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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
62/336,989	05/16/2016	Brian Michael Green	098816-1011000(ASP-006P2)

CONFIRMATION NO. 2855

23342
KILPATRICK TOWNSEND & STOCKTON LLP
1001 WEST FOURTH STREET
WINSTON-SALEM, NC 27101

**ABANDONMENT/TERMINATION
LETTER**



OC000000088799451

Date Mailed: 01/27/2017

NOTICE OF ABANDONMENT

Express Abandonment

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A copy of this notice MUST be returned with the reply.

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/nton/

**EXPRESS ABANDONMENT UNDER
37 CFR 1.138**

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Commissioner for Patents

P.O. Box 1450, Alexandria, VA 22313-1450

Application Number	62/336,989
Filing Date	May 16, 2016
First Named Inventor	Brian Michael Green
Art Unit	Unassigned
Examiner Name	Unassigned
Attorney Docket Number	098816-1011000

Please **check only one** of boxes 1 or 2 below:*(If no box is checked, this paper will be treated as a request for express abandonment as if box 1 is checked.)*

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I request that the above-identified application be expressly abandoned as of the filing date of this paper.
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I am the:

- ☐ applicant.
- ☒ attorney or agent of record. Attorney or agent registration number is 64,668
- ☐ attorney or agent acting under 37 CFR 1.34, who is authorized under 37 CFR 1.138(b) because the application is expressly abandoned in favor of a continuing application (box 2 above must be checked). Attorney or agent registration number is _____.

/Kimberlynn B. Davis/

Signature

January 26, 2017

Date

Kimberlynn B. Davis

Typed or printed name

(404) 815-6500

Telephone Number

Note: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below.

☐ Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.138. 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: Mail Stop Express Abandonment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 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.

STATEMENT UNDER 37 CFR 3.73(c)Applicant/Patent Owner: AgriMetis, LLCApplication No./Patent No.: 62/336,989Filed/Issue Date: May 16, 2016Titled: Methods for Making L-GlutofosinateAgriMetis, LLC, a corporation

(Name of Assignee)

(Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☒ The assignee of the entire right, title, and interest.
2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):
- ☐ The extent (by percentage) of its ownership interest is ____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.
- ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

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4. ☐ The recipient, via a court proceeding or the like (e.g., bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

- 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 thereof is attached.
- B. ☒ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Brian Michael Green To: Acidophil, LLCThe document was recorded in the United States Patent and Trademark Office at
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[Page 1 of 2]

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.**

3

PGR2026-00026

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Petitioner Ex. 1014

STATEMENT UNDER 37 CFR 3.73(c)

3. From: Michelle Lorraine Gradley To: ZuvaSyntha Limited

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☒ As required by 37 CFR 3.73(c)(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.

/Kimberlynn B. Davis/

Signature

Kimberlynn B. Davis

Printed or Typed Name

January 26, 2017

Date

64,668

Title or Registration Number



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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
62/336,989	05/16/2016		130	098816-1011000(ASP-006P2)		

CONFIRMATION NO. 2855

FILING RECEIPT



0000000083319560

23342
KILPATRICK TOWNSEND & STOCKTON LLP
1001 WEST FOURTH STREET
WINSTON-SALEM, NC 27101

Date Mailed: 06/01/2016

Receipt is acknowledged of this provisional patent application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. 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**

Inventor(s)

Brian Michael Green, Lutherville, MD;
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Applicant(s)

Brian Michael Green, Lutherville, MD;
Michelle Lorraine Gradley, Canterbury, UNITED KINGDOM;

Power of Attorney:

Kimberlynn Davis--64668

Permission to Access Application via Priority Document Exchange: No

Permission to Access Search Results: No

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 06/01/2016

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 62/336,989**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

Methods for Making L-Glufosinate

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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Methods for Making L-Glufosinate

FIELD

Described herein are methods for producing a single stereoisomer of glufosinate,
5 particularly for the production of L-glufosinate.

BACKGROUND

The herbicide glufosinate is a non-selective, foliarly-applied herbicide considered to be one of the safest herbicides from a toxicological or environmental standpoint. Current commercial chemical synthesis methods for glufosinate yield a racemic mixture of L- and D-
10 glufosinate (Duke *et al.* 2010 *Toxins* 2:1943-1962). However, L-glufosinate (also known as phosphinothricin or (S)-2-amino-4-(hydroxy(methyl)phosphonoyl)butanoic acid) is much more potent than D-glufosinate (Ruhland *et al.* (2002) *Environ. Biosafety Res.* 1:29–37).

Therefore, methods are needed to produce only or primarily the active, L-glufosinate form. Previously, cost effective methods to generate pure L-glufosinate, or a mixture of D- and
15 L-glufosinate enriched for L-glufosinate, have not been available. Described herein are cost effective methods for the production of L-glufosinate.

SUMMARY

Described herein are methods for making L-glufosinate, comprising reacting D-glufosinate with a D-amino acid oxidase (DAAO) enzyme to form PPO (2-oxo-4-
20 (hydroxy(methyl)phosphinoyl)butyric acid); and aminating the PPO to L-glufosinate by a transaminase (TA) enzyme, using an amine group from one or more amine donors, wherein at least 95% of the D-glufosinate is eliminated and/or at least 85% of the D-glufosinate is converted to L-glufosinate. Optionally, the amine donor is selected from the group consisting of isopropylamine, glutamate, L-glutamate, alanine, sec-butylamine, and phenylethylamine.
25 Optionally, the D-glufosinate is originally present (i.e., in the reacting step) in a racemic mixture of D- and L-glufosinate.

The DAAO enzyme can be selected from the enzyme from *Rhodospiridium toruloides* (UniProt P80324) or *Trigonopsis variabilis* (UniProt Q99042). Optionally, the DAAO enzyme is a mutant DAAO based on the sequence from *Rhodospiridium toruloides*. The mutant DAAO
30 can optionally comprise mutations at positions 54, 56, 58, 213, and/or 238. For example, the

mutant DAAO can comprise one of the following mutations at position 54: N54C, N54L, N54T, or N54V. The mutant DAAO can optionally comprise the following mutation at position 56: T56M. The mutant DAAO can optionally comprise one of the following mutations at position 58: F58A, F58G, F58H, F58K, F58N, F58Q, F58R, F58S, or F58T. Optionally, the mutant
5 DAAO can comprise the following mutation at position 213: M213S. In some embodiments, the mutant DAAO can comprise one or more of the following combinations of mutations: F58K and M213S; N54T and T56M; N54V and F58Q; and/or N54V, F58Q, and M213S.

Optionally, the TA enzyme is a gabT transaminase from *Escherichia coli* (UniProt P22256).

10 The reacting step and the aminating step can be performed in a single container. Optionally, all reagents are substantially added at the start of the reaction. Optionally, the reagents for the reacting step and the reagents for the aminating step are added to the single container at different times. The reacting step and the aminating step can optionally be performed in separate containers.

15 Also described herein is a method for selectively controlling weeds in a field containing a crop of planted seeds or crops that may optionally be resistant to glufosinate, comprising applying an effective amount of a composition comprising L-glufosinate at an enantiomeric excess of greater than 90% over D-glufosinate to the field. Also described herein is a method for selectively controlling weeds in a field, controlling weeds in non-field areas, and/or desiccating
20 crops before harvest, comprising applying an effective amount of a composition comprising L-glufosinate at an enantiomeric excess of greater than 90% over D-glufosinate and more than 0.01% but less than 15% PPO to the field.

Further described herein are compositions. The compositions comprise D-glufosinate, PPO, and L-glufosinate. Optionally, the amount of L-glufosinate is 80% or greater, 85% or
25 greater, 90% or greater, or 95% or greater based on the combined weight of D-glufosinate, PPO, and L-glufosinate. Optionally, the amount of D-glufosinate is 10% or less, 5% or less, 2.5% or less, or 1% or less based on the combined weight of D-glufosinate, PPO, and L-glufosinate. Optionally, the amount of PPO is more than 1% but less than 20%, less than 15%, less than 10%, or less than 5% based on the weight of D-glufosinate, PPO, and L-glufosinate. These
30 compositions can optionally occur as dried powders or dissolved in aqueous or non-aqueous

carrier and additional chemical species can optionally be present. Optionally, the composition is prepared and used in an *ex vivo* environment.

Also described herein are formulations. The formulations comprise L-glufosinate ammonium in an amount from 10-30% by weight of the formulation; one or more additional components selected from the group consisting of sodium alkyl ether sulfate in an amount from 10-40% by weight of the formulation; 1-methoxy-2-propanol in an amount from 0.5-2% by weight of the formulation; dipropylene glycol in an amount from 4-18% by weight of the formulation; and alkyl polysaccharide in an amount from 4-20% by weight of the formulation; and water as the balance of the formulation. Optionally, the formulation comprises L-glufosinate ammonium in an amount of 12.25% by weight of the formulation; sodium alkyl ether sulfate in an amount of 31.6% by weight of the formulation; 1-methoxy-2-propanol in an amount of 1% by weight of the formulation; dipropylene glycol in an amount of 8.6% by weight of the formulation; alkyl polysaccharide in an amount of 9.8% by weight of the formulation; and water in an amount of 36.75% by weight of the formulation. Optionally, the formulation comprises L-glufosinate ammonium in an amount of 12.25% by weight of the formulation; sodium alkyl ether sulfate in an amount of 15.8% by weight of the formulation; 1-methoxy-2-propanol in an amount of 0.5% by weight of the formulation; dipropylene glycol in an amount of 4.3% by weight of the formulation; alkyl polysaccharide in an amount of 4.9% by weight of the formulation; and water in an amount of 62.25% by weight of the formulation.

The details of one or more embodiments are set forth in the drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic of an exemplary conversion of D-glufosinate to L-glufosinate. The amine donor and keto acid product are examples and are not intended to be limiting.

