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(54) Title: BIODIESEL PRODUCTION

(57) Abstract: A process for producing alkyl fatty acid esters suitable for use as a fuel and/or lubricant. The process includes treating a feedstock containing glycerides and/or free fatty acids with a lipase, a lower alkyl alcohol and an acid catalyst under conditions to produce said alkyl fatty acid esters.

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BIODIESEL PRODUCTION

This application claims priority from Australian provisional patent application No. 2007900488 filed on 2 February 2007, the contents of which are to be taken as
5 incorporated herein by this reference.

Field of the Invention

The present invention relates to processes for the production of alkyl fatty acid ester
10 mixtures that are suitable for use as fuels, such as biodiesel, fuel additives and lubricants. The present invention also relates to fuels, such as biodiesel, produced using the processes, and to systems for producing the fuels.

Background of the Invention

15

Over 85% of the energy demands of modern society are met by the combustion of fossil fuels, such as coal, oil, and natural gas. However, fossil fuels are a non-renewable source of energy and our supply of these fuels has a finite end.

20 The environmental, health, political and social impacts of our reliance on fossil fuels are well known. The burning of fossil fuels by humans is the largest source of emissions of carbon dioxide, which is one of the greenhouse gases that contributes to global warming.

25 Alternative forms of energy are needed to help meet the increased global energy needs. Examples of alternative energy forms that are being investigated include nuclear energy, hydrogen fuels, and solar energy. However, each of these alternatives requires substantive redevelopment of infrastructure to enable their sustained and economical use.

30

Biofuels are an alternative to fossil fuels that do not have the same negative environmental impacts as fossil fuels because they are derived from atmospheric carbon dioxide, and thus do not increase the net amount of carbon dioxide in the atmosphere. Furthermore, the use of biofuels may not require substantive changes to
35 existing infrastructure and machinery as may be required from some other alternative energy sources.

Biodiesel is a biodegradable transportation fuel for use in diesel engines that is produced from plant- or animal-derived oils or fats. Biodiesel is used as a component of diesel fuel or as a replacement for diesel fuel. Biodiesel can be readily used in diesel-engine vehicles, which distinguishes biodiesel from the straight vegetable oils (SVO) or waste vegetable oils (WVO) used as fuels in some modified diesel vehicles. Biodiesel is biodegradable and non-toxic, and has significantly fewer emissions than petroleum-based diesel when burned and, therefore, its use can result in substantial environmental benefits.

Biodiesel manufactured from animal-derived fats show greater lubricity indexes than mineral- or plant-derived diesel/biodiesel fuels or mixtures, having a significant influence on engine maintenance by reducing engine component wear (for example see "Diesel Fuel Lubricity Reviewed", Paul Lacey, Sowthwest Research Institute, Steve Howell, MARC-IV Consulting, Inc., SAE paper 982567, International Fall Fuels and Lubricants Congress and Exposition, Oct. 19-22, 1998, San Francisco, California.)

Furthermore, biodiesel manufactured from animal-derived fats and high saturated plant fats have Cetane Index levels in excess of the ASTM D6751-07B Biodiesel Standard, which specifies a minimum requirement of a Cetane No. of 47. The average Cetane Index levels indicated by numerous studies by Universities and Research Institutes world wide indicate Cetane Numbers for biodiesel from animal fats ranging from approx 72 for biodiesel from lard to 61 for yellow grease biodiesel (for example see National Biodiesel Board "NBB, 2005a).

The use of biodiesel as an alternative to petroleum diesel is widely welcomed by environmental groups and the community. Biodiesel is Europe's dominant renewable fuel and, as part of a range of measures to reduce greenhouse gas emissions, the European Union is encouraging the use of biofuels. For example, the 2003 EU Biofuels Directive requires 5.75% of the energy for transport to come from renewable sources by the end of 2010, rising to 20% by 2020.

Biodiesel is comprised of a mix of mono-alkyl esters of long chain fatty acids, and is typically produced by transesterification of vegetable oil or animal fat with methanol using acid or basic catalysts. For example, International patent application

WO03/022961 describes a method for producing biofuel from waste oil or oil by-products by transesterification with methanol in the presence of sulfuric acid. In another example, International patent application WO2006/128881 describes a method for producing biodiesel from rapeseed oil or sunflower oil by transesterification with methanol in the presence of sodium hydroxide. There have also been suggestions that biodiesel can be produced from vegetable oils by an enzyme (*Candida cylindracea*) catalysed transesterification reaction (for example see published United States patent application 2005/0084941).

Whilst the integration of biodiesel as a transport fuel has generally been successful, there remain some problems with its use. For example, one of the physical properties of biodiesel that can lead to problems in use is the temperature at which the fuel starts to gel (the 'gel temperature'). The viscosity of biodiesel increases as it is cooled and the solution eventually crystallizes. Eventually, the crystals formed become large enough to block the pores of a fuel filter. The gel temperature of pure biodiesel varies depending on the glyceride composition from which it is formed and, therefore, the feedstock oil used to produce the biodiesel. Generally, the higher the saturated fatty acid content of the glyceride composition, the higher the gel temperature of the biodiesel formed from that material. For example, biodiesel produced from tallow, palm oil or coconut oil using the prior art methods tends to gel at around +15 to 16 °C. Many of these biodiesel fuels also have poor long term stability.

There is a need for processes for the production of new biodiesel fuels that do not have some of the disadvantages of prior art biodiesel fuels.

A reference herein to a patent document or other matter which is given as prior art is not to be taken as an admission that that document or matter was known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

Summary of the Invention

The present invention arises out of studies into processes for producing biodiesel from beef tallow. We have found that beef tallow, and other suitable animal fats and plant oils, can be treated with lipases and transesterified with ethanol or other lower alkyl

alcohols to produce a biodiesel fuel that has superior properties to many currently available biodiesel fuels.

5 The present invention provides a process for producing alkyl fatty acid esters suitable for use as a fuel and/or lubricant, the process including treating a feedstock containing glycerides and/or free fatty acids with a lipase, a lower alkyl alcohol and an acid catalyst under conditions to produce said alkyl fatty acid esters.

10 The feedstock containing glycerides and/or free fatty acids may be initially treated with the lipase in the presence of the lower alkyl alcohol to produce a first alkyl fatty acid ester mixture which is subsequently reacted with the lower alkyl alcohol in the presence of the acid catalyst to produce a second alkyl fatty acid ester mixture which is suitable for use as a fuel and/or lubricant.

15 The present invention also provides an alkyl fatty acid ester mixture prepared according to the process of the invention.

The present invention further provides a biodiesel fuel prepared according to the process of the invention.

20

The present invention also provides a system for use in the process for producing alkyl fatty acid esters suitable for use as a fuel and/or lubricant according to the invention.

25 The feedstock containing glycerides and/or free fatty acids may be an animal fat or plant oil. Indeed, the process of the present invention is particularly suitable for producing biodiesel from beef tallow.

30 The lipase may be a latex lipase and/or a plant lipase from a high lipid content grain, legume or seed. The latex lipase from the papaya plant is suitable in combination with a plant lipase from rice bran. The active latex lipase may be a papain lipase, such as *Carica papaya* latex lipase, and the plant lipase may be rice bran lipase. However, it will also be appreciated that the latex lipase and/or the plant lipase may not be a single lipase and may indeed be a mixture of lipases. The active lipases may be partially or wholly purified and used in the process of the present invention. Latex
35 lipases and plant lipases from a number of other plant species may also be used in

the process of the present invention. The lipase may also be a suitable lipase of microbial origin.

Without intending to be bound by any specific theory, we believe that the lipase may display some selectivity for smaller chain length fatty acids in the feedstock. The lipase may also catalyse the transesterification of the naturally occurring glycerides in the plant oil or animal fat. Furthermore, the effect of the acid catalyst is to further assist in the transesterification reaction. We believe that the effect of the lipase and acid catalyst is to provide a biodiesel fuel product that is stable and which has a lower gel temperature than an equivalent product that is formed without the use of the lipase and acid catalyst combination.

The lipase may be immobilized on a substrate. For example, the latex lipase may be immobilised on a substrate formed from a saccharide and lignite. In an embodiment of the invention, the substrate is a pyrolised mixture of a saccharide and an acid precipitate of an alkaline lignite extract.

