. Preferably the treatment to break the emulsion comprises mixing the emulsion with water, alcohol, acetone or mixtures thereof and subjecting the mixture to gravity separation. Preferably, the process is performed without using nonpolar organic solvents such as hexane.

When the lipid extraction process of the present invention includes using microorganisms from a fermentation process, the extraction process can also include solubilizing at least part of proteinaceous compounds in a fermentation broth by adding a base selected from the group consisting of hydroxides, carbonates, bicarbonates, phosphates and mixtures thereof.

The process of the present invention can also include heating the microorganisms to temperature of at least about 50 °C. Preferably, a chemical compound, such as a base, is added to the culture medium to aid in the lysing of the cells.

As an alternative to heating, the cells can be lysed with the assistance of a chelating compound such as EDTA. In addition to assisting in lysing or rupturing the cells, the chelators assist in preventing oxidation of the lipids during processing by chelating (binding with) free radical-producing metal ions in the fermentation broth such as iron or copper. Preferred forms of chelators are those that are considered food grade or GRAS (Generally Recognized As Safe). Effective chelating compounds include EDTA, citric acid or citrate, lactic acid, trisodium phosphate, polyphosphate, hexametaphosphate, EGTA, DTPA, phytic acid, or CDTA and other salt forms of these compounds. In one embodiment, sodium EDTA is added to the cells to degrade the cell walls by chelating the divalent cations which assist in holding the cell walls together. The process can be performed at higher temperatures with less EDTA or lower temperatures with higher concentrations of EDTA. For example, we have found that DHA-rich cells of *Schizochytrium* sp. can be permeabilized and/or ruptured by addition of EDTA to the cultures at the end of the fermentation process. A concentration of 10,000 ppm is required to assist in rupturing the cells at 30°C, a concentration of 5,000 ppm at 50°C and at temperatures above 70°C concentrations of lower than 1000 ppm are effective. Chelators can be added to the fermentation broth to make the cells easier to break by

physical processes such as homogenization. In addition to a chelator, water can also be added to increase the internal osmotic pressure in order to lyse the cells.

Preferably, the microorganisms are capable of growth at salinity level of less than about 12 g/L of sodium chloride, more preferably less than about 5 g/L of sodium chloride and most preferably less than about 3 g/L of sodium chloride. Preferably, the microorganisms are capable of growth at salinity levels of less than about 7 g/L of sodium and less than about 250 mg/L of chloride. Preferably, the chloride is present in an amount from about 70 to about 150 mg/L.

Preferably, the microorganisms comprise at least about 20% by weight of lipid, more preferably at least about 30% by weight, and most preferably at least about 40%. Alternatively at least about 20% of the lipid is cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof, preferably at least about 30%, and more preferably at least about 40%.

In one particular aspect of the present invention the microorganisms are capable of producing at least about 0.1 grams per liter per hour of a mixture of lipids, preferably including cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof, more preferably at least about 0.2 g/L/h, still more preferably at least about 0.3 g/L/h, and most preferably at least about 0.4 g/L/h.

In another aspect of the present invention, the microorganism is selected from the group consisting of algae, fungi, bacteria and protists. Preferably, the microorganisms are of the order Thraustochytriales. More preferably the microorganisms are selected from the genus *Thraustochytrium*, *Schizochytrium* and mixtures thereof. And most preferably, the microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and ATCC number 20892, mutant strains derived from any

of the foregoing, and mixtures thereof. Preferably, the microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888 and ATCC number 20889, and more preferably ATCC number 20888, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a flow diagram of one embodiment of a solventless extraction process of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a process for extracting, recovering, isolating or obtaining lipids from microorganisms. The process of the present invention is applicable to extracting a variety of lipids from a variety of microorganisms, for example, extracting lipids containing cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof, more preferably, omega-3 highly unsaturated fatty acids, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and/or docosapentaenoic acid (DPA) (i.e., the omega-3 form of DPA), in particular lipids containing a relatively large amount of DHA, from microorganisms producing the same; and extracting lipids containing omega-6 highly unsaturated fatty acids, such as arachidonic acid and docosapentaenoic acid (DPA) (i.e., the omega-6 form of DPA)from microorganisms producing the same. Exemplary microorganisms which produce a relatively large amount of omega-3 highly unsaturated fatty acids are disclosed in commonly assigned U.S. Patent Nos. 5,340,594 and 5,340,742, both issued to Barclay, and exemplary microorganisms which produce a relatively large amount of arachidonic acid are disclosed in commonly assigned U.S. Patent No. 5,583,019, issued to Barclay. All of the above disclosed patents are incorporated herein by reference in their entirety.