Figure 2 contains graphs showing the specific activity of mutant variants of *Rhodospiridium toruloides* DAAO. The activity for mutant variant AC201 is presented in the upper panel and the activity for mutant variant AC263 is presented in the lower panel. The indicated mutant variants are examples and are not intended to be limiting.

Figure 3 is a graph showing concentrations of L-glufosinate (first histogram bar of each set), D-glufosinate (second histogram bar of each set), and PPO (third histogram bar of each set)

before, during, and after a two-step de-racemization by the F58K, M213S mutant variant of *Rhodospiridium toruloides* DAAO and the *E. coli* gabT transaminase.

Figure 4 is a graph showing concentrations of L-glufosinate (circles), D-glufosinate (triangles), and PPO (squares) during a one-step de-racemization by a N54T, T56M, F58K, and M213S mutant variant of *Rhodospiridium toruloides* DAAO and the *E. coli* gabT transaminase.

DETAILED DESCRIPTION

Methods for the production of L-glufosinate (also known as phosphinothricin or (S)-2-amino-4-(hydroxy(methyl)phosphonoyl)butanoic acid) are provided. The methods comprise a two-step process, which may optionally occur in a single vessel and nearly simultaneously. The first step involves the oxidative deamination of D-glufosinate to PPO (2-oxo-4-(hydroxy(methyl)phosphinoyl)butyric acid). The second step involves the specific amination of PPO to L-glufosinate, using an amine group from one or more amine donors. By combining these two reactions, the proportion of L-glufosinate can be substantially increased in a racemic glufosinate mixture. Thus, provided herein are methods to obtain a composition consisting substantially of L-glufosinate. Since L-glufosinate is more potent than D-glufosinate, smaller amounts of the composition are needed to be effective. In one embodiment, described herein is a composition comprising a mixture of L-glufosinate, PPO, and D-glufosinate, where L-glufosinate is the predominant compound among the mixture of L-glufosinate, PPO, and D-glufosinate. In such a composition, PPO can contribute herbicidal activity (EP0030424).

I. Methods of Synthesis

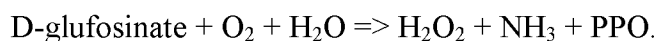
Methods for the conversion of D-glufosinate to L-glufosinate are provided. The methods described herein provide a means for converting a low cost feedstock of a racemic mixture of D- and L-glufosinate into a more valuable product that has been enriched for L-glufosinate. The methods for conversion includes two steps, which can occur in one or more separate containers. The first step is the oxidative deamination of D-glufosinate to PPO (2-oxo-4-(hydroxy(methyl)phosphinoyl)butyric acid). This step can be catalyzed by a D-amino acid oxidase (DAAO) enzyme, a D-amino acid dehydrogenase (DAAD) enzyme, or by chemical conversion. The second step is the specific amination of PPO to L-glufosinate, using an amine group from one or more amine donors. This step can be catalyzed by a transaminase (TA)

enzyme, a L-amino acid dehydrogenase (LAAD) enzyme, or chemical conversion. Using the methods described herein, compositions of substantially purified L-glufosinate can be obtained.

Figure 1 sets forth an example of the conversion of D-glufosinate to L-glufosinate. As noted above, the method involves a two-step process. As illustrated, the first step is an oxidative
 5 deamination of D-glufosinate to PPO and the second step is an amination of PPO to L-glufosinate.

The first step, i.e., the oxidative deamination of D-glufosinate to PPO, can be catalyzed by several classes of enzymes or can occur non-enzymatically. Such enzymes include DAAO, DAAD, and D-amino acid dehydratase.

10 In one embodiment a DAAO enzyme is used to catalyze the conversion of D-glufosinate to PPO. Such a reaction has the following stoichiometry:



Several DAAO enzymes are known in the art and can be used in the methods described herein, as long as they are capable of accepting D-glufosinate as a substrate. Such DAAO
 15 enzymes that can be used in the method include those from *Rhodospiridium toruloides*, *Trigonopsis variabilis*, *Fusarium sp*, *Candida sp*, *Schizosaccharomyces sp*, *Verticillium sp* and the like. Particular enzymes can be selected from proteins having sequences corresponding to Swissprot accession numbers P80324, Q99042, P00371, and P24552 or SPTREMBL numbers Q9HGY3 and Q9Y7N4. The DNA sequences which encode the DAAO may be selected from
 20 sequences set forth in EMBL accessions A56901, RGU60066, Z50019, SSDA04, D00809, AB042032, RCDAAOX, A81420, and SPCC1450. U.S. Patent No. 8,227,228 describes DAAO enzymes from *Candida intermedia*. Such sequences are herein incorporated by reference.

Additional DAAO enzymes can be identified in a variety of ways, including sequence similarity and functional screens. Optionally, the DAAO enzyme is a wild type enzyme.

25 Optionally, the DAAO enzyme is a mutant DAAO enzyme that is capable of accepting D-glufosinate as a substrate. In Hawkes *et al.* a mutant DAAO based on the sequence from *Rhodospiridium toruloides* (consisting of the F58K and M213S mutations) has been shown to accept D-glufosinate as a substrate (Hawkes *et al.* (2011) *Plant Biotechnol J.* 9(3):301-14). Other DAAO enzymes can be similarly modified to accept D-glufosinate. In the same manner,
 30 known DAAO enzymes may be improved by mutagenesis, and/or novel DAAO enzymes could be identified.

In some embodiments, mutant enzymes can be made and tested in the methods described herein. Mutants DAAO enzymes (e.g., from *Rhodotorula gracilis*) can include one mutation, two mutations, three mutations, or more than three mutations (e.g., four mutations, five mutations, six mutations, seven mutations, eight mutations, nine mutations, or ten mutations) at positions in the mutant sequence as compared to the wild type sequence. The mutant DAAO can optionally comprise mutations at positions 54, 56, 58, 213, and/or 238. In some embodiments, such mutants can comprise amino acid substitutions at positions 54 and 56 when compared with the wild type sequence. In other embodiments, such mutants can comprise amino acid substitutions at positions 54 and 58 when compared to the wild type sequence. In other embodiments, such mutants can include amino acid substitutions at positions 54, 213, and 238 when compared with the wild type sequence.

Optionally, at position 54, the wild type asparagine may be replaced by Ala, Cys, Gly, Ile, Ser, Leu, or, more preferably, Thr or Val. For example, the mutant DAAO can comprise one of the following mutations at position 54: N54C, N54L, N54T, or N54V.

Optionally, at position 56, the wild type threonine can be replaced by Ala, Cys, Gly, Ile, Asn, Arg, Ser, Thr, Met, or Val. See U.S. Patent No. 7,939,709, which is incorporated herein by reference. For example, the mutant DAAO can comprise the T56M mutation.

For example, at position 58, the wild type Phe can be replaced by Lys, Arg, Gln, Thr, Gly, Ser, Ala, Arg, Asn, or His. The mutant DAAO can optionally comprise one of the following mutations at position 58: F58A, F58G, F58H, F58K, F58N, F58Q, F58R, F58S, or F58T.

Optionally, at position 213, the wild type methionine is replaced by Arg, Lys, Ser, Cys, Asn, or Ala. In some examples, the mutant DAAO can comprise the mutation M213S.

Optionally, at position 238, the wild type tyrosine is replaced by His, Ser, Gys, Asn, or Ala.

In some embodiments, the mutant DAAO can comprise one or more of the following combinations of mutations: F58K and M213S; N54T and T56M; N54V and F58Q; N54C and F58H; N54T and F58T; N54T and F58G; N54T and F58Q; N54T and F58A; N54L and F58R; N54V and F58R; N54V and F58N; and/or N54V, F58Q, and M213S.

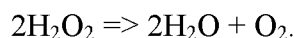
In one embodiment, the mutant DAAO comprises mutations in other DAAO enzymes in positions equivalent to positions 54, 56, 58, 213, and/or 238 of *Rhodospiridium toruloides* DAAO or *Trigonopsis variabilis* DAAO.

Other suitable D amino acid oxidases may be obtained preferably from fungal sources.

As indicated, DAAO enzymes can be identified and tested for use in the methods of the invention. To determine if the enzyme will accept D-glufosinate as a substrate, an oxygen electrode assay (Hawkes, 2011), colorimetric assay (Berneman A, Alves-Ferreira M, Coatnoan N, Chamond N, Minoprio P (2010) Medium/High Throughput D-Amino Acid Oxidase Colorimetric Method for Determination of D-Amino Acids. Application for Amino Acid Racemases. *J Microbial Biochem Technol* 2: 139-146), and/or direct measurement (via high performance liquid chromatography (HPLC), liquid chromatography mass spectrometry (LC-MS), or similar) of product formation can be employed.

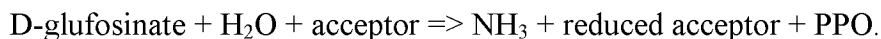
The reaction catalyzed by the DAAO enzyme requires oxygen. In some embodiments, atmospheric oxygen is sufficient to allow for the full reaction rate. In other embodiments, oxygen, oxygen enriched air, an oxygen enriched gas stream, or air, is introduced to the reaction, either in the head space or by sparging gas through the reaction vessel, to enhance the rate of reaction.

When a DAAO enzyme catalyzes the conversion of D-glufosinate to PPO, hydrogen peroxide evolves. This may be damaging to enzymes and other components of the biotransformation. Therefore, in one embodiment, an enzyme, such as catalase, can be used to catalyze the elimination of hydrogen peroxide. Catalase catalyzes the decomposition of hydrogen peroxide with the following stoichiometry:



In some embodiments, hydrogen peroxide can be eliminated using catalyzed and non-catalyzed decomposition reactions. For example, hydrogen peroxide can be eliminated by a non-catalyzed decomposition reaction using increased heat and/or pH. Hydrogen peroxide can also be eliminated by a catalyzed decomposition reaction using, for example, transition metals and other agents, such as potassium iodide.