The lower alkyl alcohol may be selected from one or more of the group consisting of: methanol, ethanol, n-propanol, i-propanol, n-butanol, s-butanol, and t-butanol. In an embodiment of the invention, the lower alkyl alcohol is ethanol.

The acid catalyst may be a mineral acid, such as sulphuric acid.

The reaction of the first alkyl fatty acid ester mixture with the lower alkyl alcohol and the acid catalyst may be carried out under continuous reactive distillation conditions whereby any unreacted lower alkyl alcohol that is distilled from the reaction is returned to the reaction mixture and the second alkyl fatty acid ester mixture is distilled from the reaction mixture.

The distilled second alkyl fatty acid ester mixture may be treated, such as by gravity separation, to remove unreacted glycerine and soap. The purified second alkyl fatty acid ester mixture thus obtained may be used as a biofuel. However, we have also found that it is advantageous to mix the second alkyl fatty acid ester mixture with a rice bran oil ester mixture to form an alkyl fatty acid ester mixture suitable for use as a fuel. The rice bran oil ester mixture may be prepared by reacting rice bran oil with a lower alkyl alcohol in the presence of methane sulphonic acid. The lower alkyl alcohol

may be n-butanol. The n-butanol may be formed by enzymatic hydrolysis of the glycerine and soap mixture that is separated from the distilled second alkyl fatty acid mixture.

5 Brief Description of the Figures

The present invention will now be described in relation to various embodiments of which some aspects are illustrated in the accompanying figures. In the figures:

10 Figure 1 is a flow diagram showing details of a process for producing biodiesel in accordance with an embodiment of the present invention.

Figure 2(a) is a first part of a flow diagram showing details of an early part of a process for producing biodiesel in accordance with an embodiment of
15 the present invention.

Figure 2(b) is a second part of a flow diagram that is a continuation of Figure 2(a) showing details of a later part of the process shown in Figure 2(a).

Description of Embodiments of the Invention

20

Various embodiments of the present invention will now be described in more detail. However, it must be appreciated that the following description is not to limit the generality of the above description. Numerals corresponding to reference numerals used in Figures 1 to 2 are used in the following description.

25

The present invention provides a process for producing alkyl fatty acid esters suitable for use as a fuel and/or lubricant. The process is particularly useful for the production of high quality biodiesel from relatively low grade feedstocks.

30 The process includes treating a feedstock 12 containing glycerides and/or free fatty acids with a lipase 14, a lower alkyl alcohol 16 and an acid catalyst 18 under conditions to produce alkyl fatty acid esters.

The feedstock 12 containing glycerides and/or free fatty acids is treated with the
35 lipase 14, the lower alkyl alcohol 16 and the acid catalyst 18 sequentially. In the embodiment that is illustrated in Figure 1, the feedstock 12 is reacted in a first step

with the lipase 14 in the presence of the lower alkyl alcohol 16 to produce a first alkyl fatty acid ester mixture 20 which is then reacted with the lower alkyl alcohol 16 in the presence of the acid catalyst 18 to produce a second alkyl fatty acid ester mixture 46.

5 Glyceride containing plant oils or animal fats are particularly suitable as the feedstock 12. As used herein the term "glyceride containing plant oil or animal fat" means any oil or fat product of plant or animal origin that contains esters formed from glycerol and fatty acids (i.e. glycerides). Glycerol has three hydroxyl functional groups which can be esterified with one, two or three fatty acids to form monoglycerides, diglycerides, 10 and triglycerides, respectively. Plant oils and animal fats contain mostly triglycerides, although they typically also contain some monoglycerides and diglycerides. The glyceride containing plant oil or animal fat may be selected from the group consisting of: animal tallow, vegetable oils, used cooking oils and fats, seeds, seed residue feedstocks, and grease trap oils.

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Animal fats are fats obtained from animal sources. Suitable animal fats that may be used in the production of biodiesel include: tallow (beef fat), lard (pork fat), schmaltz (chicken fat), blubber, cod liver oil, yellow grease, and the by-products of the production of omega-3 fatty acids from fish oil. For example, we have found that the 20 process of the present invention is particularly suitable for converting tallow to biodiesel fuel.

Suitable vegetable oils that may be used in the production of biodiesel include: rapeseed oil, soybean oil, palm oil, mustard oil, castor oil, coconut oil (copra oil), corn 25 oil, cottonseed oil, false flax oil, hemp oil, peanut oil, radish oil, ramtil oil, rice bran oil, safflower oil, sunflower oil, tung oil, algae oil, copaiba oil, honge oil, jatropha oil, jojoba oil, milk bush oil, petroleum nut oil, walnut oil, sunflower oil, dammar oil, linseed oil, poppyseed oil, stillingia oil, vernonia oil, amur cork tree fruit oil, apple seed oil, balanos oil, bladderpod oil, brucea javanica oil, burdock oil (bur oil), candlenut oil 30 (kukui nut oil), carrot seed oil, chaulmoogra oil, crambe oil, cuphea oil, lemon oil, orange oil, mango oil, mowrah butter, neem oil, rosehip seed oil, sea buckthorn oil, shea butter, snowball seed oil (viburnum oil), tall oil, tamanu oil, and tonka bean oil (cumaru oil).

35 The lower alkyl alcohol 16 may be a C₁-C₆ alcohol. In an embodiment of the invention the lower alkyl alcohol 16 is selected from one or more of the group consisting of:

methanol, ethanol, n-propanol, i-propanol, n-butanol, s-butanol, and t-butanol. Methanol is an alcohol that is commonly used in biodiesel production but we have found ethanol to be particularly suitable for use in the process of the present invention. The ratio of lower alkyl alcohol 16 to glyceride containing plant oils or animal fats 12 in the combined stream 28 is about 1:1 mol ratio.

A feedstock supply stream 22 of the feedstock 12 containing glycerides and/or free fatty acids is fed through a flow control device 24 and is combined with a regulated stream 26 of the lower alkyl alcohol 16, which is fed from an injection pump. The combined stream 28 is mixed via a static mixer 30. The combined stream 28 containing lower alkyl alcohol 16 and plant oil or animal fat 12 is reacted in the presence of the lipase 14 in a suitable reaction vessel 32. The reaction vessel 32 may be temperature controlled.

The lipase 14 may be a latex lipase and/or a plant lipase from a high lipid content grain, legume or seed. In an embodiment of the invention the lipase 14 is a combination of a latex lipase and a plant lipase from a high lipid content grain, legume or seed. As used herein, the term "lipase" means an enzyme that catalyzes the hydrolysis of triglycerides. The term "latex lipase" means a lipase found naturally in the latex of a plant. Many plant species have a latex that contains a lipase. Such plant species include, without limitation, papaya, mango, sapote, mulberry, fig, kiwifruit, and blackberry. The latex lipase may be used as a crude mixture, a semi-purified mixture, or the lipase may be purified. The latex can be collected from plants using standard methods, such as tapping the fruits of the plant and collecting the exudate latex. The latex may be frozen for storage and/or it may be freeze dried to obtain a crude solid latex lipase preparation suitable for use. Alternatively, the latex lipase may be purified using known protein purification techniques, such as chromatography.

In an embodiment of the invention the latex lipase from the papaya plant is used. Latex from papaya is used in the food and beverage industries in the form of a protease preparation, papain. Papain (EC 3.4.22.2) from papaya latex (CAS Number: 9001-73-4) is commercially available in crude form or in a lyophilized form from Sigma-Aldrich (for example under Catalog No. P4762). The active latex lipase may be a papain lipase, such as *Carica papaya* latex lipase.

The term "plant lipase from a high lipid content grain, legume or seed" means a lipase found naturally in any grain, legume or seed having a relatively high lipid content. Such grains, legumes or seeds include, without limitation, castor beans, rice bran, wheat bran, oat bran, rye, oily legumes, and oily seeds (such as canola). The rice
5 bran lipase may be used in the form of ground rice bran, or as a semi-purified or purified preparation. Methods for the extraction of lipases from rice bran are known (for example see Prabhu, A.V. *et al.*, *Biotechnol Prog.* 1999;15(6):1083-9).

The lipase used may not be a single or homogenous lipase and may indeed be a
10 mixture of different lipases. The lipase may also contain some microbial lipases. Furthermore, the active lipases may be partially or wholly purified and used in the process of the present invention.