For the sake of brevity, however, this detailed description of the invention is presented for purposes of convenience and illustration for the case of extracting lipids

comprising omega-3 highly unsaturated fatty acid from microorganisms producing the same, in particular extracting lipids from microorganisms that produce a relatively high amount of DHA. It is to be understood, however, that the invention as a whole is not intended to be so limited, and that one skilled in the art will recognize that the concept of the present invention will be applicable to other microorganisms producing a variety of lipid compositions in accordance with the techniques discussed herein. These microorganisms include microorganisms, such as fungi, protists, algae and bacteria, which produce a variety of lipids, such as phospholipids; free fatty acids; esters of fatty acids, including triglycerides of fatty acids; sterols; pigments (*e.g.*, carotenoids and oxycarotenoids) and other lipids, and lipid associated compounds such as phytosterols, ergothionine, lipoic acid and antioxidants including beta-carotene, tocotrienols, and tocopherol. Preferred lipids and lipid associated compounds include, but are not limited to, cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof. For the sake of brevity, unless otherwise stated, the term "lipid" refers to lipid and/or lipid associated compounds. Other lipids and microorganisms which may be suitable for use in the instant invention will be readily apparent to those skilled in the art.

Typical microbial lipid (in particular an oil containing an omega-3 highly unsaturated fatty acid such as DHA) manufacturing processes involve growing microorganisms which produce DHA in a fermentor, isolating the microorganisms, drying the microbial biomass and extracting the intracellular lipids with a non-polar organic solvent, *e.g.*, hexane. The extracted lipid is generally further refined to produce a high purity and/or quality lipid. The isolation of microorganisms involves diluting the fermentation broth with water and centrifuging the mixture to isolate microorganisms. When lipids are not extracted immediately or soon after isolating the microorganisms, the isolated microorganisms are typically dried, *for example*, on a drum dryer, and sealed in a package, *e.g.*, in vacuum-sealed bags, to prevent degradation of lipids. Unfortunately, the drying process exposes the microorganisms to heat, which can damage, *i.e.*, degrade the quality of, the lipid if done incorrectly. The package may develop leaks, which can further degrade the quality of the lipids. Furthermore, if the dried microorganisms are not

treated with an antioxidant, the exposure of microorganisms to air and/or light can further degrade the lipids.

Recovering the crude oil directly from the fermentation broth avoids these problems. Avoiding the non-polar organic solvent extraction step reduces manufacturing costs and also eliminates operator exposure to the dried microorganisms, which can cause an allergic response in some individuals.

The present invention provides a method for obtaining lipids from microorganisms using a substantially nonpolar organic solvent free extraction process, *i.e.*, a "solventless" extraction process. The term "solventless extraction process" refers to an extraction process which, when an aqueous or polar solvent is used, the aqueous or polar solvent includes less than about 5% of a nonpolar organic solvent, preferably less than about 4%, more preferably less than about 2%, and most preferably less than 1%. However, solvents can be employed in downstream steps such as in a refining process. The process of the present invention can include obtaining or isolating microorganisms, preferably from a fermentation process. In contrast to the prior art processes such as the extraction of oils from soybeans in which the soybeans must be dried, the process of the present invention does not require a drying step prior to the extraction process. Thus, processes of the present invention are applicable to extracting lipids from a microbial biomass containing at least about 10% by weight entrained water, preferably at least about 20%, more preferably at least about 30%, and most preferably at least about 50%. When the microorganisms are obtained from a fermentation process, the process of the present invention can include adding a base to the fermentation broth to dissolve any proteinaceous compound that may be present in the broth. "Bases" are substances which show alkali (basic) reactions in watery solutions, i.e. they attach protons and dissociate hydroxide ions. The base should be strong enough to hydrolyze or solubilize at least a portion of proteinaceous compounds that may be present in the broth. Bases which are useful for solubilizing proteins are well known to one of ordinary skill in the art of chemistry. Exemplary bases which are useful in the processes of the present invention include, but are not limited to, hydroxides, carbonates and bicarbonates of lithium, sodium, potassium, calcium, and magnesium carbonate. Other very basic compounds like basic phosphate salts, such as trisodium phosphate, can also be used.