Other enzymes can be used to catalyze the conversion of D-glufosinate to PPO. For example, a DAAD enzyme that accepts D-glufosinate as a substrate can be used with the following stoichiometry:

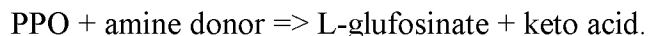


5 It is recognized that in methods where a DAAD is used, the DAAD catalyzed reaction can include redox cofactor recycling. This involves oxidizing the reduced acceptor so that it can accept more electrons from D-glufosinate.

In one embodiment, chemical oxidative deamination, wherein an intermediate α -keto acid is produced from the parent amino acid, can be used in the methods described herein to convert
 10 D-glufosinate to L-glufosinate. Chemical oxidative deamination involves the conversion of an amine group to a keto group with concomitant release of ammonia typically using metal ions such as those of copper or cobalt in an aqueous solution at temperatures between room temperature and the boiling point of the solution and at pH's in the range of 4-10. See, for example, Ikawa and Snell (1954) *J. Am. Chem. Soc.* 76 (19): 4900–4902, herein incorporated by
 15 reference.

The substantially complete (greater than 80%, greater than 85%, greater than 90%, or greater than 95%) conversion of D-glufosinate to PPO can occur within 24 hours, within 18 hours, within 12 hours, or within 8 hours.

The second step of the method described herein involves the conversion of PPO to L-
 20 glufosinate using a transaminase (TA) enzyme, an L-amino acid dehydrogenase (LAAD) enzyme, or by chemical conversion. In one embodiment, the method is a reaction catalyzed by a TA. A TA with the required stereospecificity that accepts PPO as a substrate catalyzes the amination of PPO to L-glufosinate with the following stoichiometry:



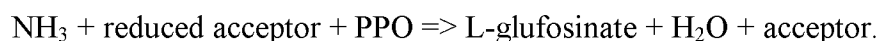
25 TAs useful in the methods described herein include the gabT transaminase from *Escherichia coli* (UniProt P22256), which has been shown to catalyze the desired reaction with PPO as a substrate (Bartsch *et al.* (1990) *Appl Environ Microbiol.* 56(1):7-12). Another enzyme has been evolved to catalyze the desired reaction at a higher rate using isopropylamine as an amine donor (Bhatia *et al.* (2004) *Peptide Revolution: Genomics, Proteomics & Therapeutics*,
 30 Proceedings of the Eighteenth American Peptide Symposium, Ed. Michael Chorev and Tomi K.

Sawyer, July 19-23, 2003, pp. 47-48). Additionally, TA enzymes from numerous microorganisms, such as *Streptomyces hygroscopicus*, *Streptomyces viridochromogenes*, *Candida albicans*, and others can be used in the practice of the methods described herein. In particular, see, for example, EP0249188, and U.S. Patent No. 5,162,212, which are incorporated
5 herein by reference. Where desired, the enzymes can be evolved by mutagenesis to increase their activities. Mutant TA enzymes can be selected for desired activity by the assays outlined in Schulz *et al.*, Appl Environ Microbiol. (1990) Jan. 56(1):1-6, and/or by direct measurement of the products by HPLC, LC-MS, or similar products.

Additional TA enzymes for use in the methods can be identified by screening collections
10 of TAs, such as those sold by Prozomix Limited (Northumberland, United Kingdom), SyncoZymes (Shanghai, China), Evocatal (Monheim am Rhein, Germany), Codexis (Redwood City, CA), or Abcam (Cambridge, United Kingdom) for the desired activity. Alternatively, sequence similarity can be used to identify novel TA enzymes. Finally, TA enzymes can also be identified from organisms capable of catalyzing the desired reaction.

The selection of an appropriate amine donor is important for an economical conversion of D-glufosinate to L-glufosinate. A variety of issues should be considered, including the cost of the donor, equilibrium thermodynamics, potential recovery of the donor, separation of the keto acid product from the desired L-glufosinate, and others. Consequently, TA enzymes that accept several different amine donors can be used, including low cost amine donors such as
15 isopropylamine (which can be advantageous since removal of the co-product, acetone, can drive the reaction to completion), L-glutamate or racemic glutamate, L-alanine or racemic alanine, sec-butylamine, and phenylethylamine. A wild type TA that accepts a desired amine donor can be identified, or a TA that does not normally accept a desired amine donor can be evolved to accept the desired substrate.

25 Other enzymes for use to catalyze the conversion of PPO to L-glufosinate include LAAD enzymes that accept PPO as a substrate. Such LAAD enzymes use the following stoichiometry:



LAAD catalyzed reactions can include redox cofactor recycling, which involves oxidizing the reduced acceptor so that it can accept more electrons from D-glufosinate.

Chemical reductive amination, wherein an amino group is produced from the parent keto-compound, can also be used to produce glufosinate, in the case where no chiral reductant or ligand is used, or L-glufosinate, in the case where a chiral reductant or ligand is used. Chemical reductive amination involves the conversion of an amine group to a keto group via an imine compound typically by treatment of the keto compound with a suitable amine in an organic solution. Suitable amines include, for example, methylamine or ammonia. Suitable organic solvents for use in the organic solution include ether or dichloromethane (DCM). The reductive amination can be performed at temperatures between room temperature and the boiling point of the solution. The thus produced imine can then be reduced using a reducing agent in an organic solution. Suitable reducing agents include, for example, LiBH_4 or $\text{Na}(\text{CN})\text{BH}_3$. Suitable organic solvents for use in the organic solution include ether or DCM. The reduction reaction can be performed at temperatures between 0 °C and the boiling point of the solution. Those skilled in the art will know that the process may be done in “one-pot” or in multiple containers, i.e., in separate transformations. Additionally, those skilled in the art will recognize that the described procedure will provide racemic amino material where possible. The use of a chiral reducing agent such as $\text{RuCl}_2[(S)\text{-BINAP}]$ or achiral reducing agents in the presence chiral ligands such as (S)- or (R)-VAPOL in a ratio between 1:1 to 1:0.05 can produce enantiomerically pure and/or enriched amino material. See, for example, Mignonac (1921) *Compt. Rend.* 172:223 and G. Li, Y. Liang, J. C. Antilla (2007) *J. Am. Chem. Soc.*, 129:5830-5831.

The substantially complete conversion of PPO to L-glufosinate may occur within 24 hours, within 18 hours, within 12 hours, or within 8 hours. Substantially complete, in this context, means that the conversion of PPO to L-glufosinate is greater than about 80%, greater than about 85%, greater than about 90%, greater than about 95%, greater than about 98%, or greater than about 99%.

If the reaction occurs in a single container or vessel, the TA enzyme can be added with the DAAO enzyme or added at a later time, e.g., after the DAAO enzyme has been allowed to catalyze some or substantially all of the oxidative deamination.

Enzymes can be added to the reaction by a number of methods. One approach is to express the enzyme(s) in microorganism(s) such as *E. coli*, *S. cerevisiae*, *P. pastoris*, and others, and to add the whole cells to the reactions as whole cell biocatalysts. Another approach is to express the enzyme(s), lyse the microorganisms, and add the cell lysate. Yet another approach is

to purify, or partially purify, the enzyme(s) from a lysate and add pure or partially pure enzyme(s) to the reaction. A further approach, which can be combined with the above approaches, is to immobilize enzyme(s) to a support (exemplary strategies are outlined in Datta *et al.* (2013) 3 Biotech. Feb; 3(1): 1–9). If multiple enzymes are required for a reaction, the enzymes can be expressed in one or several microorganisms, including expressing all enzymes within a single microorganism.

The DAO, TA, and/or other reactions can occur in a buffer. Exemplary buffers commonly used in biotransformation reactions include Tris, phosphate, or any of Good's buffers, such as 2-(N-morpholino)ethanesulfonic acid (MES); N-(2-Acetamido)iminodiacetic acid (ADA); piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES); N-(2-Acetamido)-2-aminoethanesulfonic acid (ACES); β -Hydroxy-4-morpholinepropanesulfonic acid (MOPSO); choline chloride; 3-(N-morpholino)propanesulfonic acid (MOPS); N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES); 2-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]ethanesulfonic acid (TES); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES); 3-(Bis(2-hydroxyethyl)amino)-2-hydroxypropane-1-sulfonic acid (DIPSO); acetamidoglycine, 3-(N-Tris(hydroxymethyl)methylamino(-2-hydroxypropanesulfonic acid (TAPSO); Piperazine-N,N'-bis(2-hydroxypropanesulfonic acid) (POPSO); 4-(2-Hydroxyethyl)piperazine-1-(2-hydroxypropanesulfonic acid) (HEPPSO); 3-[4-(2-Hydroxyethyl)-1-piperazinyl]propanesulfonic acid (HEPPS); tricine; glycylglycyl-L-glutamate; or 3-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]propane-1-sulfonic acid (TAPS). Additional exemplary buffer recipes can be found in Whittall, J. and Sutton, P. W. (eds) (2012) Front Matter, in Practical Methods for Biocatalysis and Biotransformations 2, John Wiley & Sons, Ltd, Chichester, UK. One or more organic solvents can also be added to the reaction.

In some embodiments, the reaction occurs within a defined pH range, which can be between pH 4 to pH 10 (e.g., between pH 6 and pH 9, such as approximately pH 7.5 to pH 8).

In some embodiments, the reaction occurs at a defined temperature. The temperature can be kept at a point between room temperature and the boiling point of the solvent, most typically between room temperature and 50 °C.