The lipase 14 may be immobilised on a substrate. An important factor for the
15 economic efficiency of an enzymatic process is a suitable method for the immobilisation of the enzyme in order to allow ready recovery and multiple use of the enzyme as well as to achieve, if possible, an increase of its stability under the reaction conditions. For a review on immobilisation of lipases see, for example F. X. Malcata *et al.*, *J. Am. Oil Chem. Soc.* 1990, 67, 890-910.

20 In the illustrated embodiments of the present invention the lipase 14 is introduced into the combined stream 28 via a static mixer 34 and fed into the reaction vessel 32. The ratio of lipase to glycerol ester may be about 1:10 w/w to about 1:30 w/w. The reactants are continuously mixed and the reaction is carried out at a temperature of
25 from about 30°C to about 70°C. The reaction may be carried out for a time period of from about 30 minutes to about 90 minutes.

After the required time in the reaction vessel 32 the first alkyl fatty acid ester mixture
20 is pumped from the reaction vessel 32 through a flow control valve 38. The first
30 alkyl fatty acid ester mixture 20 may be separated from solid material, such as immobilised lipase 14. Separation of the first alkyl fatty acid ester mixture 20 from immobilised lipase 14 and/or other solid material may be accomplished using any suitable technique, including (but not limited to) mechanical separation, filtration, gravity separation, centrifugation, etc. In one embodiment of the invention the
35 separation is achieved by centrifugation. Thus, the first alkyl fatty acid ester mixture 20 may be pumped from the reaction vessel 32 through a flow control valve 38 to a

centrifuge 40. The purified first alkyl fatty acid ester mixture 20 may be drawn from the centrifuge in the usual manner. The centrifuge reject 41 may be pumped to a stripping tank 43, where the immobilized lipases may be extracted and re-cycled back to the reaction vessel 32.

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The purified first alkyl fatty acid ester mixture 20 that is obtained after centrifugation is then reacted with the lower alkyl alcohol 16 in the presence of the acid 18 to form a second alkyl fatty acid ester mixture 46. The lower alkyl alcohol 16 may be any of the alcohols discussed previously herein and in one embodiment it is ethanol. The acid catalyst 18 may be any suitable mineral acid, such as hydrochloric acid, sulphuric acid, etc. In one embodiment of the invention the mineral acid is sulphuric acid.

The reaction of the purified first alkyl fatty acid ester mixture 20 that is obtained after centrifugation with the lower alkyl alcohol 16 in the presence of a mineral acid 18 may be carried out under suitable conditions. The lower alkyl alcohol and the mineral acid may be mixed in a ratio of about 1.5% mineral acid w/w of glycerol ester, in volume of lower alkyl alcohol that is calculated at a 12:1 molar ratio of the glycerol ester. Typically, the reaction will require heating. In one embodiment of the invention the reaction is carried out under reactive distillation conditions, such as continuous reactive distillation conditions. Thus, the purified alkyl fatty acid ester mixture 20, lower alkyl alcohol 16, and the mineral acid 18 are fed through a heated static mixer 48 into a reactive distillation tower 50. The heated static mixer 50 may be at a temperature of about 50°C to about 70°C. In one embodiment of the invention the heated static mixer is at a temperature of about 60°C.

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Continuous reactive distillation is carried out in a tower and reboiler arrangement 50 with the reacting product flowing across a plate system via downcomers to the reboiler. We have found that a plate system comprising plates formed from expanded mesh is particularly effective. The expanded mesh maybe stainless steel mesh, zirconium coated steel mesh or nickel type steel mesh. Using this system we have been able to achieve 97% conversion. In the reboiler 50, the reaction is carried out a temperature of about 110°C to about 180°C. In one embodiment of the invention the reaction is carried out a temperature of about 125°C to about 165°C. Unreacted lower alkyl alcohol is vaporised off and flows up and is condensed and recycled back into a heated static mixer 48 inflow. In this way unreacted lower alkyl alcohol that is distilled

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from the reaction is returned to the reaction mixture. The second alkyl fatty acid ester mixture 46 is distilled from the reaction mixture.

5 In an embodiment of the invention that is illustrated in Figures 2(a) and 2(b), the lipase 14 is a combination of a latex lipase 14a in combination with a plant lipase from a high lipid content grain, legume or seed 14b. We have found that a latex lipase 14a from the papaya plant in combination of a plant lipase 14b from rice bran is effective.

10 The latex lipase 14a may be immobilised on a substrate. We have found that a substrate formed from a saccharide and lignite is particularly suitable. The lignite may be an acid precipitate from an alkaline extract of lignite. We have found that a micronised, pyrolised mixture of a saccharide and the lignite extract is particularly suitable as a substrate for the papaya latex lipase 14a. The substrate may be formed as follows. Lignite is firstly micronised and the acid content of the lignite (e.g. humic
15 acids, fulvic acids, etc) is then extracted under alkaline conditions. Suitable bases for the alkaline extraction include potassium hydroxide, magnesium hydroxide, and magnesium sulphate. The pH of the resultant extract is then adjusted to about 3 to about 5. We have found that a pH of about 4.0 to about 4.5 is particularly suitable. In this way the extracted acid content of the lignite precipitates and the precipitate is
20 removed by filtration. The isolated precipitate is then mixed with a saccharide, such as (but not limited to) molasses, sucrose, etc, and the mixture is pyrolised at a temperature of between about 240°C and about 800°C. The pyrolised mixture of saccharide and lignite extract thus formed is then used as a substrate for the papaya latex lipase.

25

As best seen in Figure 2(a), the papaya latex lipase 14a and plant lipase from a high lipid content grain, legume or seed 14b (in the form of rice bran 19) are introduced into the combined stream 28 via a static mixer 30 and fed into the reaction vessel 32 containing the lower alkyl alcohol 16 and plant oil or animal fat 12. The rice bran 19
30 may be a rice bran residue that is from the in-situ esterification of the rice bran oil in a process that is described later.

The ratio of papaya latex lipase 14a to plant oil or animal fat 12 may be about 1:10 w/w to about 1:30 w/w. In one embodiment of the invention the ratio of papaya latex
35 lipase 14a to plant oil or animal fat 12 is about 1:20 w/w. The reactants are continuously mixed and the reaction is carried out at a temperature of from about

30°C to about 70°C. In one embodiment of the invention the reaction is carried out at a temperature of about 50°C. The reaction may be carried out for a time period of from about 30 minutes to about 90 minutes. In one embodiment of the invention the reaction is carried out for a time period of about 60 minutes.

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After the required time in the reaction vessel 32 the first alkyl fatty acid ester mixture 20 is separated from solid material, such as immobilised lipase 14a and rice bran 19, by centrifugation as described previously. The purified first alkyl fatty acid ester mixture 20 that is obtained after centrifugation is then reacted with the lower alkyl alcohol 16 in the presence of the acid 18 to form a second alkyl fatty acid ester mixture 46 as described previously. The second alkyl fatty acid ester mixture 46 is distilled from the reaction mixture and is continuously drawn from the reboiler 50 by a pump 52 through a flow control valve 54.

15 The second alkyl fatty acid ester mixture 46 may be used as is or purified for use as a biofuel, or it may be further treated. For example, the distilled second alkyl fatty acid ester mixture may be treated to remove unreacted glycerine. The treatment may comprise gravity separation. Thus, the second alkyl fatty acid ester mixture 46 may be drawn from the reboiler 50 by a pump 52 through a flow control valve 54 to a static glycerin/alkyl ester separator vessel 56. The separator vessel 56 is used to separate raw glycerin and un-reacted glycerol esters 58 from alkyl fatty acid ester mixture 46, which are drawn off continuously from the top layers of the separator vessel 56.

25 The raw glycerin and un-reacted glycerol esters 58 (i.e. the bottoms from the separating vessel 56) may be recycled. For example, unreacted glycerol esters and unreacted ethanol may be separated from raw glycerin and soap based on the specific gravity of each component. Thus, the raw glycerin and un-reacted glycerol esters 58 may be pumped from the separating vessel 58 to a centrifuge 60 where they are separated based on specific gravity. The centrifuge extract 62 (i.e. primarily unreacted glycerol esters and unreacted ethanol) may be pumped back to the reboiler 50.