The process of the present invention can also include rupturing or lysing the cells of microorganisms to release the lipids which are present within the cells. Cells can be lysed using any of the known methods including chemical; thermal; mechanical,

including, but not limited to, french press, mills, ultrasonication, homogenization, and steam explosion; and combinations thereof. In a thermal lysing of cells, the fermentation broth containing microorganisms are heated until cells, *i.e.*, cell walls, of microorganisms degrade or breakdown. Typically, the fermentation broth is heated to a temperature of at least about 50°C, preferably at least about 75°C, more preferably to at least about 100°C, and most preferably to at least about 130°C. An important aspect of the process is to maintain the temperature below that temperature at which the extracted lipids can be degraded. Thermally lysing the cell walls of microorganisms is particularly useful for microorganisms whose cell walls are composed of proteins. During this process the headspace of the fermentor can be filled with nitrogen or another inert gas to prevent oxidation of the lipids by oxygen.

Heating the broth also denatures proteins and helps solubilize organic materials, including proteins. Heating of the fermentation broth step can be achieved by any of the known methods, including the use of an in-line heat exchanger, and preferably by sparging steam into the fermentation broth and maintaining the broth at a desired temperature for less than about 90 minutes, preferably less than about 60 minutes, and more preferably less than about 30 minutes.

The solventless extraction process of the present invention can also include at least partially separating the spent fermentation broth from the lipids. Typically, this is achieved by centrifuging, *e.g.*, by passing the broth through a stacked-disc, separator or decantor centrifuge, and collecting lipids as an emulsion phase. Centrifuging the mixture results in a two phase mixture comprising a heavy layer and a light layer. Typically, the heavy layer is an aqueous phase, which contains the majority of cellular debris. The light layer which contains emulsified lipids is then diluted with water, again separated into two phase mixture and the light layer is again isolated. This dilution with water, separation and isolation processes (*i.e.*, washing process) can be achieved continuously by feeding water and removing the heavy layer throughout the process or it can be conducted in discreet steps. The washing process is generally repeated until a substantially non-emulsified lipid layer is obtained, although minor amounts of emulsion can remain. It is believed that the oil-water interface of the emulsion is stabilized by residual cellular debris which is removed by the washing process. During the washing process, the successive amount of water added is reduced to increase the lipid content. While reducing the amount of feed water too quickly can result in loss of lipids to the aqueous phase, reducing the amount of feed water too slowly results in an inefficient washing

AMENDED SHEET

process. One can readily determine an appropriate rate of feed water reduction by observing or analyzing the separated aqueous layer. Generally, the lipid layer, *i.e.*, the light layer, is colored; therefore, in many cases one can determine an appropriate rate of feed water reduction by simply analyzing or observing the color of the aqueous layer which is separated from the lipid layer.

Alternatively, and preferably, the emulsion can be broken, and the oil recovered using the deoiling process outlined in WO 96/05278 which is incorporated herein by reference in its entirety. In this process a water soluble compound, e.g., alcohol and/or acetone, is added to the oil/water emulsion to break the emulsion and the resulting mixture is separated by centrifugation. The isolated lipid can be further refined using a process similar to that used to refine standard vegetable oils. Briefly, the lipid refining process generally involves hydrating phospholipids by adding phosphoric acid to the lipid followed by adding sodium hydroxide to neutralize free fatty acids. These compounds are removed via centrifugation. This is then followed by a water wash step to further remove any remaining amounts of hydrated phospholipids ("gums") and neutralized fatty acids ("soapstock") in the lipid. The resulting lipid is bleached using Trysil™ and a standard bleaching clay. Citric acid is also added to remove divalent metal ions by chelation. The Trysil™ and bleaching clay are then removed via filtration to produce refined lipid. The bleached lipid can be cold filtered to remove high melting point compounds that may be present in the lipid; however, this step is generally seldom required.

The resulting lipid can be further refined by removing any low molecular weight components that may be present. Typically, these components are removed by sparging with steam at high temperatures, under high vacuum. This process also destroys any peroxide bonds which may be present and reduces or removes off- odors and helps improve the stability of the oil. An antioxidant may then be added to the resulting deodorized lipid to improve product stability.

During the refining process, the isolated lipid can be winterized to remove high melting compounds, such as saturated fatty acids. The winterization process generally involves dissolving the isolated lipid in an organic solvent, *e.g.*, hexane, cooling the resulting organic solution, and filtering the solution to remove the high melting point components of the lipid or stearine phase. The winterization process generally produces a clear lipid, especially when the isolated lipid is cloudy or opaque. As will be appreciated, the use of a solvent such as hexane is acceptable in processes such as the above described

"refining" process. Alternatively, the isolated lipid can be chilled and solidified impurities can be filtered out, without employing a solvent.

The refining, bleaching, deodorizing process outlined above would be used for triglyceride-rich lipid mixtures. Alternatively, or in addition to this process, other lipids, for example, pigments or carotenoids can be separated and purified, e.g., by partition into various solvents, chromatographic methods, etc.

