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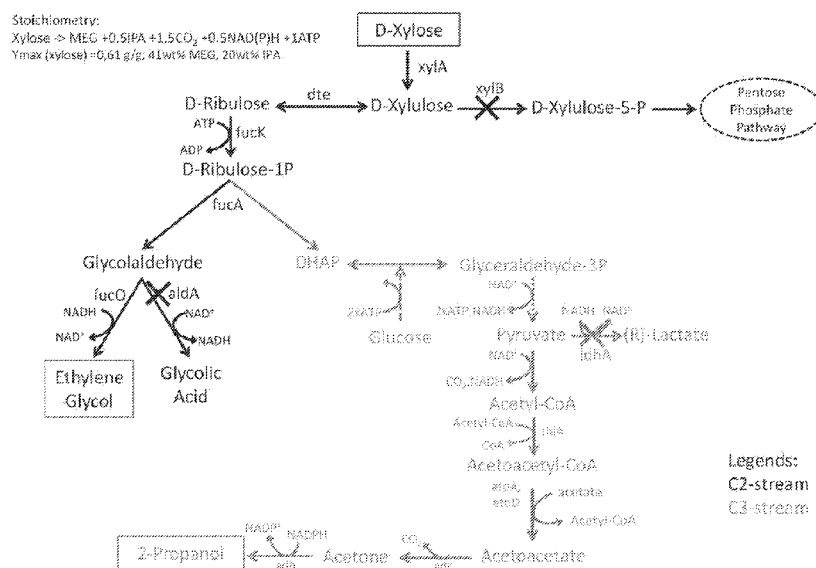
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(54) Title: MODULATION OF CARBON FLUX THROUGH THE MEG AND C3 PATHWAYS FOR THE IMPROVED PRODUCTION OF MONOETHYLENE GLYCOL AND C3 COMPOUNDS

FIG. 1



(57) Abstract: The present disclosure provides methods of modulating the flux of carbon through the monoethylene glycol (MEG) biosynthesis pathway and one or more C3 compound biosynthesis pathways by expressing enzymes that are essential for improving C3 compounds and modulating other genetic aspects of MEG and C3 compound biosynthesis. The disclosure is further drawn to modified microbes comprising the disrupted sequences and overexpressed sequences, and compositions thereof.

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MODULATION OF CARBON FLUX THROUGH THE MEG AND C3 PATHWAYS FOR THE IMPROVED PRODUCTION OF MONOETHYLENE GLYCOL AND C3 COMPOUNDS

CROSS-REFERENCE TO RELATED APPLICATION

5 [0001] This application claims priority to U.S. Provisional Application No. 62/786,294 filed December 28, 2018, entitled "METABOLIC ENGINEERING FOR THE IMPROVED PRODUCTION OF MONOETHYLENE GLYCOL AND C3 COMPOUNDS"; U.S. Provisional Application No. 62/786,298 filed December 28, 2018, entitled "METABOLIC ENGINEERING OF THE ACETATE PATHWAY FOR THE IMPROVED PRODUCTION OF MONOETHYLENE GLYCOL AND C3 COMPOUNDS"; U.S. Provisional Application No. 62/786,282 filed December 28, 2018, entitled "METABOLIC ENGINEERING OF XYLONATE PATHWAY FOR THE IMPROVED PRODUCTION OF MONOETHYLENE GLYCOL AND C3 COMPOUNDS"; U.S. Provisional Application No. 62/786,283 filed December 28, 2018, entitled "MODULATION OF ENZYMES FOR IMPROVED FLUX THROUGH THE C3 PATHWAY FOR THE IMPROVED PRODUCTION OF MONOETHYLENE GLYCOL AND C3 COMPOUNDS"; U.S. Provisional Application No. 62/786,304 filed December 28, 2018, entitled "MODULATION OF CARBON FLUX THROUGH THE MEG AND C3 PATHWAYS FOR THE IMPROVED PRODUCTION OF MONOETHYLENE GLYCOL AND C3 COMPOUNDS", the disclosures of which are
10
15
20 incorporated by reference herein.

FIELD

[0002] The present disclosure relates to recombinant microorganisms useful in the biosynthesis of monoethylene glycol (MEG) and one or more three-carbon (C3) compounds. The application further relates to the methods of producing MEG and one
25 or more C3 compounds using the recombinant microorganisms, as well as compositions comprising MEG, one or more C3 compound, and/or the recombinant microorganisms.

STATEMENT REGARDING SEQUENCE LISTING

[0003] The sequence listing associated with this application is provided in text
30 format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the sequence listing is BRSK_018_01WO_ST25.txt. The text file is about 232 kilobytes, was created on December 17, 2019, and is being submitted electronically via EFS-Web.

BACKGROUND OF THE DISCLOSURE

[0004] The expression of enzymes corresponding to the complete monoethylene glycol (MEG) and C3 pathways and their corresponding products is not enough to reach yields and productivities of MEG and C3 compounds needed for an advantageous industrial process.

[0005] There exists a need for improved biosynthesis pathways for the production of MEG and other chemical compounds useful in industrial and pharmaceutical applications.

SUMMARY OF THE DISCLOSURE

10 [0006] The present application generally relates to metabolic engineering strategies to improve carbon flux through MEG and C3 pathways, thus increasing yield, titer and/or productivity (rate of production) of MEG, C3 compounds, and the co-production of MEG and C3 compounds. The present application relates to recombinant microorganisms having one or more biosynthesis pathways for the production of monoethylene glycol (MEG) and one or more C3 compound biosynthesis pathways modified such that
15 the MEG and/or the one or more C3 compounds are produced at a faster rate and/or exhibits an increased yield or titer as compared to a microbe lacking the genetic modification (disruption and/or the overexpression of the endogenous or exogenous polynucleotides).

20 [0007] In some aspects of the present disclosure, the subject matter is drawn to a recombinant method of modulating the flux of carbon through the monoethylene glycol (MEG) biosynthesis pathway and one or more C3 compound biosynthesis pathways, the method comprising: modifying a microbe capable of coproducing MEG and one or more C3 compounds, wherein the microbe comprises (i) a disruption of one or more
25 nucleic acid sequences encoding methylglyoxal synthase (mgsA), and/or (ii) a disruption of one or more nucleic acid sequences encoding glyoxylate carboligase (gcl); wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding methylglyoxal synthases and/or
30 glyoxylate carboligases.

[0008] In some aspects of the present disclosure, the subject matter is drawn to a recombinant microbe capable of coproducing MEG and one or more C3 compounds, wherein the microbe comprises one or more of the following (i) a disruption of one or

more exogenous polynucleotide sequences encoding a phosphate acetyltransferase, (ii) a disruption of one or more endogenous polynucleotide sequences encoding an acetate kinase, (iii) a disruption of one or more endogenous polynucleotide sequences encoding a pyruvate oxidase, (iv) a disruption of one or more endogenous polynucleotide sequences encoding an ArcA regulator, (v) a disruption of one or more endogenous polynucleotide sequences encoding a lysine acetyltransferase, (vi) one or more overexpressed endogenous or exogenous polynucleotide sequences encoding a CobB regulator, and (vii) one or more overexpressed endogenous or exogenous polynucleotide sequences encoding an acetyl-CoA synthetase; wherein the MEG and/or the one or more C3 compounds are produced at a faster rate and/or exhibit an increased yield and/or titer; as compared to a microbe lacking the disruption and/or the overexpression of the endogenous or exogenous polynucleotides of any one or more of i-vii.