35 The centrifuge reject 64 (i.e. primarily raw glycerin and soap) may be converted to butanol by fermentation. As illustrated in Figure 2(b), the centrifuge reject 64 may be mixed with an appropriate enzyme and pumped via flow control valve 66 to a multi compartmented rotating biological disc bioreactor 68 with immobilized specific

bacteria and fermented to alcohols. Butanol is preferably produced using this method. Many microorganisms are known to produce butanol through fermentation. Several *Clostridium* spp (e.g. *Clostridium acetobutylicum*, *Clostridium beijerinckii* etc.) have been shown to catalyse the production of butanol may be used (see Jones, D.T. and Woods, D.R. Microbiol Rev. 1986 December; 50(4): 484–524). Other enzymes that could be used for this transformation include purified or crude mixtures containing microbial and/or fungal dehydrogenases. The alcohol produced may be distilled via a distillation apparatus 70 and used in the reactions discussed in more detail below.

- 10 We have found that a superior biodiesel product is formed if the alkyl fatty acid ester mixture 46 that has been separated from raw glycerin and un-reacted glycerol esters 58 is mixed with esters of rice bran oil.

Rice bran oil esters may be prepared using any suitable esterification or transesterification technique. However, we have found that it is advantageous to prepare rice bran oil esters using a sulphonic acid as the catalyst for the transesterification reaction. Suitable sulphonic acids include alkyl sulphonic acids, such as methane sulphonic acids. Thus, rice bran may be reacted with a lower alkyl alcohol in the presence of methane sulphonic acid to form a rice bran oil ester mixture. The lower alkyl alcohol may be selected from one or more of the group consisting of methanol, ethanol, n-propanol, i-propanol, n-butanol, s-butanol, and t-butanol. Preferably, the lower alkyl alcohol is n-butanol. The n-butanol may be formed by enzymatic hydrolysis of a glycerine and soap mixture that is formed during the production of the second alkyl fatty acid mixture, as described previously.

25 Rice bran 19 with an oil content of about 19 to about 23% w/w is continuously charged into a reactor vessel 74 and mixed with a regulated flow of butanol 76 mixed with methane sulphonic acid 78. The butanol 76 and methane sulphonic acid 78 are pumped to a static mixer 80 prior to being injected into the reactor vessel 74. The ratio of methane sulphonic acid to n-butanol that is injected into the reactor vessel 74 may be about 1:40 w/w to about 1:50 w/w. In one embodiment the ratio of methane sulphonic acid to n-butanol is about 1:40 w/w to about 1:50 w/w.

110 Kg of rice bran per 1Kl of alkyl ester may be charged into the reactor vessel 74. The reactor vessel 74 may be a continuously stirred temperature controlled jacketed bio-reactor. The ratio of n-butanol to rice bran may be about 1:3 w/w to about 1:4

w/w. In one embodiment the ratio of n-butanol to rice bran in the starting reaction mixture is about 1:3.5 w/w.

5 The rice bran oil ester mixture 82 may be separated from solid material prior to mixing with the second alkyl fatty acid mixture 46. For example, the rice bran oil ester mixture 82 may be separated from the solid material by centrifugation. Thus, the rice bran oil ester mixture 82 may be drawn off and through a regulating flow control valve 84 to a centrifuge 86. The extract from the centrifuge (i.e. rice bran oil ester mixture 82) may be pumped to a mixing vessel 88 for mixing with the second alkyl fatty acid
10 mixture 46.

Reject 90 from the centrifuge 86 (i.e. primarily rice bran) may be reused in the reaction to produce alkyl fatty acid ester mixture 20.

15 The alkyl fatty acid ester mixture 46, which is drawn off continuously from the top layers of the separator vessel 56, may be mixed with the rice bran oil ester mixture 82 in a static mixer 92 and pumped into a continuously pump mixed vessel 94. The pH of the mixture may be adjusted to about 6 to about 8. In one embodiment of the invention the pH of the mixture is adjusted to about 7.

20 The pH of the second alkyl fatty acid ester and rice bran oil ester mixture can be adjusted by adding an agent 95 selected from the group consisting of: potassium hydroxide, sodium hydroxide, magnesium hydroxide, ammonia, and calcium hydroxide. In one embodiment of the invention the pH of the mixture is adjusted by
25 adding magnesium hydroxide.

The second alkyl fatty acid ester and rice bran oil ester mixture may be treated with a filtering agent 96. Suitable filtering agents 96 may be selected from the group consisting of diatomaceous earth, activated charcoal, and silicates. The silicate may
30 be an aluminosilicate or a magnesium silicate. In one embodiment of the invention the filtering agent 96 is a magnesium silicate. Magnesium silicates are particularly beneficial as they not only act as filtering agent but they also extract residual water from the second alkyl fatty acid ester and rice bran oil ester mixture.

35 The filtering agent 96 may be separated from the second alkyl fatty acid ester and rice bran oil ester mixture by mechanical filtration. For example, the second alkyl fatty

acid ester and rice bran oil ester mixture, filtering agent and reacted pH adjusting agent may be pumped through a continuous belt filter press 97.

5 Filtered alkyl esters are the finished product 98 and may be retained for storage. We have found that the biodiesel produced has a gelling temperature somewhere between about 0°C and about 10°C depending on the starting feedstock material. For example, when the starting feedstock is beef tallow (which is a readily available, inexpensive starting material) we are able to produce biodiesel having a gelling temperature in this range. This is in contrast to prior art biodiesel fuels formed from
10 beef tallow, which we have found have a gelling temperature of about 16°C. Thus, using the processes of the present invention we are able to form a high grade biodiesel fuel from relatively low grade starting materials, such as tallow.

Furthermore, the biodiesel produced using the processes of the present invention has
15 excellent long term stability. For example, we have found that there is no evidence of oxidation of the sample after storage at room temperature in an open vessel for about 12 months. Without intending to be bound by theory, we believe that the oxidative stability of the biodiesel produced using the processes of the present invention may be due, at least in part, to the presence of alpha-tocopherol in the final product. Rice
20 bran is known to contain relatively high levels of alpha-tocopherol, which is a naturally occurring antioxidant. We believe, that at least some of the alpha-tocopherol present in the rice bran 19 used in the initial lipase catalysed reaction and/or the rice bran 74 used in the production of rice bran oil ester mixture 82 is carried through into the finished product 98. It is postulated that the use of methanesulphonic acid as the
25 catalyst in the preparation rice bran esters is advantageous because it does not appreciably degrade the alpha-tocopherol in the same way that some other acid catalysts might.

The process of the present invention may be operated in a continuous, zero-waste
30 manner.

The finished product is suitable for use as biodiesel and may be used as is or in admixture with mineral diesel. For example, prior art biodiesel fuels are typically mixed with mineral diesel in a ratio of 9:1 mineral to biodiesel. This is primarily done
35 because the high gelling point of the biodiesel limits the amount that can be used in

the mixture. However, the biodiesel produced according to the present invention may be mixed with mineral diesel in a ratio of about 7:3.

Advantageously, we have found that the cetane index of a biodiesel product can be increased by adding butyl esters of rice bran oil to the product.

Description of specific embodiment(s)

Example 1 – *Production of biodiesel*

10

Biodiesel is produced using the procedures and apparatus as described previously. Specifically the following steps are carried out.

15 A feedstock supply stream 22 of tallow 12 is fed through a flow control device 24 and is combined with a regulated stream 26 of ethanol 16, which is fed from an injection pump. The combined stream 28 is mixed via a static mixer 30. The combined stream 28 is reacted in the presence of *Carica papaya* latex lipase immobilised on a substrate formed from a saccharide and lignite and rice bran lipase 14 in a temperature controlled reaction vessel 32. The ratio of lipase to tallow is between 1:10 w/w and 20 1:30 w/w. The reactants are continuously mixed and the reaction is carried out at a temperature of between 30°C and 70°C for 30 to 90 minutes.

The reaction mixture (containing the first alkyl fatty acid ester mixture 20) is then pumped from the reaction vessel 32 through a flow control valve 38 to a centrifuge 40 25 where it is separated from solid material, such as immobilised lipase 14. The purified first alkyl fatty acid ester mixture 20 is drawn from the centrifuge in the usual manner. The centrifuge reject 41 is pumped to a stripping tank 43, where the immobilized lipases are extracted and re-cycled back to the reaction vessel 32.