While, the process of the present invention can include isolating microorganisms from a fermentation process, one of the advantages of the present invention is that it allows fermentation of microorganisms and isolation of lipids to be carried out in a single vessel. For example, after the fermentation, one can add base to the fermentation vessel and heat the mixture to lyse cells. After separating the phase into a heavy layer and a light layer, the light layer can be transferred to another vessel for further processing or the heavy layer can be removed from the fermentation vessel, for example, by draining through the bottom of the fermentation vessel, and the remaining light layer can be further processed within the same fermentation vessel.

If the concentration of lipids in the cells of the microbial culture is high (e.g., greater than about 20%) but the concentration of cells is low (e.g., less than about 40 g/L), as in cells grown in continuous fermentation systems, or cultures of difficult (e.g., fragile) to grow cells, or cultures produced in photosynthetically-based culture systems, the cells can be concentrated, e.g., by centrifugation, filtration, or settling, prior to employing the methods of the invention if necessary.

Additional objects, advantages, and novel features of this invention will become apparent to those skilled in the art upon examination of the following examples thereof, which are not intended to be limiting.

Examples

Process reproducibility was characterized by producing three samples of fully refined oil using crude oil from the new solventless extraction process. A hexane-extracted sample was also fully refined to serve as a control. The fermentation, extraction and oil isolation steps were performed at a large scale, while the oil refining studies were performed at a small scale.

The fully refined oil samples were analyzed to demonstrate process reproducibility.

Fermentation:

An oil-rich microorganism *(Schizochytrium sp.)* was grown in a 1200 gallon fermentor to produce a fermentation broth for the following extraction processes. A single batch was used to generate the starting broth for the three solventless extraction processes. The fermentation was allowed to run for 94 hours, while controlling the glucose levels at 13 g/L, after which time the corn syrup feed was terminated. Residual glucose levels dropped to <5 g/L four hours later. This resulted in a final age of 98 hours. The final broth volume was 958 gallons. The final yield was 146 g/L dry weight of cells. Both in-process contamination checks and a thorough analysis of a final broth sample failed to show any signs of contamination.

Hexane-Extracted Control Sample:

A small aliquot of broth from the fermentation batch was drum-dried and extracted with hexane to serve as a control sample. The biomass intermediate was recovered using a double-drum dryer. Analysis of this lipid is shown in Table 1.

Table 1. Analysis of drum dried *Schizochytrium* sp. biomass.

Parameter	Value
DHA Content (FAME basis)	35.7%
Oil Content	62.7%
Peroxide Value (meq/kg)	2.6
Total Plate Count (cfu/g)	<50
DHA Content*	20.3%
FAME Content	56.9%

* cellular dry weight basis

Solventless Extraction Process:

Crude oil was obtained by treating three 400-gallon aliquots (approx.) of the fermentation broth. Each 400-gallon aliquot from the fermentor was processed separately, starting with the caustic/heat treatment steps. Each aliquot was treated with 20 grams of 45% KOH per liter and heated to 130°C for about 30 minutes by passing steam through the fermentation broth. The crude oil was recovered from the treated broth using a commercial-scale Westfalia HFA-100 stacked-disc centrifuge. Summary results for various process parameters are reported in Table 2, and the final crude oil analysis results are shown in Table 3.

Table 2. Process Data from the Solventless Extraction Process.

	SFE-1	SFE-2	SFE-3
Broth Treatment			
Volume of Broth Processed	288 gal	288 gal	258 gal
Final Treated pH	7.5	8.0	8.7
Final Volume After Heat Treatment	388 gal	398 gal	308 gal
Volume Increase From Condensate	34.7%	38.2%	19.4%
1st Pass Emulsion			
Total Volume (gal)	180	133	149
Est. Oil Concentration (w/w)	12.0%	24.5%	16.1%
Apparent Density (g/mL)	0.986	0.991	0.999
Oil Isolation			
Total Crude Oil Recovered (lb)	182	165	174
DHA Oil Lot Number Assigned	SF1A	SF2A	SF3A

Table 3. Analysis of Lots of DHA oil from the Solventless Extraction Process.