[0009] In some aspects of the present disclosure, the subject matter is drawn to a recombinant microbe capable of coproducing MEG and one or more C3 compounds, wherein the microbe comprises one or more of the following: (i) one or more exogenous polynucleotide sequences encoding a xylose dehydrogenase, (ii) one or more endogenous or exogenous polynucleotide sequences encoding a xylonolactonase, (iii) one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase, (iv) one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase, (v) one or more overexpressed endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase, (vi) one or more overexpressed endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase, and (vii) one or more overexpressed endogenous or exogenous polynucleotide sequences encoding a glycoaldehyde reductase; wherein the MEG and/or the one or more C3 compounds are produced at a faster rate and/or exhibit an increased yield or titer; as compared to a microbe lacking the endogenous or exogenous introduced enzymes and/or the overexpression of the endogenous or exogenous enzymes of any one or more of i-vii.

[0010] In some aspects of the present disclosure, the subject matter is drawn to a recombinant microbe capable of coproducing MEG and one or more C3 compounds, wherein the microbe comprises: (i) introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or (ii) introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-

CoA lyase; wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe not having been introduced an acetoacetyl CoA synthase, hydroxymethylglutaryl-CoA synthase, and/or hydroxymethylglutaryl-CoA lyase.

5 [0011] In some aspects, the recombinant microbe further comprises any one or more modifications described herein.

[0012] In some aspects of the present disclosure, the subject matter is drawn to a recombinant microbe capable of coproducing MEG and one or more C3 compounds wherein the microbe comprises one or more of the following: (i) one or more disrupted
10 nucleic acid sequences encoding methylglyoxal synthase (mgsA), (ii) one or more disrupted nucleic acid sequences encoding glyoxylate carboligase (gcl), (iii) one or more disrupted polynucleotide sequences encoding a phosphate acetyltransferase, (iv) one or more disrupted polynucleotide sequences encoding an acetate kinase, (v) one or more disrupted polynucleotide sequences encoding a pyruvate oxidase, (vi) one or
15 more disrupted endogenous polynucleotide sequences encoding an ArcA regulator, (vii) one or more disrupted endogenous polynucleotide sequences encoding a lysine acetyltransferase, (viii) one or more overexpressed endogenous or exogenous polynucleotide sequences encoding a CobB regulator, (ix) one or more overexpressed endogenous or exogenous polynucleotide sequences encoding an acetyl-CoA synthetase, (x) one or
20 more exogenous polynucleotide sequences encoding a xylose dehydrogenase, (xi) one or more endogenous or exogenous polynucleotide sequences encoding a xylonolactonase, (xii) one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase, (xiii) one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase, (xiv) one or
25 more overexpressed endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase, (xv) one or more overexpressed endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase, (xvi) one or more overexpressed endogenous or exogenous polynucleotide sequences encoding a glycoaldehyde reductase, (xvii) one or more polynucleotide sequences en-
30 coding acetoacetyl CoA synthase, and (xviii) one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase; wherein the MEG and/or the one or more C3 compounds is produced at a faster rate

and/or exhibits an increased yield or titer; as compared to a microbe lacking the modification.

[0013] In some aspects of the present disclosure, the subject matter is drawn to a recombinant microbe capable of coproducing MEG and one or more C3 compounds, wherein the microbe comprises one or more of the following: one or more disrupted nucleic acid sequences encoding methylglyoxal synthase (mgsA), and one or more disrupted nucleic acid sequences encoding glyoxylate carboligase (gcl) and/or; one or more disrupted polynucleotide sequences encoding a phosphate acetyltransferase, and/or one or more disrupted polynucleotide sequences encoding an acetate kinase, and/or one or more endogenous or exogenous polynucleotide sequences encoding a xylolactonase, and/or one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase, and/or one or more endogenous or exogenous a deletion of one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or transferase (AtoDA). In some aspects, the microbe comprises a functional acetoacetyl-CoA transferase (AtoDA).

[0014] In some aspects, the deletion comprises the deletion of the one or more endogenous or exogenous polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase; wherein.

[0015] In some aspects, the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding methylglyoxal synthases and any of the modifications above.

[0016] In some aspects of the present disclosure, the subject matter is drawn to a recombinant microbe capable of coproducing MEG and one or more C3 compounds, the microbe comprising one or more of the following: one or more disrupted nucleic acid sequences encoding glyoxylate carboligase (gcl); and one or more disrupted polynucleotide sequences encoding a phosphate acetyltransferase, and/or one or more disrupted polynucleotide sequences encoding an acetate kinase, and/or one or more endogenous or exogenous polynucleotide sequences encoding a xylolactonase, and/or one or more endogenous or exogenous polynucleotide sequences or overexpressing an endogenous polynucleotide o sequences encoding a xylonate dehydratase, and/or one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hy-

droxymethylglutaryl-CoA lyase; wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding glyoxylate carboligase (gcl) and any of the modifications above.

5 [0017] In some aspects of the present disclosure, the subject matter is drawn to a recombinant microbe capable of coproducing MEG and one or more C3 compounds, the microbe comprising one or more of the following: one or more disrupted polynucleotide sequences encoding a phosphate acetyltransferase and disrupting one or more endogenous polynucleotide sequences encoding an acetate kinase, and/or introducing
10 one or more endogenous or exogenous polynucleotide sequences encoding a xylolactonase, and/or introducing one or more endogenous or exogenous polynucleotide sequences or overexpressing an endogenous polynucleotide o sequence encoding a xylonate dehydratase, and/or introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or introducing one or more polynucleotide sequences
15 encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase, and/or; disrupting one or more endogenous polynucleotide sequences encoding an ArcA regulator, and/or, disrupting one or more endogenous polynucleotide sequences encoding a lysine acetyltransferase, and/or overexpressing one or more endogenous or exogenous polynucleotide sequences encoding an acetyl-CoA synthetase; wherein the
20 MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding a phosphate acetyltransferase and any of the modifications above.

[0018] In some aspects of the present disclosure, the subject matter is drawn to a
25 recombinant microbe capable of coproducing MEG and one or more C3 compounds by: modifying a microbe coproducing MEG and one or more C3 compounds by: disrupting one or more endogenous polynucleotide sequences encoding an acetate kinase and introducing one or more endogenous or exogenous polynucleotide sequences encoding a xylolactonase, and/or one or more endogenous or exogenous polynucleotide se-
30 quence or overexpressing an endogenous polynucleotide sequence encoding a xylonate dehydratase, and/or one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase; wherein the

MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding acetate kinase and any of the modifications above.

5 [0019] In some aspects of the present disclosure, the subject matter is drawn to a recombinant microbe capable of coproducing MEG and one or more C3 compounds, the microbe comprising one or more of the following: one or more endogenous or exogenous polynucleotide sequences encoding a xylolactonase and one or more endogenous or exogenous polynucleotide sequence or overexpressing an endogenous polynucleotide sequence encoding a xylonate dehydratase, and/or one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase; wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe lacking the exogenous introduced or endogenous overexpressed xylolactonase, and any of the modifications above.

[0020] In some aspects of the present disclosure, the subject matter is drawn to a recombinant microbe capable of coproducing MEG and one or more C3 compounds, the microbe comprising one or more of the following: one or more endogenous or exogenous polynucleotide sequence or overexpressing an endogenous polynucleotide sequence encoding a xylonate dehydratase and one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase; wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe lacking the exogenous introduced or endogenous overexpressed xylonate dehydratase, any of the modifications above.