30 The purified first alkyl fatty acid ester mixture 20 that is obtained after centrifugation is then reacted with methanol 16 in the presence of sulphuric acid 18 to form a second alkyl fatty acid ester mixture 46. The methanol 16 and sulphuric acid 18 are mixed in a ratio of about 1.5% sulphuric acid w/w, in a volume of methanol that is calculated at a 12:1 molar ratio. The reaction is carried out under continuous reactive distillation 35 conditions at a temperature of about 60°C. The second alkyl fatty acid ester mixture

46 is distilled from the reaction mixture and is continuously drawn from the reboiler 50 by a pump 52 through a flow control valve 54.

5 The second alkyl fatty acid ester mixture 46 is passed in to a static glycerin/alkyl ester separator vessel 56 where raw glycerin and un-reacted glycerol esters 58 are separated from the alkyl fatty acid ester mixture 46 by gravity filtration. The alkyl fatty acid ester mixture 46 is drawn off continuously from the top layers of the separator vessel 56.

10 The raw glycerin and un-reacted glycerol esters 58 (i.e. the bottoms from the separating vessel 56) are recycled by pumping from the separating vessel 58 to a centrifuge 60 where the unreacted glycerol esters and unreacted ethanol are separated from raw glycerin and soap based on the specific gravity of each component. The centrifuge extract 62 (i.e. primarily unreacted glycerol esters and
15 unreacted ethanol) is pumped back to the reboiler 50.

The centrifuge reject 64 (i.e. primarily raw glycerin and soap) is pumped via flow control valve 66 to a multi compartmented rotating biological disc bioreactor 68 containing immobilized *Clostridium* spp bacteria which ferment the material to butanol.
20 The butanol is distilled via a distillation apparatus 70 and used in the production of rice bran oil esters 82.

To produce rice bran oil esters 82, rice bran 74 with an oil content of about 19 to about 23% w/w is continuously charged into a reactor vessel 74 and mixed with a
25 regulated flow of butanol 76 mixed with methanesulphonic acid. The butanol 76 and methane sulphonic acid 78 are pumped to a static mixer 80 prior to being injected into the reactor vessel 74. The ratio of methane sulphonic acid to n-butanol that is injected into the reactor vessel 74 is about 1:40 w/w to about 1:50 w/w. The ratio of n-butanol to rice bran is 1:3 w/w to 1:4 w/w. 110 Kg of rice bran per 1Kl of alkyl ester is
30 charged into the reactor vessel 74. The reactor vessel 74 is continuously stirred and temperature controlled.

The rice bran oil ester mixture 82 is drawn off from the vessel 74 and through a regulating flow control valve 84 to a centrifuge 86. The extract from the centrifuge (i.e.
35 rice bran oil ester mixture 82) is pumped to a mixing vessel 88 for mixing with the

second alkyl fatty acid mixture 46. Reject 90 from the centrifuge 86 (i.e. primarily rice bran) is reused in the reaction to produce alkyl fatty acid ester mixture 20.

5 The alkyl fatty acid ester mixture 46 is mixed with the rice bran oil ester mixture 82 in a static mixer 92 and pumped into a continuously pump mixed vessel 94. The pH of the mixture is adjusted to about 7 by adding magnesium hydroxide. Magnesium silicate is then added as a filtering agent and a drying agent and it is then separated from the second alkyl fatty acid ester and rice bran oil ester mixture pumping it through a continuous belt filter press 96.

10

The filtered alkyl esters are the finished product 98 and are retained for storage.

15 Example 2 – *Characterisation of biodiesel*

The alkyl fatty acid ester composition of the finished product 98 obtained from the reaction sequence described were analysed by gas chromatography (GC) essentially as described in British Standard Institution (BSI) reference method BS EN
20 14103:2003; "Fat and oil derivatives-Fatty Acid Methyl Esters (FAME) - Determination of ester and linolenic acid methyl ester contents".

The present invention however relates to the formation of mixtures which are produced by way of transesterification reactions of animal fat and rice bran oil
25 performed in the presence of ethanol and n-butanol, respectively, to produce fatty acyl ethyl esters and fatty alkyl butyl esters, respectively. Therefore in the spirit of the EN 14103:2003 Standard, the present invention reports all information necessary for the complete identification of the sample; the sampling method used; the test method used with reference to the European Standard; and the test results obtained.

30

Both fatty alkyl ethyl esters (FAEE) and fatty alkyl butyl esters (FABE) were analysed using an Agilent 7890A GC system (Agilent Technologies Wilmington, DE, USA), equipped with flame ionization detector (FID) and an HP-Innowax capillary column (1909N-113; J & W Scientific; 30m x 320um x 0.25um) using high-purity Helium as
35 carrier gas. FAEE and FABE were analysed using identical temperature

programming conditions of 140°C for 0min, then 5°C / min to 220°C for 3min, then 20°C / min to 260°C for 8 mins.

FAEEs were identified from their retention times against mixes of commercially obtained FAEE standards (Nu-Chek Prep, Inc., Elysian, MN, USA) which were aliquoted in heptane containing 0.005% (w/v) butylated hydroxy anisole as antioxidant. FABLEs were identified from their retention times against FABLE mixes prepared from commercially available triacylglycerides (TAG) (Nu-Chek). To prepare FABLEs, 10mg of homogeneous acyl fatty acid (with respect to the sn-1, sn-2 and sn-3 positions) TAGs dissolved in chloroform / methanol (9:1; v/v, containing 0.005% (w/v) butylated hydroxy anisole as antioxidant), were dried under nitrogen and reacted with 2ml of 2.5% (v/v) methanesulfonic acid in n-butanol (final ratio methanesulfonic acid to n-butanol (1:40; v/v)) for 3 hours at 80°C. After cooling, FABLEs were extracted in the heptane phase by separation of the phases using the ratio of (approximately), n-butanol : water : methanol : heptane of 1:1:1.2:1.3 (by volume) or by selectively breaking the phase by changing the proportions of each solvent as required on a sample by sample basis. The FABLEs in the heptane phase were dried overnight using anhydrous sodium sulphate and then each FABLE made up in 2ml heptane, capped under nitrogen. For both the FAEEs and the FABLEs, appropriate mixes of individual esters were prepared to enable identification of all major peaks from chromatograms of all biodiesel samples.

Fatty acids are designated by their carbon length followed by the number of unsaturated double bonds and expressed as wt (%) of the total fatty acids. FA(ee); fatty acid ethyl esters; be; FA(be): fatty acid butyl esters. Other fatty acids represent fatty acids with retention times greater than C22:6 n-3ee or C22:6 n-3be.

Table 1 shows the fatty acid composition of the mixture derived from the esterification of animal fat in stage 1 of the biodiesel process in which this material is treated with an immobilised plant lipase in the presence of ethanol and later sulphuric acid. The fatty alkyl esters identified in this mixture were only of the ethyl ester type as opposed to methyl esters described for other biodiesel mixtures. Ethyl esters were primarily C16:0 (palmitate) 23.8%, C18:0 (stearate) 22.2%, and C18:1cis 9 (oleate) 37.9%, with an overall degree of saturation of 48.2% consistent with the fatty acids of the starting material being of animal origin. Minor fatty acid ethyl esters detected comprised isomeric forms of C18:1 likely to be elaidic and/or vaccenic ethyl esters, as well as

ethyl esters with retention times longer than C22:6 likely to be of the C24 ethyl ester type.

Table 1 - Fatty acid analysis of biodiesel mixtures produced by esterification of animal fat (Biodiesel sample # 2).

Fatty acid	% (w/w)
14:0 ee	2.2
16:0 ee	23.8
16:1 ee	2.8
18:0 ee	22.2
18:1 ee (cis 9)	37.9
18:1 ee (other isomers)	3.7
18:2 ee	2.3
Other fatty acids (ee)	5.0
Σ Saturated fatty acids	48.2

Table 2 shows the fatty acid composition of the mixture derived from the esterification of rice bran oil in stage 2 of the biodiesel process in which this material is treated in the presence of n-butanol and methanesulphonic acid. The fatty alkyl esters identified in this mixture were only of the butyl ester type as opposed to methyl esters described for other biodiesel mixtures and the ethyl esters described for stage 1 of the present invention. Butyl esters were primarily C16:0 (palmitate) 19.1%, C18:1 cis 9 (oleate) 32.7% and C18:2 (linoleate) 28.7%, with an overall degree of saturation of 23.6% consistent with the fatty acids of the starting material being of plant origin. Several other fatty alkyl ethyl esters were detected but these were difficult to unequivocally identify with the butyl ester standards available, and together comprised about 14.5% of the total fatty acid esters in this sample.