Parameter	SF1A	SF2A	SF3A
DHA Content (%FAME)	39.0%	38.6%	39.2%
Peroxide Value (meq/kg)	4.6	1.8	2.0
Acid Value (mg KOH/g)	N/D	N/D	N/D
Moisture Content	N/D	N/D	N/D

Refining:

A sample from each aliquot of crude oil was winterized, refined, bleached and deodorized at a small scale, as was a sample of the crude oil from the hexane-extracted control. Miscellaneous process data from these small scale experiments is shown in Table 4, including recovery efficiencies for the various processing steps. While it is difficult to read too much into recovery efficiencies for bench-scale processes, as losses tend to be disproportionately large, the values listed in Table 4 show that values for the solventless-extracted samples tend to bracket the values measured for the hexane-extracted control, with the one exception being the winterization step. While the recovery efficiency during the winterization step for the hexane control was lower than those observed for the other three samples, this difference is insignificant from a statistical perspective. The high losses during the winterization step caused the overall recovery efficiency for the hexane-control sample to be lower as well. The lower yield would not be expected to have a significant impact on the overall quality of the oil. All in all, differences in the processing of the various oil samples were minimal.

AMENDED SHEET

Table 4. Miscellaneous Process Data from the Oil Refining Steps.

	HEX-1	SF1A	SF2A	SF3A
Processing Conditions				
Miscella Concentration	45.0%	52.9%	52.8%	45.0%
Steam Sparge Rate	3.4%	3.4%	2.5%	2.2%
Recovery Efficiencies				
Winterization	80.6%	92.3%	87.7%	85.5%
Refining	89.4%	84.8%	91.8%	95.0%
Water Wash	90.6%	94.5%	95.8%	81.2%
Bleaching	86.1%	89.2%	87.3%	84.1%
Deodorization	97.4%	96.1%	97.2%	97.5%
Packaging	88.2%	89.7%	89.3%	95.8%
Overall	**48.2%**	**56.9%**	**58.5%**	**51.8%**

Fully refined oil samples from the three solventless extraction runs, and the hexane-extracted control, were analyzed and the results are shown in Table 5. Also shown are the corresponding release specifications for each parameter.

A sample of the starting crude oil from the solventless extraction run was also analyzed for iron content. The iron content of this sample was 0.08 ppm. The concentrations of the other trace metals were all below their respective detection limits.

Table 5. QC Results for RBD DHA Oil from the Solventless Extraction Process compared to hexane extracted oil.

	Hexane	Solventless Extrac. Process		
Run ID #	HEX-1	SFA1	SFA2	SFA3
Peroxide Value (meq/kg)	0.28	0.69	0.35	0.34
Acid Value (mg KOH/g)	0.17	0.11	0.57	0.24
Moisture & Volatiles	0.00%	0.06%**	0.00%	0.00%
Trace Metals (ppm)				
Lead	<0.20	<0.20	<0.20	<0.20
Arsenic	<0.20	<0.20	<0.20	<0.20
Iron	0.22	0.21	0.56***	0.02
Copper	<0.05	<0.05	<0.05	<0.05
Mercury	<0.20	<0.20	<0.20	<0.20
DHA (%FAME)	36.9	37.3	37.0	37.7
DHA (mg/g oil)	342	345	343	351
Hexane (ppm)	<3	<3	<3	<3

* Value was reduced to 0.22 mg KOH/g after repeating the refining and bleaching steps

** Sample analyzed by the San Diego Fermentation Sciences Analytical Group.

*** Value was reduced to <0.02 ppm after repeating the refining and bleaching steps

AMENDED SHEET

Shown in Table 6 is a more direct comparison of the average analysis results for the three samples from the solventless extraction process versus those for the hexane control.

Table 6. Comparison of Average Values.

	Hexane	Solventless Extraction			
Parameter	Control	Mean	Std Dev	CV	%Diff
Peroxide Value (meq/kg)	0.28	0.46	0.20	43.3%	64.3%
Acid Value (mg KOH/g)	0.17	0.19*	0.06	33.3%	11.2%
Moisture & Volatiles	0.00%	0.02%	0.03%	173%	ND
Trace Metals (ppm)					
Lead	<0.20	<0.20	N/A	N/A	0.0%
Arsenic	<0.20	<0.20	N/A	N/A	0.0%
Iron	0.22	0.26	0.27	104%	18.2%
Copper	<0.05	<0.05	N/A	N/A	0.0%
Mercury	<0.20	<0.20	N/A	N/A	0.0%
DHA Content (%FAME)	36.9%	37.3%	0.4%	0.9%	1.1%
DHA Content (mg/g)	342	346	4	1.2%	1.2%
Hexane (ppm)	<3	<3	N/A	N/A	0.0%

* Calculated using the acid value for the re-worked sample.

The results from this experiment clearly demonstrate that the solventless extraction process is both reproducible and lipids from solventless extraction are relatively indistinguishable from the lipids obtained from hexane extraction process in terms of process performance and product quality. The final product from the solventless extraction process is substantially equivalent to lipids from a current hexane-based extraction process, as determined by similarities between the fatty acid and sterol profiles of the product from these two processes.