As indicated, the methods described herein provide a composition of substantially pure L-glufosinate (rather than a racemic mixture of L-glufosinate and D-glufosinate). Substantially pure L-glufosinate means that greater than about 80%, greater than about 85%, greater than

about 90%, greater than about 95%, greater than about 96%, greater than about 97%, greater than about 98%, or greater than about 99% of the D-glufosinate has been converted to L-glufosinate resulting in a composition having greater than about 80%, greater than about 85%, greater than about 90%, greater than about 95%, greater than about 96%, greater than about 97%, greater than about 98%, or greater than about 99% L-glufosinate compared to the sum of the D-glufosinate and the L-glufosinate present in the composition.

Optionally, the L-glufosinate can be partially or completely purified from the biotransformation reaction mixture. A number of alternative procedures can be employed, including, but not limited to: a) crystallization from the biotransformation mixture using suitable solvents such as ether or water at temperatures varying between -20 °C to the boiling point of the solution, b) extraction of L-glufosinate from the biotransformation mixture through repeated treatment with and separation of an organic solvent such as DCM or ethyl acetate (EtOAc) followed by concentration *in vacuo*, c) extraction of L-glufosinate from the biotransformation mixture through repeated treatment with and separation of an organic solvent such as DCM or EtOAc followed by concentration *in vacuo* and crystallization of this material mixture using suitable solvents such as ether or water at temperatures varying between -20 °C to the boiling point of the solution, d) concentration *in vacuo* of the biotransformation mixture followed by crystallization of the thus produced concentrate using suitable solvents such as ether or water at temperatures varying between -20 °C to the boiling point of the solution, or e) concentration *in vacuo* of the biotransformation mixture followed by fractional purification over an immobilized phase such as silica gel or octadecyl silane ligands of the thus produced concentrate as a mobile phase in a solvent such as DCM, methanol (MeOH), EtOAc, ammonia (NH₃), or mixtures thereof, such as DCM/MeOH or EtOAc/MeOH/NH₃. Alternatively, the biotransformation mixture can be used directly (and/or with the addition of various adjuvants) for the prevention or control of weeds.

In one embodiment, the L-glufosinate is not isolated from the biotransformation mixture and a composition comprising D-glufosinate, PPO, and L-glufosinate is obtained. This composition may be used directly as a herbicidal composition.

II. Compositions

Also described herein are compositions comprising the reaction products described above. In some embodiments, the composition substantially includes L-glufosinate. In some embodiments, the composition comprises a mixture of L-glufosinate, PPO, and D-glufosinate.

Optionally, L-glufosinate is the predominant compound among L-glufosinate, PPO, and D-glufosinate. For example, L-glufosinate can be present in the composition in an amount of at least 80% by weight of the sum of L-glufosinate, PPO, and D-glufosinate, at least 85 % by weight of the sum of L-glufosinate, PPO, and D-glufosinate, at least 90% by weight of the sum of L-glufosinate, PPO, and D-glufosinate, at least 95% by weight of the sum of L-glufosinate, PPO, and D-glufosinate, at least 96% by weight of the sum of L-glufosinate, PPO, and D-glufosinate, at least 97% by weight of the sum of L-glufosinate, PPO, and D-glufosinate, at least 98% by weight of the sum of L-glufosinate, PPO, and D-glufosinate, or at least 99% by weight of the sum of L-glufosinate, PPO, and D-glufosinate.

The composition can include PPO in an amount up to 20% by weight of the sum of L-glufosinate, PPO, and D-glufosinate. Optionally, the composition includes from 0.001% to 20% PPO (e.g., from 0.05% to 15% or from more than 0.01% to less than 5% PPO). For example, the composition can include PPO in an amount of less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, 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 0.01% by weight of the sum of the masses of L-glufosinate, PPO, and D-glufosinate.

D-Glufosinate can be present in the composition in an amount of 15% or less by weight of the sum of L-glufosinate, PPO, and D-glufosinate. For example, D-glufosinate can be present in an amount of 14 % or less, 13 % or less, 12 % or less, 11 % or less, 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, 1 % or less, or 0.5 % or less by weight of the sum of L-glufosinate, PPO, and D-glufosinate.

In some embodiments, the composition can contain small amounts (e.g., about 10% or less, about 8% or less, about 5% or less, about 2% or less, or about 1% or less by weight of the composition) of D-glufosinate. In some embodiments, the composition can contain small amounts (e.g., about 15% or less, about 10% or less, about 8% or less, about 5% or less, about 2% or less, or about 1% or less by weight of the composition) of PPO.

The compositions described herein are useful for application to a field of crop plants for the prevention or control of weeds. The composition may be formulated as a liquid for spraying on a field. The L-glufosinate is provided in the composition in effective amounts. As used herein, effective amount means from about 10 grams active ingredient per acre to about 1,500 grams active ingredient per acre, e.g., from about 50 grams to about 400 grams or from about 100 grams to about 350 grams. In some embodiments, the active ingredient is L-glufosinate. For example, the amount of L-glufosinate in the composition can be about 10 grams, about 50 grams, about 100 grams, about 150 grams, about 200 grams, about 250 grams, about 300 grams, about 350 grams, about 400 grams, about 500 grams, about 550 grams, about 600 grams, about 650 grams, about 700 grams, about 750 grams, about 800 grams, about 850 grams, about 900 grams, about 950 grams, about 1,000 grams, about 1,050 grams, about 1,100 grams, about 1,150 grams, about 1,200 grams, about 1,250 grams, about 1,300 grams, about 1,350 grams, about 1,400 grams, about 1,450 grams, or about 1,500 grams L-glufosinate per acre.

The herbicidal compositions (including concentrates which require dilution prior to application to the plants) described herein contain L-glufosinate (i.e., the active ingredient), optionally some residual D-glufosinate and/or PPO, and one or more adjuvant components in liquid or solid form.

The compositions are prepared by admixing the active ingredient with one or more adjuvants, such as diluents, extenders, carriers, surfactants, organic solvents, humectants, or conditioning agents, to provide a composition in the form of a finely-divided particulate solid, pellet, solution, dispersion, or emulsion. Thus, the active ingredient can be used with an adjuvant, such as a finely-divided solid, a liquid of organic origin, water, a wetting agent, a dispersing agent, an emulsifying agent, or any suitable combination of these. From the viewpoint of economy and convenience, water is the preferred diluent. However, not all the compounds are resistant to hydrolysis and in some cases this may dictate the use of non-aqueous solvent media, as understood by those of skill in the art.

Optionally, one or more additional components can be added to the composition to produce a formulated herbicidal composition. Such formulated compositions can include L-glufosinate, carriers (e.g., diluents and/or solvents), and other components. The formulated composition includes an effective amount of L-glufosinate. Optionally, the L-glufosinate can be present in the form of L-glufosinate ammonium. The L-glufosinate ammonium can be present in

an amount ranging from 10 % to 30 % by weight of the formulated composition. For example, the L-glufosinate ammonium can be present in an amount of 10 %, 12 %, 14 %, 16 %, 18 %, 20 %, 22 %, 24 %, 26 %, 28 %, or 30 % by weight of the formulated composition. Optionally, the L-glufosinate ammonium is present in an amount of 12.25 %.

5 In some examples, the formulated composition can include one or more surfactants. A suitable surfactant for use in the formulated composition includes sodium alkyl ether sulfate. The surfactant can be present in an amount from 10 % to 40 % by weight of the formulated composition. For example, the surfactant can be present in an amount of 10 %, 12 %, 14 %, 16 %, 18 %, 20 %, 22 %, 24 %, 26 %, 28 %, 30 %, 32 %, 34 %, 36 %, 38 %, or 40 % by weight of
10 the formulated composition. Optionally, the sodium alkyl ether sulfate is present in an amount of 15.8 % or 31.6 %.

The formulated composition can optionally include one or more solvents (e.g., organic solvents). Optionally, the solvent can be 1-methoxy-2-propanol, dipropylene glycol, ethylene glycol, and mixtures thereof. The one or more solvents can be present in an amount ranging
15 from 4 % to 20 % by weight of the formulated composition. For example, the total amount of solvents in the composition can be present in an amount of 4 % to 18 %, 5 % to 15 %, or 7.5 % to 10 % by weight of the formulated composition.

Optionally, the solvent includes a combination of two solvents. For example, the solvents in the formulation can include 1-methoxy-2-propanol and dipropylene glycol. The 1-
20 methoxy-2-propanol can be present, for example, in an amount of 0.5 % to 2 % by weight of the formulated composition. For example, the 1-methoxy-2-propanol can be present in the amount of 0.5 %, 0.6 %, 0.7 %, 0.8 %, 0.9 %, 1.0 %, 1.1 %, 1.2 %, 1.3 %, 1.4 %, 1.5 %, 1.6 %, 1.7 %, 1.8 %, 1.9 %, or 2.0 % by weight of the formulated composition. Optionally, the 1-methoxy-2-propanol is present in an amount of 0.5 % or 1.0 % by weight of the formulated composition.

25 The dipropylene glycol can be present in an amount of from 4 % to 18 % by weight of the formulated composition. For example, the dipropylene glycol can be present in an amount of 4 %, 6 %, 8 %, 10 %, 12 %, 14 %, 16 %, or 18 % by weight of the formulated composition. Optionally, the dipropylene glycol is present in an amount of 4.3 % or 8.6 % by weight of the formulated composition.

30 The formulated composition can also include one or more polysaccharide humectants. Examples of suitable polysaccharide humectants include, for example, alkyl polysaccharides,

pentoses, high fructose corn syrup, sorbitol, and molasses. The polysaccharide humectant, such as alkyl polysaccharide, can be present in the formulated composition in an amount ranging from 4 % to 20 % by weight of the formulated composition. For example, the total amount of polysaccharide humectant in the composition can be present in an amount of 4 % to 18 %, 4.5 % to 15 %, or 5 % to 10 % by weight of the formulated composition. In some examples, the total amount of polysaccharide humectant, such as the alkyl polysaccharide, present in the formulated composition can be 4 %, 5 %, 6 %, 7 %, 8 %, 9 %, 10 %, 11 %, 12 %, 13 %, 14 %, 15 %, 16 %, 17 %, or 18 %. Optionally, the alkyl polysaccharide can be present in an amount of 4.9 % or 9.8 %.