Table 2 - Fatty acid analyses of biodiesel mixtures produced by esterification of rice bran oil (Biodiesel sample # 3).

5	Fatty acid	% (w/w)
10	16:0 be	19.1
	18:0 be	2.2
	18:1 be (cis 9)	32.7
	18:2 be	28.7
	18:3 be	0.8
	22:0 be	2.3
15	Other fatty acids (be)	14.5
	Σ Saturated fatty acids	23.6

Table 3 shows the fatty acid composition of the combined mixture derived from both the esterification of animal fat in stage 1 of the biodiesel process in which this material is treated with an immobilised plant lipase in the presence of ethanol and later sulphuric acid, and the esterification of rice bran oil in stage 2 of the biodiesel process in which this material is treated with in the presence of n-butanol and methane sulphonic acid. Both ethyl esters and butyl esters are identified in this mixture with the ethyl esters comprising approximately 91% and the butyl esters 9% of the total fatty alkyl esters. The complete fatty alkyl ester composition of this biodiesel mixture is shown as both total % FAEE plus FBE (left hand column) and % FAEE or % FBE (right hand column). The FAEE profile compares well with that shown in Table 1, while for the butyl esters, the major species present, C 16:1 (18.0%), C 18:1 (30.1%) and C 18:2 (30.0%), are also similar in proportion to that shown in Table 2 for the rice bran derived fatty alkyl butyl esters. The other fatty alkyl butyl esters present in Biodiesel sample #3, for which identification was difficult due to the relatively lower proportion of butyl to ethyl esters in this mixture, comprised approximately 2.0% of the total ester content or 21.9% of the total butyl esters.

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Table 3 - Fatty acid analysis of biodiesel produced by esterification of animal fat and rice bran oil. (Biodiesel sample # 4).

Fatty acid (ethyl esters)	% (w/w) (total FAEE plus FABE)	% (w/w) (% of FAEE or FABE)
Fatty acid ethyl esters (FAEE)		
14:0 ee	2.1	2.3
16:0 ee	22.5	24.8
16:1 ee	2.7	2.9
18:0 ee	20.5	22.6
18:1 ee (cis 9)	35.1	38.6
18:1 ee (other isomers)	3.4	3.7
Other fatty acids (ee)	4.7	5.1
Σ Fatty acids	91.0	100.0
Σ Saturated fatty acids (FAEE)		49.6
Fatty acid butyl esters (FABE)		
16:0 be	1.6	18.0
18:1 be (cis 9)	2.7	30.1
18:2 be	2.7	30.0
Other fatty acids (be)	2.0	21.9
Σ Fatty acids	9.0	100.0
Σ Saturated fatty acids (FABE)		18.0
Σ Saturated fatty acids (FAEE + FABE)		46.7

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The embodiments have been described by way of example only and modifications within the spirit and scope of the invention are envisaged. Thus, it must be appreciated that there may be other various and modifications to the configurations described herein which are also within the scope of the present invention.

5

Claims

1. A process for producing alkyl fatty acid esters suitable for use as a fuel and/or lubricant, the process including treating a feedstock containing glycerides and/or free fatty acids with a lipase, a lower alkyl alcohol and an acid catalyst under conditions to produce said alkyl fatty acid esters.
2. A process according to claim 1, wherein the feedstock containing glycerides and/or free fatty acids is treated with the lipase in the presence of the lower alkyl alcohol to produce a first alkyl fatty acid ester mixture which is then reacted with the lower alkyl alcohol in the presence of the acid catalyst to produce a second alkyl fatty acid ester mixture which is suitable for use as a fuel and/or lubricant.
3. A process according to either claim 1 or claim 2, wherein the lipase is selected from one or more of the group consisting of: a latex lipase and a plant lipase from a high lipid content grain, legume or seed.
4. A process according to claim 3, wherein the lipase is a combination of a latex lipase and a plant lipase.
5. A process according to either claim 3 or claim 4, wherein the latex lipase is a papaya latex lipase.
6. A process according to claim 5, wherein the papaya latex lipase is immobilized on a substrate formed from sucrose and lignite.
7. A process according to claim 6, wherein the substrate is a pyrolised mixture of a saccharide and an acid precipitate from an alkaline extract of lignite.
8. A process according to any one of claims 3 to 7, wherein the plant lipase from a high lipid content grain, legume or seed is a rice bran lipase.
9. A process according to any one of the preceding claims, wherein the feedstock containing glycerides and/or free fatty acids is a plant oil or animal fat.

10. A process according to claim 9, wherein the feedstock containing glycerides and/or free fatty acids is tallow.
11. A process according to any one of the preceding claims, wherein the lower alkyl alcohol is selected from one or more of the group consisting of methanol, ethanol, n-propanol, i-propanol, n-butanol, s-butanol, and t-butanol.
12. A process according to claim 11, wherein the lower alkyl alcohol is ethanol.
13. A process according to any one of claims 5 to 12, wherein the ratio of papaya latex lipase to feedstock containing glycerides and/or free fatty acids is about 1:10 w/w to about 1:30 w/w.
14. A process according to claim 13, wherein the ratio is about 1:20 w/w.
15. A process according to any one of claims 2 to 14, wherein the reaction to produce the first alkyl fatty acid ester mixture is carried out at a temperature of from about 30 °C to about 70 °C.
16. A process according to claim 15, wherein the reaction is carried out at a temperature of about 50 °C.
17. A process according to any one of claims 2 to 16, wherein the reaction to produce the first alkyl fatty acid ester mixture is carried out for a time period of from about 30 minutes to about 90 minutes.
18. A process according to claim 17, wherein the reaction is carried out for a time period of about 60 minutes.
19. A process according to any one of claims 2 to 18, further including separating the first alkyl fatty acid ester mixture from solid material.
20. A process according to claim 19, wherein the first alkyl fatty acid ester mixture is separated from solid material by centrifugation.

21. A process according to any one of the preceding claims, wherein the acid catalyst is a mineral acid.
22. A process according to claim 21, wherein the mineral acid is sulphuric acid.
- 5 23. A process according to any one of claims 2 to 22, wherein the reaction to produce the second alkyl fatty acid mixture is carried out under reactive distillation conditions.
- 10 24. A process according to claim 23, wherein the reaction is carried out under continuous reactive distillation conditions.
25. A process according to either claim 23 or claim 24, wherein the reaction is carried out a temperature of about 110 °C to about 180 °C.
- 15 26. A process according to claim 25, wherein the reaction is carried out a temperature of about 125 °C to about 165 °C.
27. A process according to any one of claims 23 to 26, wherein unreacted lower alkyl alcohol that is distilled from the reaction is returned to the reaction mixture.
- 20 28. A process according to any one of claims 23 to 27, wherein the second alkyl fatty acid ester mixture is distilled from the reaction mixture.
- 25 29. A process according to claim 28, wherein the distilled second alkyl fatty acid ester mixture is treated to remove unreacted glycerine.
30. A process according to claim 29, wherein said treatment comprises gravity separation.
- 30 31. A process according to any one of claims 2 to 30, wherein the pH of the second alkyl fatty acid ester mixture is adjusted to between about 6 and about 8.
32. A process according to claim 31, wherein the pH of the second alkyl fatty acid ester mixture is adjusted to about 7.
- 35

33. A process according to either claim 31 or claim 32, wherein the pH of the second alkyl fatty acid ester mixture is adjusted by adding an agent selected from the group consisting of: potassium hydroxide, sodium hydroxide, magnesium hydroxide, ammonia, and calcium hydroxide.

5

34. A process according to claim 33, wherein the pH of the second alkyl fatty acid ester mixture is adjusted by adding magnesium hydroxide.

35. A process according to any one of claims 2 to 34, wherein the second alkyl fatty acid ester mixture is filtered to remove suspended solids.

36. A process according to any one of claims 2 to 35, wherein the second alkyl fatty acid ester mixture is treated with a filtering agent.

37. A process according to claim 36, wherein the filtering agent is selected from the group consisting of diatomaceous earth, activated charcoal, and silicates.