The present invention, in various embodiments, includes components, methods, processes, systems and/or apparatus substantially as depicted and described herein, including various embodiments, subcombinations, and subsets thereof. Those of skill in the art will understand how to make and use the present invention after understanding the present disclosure. The present invention, in various embodiments, includes providing devices and processes in the absence of items not depicted and/or described herein or in various embodiments hereof, including in the absence of such items as may have been used in previous devices or processes, *e.g.*, for improving performance, achieving ease and/or reducing cost of implementation.

The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, *e.g.*, as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

WHAT IS CLAIMED IS:

1. A process for obtaining lipid from microorganisms comprising:

(a) lysing cells of the microorganisms to produce a lysed cell mixture;

(b) treating said lysed cell mixture to produce a phase separated mixture comprising a heavy layer and a light layer, wherein said heavy layer comprises an aqueous solution and said light layer comprises said lipid;

(c) separating said heavy layer from said light layer; and

(d) obtaining said lipid from said light layer.

2. The process of Claim 1, wherein said step (b) comprises centrifuging said lysed cell mixture.

3. The process of Claim 2, wherein said light layer comprises an emulsified lipid.

4. The process of Claim 3 further comprising:

(e) adding an aqueous extraction solution to said light layer of step (c); and

(f) repeating said steps (c), (d) and (e) until said lipid becomes substantially non-emulsified prior to said step (d).

5. The process of Claim 3, wherein said emulsified lipid comprises a suspension of said lipid in an aqueous solution.

6. The process of Claim 1, wherein said aqueous solution comprises solid cell materials.

7. The process of Claim 1, wherein said microorganisms are obtained from a fermentation process.

8. The process of Claim 7 further comprising adding a base to a fermentation broth.

9. The process of Claim 8, wherein said base is selected from the group consisting of hydroxides, carbonates, bicarbonates, phosphates and mixtures thereof.

10. The process of Claim 7 further comprising solubilizing at least part of proteinaceous compounds in a fermentation broth.

11. The process of Claim 1, wherein said step (a) comprises heating said microorganisms to temperature of at least about 50°C.

12. The process of Claim 1, wherein said microorganism is capable of growth at salinity level of less than about 12 g/L of sodium chloride.

13. The process of Claim 1, wherein said microorganism comprises at least about 20% by weight of lipid.

AMENDED SHEET

14. The process of Claim 1, wherein said microorganism is selected from the group consisting of algae, fungi, bacteria and protists.

15. The process of Claim 14, wherein said microorganisms comprise microorganisms of the order Thraustochytriales.

16. The process of Claim 15, wherein said microorganisms are selected from the genus *Thraustochytrium*, *Schizochytrium* and mixtures thereof.

17. The process of Claim 16, wherein said microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and ATCC number 20892, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

18. The process of Claim 1, wherein said microorganisms are capable of producing at least about 0.1 grams per liter per hour of cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

19. The process of Claim 1, wherein at least about 20% of said lipid is cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

20. A process for obtaining lipids from microorganisms comprising:

(a) growing said microorganisms in a fermentation medium to produce a fermentation broth;

(b) solubilizing at least a part of any proteins present in said fermentation broth;

(c) lysing cells of said microorganisms to produce a lysed cell mixture;

(d) treating said lysed cell mixture to produce a phase separated mixture comprising a heavy layer and a light layer, wherein said heavy layer comprises an aqueous solution and said light layer comprises emulsified lipids;

(e) separating said heavy layer from said light layer; and

(f) obtaining said lipids from said light layer.

21. The process of Claim 20, wherein said step of dissolving proteins comprises contacting said fermentation broth with a base.

22. The process of Claim 21, wherein said base is selected from the group consisting of hydroxides, carbonate, bicarbonates, phosphates and mixtures thereof.

23. The process of Claim 20, wherein said step of lysing cells comprises heating said microorganisms to a temperature of at least about 50°C.

24. The process of Claim 20, wherein said step of producing the phase separated mixture comprises centrifuging said lysed cell mixture.

25. The process of Claim 20, wherein said step of obtaining said lipids from said light layer comprises:

(a) adding an aqueous washing solution to said light layer;

(b) separating said aqueous washing solution from said light layer; and

(c) repeating said steps (a) and (b) until said lipid becomes substantially non-emulsified.

26. The process of Claim 20, wherein said aqueous solution comprises solid cell materials.

27. The process of Claim 20, wherein said microorganism is capable of growth at salinity level of less than about 12 g/L of sodium chloride.

28. The process of Claim 20, wherein said microorganism comprises at least about 20% by weight of lipid.

29. The process of Claim 20, wherein said microorganism is selected from the group consisting of algae, fungi, bacteria and protists.