[0021] In some aspects of the present disclosure, the subject matter is drawn to a recombinant microbe capable of coproducing MEG and one or more C3 compounds, the microbe comprising one or more of the following: one or more overexpressed endogenous or exogenous polynucleotide sequences encoding an acetyl-CoA synthetase; and one or more disrupted nucleic acid sequences encoding methylglyoxal synthase (mgsA), one or more disrupted nucleic acid sequences encoding glyoxylate carboligase

(gcl) and/or; one or more disrupted polynucleotide sequences encoding an acetate kinase, and/or one or more endogenous or exogenous polynucleotide sequences encoding a xylolactonase, and/or one or more endogenous or exogenous polynucleotide sequences or overexpressing an endogenous polynucleotide sequences encoding a xylonate dehydratase, and/or one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase; one or more disrupted polynucleotide sequences encoding an ArcA regulator, and/or, one or more disrupted polynucleotide sequences encoding a lysine acetyltransferase, and/or where-
10 in the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe lacking the endogenous overexpressed acetyl-CoA synthetase and any of the modifications above.

[0022] In some aspects of the present disclosure, the subject matter is drawn to a method of making a recombinant microbe capable of coproducing MEG and one or
15 more C3 compounds by: modifying a microbe coproducing MEG and one or more C3 compounds by: (i) disrupting one or more nucleic acid sequences encoding methylglyoxal synthase (mgsA), and/or (ii) disrupting one or more nucleic acid sequences encoding glyoxylate carboligase (gcl); wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding methylglyoxal synthases and/or glyoxylate carboligases.
20

[0023] In some aspects of the present disclosure, the subject matter is drawn to a method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by: modifying a microbe coproducing MEG and one or more C3
25 compounds by performing one or more of the following: (i) disrupting one or more polynucleotide sequences encoding a phosphate acetyltransferase, (ii) disrupting one or more polynucleotide sequences encoding an acetate kinase, (iii) disrupting one or more polynucleotide sequences encoding a pyruvate oxidase, (iv) disrupting one or more polynucleotide sequences encoding an ArcA regulator, (v) disrupting one or more polynucleotide sequences encoding a lysine acetyltransferase, (vi) overexpressing one or
30 more endogenous or exogenous polynucleotide sequences encoding a CobB regulator, and (vii) overexpressing one or more endogenous or exogenous polynucleotide sequences encoding an acetyl-CoA synthetase; wherein the MEG and/or the one or more

C3 compounds are produced at a faster rate and/or exhibit an increased yield or titer; as compared to a microbe lacking the disruption and/or the overexpression of the endogenous or exogenous polynucleotides of any one or more of i-vii.

[0024] In some aspects of the present disclosure, the subject matter is drawn to a method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by: modifying a microbe coproducing MEG and one or more C3 compounds by performing one or more of the following: (i) introducing one or more endogenous or exogenous polynucleotide sequences encoding a xylose dehydrogenase, (ii) introducing one or more exogenous polynucleotide sequences encoding a xylolactonase, (iii) introducing one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase, (iv) introducing one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase, (v) overexpressing one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase, (vi) overexpressing one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase, and (vii) overexpressing one or more endogenous or exogenous polynucleotide sequences encoding a glycoaldehyde reductase; wherein the MEG and/or the one or more C3 compounds are produced at a faster rate and/or exhibit an increased yield or titer; as compared to a microbe lacking the exogenous introduced enzymes and/or the overexpression of the endogenous or exogenous enzymes of any one or more of i-vii.

[0025] In some aspects of the present disclosure, the subject matter is drawn to a method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by: modifying a microbe coproducing MEG and one or more C3 compounds by: (i) introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or (ii) introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase; wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe not having been introduced an acetoacetyl CoA, hydroxymethylglutaryl-CoA synthase, or hydroxymethylglutaryl-CoA lyase.

[0026] In some aspects the microbe comprises any one or more modifications set forth herein.

[0027] In some aspects of the present disclosure, the subject matter is drawn to a method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by: modifying a microbe coproducing MEG and one or more C3 compounds by performing one or more of the following: (i) disrupting one or more nucleic acid sequences encoding methylglyoxal synthase (mgsA), (ii) disrupting one or more nucleic acid sequences encoding glyoxylate carboligase (gcl), (iii) disrupting one or more exogenous polynucleotide sequences encoding a phosphate acetyltransferase, (iv) disrupting one or more polynucleotide sequences encoding an acetate kinase, (v) disrupting one or more polynucleotide sequences encoding a pyruvate oxidase, (vi) disrupting one or more polynucleotide sequences encoding an ArcA regulator, (vii) disrupting one or more polynucleotide sequences encoding a lysine acetyltransferase, (viii) overexpressing one or more endogenous or exogenous polynucleotide sequences encoding a CobB regulator, (ix) overexpressing one or more endogenous or exogenous polynucleotide sequences encoding an acetyl-CoA synthetase, (x) introducing one or more exogenous polynucleotide sequences encoding a xylose dehydrogenase, (xi) introducing one or more endogenous or exogenous polynucleotide sequences encoding a xylolactonase, (xii) introducing one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase, (xiii) introducing one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase, (xiv) overexpressing one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase, (xv) overexpressing one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase, (xvi) overexpressing one or more endogenous or exogenous polynucleotide sequences encoding a glycoaldehyde reductase, (xvii) introducing one or more endogenous or exogenous polynucleotide sequences encoding acetoacetyl CoA synthase, and (xviii) introducing one or more endogenous or exogenous polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase; wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe lacking the modification.

[0028] In some aspects, the microbe is a bacterium or a fungus. In some aspects, the bacterium is an *Escherichia coli*. In some aspects, the MEG exhibits an increased

yield or titer. In some aspects, the increased yield or titer is an increase of at least 2%. In some aspects, the increased yield or titer is an increase of at least 15%.

[0029] In some aspects, the one or more C3 compounds is acetone. In some aspects, the acetone exhibits an increased yield or titer. In some aspects, the increased yield or titer is an increase of at least 2%. In some aspects, the increased yield or titer is an increase of at least 15%.

[0030] In some aspects, the microbe utilizes xylose, cellobiose, arabinose, mannose, and/or glucose in the coproduction of the MEG and the one or more C3 compounds. In some aspects, the C3 compounds are selected from acetone, isopropanol, and propene.

[0031] In some aspects of the present disclosure, the subject matter is drawn to a method of modulating the flux of carbon through the monoethylene glycol (MEG) biosynthesis pathway and one or more C3 compound biosynthesis pathways, the method comprising: modifying a microbe coproducing MEG and one or more C3 compounds by: (i) introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or (ii) introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase; wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield and/or titer; as compared to a microbe not having been introduced an acetoacetyl CoA, hydroxymethylglutaryl-CoA synthase, or hydroxymethylglutaryl-CoA lyase.

[0032] In some aspects, the microbe comprises a deletion of one or more polynucleotide sequences encoding acetoacetyl-CoA thiolase. In some aspects, the microbe lacks a functional acetoacetyl-CoA thiolase. In some aspects, the microbe comprises a functional acetoacetyl-CoA thiolase.

[0033] In some aspects, the microbe comprises a deletion of one or more polynucleotide sequences encoding acetoacetyl-CoA transferase (AtoDA). In some aspects, the microbe comprises a functional acetoacetyl-CoA transferase (AtoDA).

[0034] In some aspects, the deletion comprises the deletion of the one or more polynucleotide sequences.

[0035] In some aspects, the MEG and/or the one or more C3 compounds is produced at a faster rate and/or an increased yield and/or titer. In some aspects, the microbe is a bacterium or a fungus. In some aspects, the bacterium is an *Escherichia coli*.