A diluent can also be included in the formulated composition. Suitable diluents include water and other aqueous components. Optionally, the diluents are present in an amount necessary to produce compositions ready for packaging or for use.

In one example, the formulated composition includes L-glufosinate ammonium in an amount of 12.25% by weight of the formulation; sodium alkyl ether sulfate in an amount of 31.6% by weight of the formulation; 1-methoxy-2-propanol in an amount of 1% by weight of the formulation; dipropylene glycol in an amount of 8.6% by weight of the formulation; alkyl polysaccharide in an amount of 9.8% by weight of the formulation; and water in an amount of 36.75% by weight of the formulation.

In another example, the formulated composition includes L-glufosinate ammonium in an amount of 12.25% by weight of the formulation; sodium alkyl ether sulfate in an amount of 15.8% by weight of the formulation; 1-methoxy-2-propanol in an amount of 0.5% by weight of the formulation; dipropylene glycol in an amount of 4.3% by weight of the formulation; alkyl polysaccharide in an amount of 4.9% by weight of the formulation; and water in an amount of 62.25% by weight of the formulation.

Further components suitable for use in the formulated compositions provided herein are described in U.S. Patent Nos. 4,692,181 and 5,258,358, both of which are incorporated by reference herein in their entireties.

The herbicidal compositions described herein, particularly liquids and soluble powders, can contain as further adjuvant components one or more surface-active agents in amounts sufficient to render a given composition readily dispersible in water or in oil. The incorporation of a surface-active agent into the compositions greatly enhances their efficacy. Surface-active

agent, as used herein, includes wetting agents, dispersing agents, suspending agents, and emulsifying agents are included therein. Anionic, cationic, and non-ionic agents can be used with equal facility.

Suitable wetting agents include alkyl benzene and alkyl naphthalene sulfonates, sulfated
5 fatty alcohols, amines or acid amides, long chain acid esters of sodium isothionate, esters of sodium sulfosuccinate, sulfated or sulfonated fatty acid esters petroleum sulfonates, sulfonated vegetable oils, ditertiary acetylenic glycols, polyoxyethylene derivatives of alkylphenols (particularly isooctylphenol and nonylphenol), and polyoxethylene derivatives of the mono-
higher fatty acid esters of hexitol anhydrides (e.g. sorbitan). Exemplary dispersants include
10 methyl cellulose, polyvinyl alcohol, sodium lignin sulfonates, polymeric alkyl naphthalene sulfonates, sodium naphthalene sulfonate, polymethylene bisnaphthalenesulfonate, and sodium N-methyl-N- (long chain acid) laurates.

Water-dispersible powder compositions can be made containing one or more active ingredients, an inert solid extender, and one or more wetting and dispersing agents. The inert
15 solid extenders are usually of mineral origin, such as the natural clays, diatomaceous earth, and synthetic minerals derived from silica and the like. Examples of such extenders include kaolinites, attapulgite clay, and synthetic magnesium silicate. Water-dispersible powders described herein can optionally contain from about 5 to about 95 parts by weight of active ingredient (e.g., from about 15 to 30 parts by weight of active ingredient), from about 0.25 to 25
20 parts by weight of wetting agent, from about 0.25 to 25 parts by weight of dispersant, and from 4.5 to about 94.5 parts by weight of inert solid extender, all parts being by weight of the total composition. Where required, from about 0.1 to 2.0 parts by weight of the solid inert extender can be replaced by a corrosion inhibitor or anti-foaming agent or both.

Aqueous suspensions can be prepared by dissolution or by mixing together and grinding
25 an aqueous slurry of a water-insoluble active ingredient in the presence of a dispersing agent to obtain a concentrated slurry of very finely-divided particles. The resulting concentrated aqueous suspension is characterized by its extremely small particle size, so that when diluted and sprayed, coverage is very uniform.

Emulsifiable oils are usually solutions of active ingredient in water-immiscible or
30 partially water-immiscible solvents together with a surface active agent. Suitable solvents for the active ingredient described herein include hydrocarbons and water-immiscible ethers, esters, or

ketones. The emulsifiable oil compositions generally contain from about 5 to 95 parts active ingredient, about 1 to 50 parts surface active agent, and about 4 to 94 parts solvent, all parts being by weight based on the total weight of emulsifiable oil.

Compositions described herein can also contain other additaments, for example, fertilizers, phytotoxicants and plant growth regulants, pesticides, and the like used as adjuvants or in combination with any of the above-described adjuvants. The compositions described herein can also be admixed with the other materials, e.g., fertilizers, other phytotoxicants, etc., and applied in a single application.

In each of the formulation types described herein, e.g., liquid and solid formulations, the concentration of the active ingredients are the same.

III. Methods of Use

The compositions described herein can be used in methods for selectively controlling weeds in a field or any other area, including, for example, a railway, lawn, golf course, and others where the control of weeds is desired. Optionally, the weeds in the field or other area can contain a crop of planted seeds or crops that are resistant to glufosinate. The methods can include applying an effective amount of a composition comprising L-glufosinate as described herein to the field.

The compositions described herein are useful for application to a field of crop plants for the prevention or control of weeds. The composition may be formulated as a liquid for spraying on a field. The L-glufosinate is provided in the composition in effective amounts. As used herein, effective amount means from about 10 grams active ingredient per acre to about 1,500 grams active ingredient per acre, e.g., from about 50 grams to about 400 grams or from about 100 grams to about 350 grams. In some embodiments, the active ingredient is L-glufosinate. For example, the amount of L-glufosinate in the composition can be about 10 grams, about 50 grams, about 100 grams, about 150 grams, about 200 grams, about 250 grams, about 300 grams, about 350 grams, about 400 grams, about 500 grams, about 550 grams, about 600 grams, about 650 grams, about 700 grams, about 750 grams, about 800 grams, about 850 grams, about 900 grams, about 950 grams, about 1,000 grams, about 1,050 grams, about 1,100 grams, about 1,150 grams, about 1,200 grams, about 1,250 grams, about 1,300 grams, about 1,350 grams, about 1,400 grams, about 1,450 grams, or about 1,500 grams L-glufosinate per acre.

IV. Exemplary Embodiments

Non-limiting embodiments include:

1. A method for making L-glufosinate, comprising:
reacting D-glufosinate with a D-amino acid oxidase (DAAO) enzyme to form PPO (2-
5 oxo-4-(hydroxy(methyl)phosphinoyl)butyric acid); and
aminating the PPO to L-glufosinate by a transaminase (TA) enzyme, using an amine
group from one or more amine donors,
wherein at least 85% of the D-glufosinate is converted to L-glufosinate.
2. The embodiment of paragraph 1, wherein the amine donor is selected from the
10 group consisting of isopropylamine, glutamate, L-glutamate, alanine, sec-butylamine, and
phenylethylamine.
3. The embodiment of paragraph 1, wherein the D-glufosinate is originally present
in a racemic mixture of D- and L-glufosinate.
4. The embodiment of paragraph 1, wherein the DAAO enzyme is selected from the
15 enzyme from *Rhodospiridium toruloides* or *Trigonopsis variabilis*. In an embodiment, the
Rhodospiridium toruloides DAAO enzyme is UniProt P80324. In an embodiment, the
Trigonopsis variabilis DAAO enzyme is UniProt Q99042.
5. The embodiment of paragraph 1, wherein the DAAO enzyme is a mutant DAAO
based on the sequence from *Rhodospiridium toruloides*.
6. The embodiment of paragraph claim 5, wherein the mutant DAAO comprises one
or more mutations at positions 54, 56, 58, 213, and 238.
7. The embodiment of paragraph 6, wherein the mutation at position 54 is selected
from the group consisting of N54C, N54L, N54T, and N54V.
8. The embodiment of paragraph 6, wherein the mutation at position 56 is T56M.
9. The embodiment of paragraph 6, wherein the mutation at position 58 is selected
from the group consisting of F58A, F58G, F58H, F58K, F58N, F58Q, F58R, F58S, and F58T.
10. The embodiment of paragraph 6, wherein the mutation at position 213 is M213S.
- 20 11. The embodiment of paragraph 5, wherein the mutant DAAO comprises mutations
F58K and M213S.
12. The embodiment of paragraph 5, wherein the mutant DAAO comprises mutations
at positions 54 and 56.

13. The embodiment of paragraph 5, wherein the mutant DAAO comprises mutations N54T and T56M.

14. The embodiment of paragraph 5, wherein the mutant DAAO comprises mutations F58Q or F58H.

15. The embodiment of paragraph 5, wherein the mutant DAAO comprises mutations N54V and F58Q.

16. The embodiment of paragraph 5, wherein the mutant DAAO comprises mutations N54V, F58Q, and M213S.

17. The embodiment of paragraph 1, wherein the TA enzyme is a gabT transaminase from *Escherichia coli*. In an embodiment, the *Escherichia coli* gabT transaminase is UniProt
5 P22256.

18. The embodiment of paragraph 1, wherein the reacting step and the aminating step are performed in a single container.

19. The embodiment of paragraph 18, wherein all reagents are substantially added at the start of the reaction.

10 20. The embodiment of paragraph 18, wherein the reagents for the reacting step and the reagents for the aminating step are added to the single container at different times.

21. The embodiment of paragraph 1, wherein the reacting step and the aminating step are performed in separate containers.