30. The process of Claim 29, wherein said microorganisms comprise microorganisms of the order Thraustochytriales.

31. The process of Claim 30, wherein said microorganisms are selected from the genus *Thraustochytrium*, *Schizochytrium* and mixtures thereof.

32. The process of Claim 31, wherein said microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and

AMENDED SHEET

JT/US 00/01806
IPEA/US 26 NOV 2001

ATCC number 20892, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

33. The process of Claim 20, wherein said microorganisms are capable of producing at least about 0.1 grams per liter per hour of cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

34. The process of Claim 20, wherein at least about 20% of said lipid is cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

35. A process for obtaining lipids from microorganisms comprising:

(a) growing said microorganisms in a fermentation medium to produce a fermentation broth;

(b) contacting said fermentation broth with a base to dissolve at least a part of any proteins present in said fermentation broth;

(c) increasing the temperature of said fermentation broth to at least about 50°C to lyse cells of said microorganisms to produce a lysed cell mixture;

(d) separating substances of different densities from said lysed cell mixture to produce a phase separated mixture comprising a heavy layer and a light layer, wherein said heavy layer comprises an aqueous solution and said light layer comprises emulsified lipids;

(e) removing said heavy layer from said phase separated mixture;

(f) adding an aqueous washing solution to said light layer;

(g) separating substances of different densities from said mixture of step (f);

(h) removing said heavy layer from said phase separated mixture; and

(i) repeating said steps (f)-(h) until said lipid becomes substantially non-emulsified.

AMENDED SHEET

36. The process of Claim 35, wherein said base is selected from the group consisting of hydroxides, carbonates, bicarbonates, and mixtures thereof.

37. The process of Claim 35, wherein said step of producing the phase separated mixture comprises centrifuging said lysed cell mixture.

38. The process of Claim 35, wherein said aqueous solution of step (d) comprises solid cell materials.

39. The process of Claim 35, wherein said microorganism is capable of growth at salinity level of less than about 12 g/L of sodium chloride.

40. The process of Claim 35, wherein said microorganism comprises at least about 20% by weight of lipid.

41. The process of Claim 35, wherein said microorganism is selected from the group consisting of algae, fungi, bacteria and protists.

42. The process of Claim 35, wherein said microorganisms comprise microorganisms from the group consisting of golden algae, green algae, dinoflagellates, yeast, fungi of the genus *Mortierella*, and Stramenopiles.

43. The process of Claim 35, wherein said microorganisms comprise microorganisms of the order Thraustochytriales.

44. The process of Claim 43, wherein said microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and ATCC number 20892, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

45. The process of Claim 35, wherein said microorganisms are capable of producing at least about 0.1 grams per liter per hour of cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

46. The process of Claim 35, wherein at least about 20% of said lipid is cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-

PCT/US 01/01806
PEA/US 08 MAY 2002

6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

47. A process for obtaining lipids from microorganisms comprising:

(a) growing microorganisms in a culture medium;

(b) treating said culture medium and the microorganism cells to release intracellular lipids;

(c) subjecting the culture medium containing the released intracellular lipids to gravity separation to form a light lipid-containing phase and a heavy phase;

(d) separating said light phase from said heavy phase;

(e) treating said light phase to break an emulsion formed between said lipid and water; and

(f) recovering a crude lipid.

48. The process of Claim 47 wherein said microorganisms are lipid-rich microorganisms.

49. The process of Claim 47 wherein said microorganisms are selected from the group consisting of algae, bacteria, fungi and protists.

50. The process of Claim 47 wherein said microorganisms are selected from the group consisting of golden algae, green algae, dinoflagellates, yeast, fungi of the genus *Mortierella*, and Stramenopiles.

51. The process of Claim 47, wherein said microorganisms comprise microorganisms of the order Thraustochytriales.

52. The process of Claim 51, wherein said microorganisms are selected from the genus *Thraustochytrium*, *Schizochytrium* and mixtures thereof.

53. The process of Claim 52, wherein said microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and ATCC number 20892, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

54. The process of Claim 47, wherein said microorganisms are capable of producing at least about 0.1 grams per liter per hour of cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty

AMENDED SHEET

acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

55. The process of Claim 54, wherein at least about 20% of said lipid is cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

56. The process of Claim 47, wherein said treatment includes a treatment selected from the group consisting of heating said cells, exposing said cells to a basic compound, exposing said cells to a chelating compound or combinations thereof.

57. The process of Claim 47, wherein said treatment comprises heating the cells to at least 50°C before, during or after exposing the cells to a basic compound, a chelating compound or mixtures thereof.