In some aspects, the MEG exhibits an increased yield or titer. In some aspects, the increased yield or titer is an increase of at least 2%. In some aspects, the increased yield or titer is an increase of at least 15%. In some aspects, the one or more C3 compounds is acetone. In some aspects, the acetone exhibits an increased yield or titer. In some aspects, the increased yield or titer is an increase of at least 2%. In some aspects, the increased yield or titer is an increase of at least 15%.

[0036] In some aspects, the one or more C3 compounds is acetone. In some aspects, the acetone exhibits an increased yield and/or titer. In some aspects, the increased yield and/or titer is an increase of at least 2%. In some aspects, the increased yield and/or titer is an increase of at least 15%. In some aspects, the acetone is produced at a faster rate. In some aspects, the faster rate is an increase of at least 2%. In some aspects, the faster rate is an increase of at least 15%. In some aspects, (i) the MEG exhibits an increased yield and/or titer of at least 2%, and/or (ii) the one or more C3 compounds exhibits an increased yield and/or titer of at least 2%. In some aspects, (i) the MEG exhibits an increased yield and/or titer of at least 15%, and/or (ii) the one or more C3 compounds exhibits an increased yield and/or titer of at least 15%. In some aspects, (i) the rate of MEG production exhibits an increase of at least 2%, and/or (ii) the rate of the one or more C3 compound production exhibits an increase of at least 2%. In some aspects, (i) the rate of MEG production exhibits an increase of at least 15%, and/or (ii) the rate of the one or more C3 compound production exhibits an increase of at least 15%.

[0037] In some aspects, the microbe utilizes xylose, cellobiose, arabinose, mannose, and/or glucose in the coproduction of the MEG and the one or more C3 compounds, wherein the C3 compounds are selected from acetone, isopropanol, and propene.

BRIEF DESCRIPTION OF THE FIGURES

[0038] Illustrative embodiments of the disclosure are illustrated in the drawings, in which:

[0039] **FIG. 1** illustrates MEG and isopropanol co-production pathway via xylulose-1-phosphate.

[0040] **FIG. 2** illustrates MEG and isopropanol co-production pathway via xylonate.

[0041] **FIG. 3** illustrates possible three carbon co-products for MEG.

[0042] FIG. 4 illustrates improved MEG production from xylose in *E. coli*.

[0043] FIG. 5 illustrates overall yield (g products/g xylose) of ethylene glycol, isopropanol and acetone produced using a xylulose-1-phosphate pathway.

[0044] FIG. 6 illustrates co-production of MEG, isopropanol and acetone using a
5 xylulose-1-phosphate pathway in *E. coli*.

[0045] FIG. 7 illustrates overall yield (g products/g xylose) of ethylene glycol, isopropanol and acetone produced using a xylulose-1-phosphate pathway.

[0046] FIG. 8 illustrates co-production of MEG, isopropanol, and acetone using a xylonate pathway in *E. coli*.

10 [0047] FIG. 9 illustrates overall yield (g products/g xylose) of ethylene glycol, isopropanol and acetone produced using a xylulose-1-phosphate pathway.

[0048] FIG. 10 shows an SDS-PAGE of soluble fraction of assays (a) to (e) as described in Example 3. The arrow indicates LinD expression in (b), (c), (d) and (e).

[0049] FIG. 11 illustrates that assays (d) and (e) showed the production of propylene and isopropanol in IPA+LinD candidates. Assay (a) showed isopropanol production of pZs*13_IPA and a small amount of propylene. Assays (b) and (c) showed propylene production in medium supplemented with 3.0g/L isopropanol using glycerol and
15 glucose as carbon source, respectively.

[0050] FIG. 12A – FIG. 12D illustrates the increased MEG production in the strain with nphT7 expressed vs the parental strain (FIG. 12A), with increased acetone production in the strain (FIG. 12B), with increased acetic acid production (FIG. 12C), and with a decreased peak production of xylonic acid compared with the parent (FIG. 12D).
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[0051] FIG. 13A – FIG. 13D illustrates the increased MEG production in the strain with HMG-CoA expressed vs the parental strain (FIG. 13A), with increased acetone production in the strain (FIG. 13B), with little effect on acetic acid production (FIG. 13C), and with little effect on xylulose accumulation compared with the parent (FIG. 13D).
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[0052] FIG. 14A – FIG. 14B illustrates the amounts of MEG detected for Δ pta Δ atoDA atoDA::ERG13,ynG strain (FIG. 14A) and Δ pta atoDA::ERG13,ynG Δ atoDA strain (FIG. 14B) relative to the Δ pto strain.

30 [0053] FIG. 15 illustrates the co-production of MEG and acetone for Δ pta + yagF overexpression strain vs. the Δ pta strain.

[0054] FIG. 16A – FIG. 16D illustrates the increased MEG production in the mgsA deleted strain vs the parental strain (FIG. 16A), the increased acetone production in the

in the *mgsA* deleted strain vs the parental strain (**FIG. 16B**), the increased acetic acid production in the *mgsA* deleted strain vs the parental strain (**FIG. 16C**), and the decreased xylonic acid peak in the *mgsA* deleted strain vs the parental strain (**FIG. 16D**) as it pertains to Example 4 (xylonate pathway).

5 [0055] **FIG. 17A – FIG. 17D** illustrates the increased MEG production in the *mgsA* deleted strain vs the parental strain (**FIG. 17A**), the increased acetone production in the *mgsA* deleted strain vs the parental strain (**FIG. 17B**), the change in acetic acid production in the *mgsA* deleted strain vs the parental strain (**FIG. 17C**), and the xylulose accumulation in the *mgsA* deleted strain vs the parental strain (**FIG. 17D**) as it pertains to
10 Example 5 (xylulose pathway).

[0056] **FIG. 18A – FIG. 18D** illustrates the increased MEG production in the *gcl* deleted strain vs the parental strain (**FIG. 18A**), the increased acetone production in the *gcl* deleted strain vs the parental strain (**FIG. 18B**), the increase in acetic acid production in the *gcl* deleted strain vs the parental strain (**FIG. 18C**), and the decreased xy-
15 lonic acid peak in the *gcl* deleted strain vs the parental strain (**FIG. 18D**) as it pertains to Example 6 (xylonate pathway).

[0057] **FIG. 19A – FIG. 19B** illustrates the increased MEG production in the Δ *pta* strain vs the parental strain (**FIG. 19A**), and the increased MEG production in the Δ *ackA* strain vs the parental strain (**FIG. 19B**).

20 [0058] **FIG. 20A – FIG. 20B** illustrate the higher productivity of MEG (**FIG. 20A**) and acetone (**FIG. 20B**) for Δ *arcA* compared to the parental strain.

[0059] **FIG. 21A – FIG. 21B** illustrate the higher amounts of MEG (**FIG. 21A**) and acetone (**FIG. 21B**) for Δ *pta* Δ *arcA* and Δ *pta* Δ *pka* compared to the Δ *pta* strain.

[0060] **FIG. 22A – FIG. 22D** illustrates the increased MEG production in the
25 strains harboring xylonolactonase expressed in plasmids vs the parental strain (**FIG. 22A**), the increased acetone production in the strains harboring xylonolactonase expressed in plasmids vs the parental strain (**FIG. 22B**), the increased production of acetic acid in the strains harboring xylonolactonase expressed in plasmids vs the parental strain (**FIG. 22C**), and the decrease in the peak production of xylonic acid in the strains
30 harboring xylonolactonase expressed in plasmids vs the parental strain (**FIG. 22D**).