22. A method for selectively controlling weeds in a field containing a crop of planted
15 seeds or crops that are resistant to glufosinate, comprising:
applying an effective amount of a composition comprising L-glufosinate at an enantiomeric excess of greater than 90% over D-glufosinate to the field.

23. A method for selectively controlling weeds in a field, comprising
applying an effective amount of a composition comprising L-glufosinate at an
20 enantiomeric excess of greater than 90% over D-glufosinate and more than 0.01% but less than 10% PPO to the field.

24. A composition comprising D-glufosinate, PPO, and L-glufosinate.

25. The embodiment of paragraph 24, wherein the amount of L-glufosinate based on the combined weight of D-glufosinate, PPO, and L-glufosinate is 90% or greater.

26. A formulation, comprising L-glufosinate ammonium in an amount from 10-30% by weight of the formulation; one or more additional components selected from the group consisting of sodium alkyl ether sulfate in an amount from 10-40% by weight of the formulation; 1-methoxy-2-propanol in an amount from 0.5-2% by weight of the formulation; dipropylene glycol in an amount from 4-18% by weight of the formulation; and alkyl polysaccharide in an amount from 4-20% by weight of the formulation; and water as the balance of the formulation.

27. The embodiment of paragraph 26, wherein the formulation comprises: L-glufosinate ammonium in an amount of 12.25% by weight of the formulation; sodium alkyl ether sulfate in an amount of 31.6% by weight of the formulation; 1-methoxy-2-propanol in an amount of 1% by weight of the formulation; dipropylene glycol in an amount of 8.6% by weight of the formulation; alkyl polysaccharide in an amount of 9.8% by weight of the formulation; and water in an amount of 36.75% by weight of the formulation.

28. The embodiment of paragraph 26, wherein the formulation comprises: L-glufosinate ammonium in an amount of 12.25% by weight of the formulation; sodium alkyl ether sulfate in an amount of 15.8% by weight of the formulation; 1-methoxy-2-propanol in an amount of 0.5% by weight of the formulation; dipropylene glycol in an amount of 4.3% by weight of the formulation; alkyl polysaccharide in an amount of 4.9% by weight of the formulation; and water in an amount of 62.25% by weight of the formulation.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1: DAAO enzyme purification

The coding sequence of a mutant DAAO from *Rhodospiridium toruloides* (for example, consisting of a MMARIRL leader sequence and the F58K and M213S mutations) was cloned into the pET14b vector to allow for expression of an N terminally 6xHis tagged protein. This pET14b-RgDAAO plasmid was transformed into BL21 (BE3) trxB pLysS cells.

To purify DAAO enzyme, cells were grown in 400 mL autoinducing medium (LB broth base with trace elements, Formedium) at 30°C for 20 to 24 hours. Cells were harvested in precooled centrifuges and buckets, washed with cold water, centrifuged again, and stored at -80°C until purification.

Cell pellets were then thawed in lysis buffer (50 mM potassium phosphate, pH 8.0, 20 mM imidazole and 1% Sigma protease inhibitor cocktail (PIC) w/o EDTA) at a volume of 5 mL of lysis buffer per 1 g cell pellet. While on ice, cells were sonicated 4 times for 30 seconds at an amplitude of 10. The cell lysate was clarified by centrifugation and then added to cobalt resin (HisPur Cobalt, ThermoScientific) at 4 times the bed volume. The cell lysate was incubated for 1 hour, with gentle shaking, at room temperature. The resin was added to a column and washed twice with 5 bed volumes of wash buffer (50 mM Kpi, pH 8.0, 20 mM imidazole). The elution was performed 4 times with 1 bed volume of elution buffer (50 mM Kpi, 200 mM imidazole).

Example 2: Colorimetric determination of DAAO activity

DAAO activity was determined similarly to Berneman et al. In brief, 100 uL of substrate and HRP (0.1 mg/mL HRP, Sigma P8375, and the desired amount of D-glufosinate or racemic D/L-glufosinate in 50 mM potassium phosphate, pH 8) was added to a Brand UV micro cuvette. To that, 50 uL of dyes (60 ug/mL TBHBA, Sigma 439533, and 1 mg/mL 4-aminoantipyrine, Sigma A4382, in 50 mM potassium phosphate, pH 8) was added and then 50 uL of enzyme mix (DAAO concentration as desired in 100 mM potassium phosphate, pH 8). The reaction was monitored on a spectrophotometer at 510 nm over an appropriate time to determine the enzyme kinetics. Although no flavin adenine dinucleotide (FAD) was added to the purification of DAAO or the reaction, this reagent can optionally be included.

Figure 2 shows the specific activity of two exemplary mutant variants of *Rhodospiridium toruloides* DAAO, AC201 and AC263, purified as in Example 1. AC201 contains F58K and M213S and AC263 contains N54T, T56M, F58K, and M213S. The X axis shows the concentration of substrate (mM) and the Y axis shows the specific activity (mmol/min/mg).

Example 3: Purification of transaminases

To purify, for example, the *Escherichia coli* gabT transaminase (<http://www.uniprot.org/uniprot/P22256>), the gene was amplified from E. coli K12 strain ER2925 and cloned into pET-14b to generate an N-terminal 6xHis tagged version. This plasmid was then transformed into BL21 (DE3) cells for induction. After induction in autoinducing media, cells were lysed by sonication and purified as described in Example 1.

Example 4: Demonstration of transaminase activity

In a non-limiting example, the source of PPO for a transamination assay can be D-glufosinate or racemic D/L-glufosinate that has been converted by a DAAO to PPO. In the first

step, 39 mM racemic D/L-glufosinate was incubated with 0.5 mg/ml purified *Rhodospiridium toruloides* DAAO F58K M213S and 10 ug/mL catalase in 50 mM Potassium phosphate buffer, pH8 for 20 hours at 30 C. This resulted in conversion of the majority of the D-glufosinate to PPO. Subsequently, purified *E. coli* gabT was added at 20 ug/mL and L-glutamate was added at 50 mM as the amine donor. At relevant points, samples were stopped by boiling for 10 minutes followed by precipitation with an equal volume of acetonitrile. The individual chemical species were resolved on an HPLC with a Chirobiotic T2 column and quantified by comparison to authentic standards.

As shown in Figure 3, the combination of a mutant variant of DAAO and a transaminase resulted in an improvement of an enantiomeric enrichment that started at 0% for L-glufosinate over D-glufosinate (ie equal representation of D-glufosinate and L-glufosinate) to an enantiomeric enrichment of 92%. These results demonstrate that *E. coli* gabT has transaminase activity, and this assay can be used to determine the activity of any number of wild type and/or mutant potential transaminases.

Example 5: De-racemization of racemic D/L-glufosinate in a single vessel

Reactions were set up similarly as in Example 4. The system (5.45 mL, 30 °C) was run in phosphate buffer at a pH of 7.3. It was noted that 50 mM of phosphate buffer at a pH of 8.0 was inadequate to buffer the amino acid additions and that the unadjusted pH after amino acid additions in this system was pH 6.4. The pH was adjusted using 1M of the base salt K₂HPO₄ to a volume of 5.45 mL, meaning that the actual initial substrate concentration by addition was 275 mM. The following reagents were added essentially simultaneously at the start of the reaction: 271 mg D,L-glufosinate, 420 mg glutamate, 15 mg AC263 DAAO, 50 µg catalase, and 1.0 mg *E. coli* gab T transaminase. Figure 4 shows that, when all reagents were added, the amount of D-PPT (D-glufosinate) diminished with only modest accumulation of PPO. This result indicates an efficient deracemisation of D/L-glufosinate into L-glufosinate by the RgDAAO/EcgabT enzyme couple.

Example 6: Demonstration of improved DAAO enzymes

Using protein mutagenesis strategies as outlined above, improved and variant DAAO enzymes were identified. The enzymes were assayed according to the procedures described below.

Stock Solutions:

The following dye stock solutions were prepared: a 20 mg/mL stock solution of 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) in DMSO; and a 100 mg/mL stock solution of 4-aminoantipyrine (4-AAP) in water. The following enzyme stock solution was prepared: a 1 mg/mL stock solution of horseradish peroxidase (HRP) type 6 in a pH 8.0 potassium phosphate buffer. The following substrate stock solution was prepared: varying concentrations of D or DL amino acid in a pH 8.0 potassium phosphate buffer.

Reaction Mixes:

The following reaction mixtures were prepared:

Mix A is a combination of the substrate and HRP enzyme. Solutions were prepared for each substrate concentration to be assayed using reaction buffer. The solutions were two times the final substrate concentration and 0.2 mg/mL for the HRP solution.

Mix B is a dye mixture. To 5 mL of reaction buffer was added 120 μ L of TBHBA solution and 400 μ L of 4-AAP solution.

Mix C is an enzyme mixture. A 0.2 mg/mL solution of DAAO in reaction buffer was prepared. The final reaction concentration was 50 μ g/mL.

Protocol:

A spectrophotometer was used at a wavelength of 510 nm, which corresponds to the maximum absorbance for 4-AAP/TBHBA and is the point at which the extinction coefficient is 29400 $M^{-1} cm^{-1}$. The temperature for performing the assays was 30 °C. The reaction kinetics were obtained by measuring every minute for 15 minutes. Between measurements, 20 seconds of orbital shaking at normal intensity was performed, followed by 10 seconds of settling time.

Using a 96-well plate, the following mixes (with replicates) were added in the following order using multi-channel: 100 μ l mix A, 50 μ l mix B, and 50 μ l mix C. The measurements were started immediately after the enzyme addition.