58. The process of Claim 47, wherein said gravity separation of step (c) comprises passing the culture medium containing the released intracellular lipids through a stacked-disc, separator or decanter centrifuge.

59. The process of Claim 47, wherein the treatment of step to break the emulsion comprises mixing the emulsion with water, alcohol and/or acetone and subjecting the mixture to gravity separation.

60. The process of Claim 59, wherein said gravity separation comprises centrifugation.

61. The process of Claim 60, wherein said centrifugation includes treatment in a stacked-disc-, separator- or decanter-type centrifuge.

62. The process of Claim 59, wherein said treatment is repeated at least 3 times to obtain said crude lipid.

63. The process of Claim 47, wherein said crude lipid is subjected to further refining or processing to obtain a refined lipid.

64. The process of Claim 63, wherein said crude lipid is bleached and deodorized.

65. A process for recovering lipids from microorganisms comprising the steps:

AMENDED SHEET

PCT/IS 01/01806
PEA/IS 06 MAY 2002

(a) growing said microorganisms in a culture medium;

(b) treating microorganism cells from said culture medium without drying said cells to release intracellular lipids;

(c) subjecting the culture medium containing the released intracellular lipids to gravity separation to form a light lipid-containing phase and a heavy phase;

(d) separating said light phase from said heavy phase;

(e) treating said light phase to break an emulsion formed between said lipid and water; and

(f) recovering a crude lipid.

66. The process of Claim 65 wherein said microorganisms are lipid-rich microorganisms.

67. The process of Claim 65 wherein said microorganisms are selected from the group consisting of algae, bacteria, fungi and protists.

68. The process of Claim 65 wherein said microorganisms are selected from the group consisting of golden algae, green algae, dinoflagellates, yeast, fungi of the genus *Mortierella*, and Stramenopiles.

69. The process of Claim 65, wherein said microorganisms comprise microorganisms of the order Thraustochytriales.

70. The process of Claim 69, wherein said microorganisms are selected from the genus *Thraustochytrium*, *Schizochytrium* and mixtures thereof.

71. The process of Claim 70, wherein said microorganisms are selected from the group consisting of microorganisms having the identifying characteristics of ATCC number 20888, ATCC number 20889, ATCC number 20890, ATCC number 20891 and ATCC number 20892, strains of *Mortierella schmuckeri*, strains of *Crypthecodinium cohnii*, mutant strains derived from any of the foregoing, and mixtures thereof.

72. The process of Claim 65, wherein said microorganisms are capable of producing at least about 0.1 grams per liter per hour of cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

AMENDED SHEET

73. The process of Claim 72, wherein at least about 20% of said lipid is cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof.

74. The process of Claim 65, wherein said treatment of the cells includes a treatment selected from the group consisting of heating said cells, exposing said cells to basic conditions, exposing said cells to a chelating compound or combinations thereof.

75. The process of Claim 65, wherein said treatment of the cells comprises heating the cells to at least 50°C before, during or after exposing the cells to a basic condition, a chelating compound or mixtures thereof.

76. The process of Claim 65, wherein said gravity separation of step (c) comprises passing the culture medium containing the released intracellular lipids through a stacked-disc, separator or decanter centrifuge.

77. The process of Claim 65, wherein the treatment to break the emulsion comprises mixing the emulsion with water, alcohol and/or acetone and subjecting the mixture to gravity separation.

78. The process of Claim 77, wherein said gravity separation comprises centrifugation.

79. The process of Claim 78, wherein said centrifugation includes treatment in a stacked-disc-, separator- or decanter-type centrifuge.

80. The process of Claim 77, wherein said treatment is repeated at least 3 times to obtain said crude lipid.

81. The process of Claim 65, wherein said crude lipid is subjected to further refining or purification to obtain a refined lipid.

82. The process of Claim 81, wherein said crude lipid is bleached and deodorized.

83. A lipid made by the process of any one of claims 1-82.

84. A lipid derived from microorganisms with less than 0.2ppm residual nonpolar organic solvent.

85. A lipid comprising greater than 15% cholesterol, phytosterols, desmosterol, tocotrienols, tocopherols, ubiquinones, carotenoids and xanthophylls such as

beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin, canthaxanthin, and fatty acids such as conjugated linoleic acids, and omega-3 and omega-6 highly unsaturated fatty acids such as eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, arachidonic acid, stearidonic acid, dihomogammalinolenic acid and gamma-linolenic acid or mixtures thereof with less than 0.2 ppm residual nonpolar organic solvent

86. A lipid comprising greater than 15% docosahexaenoic acid and less than 0.2 ppm residual nonpolar organic solvent.