[0061] **FIG. 23A – FIG. 23D** illustrates the increased MEG production in the strains harboring xylonolactonase expressed in plasmids vs the parental strain (**FIG. 23A**), the increased acetone production in the strains harboring xylonolactonase ex-

pressed in plasmids vs the parental strain (FIG. 23B), the increased production of acetic acid in the strains harboring xylonolactonase expressed in plasmids vs the parental strain (FIG. 23C), and the decrease in the peak production of xylonic acid in the strains harboring xylonolactonase expressed in plasmids vs the parental strain (FIG. 23D).

5 DETAILED DESCRIPTION OF THE DISCLOSURE

[0062] The following definitions and abbreviations are to be used for the interpretation of the disclosure.

[0063] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an enzyme" includes a plurality of such enzymes and reference to "the microorganism" includes reference to one or more microorganisms, and so forth.

[0064] As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having," "contains," "containing," or any other variation thereof, are intended to cover a non-exclusive inclusion. A composition, mixture, process, method, article, or apparatus that comprises a list of elements is not necessarily limited to only those elements but may include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, "or" refers to an inclusive "or" and not to an exclusive "or."

[0065] The terms "polynucleotide", "nucleotide", "nucleotide sequence", "nucleic acid" and "oligonucleotide" are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), short interfering RNA (siRNA), short-hairpin RNA (shRNA), micro-RNA (miRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise one or more modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-

nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component.

[0066] "Complementarity" refers to the ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. A percent complementarity indicates the percentage of residues in a nucleic acid molecule which can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary, respectively). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. "Substantially complementary" as used herein refers to a degree of complementarity that is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, or more nucleotides, or refers to two nucleic acids that hybridize under stringent conditions. Sequence identity, such as for the purpose of assessing percent complementarity, may be measured by any suitable alignment algorithm, including but not limited to the Needleman-Wunsch algorithm (see e.g. the EMBOSS Needle aligner available at www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html, optionally with default settings), the BLAST algorithm (see e.g. the BLAST alignment tool available at blast.ncbi.nlm.nih.gov/Blast.cgi, optionally with default settings), or the Smith-Waterman algorithm (see e.g. the EMBOSS Water aligner available at www.ebi.ac.uk/Tools/psa/emboss_water/nucleotide.html, optionally with default settings). Optimal alignment may be assessed using any suitable parameters of a chosen algorithm, including default parameters.

[0067] As used herein, "expression" refers to the process by which a polynucleotide is transcribed from a DNA template (such as into and mRNA or other RNA transcript) and/or the process by which a transcribed mRNA is subsequently translated into peptides, polypeptides, or proteins. Transcripts and encoded polypeptides may be collectively referred to as "gene product." If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell.

[0068] The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-

amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component. As used herein the term "amino acid" includes natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics.

[0069] As used herein, the term "about" is used synonymously with the term "approximately." Illustratively, the use of the term "about" with regard to an amount indicates that values slightly outside the cited values, e.g., plus or minus 0.1% to 10%.

[0070] The term "biologically pure culture" or "substantially pure culture" refers to a culture of a bacterial species described herein containing no other bacterial species in quantities sufficient to interfere with the replication of the culture or be detected by normal bacteriological techniques.

[0071] As used herein, a "parent strain" or "base strain" is the strain which has been modified to produce a new resulting strain. For example, if *Escherichia coli* strain XL1-Blue were genetically modified to disrupt a genomic polynucleotide sequences, the *E. coli* strain XL1-Blue is the parent strain or base strain to the subsequent genetically modified strain. In some aspects, a parent or base strain may be naturally occurring. In other aspects, a parent or base strain may be non-naturally occurring.

[0072] As used herein, a "control sequence" refers to an operator, promoter, silencer, or terminator.

[0073] As used herein, "introduced" refers to the introduction by means of modern biotechnology, and not a naturally occurring introduction.

[0074] As used herein, a "constitutive promoter" is a promoter, which is active under most conditions and/or during most development stages. There are several advantages to using constitutive promoters in expression vectors used in biotechnology, such as: high level of production of proteins used to select transgenic cells or organisms; high level of expression of reporter proteins or scorable markers, allowing easy detection and quantification; high level of production of a transcription factor that is part of a regulatory transcription system; production of compounds that requires ubiquitous activity in the organism; and production of compounds that are required during all stages of development.

[0075] As used herein, a "non-constitutive promoter" is a promoter which is active under certain conditions, in certain types of cells, and/or during certain development stages. For example, inducible promoters, and promoters under development control are non-constitutive promoters.

5 [0076] As used herein, "inducible" or "repressible" promoter is a promoter which is under chemical or environmental factors control. Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, certain chemicals, the presence of light, acidic or basic conditions, etc.

[0077] As used herein, the term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is regulated by the other. For example, a promoter is operably linked with a coding sequence when it is capable of regulating the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in a sense or antisense orientation. In another example, the complementary RNA regions of the disclosure can be operably linked, either directly or indirectly, 5' to the target mRNA, or 3' to the target mRNA, or within the target mRNA, or a first complementary region is 5' and its complement is 3' to the target mRNA.

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[0078] The term "signal sequence" as used herein refers to an amino acid sequence that targets peptides and polypeptides to cellular locations or to the extracellular environment. Signal sequences are typically at the N-terminal portion of a polypeptide and are typically removed enzymatically. Polypeptides that have their signal sequences are referred to as being full-length and/or unprocessed. Polypeptides that have had their signal sequences removed are referred to as being mature and/or processed.

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25 [0079] The term "exogenous" as used herein with reference to various molecules, e.g., polynucleotides, polypeptides, enzymes, etc., refers to molecules that are not normally or naturally found in and/or produced by a given yeast, bacterium, organism, microorganism, or cell in nature.

[0080] On the other hand, the term "endogenous" or "native" as used herein with reference to various molecules, e.g., polynucleotides, polypeptides, enzymes, etc., refers to molecules that are normally or naturally found in and/or produced by a given yeast, bacterium, organism, microorganism, or cell in nature.

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[0081] The term "heterologous" as used herein in the context of a modified host cell refers to various molecules, e.g., polynucleotides, polypeptides, enzymes, etc., wherein at least one of the following is true: (a) the molecule(s) is/are foreign ("exogenous") to (i.e., not naturally found in) the host cell; (b) the molecule(s) is/are naturally found in (e.g., is "endogenous to") a given host microorganism or host cell but is either produced in an unnatural location or in an unnatural amount in the cell; and/or (c) the molecule(s) differ(s) in nucleotide or amino acid sequence from the endogenous nucleotide or amino acid sequence(s) such that the molecule differing in nucleotide or amino acid sequence from the endogenous nucleotide or amino acid as found endogenously is produced in an unnatural (e.g., greater than naturally found) amount in the cell.

[0082] The term "homolog," as used herein with respect to an original enzyme or gene of a first family or species, refers to distinct enzymes or genes of a second family or species which are determined by functional, structural, or genomic analyses to be an enzyme or gene of the second family or species which corresponds to the original enzyme or gene of the first family or species. Homologs most often have functional, structural, or genomic similarities. Techniques are known by which homologs of an enzyme or gene can readily be cloned using genetic probes and PCR. Identity of cloned sequences as homologs can be confirmed using functional assays and/or by genomic mapping of the genes.