38. A process according to claim 37, wherein the silicate is selected from the group consisting of aluminosilicates and magnesium silicates.

20

39. A process according to claim 38, wherein the silicate is a magnesium silicate.

40. A process according to any one of claims 36 to 39, wherein the filtering agent is separated from the second alkyl fatty acid ester mixture by mechanical filtration.

25

41. A process according to any one of claims 2 to 40, further comprising:
- reacting rice bran oil with a lower alkyl alcohol in the presence of methane sulphonic acid to form a rice bran oil ester mixture; and
- mixing the rice bran oil ester mixture with the second alkyl fatty acid ester mixture to form an alkyl fatty acid ester mixture suitable for use as a fuel.

30

42. A process according to claim 41, wherein the lower alkyl alcohol is selected from one or more of the group consisting of methanol, ethanol, n-propanol, i-propanol, n-butanol, s-butanol, and t-butanol.

35

43. A process according to claim 42, wherein the lower alkyl alcohol is n-butanol.

44. A process according to claim 43, wherein the n-butanol is formed by enzymatic hydrolysis of a glycerine and soap mixture that is formed during the production of the second alkyl fatty acid mixture.

5

45. A process according to any one of claims 36 to 39, wherein the rice bran oil ester mixture is separated from solid material prior to mixing with the second alkyl fatty acid mixture.

10 46. A process according to claim 45, wherein the rice bran oil ester mixture is separated from the solid material by centrifugation.

47. A process according to any one of claims 41 to 46, wherein the ratio of n-butanol to rice bran oil is about 1:3.5 w/w.

15

48. A process according to any one of claims 41 to 42, wherein the ratio of n-butanol to rice bran oil in the starting reaction mixture is about 1:3 w/w to about 1:4 w/w.

20 49. A process according to claim 48, wherein the ratio of n-butanol to rice bran oil is about 1:3.5 w/w.

50. A process according to any one of claims 41 to 49, wherein the ratio of methane sulphonic acid to n-butanol in the starting reaction mixture is about 1:40 w/w
25 to about 1:50 w/w.

51. A process according to claim 50, wherein the ratio of methane sulphonic acid to n-butanol is about 1:40 w/w to about 1:50 w/w.

30 52. An alkyl fatty acid ester mixture prepared according to the process of any one of claims 1 to 52.

53. A biodiesel fuel prepared according to the process of any one of claims 1 to 52.

35

54. A biodiesel fuel according to claim 53, wherein the gelling temperature of the fuel is between about 0°C and about 10°C.

55. A biodiesel fuel that is substantially stable to oxidation under ambient
5 conditions for a period of at least twelve months.

56. A system for carrying out the process of any one of claims 1 to 51.

57. A process according to claim 1 and substantially as hereinbefore described
10 with respect to the accompanying example(s).