The enzyme kinetics were measured as described above, plotted on a Michaelis Menten graph, and used to calculate V_{max} and K_M . As shown below in Table 1, variant mutant DAAO enzymes showed a range of activities:

Table 1:

Variant	Mutations	Vmax (umole/min*mg)	Km (mM)
Ac263	54T, 56M, 58K, 213S	0.6	0.7
Ac302	54V, 58Q, 213S	1.8	7.6
Ac305	54C, 58H, 213S	1.7	8.2
Ac309	54T, 58T, 213S	1.4	12.5
Ac312	54T, 58G, 213S	1.4	11.8
Ac314	54T, 58Q, 213S	1.7	5.5
Ac316	54T, 58S, 213S	1.5	10.0
Ac318	54T, 58A, 213S	1.4	3.2
Ac319	54L, 58R, 213S	1.2	5.9
Ac320	54V, 58R, 213S	1.5	9.1
Ac322	54V, 58N, 213S	1.5	4.4

Example 7: De-racemization of racemic D/L-glufosinate at a larger scale

The scale of the de-racemization is increased using approaches familiar to those skilled in the art. Reagents and their relative ratios are substantially similar to Example 5, but the amounts are significantly greater. Rather than tubes in shakers, the reactions are performed in stirred jacketed reactors, including, optionally, air or oxygen sparging of the broth or headspace. These reactors vary in size, from less than 10 mL reaction to tens or hundreds of thousands of liters. Stirring rates are chosen to increase reaction mixing and rate while minimizing power consumption and shear.

It is understood that the terminology used herein is for the purpose of describing particular embodiments only, and the terminology is not intended to be limiting. The scope of the invention will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention. Certain ranges are

presented herein with numerical values being preceded by the term "about." The term "about" is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating
5 unrecited number may be a number, which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number.

All publications, patents, and patent applications cited in this specification are incorporated herein by reference to the same extent as if each individual publication, patent, or patent application were specifically and individually indicated to be incorporated by reference.
10 Furthermore, each cited publication, patent, or patent application is incorporated herein by reference to disclose and describe the subject matter in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the invention described herein is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided might be
15 different from the actual publication dates, which may need to be independently confirmed.

It is noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only," and the like in connection with the recitation of claim elements, or use of a "negative" limitation. As will be apparent to those of skill in the art upon reading this disclosure,
20 each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the invention. Any recited method may be carried out in the order of events recited or in any other order that is logically possible. Although any methods and materials similar or equivalent to those described herein
25 may also be used in the practice or testing of the invention, representative illustrative methods and materials are now described.

WHAT IS CLAIMED IS:

1. A method for making L-glufosinate, comprising:
reacting D-glufosinate with a D-amino acid oxidase (DAAO) enzyme to form PPO (2-oxo-4-(hydroxy(methyl)phosphinoyl)butyric acid); and
aminating the PPO to L-glufosinate by a transaminase (TA) enzyme, using an amine group from one or more amine donors,
wherein at least 85% of the D-glufosinate is converted to L-glufosinate.
2. The method of claim 1, wherein the amine donor is selected from the group consisting of isopropylamine, glutamate, L-glutamate, alanine, sec-butylamine, and phenylethylamine.
3. The method of claim 1, wherein the D-glufosinate is originally present in a racemic mixture of D- and L-glufosinate.
4. The method of claim 1, wherein the DAAO enzyme is selected from the enzyme from *Rhodospiridium toruloides* (UniProt P80324) or *Trigonopsis variabilis* (UniProt Q99042).
5. The method of claim 1, wherein the DAAO enzyme is a mutant DAAO based on the sequence from *Rhodospiridium toruloides*.
6. The method of claim 5, wherein the mutant DAAO comprises one or more mutations at positions 54, 56, 58, 213, and 238.
7. The method of claim 6, wherein the mutation at position 54 is selected from the group consisting of N54C, N54L, N54T, and N54V.
8. The method of claim 6, wherein the mutation at position 56 is T56M.
9. The method of claim 6, wherein the mutation at position 58 is selected from the group consisting of F58A, F58G, F58H, F58K, F58N, F58Q, F58R, F58S, and F58T.

10. The method of claim 6, wherein the mutation at position 213 is M213S.
11. The method of claim 5, wherein the mutant DAAO comprises mutations F58K and M213S.
12. The method of claim 5, wherein the mutant DAAO comprises mutations at positions 54 and 56.
13. The method of claim 5, wherein the mutant DAAO comprises mutations N54T and T56M.
14. The method of claim 5, wherein the mutant DAAO comprises mutations F58Q or F58H.
15. The method of claim 5, wherein the mutant DAAO comprises mutations N54V and F58Q.
16. The method of claim 5, wherein the mutant DAAO comprises mutations N54V, F58Q, and M213S.
17. The method of claim 1, wherein the TA enzyme is a gabT transaminase from *Escherichia coli* (UniProt P22256).
18. The method of claim 1, wherein the reacting step and the aminating step are performed in a single container.
19. The method of claim 13, wherein all reagents are substantially added at the start of the reaction.
20. The method of claim 13, wherein the reagents for the reacting step and the reagents for the aminating step are added to the single container at different times.

21. The method of claim 1, wherein the reacting step and the aminating step are performed in separate containers.
22. A method for selectively controlling weeds in a field containing a crop of planted seeds or crops that are resistant to glufosinate, comprising:
applying an effective amount of a composition comprising L-glufosinate at an enantiomeric excess of greater than 90% over D-glufosinate to the field.
23. A method for selectively controlling weeds in a field, comprising
applying an effective amount of a composition comprising L-glufosinate at an enantiomeric excess of greater than 90% over D-glufosinate and more than 0.01% but less than 10% PPO to the field.
24. A composition comprising D-glufosinate, PPO, and L-glufosinate.
25. The composition of claim 19, wherein the amount of L-glufosinate is 90% or greater based on the total amount of D-glufosinate, PPO, and L-glufosinate.
26. A formulation, comprising:
L-glufosinate ammonium in an amount from 10-30% by weight of the formulation;
one or more additional components selected from the group consisting of sodium alkyl ether sulfate in an amount from 10-40% by weight of the formulation; 1-methoxy-2-propanol in an amount from 0.5-2% by weight of the formulation; dipropylene glycol in an amount from 4-18% by weight of the formulation; and alkyl polysaccharide in an amount from 4-20% by weight of the formulation; and
water as the balance of the formulation.
27. The formulation of claim 21, wherein the formulation comprises:
L-glufosinate ammonium in an amount of 12.25% by weight of the formulation;
sodium alkyl ether sulfate in an amount of 31.6% by weight of the formulation;
1-methoxy-2-propanol in an amount of 1% by weight of the formulation;

dipropylene glycol in an amount of 8.6% by weight of the formulation;
alkyl polysaccharide in an amount of 9.8% by weight of the formulation; and
water in an amount of 36.75% by weight of the formulation.

28. The formulation of claim 21, wherein the formulation comprises:
L-glufosinate ammonium in an amount of 12.25% by weight of the formulation;
sodium alkyl ether sulfate in an amount of 15.8% by weight of the formulation;
1-methoxy-2-propanol in an amount of 0.5% by weight of the formulation;
dipropylene glycol in an amount of 4.3% by weight of the formulation;
alkyl polysaccharide in an amount of 4.9% by weight of the formulation; and
water in an amount of 62.25% by weight of the formulation.

ABSTRACT

Methods for the production of L-glufosinate (also known as phosphinothricin or (S)-2-amino-4-(hydroxy(methyl)phosphonoyl)butanoic acid) are provided. The methods comprise a two-step process. The first step involves the oxidative deamination of D-glufosinate to PPO (2-oxo-4-(hydroxy(methyl)phosphinoyl)butyric acid). The second step involves the specific amination of PPO to L-glufosinate, using an amine group from one or more amine donors. By combining these two reactions, the proportion of L-glufosinate in a mixture of L-glufosinate and D-glufosinate can be substantially increased.

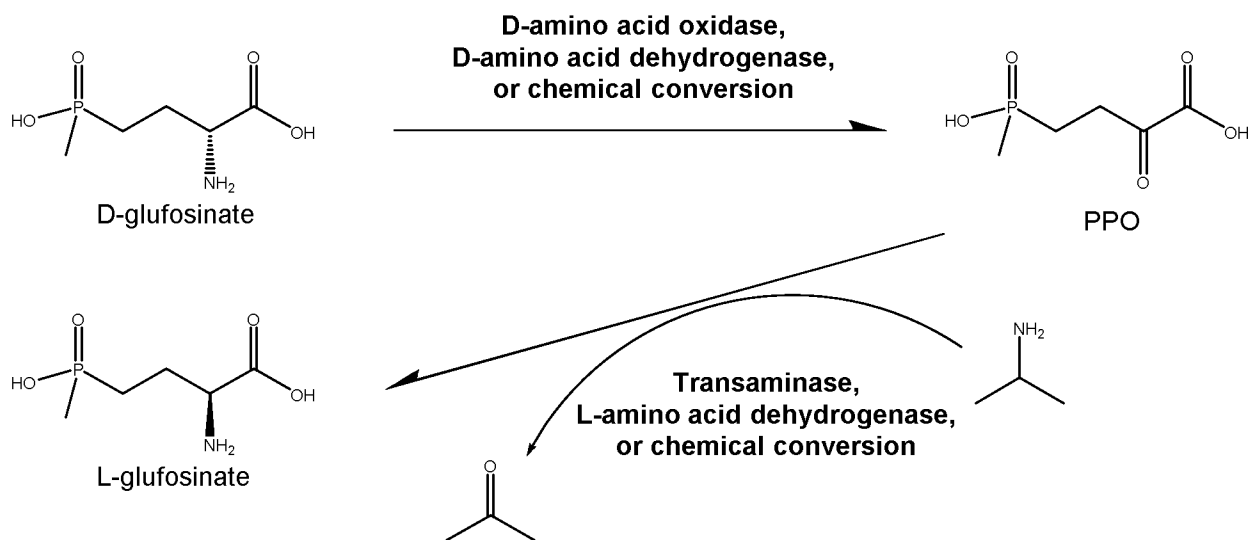
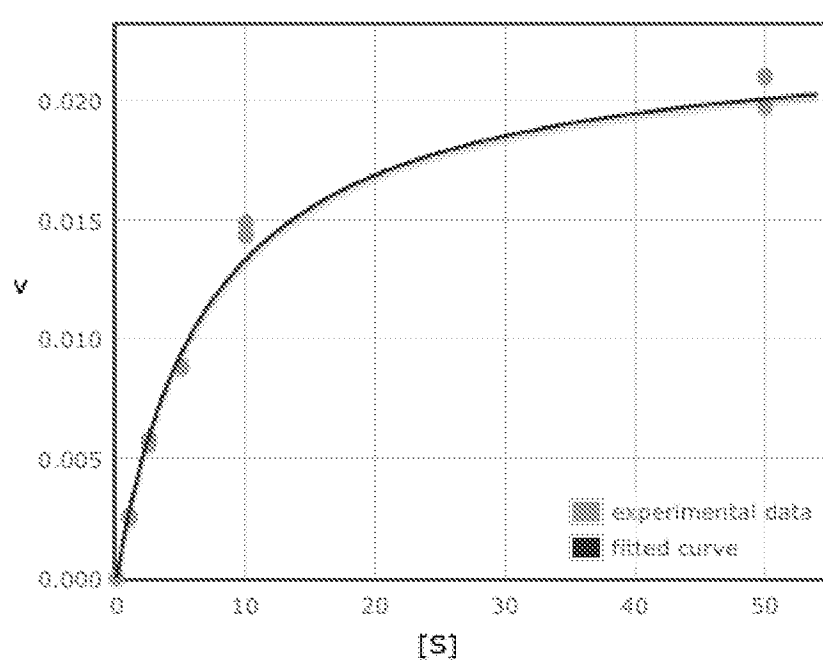