[0083] A protein has "homology" or is "homologous" to a second protein if the amino acid sequence encoded by a gene has a similar amino acid sequence to that of the second gene. Alternatively, a protein has homology to a second protein if the two proteins have "similar" amino acid sequences. Thus, the term "homologous proteins" is intended to mean that the two proteins have similar amino acid sequences. In certain instances, the homology between two proteins is indicative of its shared ancestry, related by evolution. The terms "homologous sequences" or "homologs" are thought, believed, or known to be functionally related. A functional relationship may be indicated in any one of a number of ways, including, but not limited to: (a) degree of sequence identity and/or (b) the same or similar biological function. Preferably, both (a) and (b) are indicated. The degree of sequence identity may vary, but in one embodiment, is at least 50% (when using standard sequence alignment programs known in the art), at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%,

at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least 98.5%, or at least about 99%, or at least 99.5%, or at least 99.8%, or at least 99.9%. Homology can be determined using software programs readily available in the art, such as those discussed in Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987) Supplement 30, section 7.718, Table 7.71. Some alignment programs are MacVector (Oxford Molecular Ltd, Oxford, U.K.) and ALIGN Plus (Scientific and Educational Software, Pennsylvania). Other non-limiting alignment programs include Sequencher (Gene Codes, Ann Arbor, Michigan), AlignX, and Vector NTI (Invitrogen, Carlsbad, CA). A similar biological function may include, but is not limited to: catalyzing the same or similar enzymatic reaction; having the same or similar selectivity for a substrate or co-factor; having the same or similar stability; having the same or similar tolerance to various fermentation conditions (temperature, pH, etc.); and/or having the same or similar tolerance to various metabolic substrates, products, by-products, intermediates, etc. The degree of similarity in biological function may vary, but in one embodiment, is at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least 98.5%, or at least about 99%, or at least 99.5%, or at least 99.8%, or at least 99.9%, according to one or more assays known to one skilled in the art to determine a given biological function.

[0084] The term "variant" refers to any polypeptide or enzyme described herein. A variant also encompasses one or more components of a multimer, multimers comprising an individual component, multimers comprising multiples of an individual component (e.g., multimers of a reference molecule), a chemical breakdown product, and a biological breakdown product. In particular, non-limiting embodiments, an enzyme may be a "variant" relative to a reference enzyme by virtue of alteration(s) in any part of the polypeptide sequence encoding the reference enzyme. A variant of a reference enzyme can have enzyme activity of at least 10%, at least 30%, at least 50%, at least 80%, at least 90%, at least 100%, at least 105%, at least 110%, at least 120%, at least 130% or more in a standard assay used to measure enzyme activity of a preparation of the reference

enzyme. In some embodiments, a variant may also refer to polypeptides having at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the full-length, or unprocessed enzymes of the present disclosure. In some embodiments, a variant may also refer to polypeptides having at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the mature, or processed enzymes of the present disclosure.

[0085] As used herein, the term "non-naturally occurring," when used in reference to a microorganism, organism, or enzyme activity of the disclosure, is intended to mean that the microorganism, organism, or enzyme has at least one genetic alteration not normally found in a naturally occurring strain of the referenced species, including wild-type strains of the referenced species. Genetic alterations include, for example, modifications introducing expressible nucleic acids encoding metabolic polypeptides, other nucleic acid additions, nucleic acid deletions and/or other functional disruption of the microorganism's genetic material. Such modifications include, for example, coding regions and functional fragments thereof, for heterologous, homologous, or both heterologous and homologous polypeptides for the referenced species. Additional modifications include, for example, non-coding regulatory regions in which the modifications alter expression of a gene or operon. Exemplary non-naturally occurring microorganism or enzyme activity includes the hydroxylation activity described above.

[0086] As used herein the terms "microorganism" or "microbe" should be taken broadly. These terms, used interchangeably, include but are not limited to, the two prokaryotic domains, Bacteria and Archaea.

[0087] As used herein, "isolate," "isolated," "isolated microbe," and like terms, are intended to mean that the one or more microorganisms has been separated from at least one of the materials with which it is associated in a particular environment (for example media, water, reaction chamber, etc.). Thus, an "isolated microbe" does not exist in its naturally occurring environment; rather, it is through the various techniques described herein that the microbe has been removed from its natural setting and placed into a non-naturally occurring state of existence. Thus, the isolated strain or isolated microbe may exist as, for example, a biologically pure culture, or as spores (or other

forms of the strain). In aspects, the isolated microbe may be in association with an acceptable carrier, which may be a commercially or industrial acceptable carrier.

[0088] In certain aspects of the disclosure, the isolated microbes exist as "isolated and biologically pure cultures." It will be appreciated by one of skill in the art that an isolated and biologically pure culture of a particular microbe, denotes that said culture is substantially free of other living organisms and contains only the individual microbe in question. The culture can contain varying concentrations of said microbe. The present disclosure notes that isolated and biologically pure microbes often "necessarily differ from less pure or impure materials." See, e.g. In re Bergstrom, 427 F.2d 1394, (CCPA 1970)(discussing purified prostaglandins), see also, In re Bergy, 596 F.2d 952 (CCPA 1979)(discussing purified microbes), see also, Parke-Davis & Co. v. H.K. Mulford & Co., 189 F. 95 (S.D.N.Y. 1911) (Learned Hand discussing purified adrenaline), *aff'd in part*, *rev'd in part*, 196 F. 496 (2d Cir. 1912), each of which are incorporated herein by reference. Furthermore, in some aspects, the disclosure provides for certain quantitative measures of the concentration, or purity limitations, that must be found within an isolated and biologically pure microbial culture. The presence of these purity values, in certain embodiments, is a further attribute that distinguishes the presently disclosed microbes from those microbes existing in a natural state. See, e.g., Merck & Co. v. Olin Mathieson Chemical Corp., 253 F.2d 156 (4th Cir. 1958) (discussing purity limitations for vitamin B12 produced by microbes), incorporated herein by reference.

[0089] As used herein, "individual isolates" should be taken to mean a composition, or culture, comprising a predominance of a single genera, species, or strain, of microorganism, following separation from one or more other microorganisms.

[0090] Microbes of the present disclosure may include spores and/or vegetative cells. In some embodiments, microbes of the present disclosure include microbes in a viable but non-culturable (VBNC) state. As used herein, "spore" or "spores" refer to structures produced by bacteria and fungi that are adapted for survival and dispersal. Spores are generally characterized as dormant structures; however, spores are capable of differentiation through the process of germination. Germination is the differentiation of spores into vegetative cells that are capable of metabolic activity, growth, and reproduction. The germination of a single spore results in a single fungal or bacterial vegetative cell. Fungal spores are units of asexual reproduction, and in some cases are nec-

essary structures in fungal life cycles. Bacterial spores are structures for surviving conditions that may ordinarily be nonconductive to the survival or growth of vegetative cells.

[0091] As used herein, "microbial composition" refers to a composition comprising one or more microbes of the present disclosure.

5 [0092] As used herein, "carrier," "acceptable carrier," "commercially acceptable carrier," or "industrial acceptable carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the microbe can be administered, which does not detrimentally effect the microbe.

[0093] The term "yield potential" as used herein refers to a yield of a product from a biosynthetic pathway. In one embodiment, the yield potential may be expressed as a percent by weight of end product per weight of starting compound.

[0094] The term "thermodynamic maximum yield" as used herein refers to the maximum yield of a product obtained from fermentation of a given feedstock, such as glucose, based on the energetic value of the product compared to the feedstock. In a normal fermentation, without use of additional energy sources such as light, hydrogen gas or methane or electricity, for instance, the product cannot contain more energy than the feedstock. The thermodynamic maximum yield signifies a product yield at which all energy and mass from the feedstock is converted to the product. This yield can be calculated and is independent of a specific pathway. If a specific pathway towards a product has a lower yield than the thermodynamic maximum yield, then it loses mass and can most likely be improved upon or substituted with a more efficient pathway towards the product.