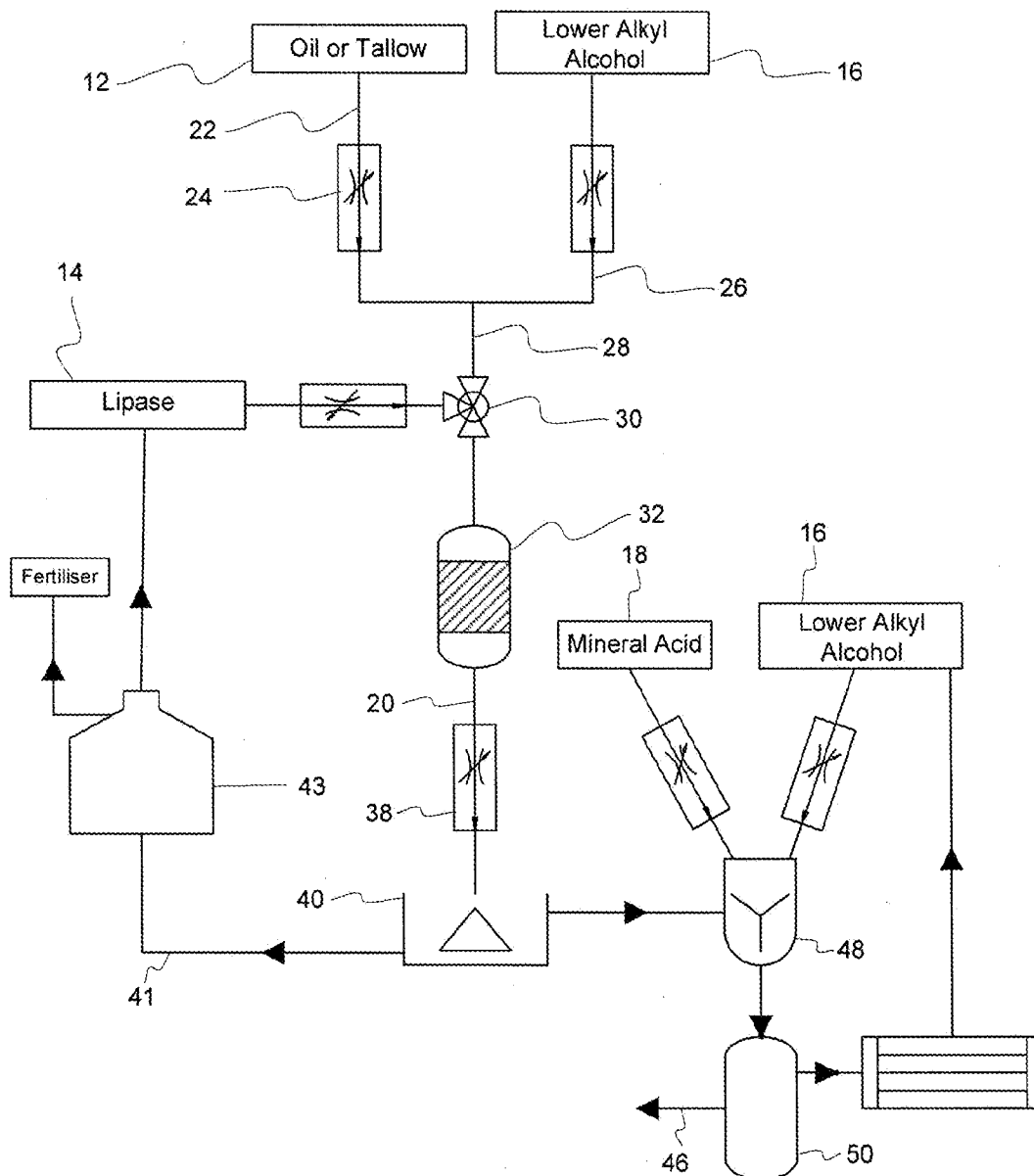


FIGURE 1

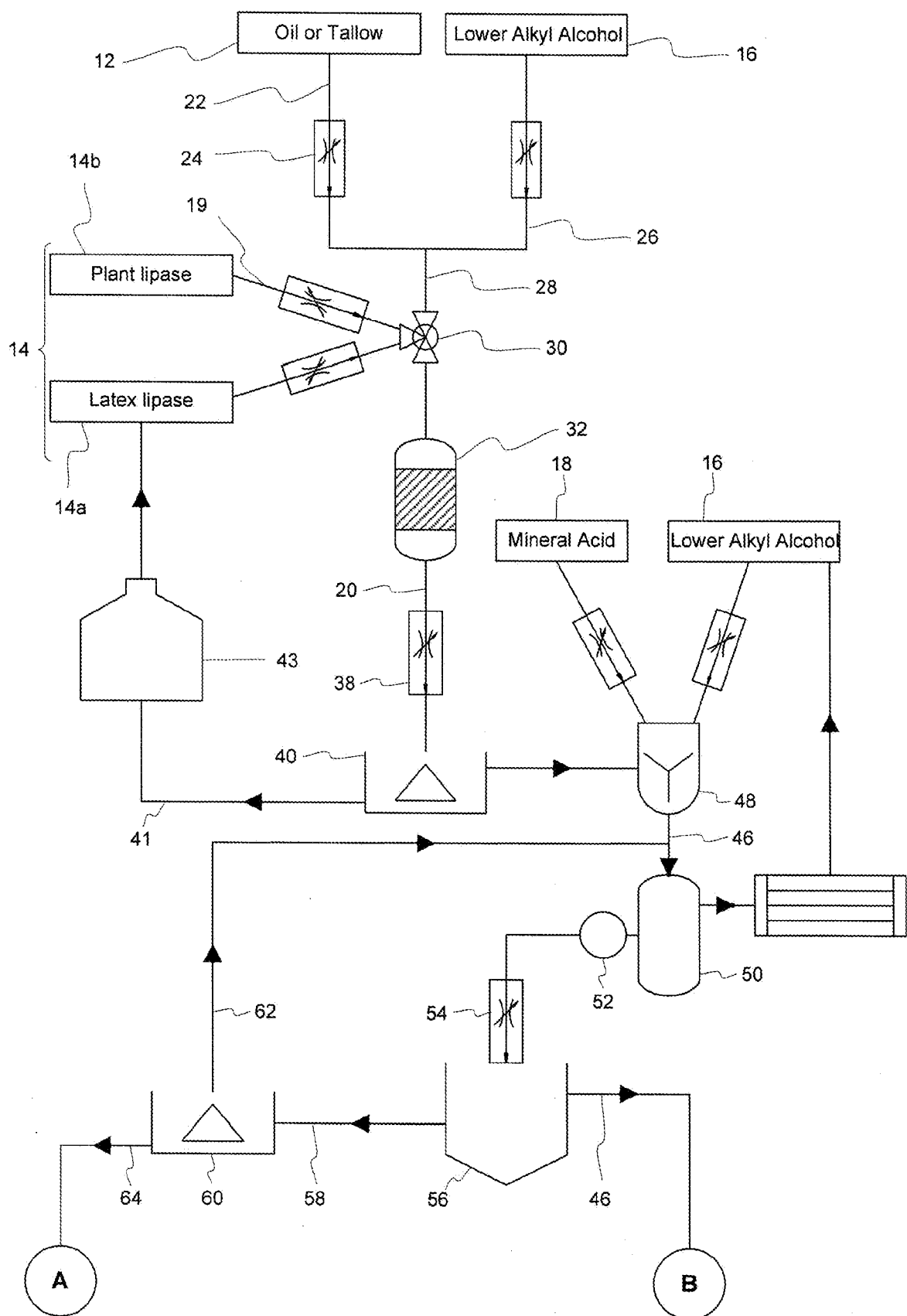


FIGURE 2(a)

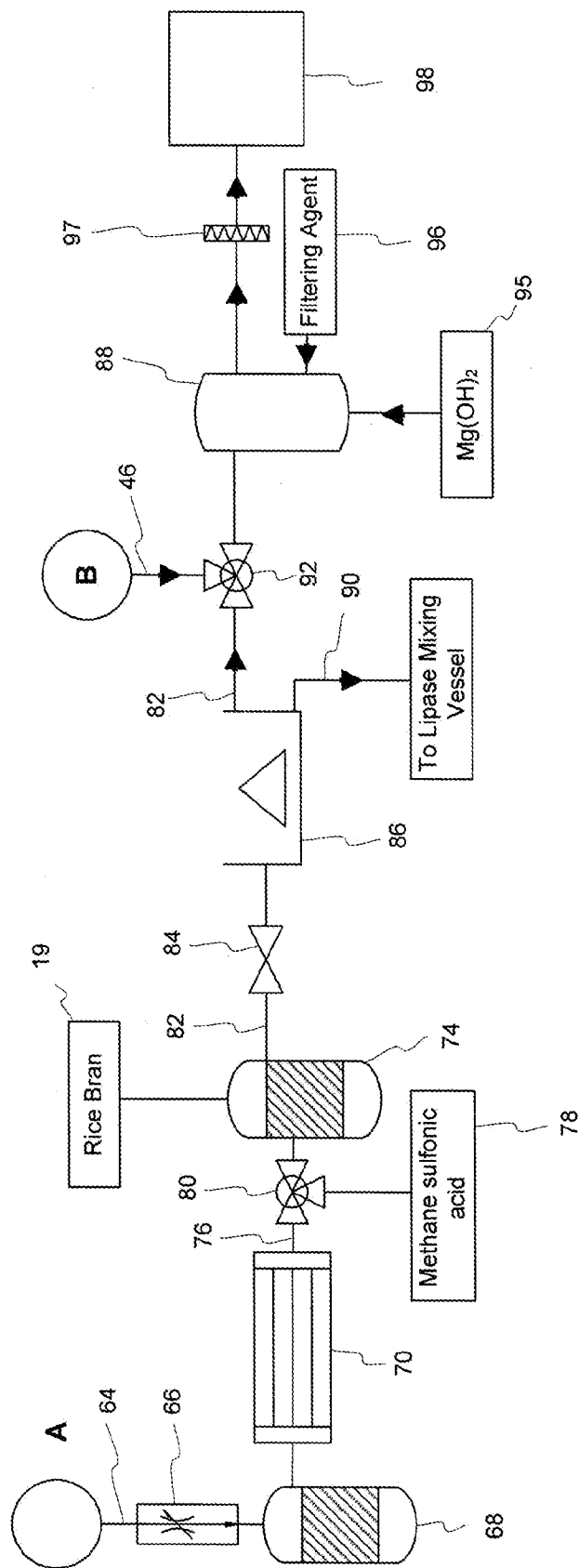


FIGURE 2(b)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2008/000112

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. *C11C 3/00* (2006.01) *C10M 107/26* (2006.01) *C11C 3/04* (2006.01) *C10L 1/02* (2006.01)
C10M 107/32 (2006.01) *C11C 3/08* (2006.01) *C10M 101/04* (2006.01) *C10M 129/95* (2006.01)
C11C 3/10 (2006.01)

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)

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)

WPAT, JAPIO and Espacenet: c11c-3/00/ic, c10l-001/02/ic, c10m-101/04/ic, c10m-107/26/ic, c10m-107/32/ic, c10m-129/95/ic, c11c-3/04/ic, c11c-3/10/ic, C11C-3/08/IC, biodiesel+ or biofuel+ or fuel+ or lubric+, lipase+, alcohol+, (acid catalyst+); biodiesel fuel and stability and oxidation

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/050589 A1 (COGNIS IP MANAGEMENT GMBH) 18 May 2006 See Abstract; claim 9; page 3, line 27 to page 4, line 20; page 5, lines 1-2	1-4, 9, 11-12, 19-22, 36-37, 40, 52-53, 55-56

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

* "A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 26 February 2008	Date of mailing of the international search report 18 MAR 2008
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. +61 2 6283 7999	Authorized officer  DEBASHIS ROY AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : (02) 6225 6125

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2008/000112

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Patent Abstracts of Japan, JP 2006-272326 A (NATIONAL UNIV CORP SHIZUOKA UNIV et al.) 12 October 2006 (machine translation, retrieved 8 May 2007 from Internet) <URL: http://www4.ipdl.inpit.go.jp/Tokujitu/PAJdetail.ipdl?N0000=60&N0120=01&N2001=2&N3001=2006-272326 See Abstract and Paragraph [0018] of machine-based translation	1-4, 9, 11-12, 19-22, 31-34, 52-53, 55-56
X	Patent Abstracts of Japan, JP 2005-171099 A (MIZUSAWA IND CHEM LTD) 30 June 2005 (machine translation, retrieved 8 May 2007 from Internet) <URL: http://www4.ipdl.inpit.go.jp/Tokujitu/PAJdetail.ipdl?N0000=60&N0120=01&N2001=2&N3001=2005-171099 See Abstract and Paragraph [0023] of machine-based translation	1-4, 9, 11-12, 19-22, 31-34, 52-53, 55-56
X	US 2005/0084941 A1 (ABE et al.) 21 April 2005 See whole document especially Paragraph [0039]	1-4, 8-12, 15-22, 31-34, 52-53, 55-56
X	US 2006/0219979 A1 (ASBAHR et al.) 05 October 2006 See Abstract and page 6	55
A	US 2003/0032826 A1 (HANNA) 13 February 2003 See whole document especially Paragraph [0019]	1-57
A	US 6982155 B1 (FUKUDA et al.) 03 January 2006 See whole document	1-57

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2008/000112

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2006050589	BR	PI0419166	CA	2586889	CN	101056985
		EP	1809755				
JP	2006272326						
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US	2005084941	AU	2003211577	BR	0303369	CN	1522293
		EP	1484384	JP	2003336082	WO	03076553
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
END OF ANNEX							