Figure 1

AC201



AC263

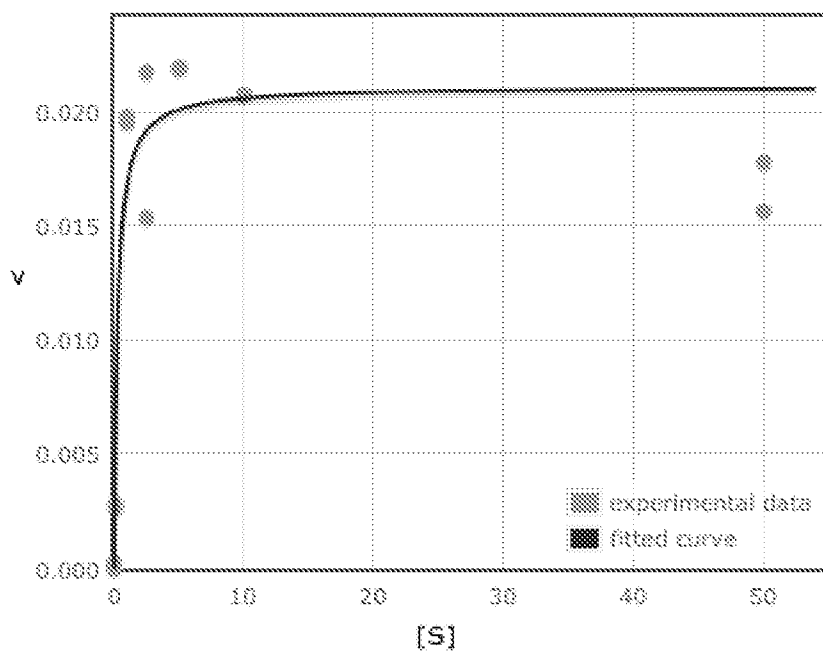


Figure 2

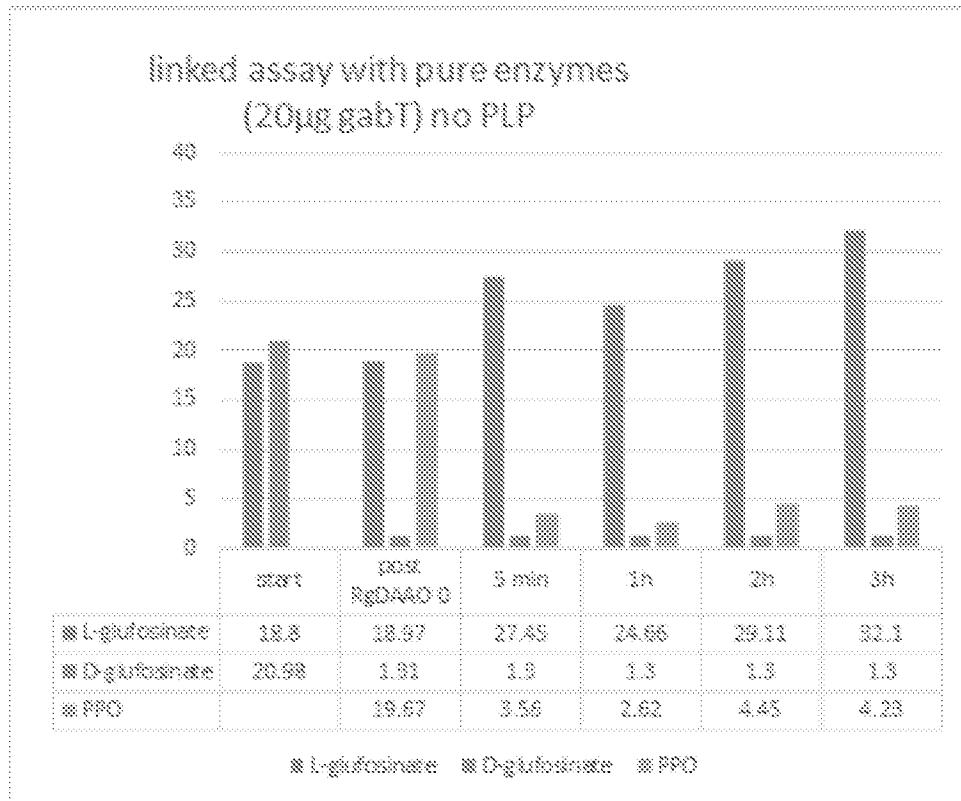


Figure 3

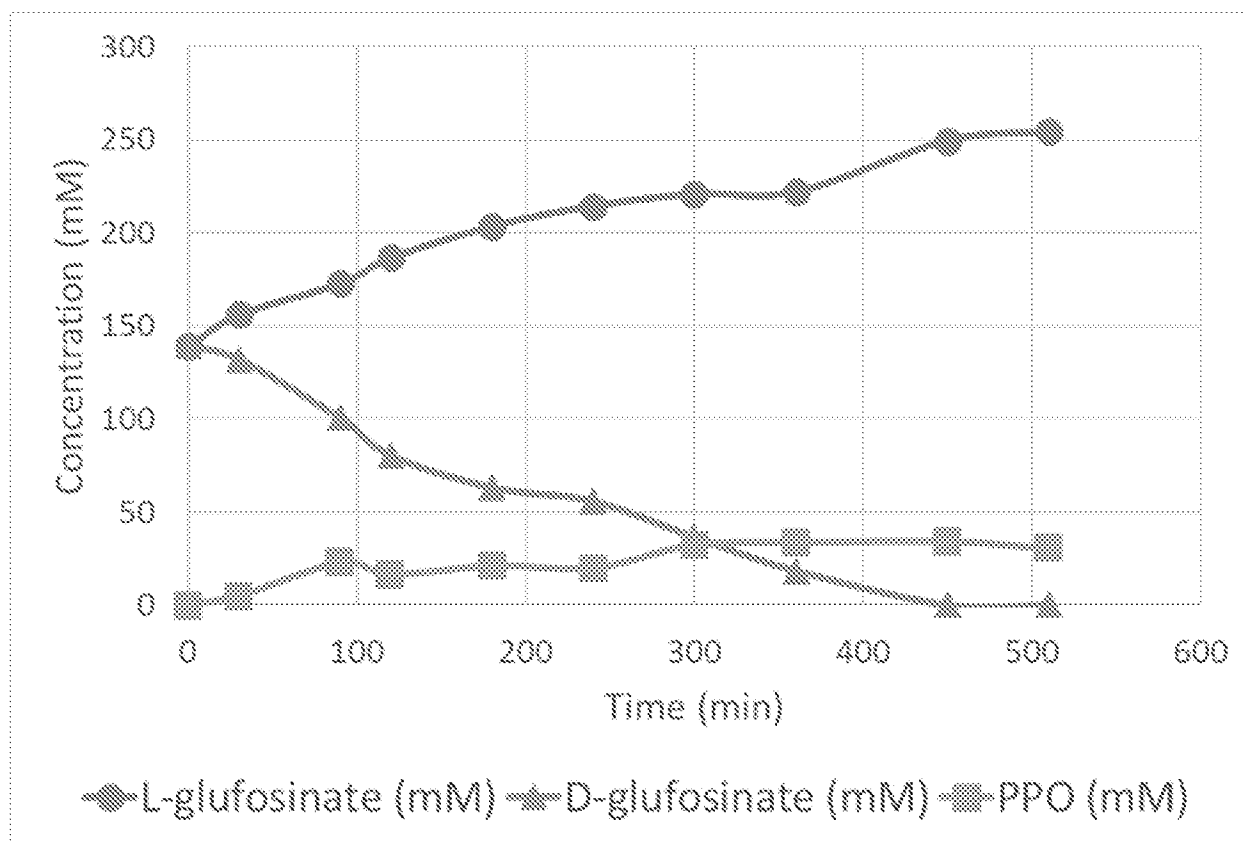


Figure 4