[0095] The term "redox balanced" refers to a set of reactions, which taken together produce as much redox cofactors as they consume. Designing metabolic pathways and engineering an organism such that the redox cofactors are balanced or close to being balanced usually results in a more efficient, higher yield production of the desired compounds. Redox reactions always occur together as two half-reactions happening simultaneously, one being an oxidation reaction and the other a reduction reaction. In redox processes, the reductant transfers electrons to the oxidant. Thus, in the reaction, the reductant or reducing agent loses electrons and is oxidized, and the oxidant or oxidizing agent gains electrons and is reduced. In one embodiment, the redox reactions take place in a biological system. Biological energy is frequently stored and released by means of redox reactions. Photosynthesis involves the reduction of carbon dioxide into

sugars and the oxidation of water into molecular oxygen. The reverse reaction, respiration, oxidizes sugars to produce carbon dioxide and water. As intermediate steps, the reduced carbon compounds are used to reduce nicotinamide adenine dinucleotide (NAD⁺), which then contributes to the creation of a proton gradient, which drives the synthesis of adenosine triphosphate (ATP) and is maintained by the reduction of oxygen. The term redox state is often used to describe the balance of GSH/GSSG, NAD⁺/NADH and NADP⁺/NADPH in a biological system such as a cell or organ. The redox state is reflected in the balance of several sets of metabolites (e.g., lactate and pyruvate, beta-hydroxybutyrate, and acetoacetate), whose interconversion is dependent on these ratios. An abnormal redox state can develop in a variety of deleterious situations, such as hypoxia, shock, and sepsis.

[0096] As used herein, the term "productivity" refers to the total amount of bioproduct produced per liter per hour.

[0097] The terms "C2 pathway", "C2 branch pathway", "C2 biochemical pathway" or "C2 stream" as used herein refers to a biochemical pathway wherein MEG can be produced via glycolaldehyde.

[0098] The terms "C3 pathway", "C3 branch pathway", "C3 biochemical pathway" or "C3 stream" as used herein refers to a biochemical pathway wherein MEG and/or one or more co-product such as acetone, isopropanol, propene, isobutene and/or serine pathway compounds can be produced via pyruvate, acetyl-CoA or dihydroxyacetonephosphate (DHAP).

[0099] The strategies described herein were evaluated for the potential of improvement considering the overall carbon flux of the MEG + C3 compound co-production pathways. The methods described herein deliver gains that are not only specific to a single pathway but have a synergistic effect on the global carbon, energy and co-factor balances. These synergistic or antagonistic results can only be predicted when focusing on the metabolic complexity of the MEG + C3 co-production pathway.

[00100] The present disclosure combines the production of monoethylene glycol (MEG) and one or more three carbon compounds in different hosts. In some embodiments, the three carbon compound is isopropanol (IPA). The present disclosure thereby avoids some of the biggest pathway engineering challenges for known MEG and IPA pathways demonstrated so far. Surprisingly, the combination of a pathway for MEG production and a pathway for production of a three carbon compound complements

each other and is highly synergistic, avoiding or overcoming the biggest challenges and shortcomings of each pathway alone, establishing a good redox balance but also delivering required ATP, without production of excess ATP.

[00101] A demonstrated fermentative production of MEG from xylose
5 (WO2013126721A1, which is herein referenced in its entirety), via ribulose-1-phosphate, has a high yield potential (79 wt% = 0.79 g MEG/g xylose). MEG is produced via two different pathways which are active in parallel, a 2-carbon (C2) stream (via glycolaldehyde) and a 3-carbon (C3) stream (via dihydroxyacetonephosphate (DHAP)). The C2 stream is easy to implement at high efficiency, but the C3 stream is
10 very difficult to implement at high efficiency via metabolic engineering. Several pathway options for DHAP → MEG exist, all of which are difficult to implement. Furthermore, the overall process is ATP neutral. Thus, some xylose and therefore yield will be lost in order to obtain some surplus ATP required for cell growth and maintenance.

[00102] A further demonstrated fermentative production of MEG from xylose (Alkim
15 *et al.*, Microb Cell Fact (2015) 14:127), via xylulose-1-phosphate, is very similar to the route described by WO2013126721A1. It has the same high yield potential (79 wt%), but the C3 stream for MEG production via DHAP is difficult to implement and there is an ATP shortage.

[00103] A further fermentative production of MEG was demonstrated from glucose
20 (Chen *et al.*, Met. Eng. (2016) 33:12–18). It uses exclusively a pathway identical to one of the C3 stream solutions of WO2013126721A1, going via DHAP and then ethanolamine to glyceraldehyde to MEG. Only in this case, DHAP is derived from glucose, not from xylose. Thus it suffers even more from the technical difficulty to implement a high productivity and high yield pathway from DHAP to MEG. It furthermore has a reduced
25 total yield potential of 69 wt% versus the thermodynamic maximum yield for the product MEG derived from glucose (82 wt%). The pathway is furthermore ATP neutral, not generating any ATP that the cells need for growth and maintenance. This pathway is also not redox balanced and has a high excess of 2 mol NADH per mol of consumed glucose, all of which needs to be re-oxidized for the cell to be viable. In an aerobic fermentation, this NADH can be used to generate ATP, which however would be in high excess
30 (2 NADH → 6 ATP), leading to excess biomass formation during the production phase and therefore reduced product formation and yield. The only described solution for the loss of yield potential for MEG production from glucose is the production of MEG

from xylose with a high yield potential. The only described solution for the excess NADH production in the MEG from glucose process is the production of MEG from xylose which can be redox neutral.

[00104] A demonstrated fermentative production of IPA via acetoacetyl-CoA (US 2010/0311135, which is herein referenced in its entirety) has excess NADH (2 mol per mol of consumed glucose) and low yield potential (34 wt%). This pathway has excess ATP (2 mol per mol of consumed glucose), more than is required for cell maintenance during the production phase, thereby favoring biomass formation over production. If the NADH is not utilized via carbon fixation, it needs to be re-oxidized for the cell to stay viable, further losing glucose in this process. Alternatively, NADH can be oxidized through ATP production, which would lead to even more unwanted excess ATP.

[00105] Other potential solutions exist for reducing NADH excess and increasing IPA yield potential (thermodynamic max yield = 47 wt%): re-capturing CO₂ produced in excess during the fermentation and in doing so also re-oxidizing excess NADH (CO₂ fixation). Or avoid excess CO₂ and NADH release altogether by diverting some flux from glycolysis to a phosphoketolase (PK)/ phosphotransacetylase (PTA) pathway to generate more acetyl-CoA and less CO₂ and NADH. However, so far none of these options have been technically demonstrated in the context of IPA production and are generally known to be very challenging.

[00106] The present disclosure combines one of three easy to implement high yield C2-streams for MEG production from xylose with an easy to implement IPA production stream via the DHAP pathway. Surprisingly, the problem of the IPA pathway, excess NADH production, complements the NADH requiring C2 part of MEG production. The combination of these pathways leads to a high total yield potential of 61 wt%, which is close to the maximum energetic yield of 65 wt% for degradation of xylose into MEG and IPA, assuming these products are produced in a 2:1 ratio. This high yield potential stems from the synergies of coupling the IPA pathway with the C2-branch of MEG production from xylose.

[00107] The proposed pathway in its basic form is not redox neutral, but has a small excess of 0.5 mol NADH per mol of consumed xylose. In an aerobic fermentation, oxidation of NADH can deliver just enough ATP to obtain sufficient, but not excessive, ATP required for growth and maintenance during the production phase without having a significantly negative impact on product formation.

[00108] The present disclosure solves a number of problems associated with MEG and/or IPA production. In one embodiment, the problem of a difficult to implement C3 pathway in production of MEG from xylose is solved. In another embodiment, the problem of ATP shortage in production of MEG from xylose is solved. In another embodiment, the problem of loss of yield potential in production of MEG from glucose is solved. In another embodiment, the problem of ATP shortage in production of MEG from glucose is solved. In another embodiment, the problem of excess NADH production in production of MEG from glucose is solved. In another embodiment, the problem of loss of yield potential in production of IPA from glucose is solved. In another embodiment, the problem of excess NADH production in production of IPA from glucose is solved.

[00109] In one embodiment, the pathway for MEG + IPA co-production in *E. coli* comprises the following enzymes for IPA production: thiolase, acetate:acetoacetyl-CoA transferase or hydrolase, acetoacetate decarboxylase and secondary alcohol dehydrogenase. The MEG pathway via ribulose-1-phosphate comprises the following enzymes: D-tagatose 3-epimerase, D-ribulokinase, D-ribulose-phosphate aldolase and glycolaldehyde reductase. In order to increase carbon flux to the desired pathway, three specific genes that could divert carbon flux were identified and deleted: xylB gene coding for a xylulokinase (this enzyme can divert carbon flux into the pentose phosphate pathway), the aldA gene coding for aldehyde dehydrogenase A (can divert carbon flux from glycolaldehyde to glycolate instead of to MEG) and the ldhA gene coding for lactate dehydrogenase (this enzyme can divert carbon flux from pyruvate to lactate instead of to acetyl-CoA).

[00110] The first step of the pathway (**FIG. 1**) is the natural conversion of D-xylose into D-xylulose. D-xylulose normally enters the pentose phosphate pathway for energy and biomass generation, which is inhibited by the deletion of the xylB gene. In the engineered pathway, all carbon will be re-directed to D-ribulose by the D-tagatose 3-epimerase enzyme. D-ribulose is then converted to D-Ribulose-1-phosphate by the native *E. coli* enzyme D-ribulokinase. D-Ribulose-1-phosphate is cleaved into glycolaldehyde and dihydroxy acetone phosphate (DHAP) by D-ribulose-phosphate aldolase. The further degradation of DHAP is termed the C3 branch, leading to IPA production. Degradation of glycolaldehyde, termed the C2-branch, can lead to ethylene glycol or glycolate formation. Glycolate is the undesired by-product that can be produced by the aldA gene product. Ethylene glycol can be produced from glycolaldehyde using the en-

zyme glycolaldehyde reductase. The conversion of DHAP to acetyl-CoA (through glyceraldehyde-3-phosphate and pyruvate) is part of natural *E. coli* metabolism. One molecule of acetyl-CoA is condensed to another molecule of acetyl-CoA by the enzyme thiolase to produce acetoacetyl-CoA. The CoA from acetoacetyl-CoA is recycled to a molecule of acetate by acetate:acetoacetyl-CoA transferase or hydrolase, generating acetyl-CoA and acetoacetate. Acetoacetate is decarboxylated by acetoacetate decarboxylase to acetone which is further reduced to IPA by a secondary alcohol dehydrogenase enzyme. IPA can further be converted to propene by a dehydratase.

[00111] In another embodiment, the pathway for MEG + IPA co-production in *E. coli* comprises the following enzymes for IPA production: thiolase, acetate:acetoacetyl-CoA transferase or hydrolase, acetoacetate decarboxylase and secondary alcohol dehydrogenase. The MEG pathway via D-xylulose-1-phosphate comprises the following enzymes: D-xylulose 1-kinase, D-xylulose-1-phosphate aldolase and glycolaldehyde reductase. In order to increase carbon flux to the desired pathway, three specific genes that could divert carbon flux were identified and deleted: xylB gene coding for a xylulokinase (this enzyme can divert carbon flux into the pentose phosphate pathway), the aldA gene coding for aldehyde dehydrogenase A (can divert carbon flux from glycolaldehyde to glycolate instead of to MEG) and the ldhA gene coding for lactate dehydrogenase (this enzyme can divert carbon flux from pyruvate to lactate instead of to acetyl-CoA).

[00112] The first step of the pathway (FIG. 2) is the natural conversion of D-xylose into D-xylulose. D-xylulose normally enters the pentose phosphate pathway for energy and biomass generation, which is inhibited by the deletion of the xylB gene. In the engineered pathway, all carbon will be re-directed to D-xylulose-1-phosphate by the D-xylulose 1-kinase enzyme. D-xylulose-1-phosphate is then cleaved into glycolaldehyde and dihydroxy acetone phosphate (DHAP) by D-xylulose-1-phosphate aldolase. Production of MEG from glycolaldehyde and a three carbon compound from DHAP (for example, acetone, IPA and/or propene) proceeds as described for FIG. 1.

[00113] In another embodiment, the pathway for MEG + IPA co-production in *E. coli* comprises the following enzymes for IPA production: thiolase, acetate:acetoacetyl-CoA transferase or hydrolase, acetoacetate decarboxylase and secondary alcohol dehydrogenase. The MEG pathway via D-xylonate comprises the following enzymes: xylose dehydrogenase, optionally xylonolactonase, xylonate dehydratase, 2-keto-3-deoxy-

D-xylonate aldolase and glycolaldehyde reductase. In order to increase carbon flux to the desired pathway, three specific genes that could divert carbon flux were identified and deleted: xylA gene coding for a D-xylose isomerase (this enzyme can divert carbon flux from D-xylose to D-xylulose instead of to D-xylonate or D-xylonolactone), the aldA
5 gene coding for aldehyde dehydrogenase A (can divert carbon flux from glycolaldehyde to glycolate instead of to MEG) and the ldhA gene coding for lactate dehydrogenase (this enzyme can divert carbon flux from pyruvate to lactate instead of to acetyl-CoA).

[00114] The first step of the pathway (**FIG. 3**) is the conversion of D-xylose into D-xylonate, either by a two-step process using a xylose dehydrogenase to convert D-xylose to D-xylonolactone followed by conversion of D-xylonolactone to D-xylonate with
10 a xylonolactonase enzyme, or by a one-step process using a xylose dehydrogenase to convert D-xylose directly to D-xylonate. The conversion of D-xylose to D-xylulose is inhibited by the deletion of the xylA gene. D-xylonate is then converted to 2-keto-3-deoxy-xylonate by a xylonate dehydratase. 2-keto-3-deoxy-xylonate is then cleaved into
15 glycolaldehyde and pyruvate by 2-keto-3-deoxy-D-xylonate aldolase. Production of MEG from glycolaldehyde and a three carbon compound from pyruvate (for example, acetone, IPA and/or propene) proceeds as described for **FIG. 1**.

[00115] The pathway for MEG + IPA co-production in *S. cerevisiae* (**FIG. 5**) comprises the following enzymes for IPA production: thiolase, acetate:acetoacetyl-CoA
20 transferase or hydrolase, acetoacetate decarboxylase and secondary alcohol dehydrogenase. The MEG pathway via D-ribulose-1-phosphate comprises the following enzymes: D-tagatose 3-epimerase, D-ribulokinase, D-ribulose-phosphate aldolase and glycolaldehyde reductase. Besides the two main pathways, *S. cerevisiae* is not capable of consuming xylose, so two different pathways were tested for xylose consumption.
25 Pathway 1 comprises 2 genes: Xyl1 converts D-Xylose to xylitol, and Xyl2 converts Xylitol to D-xylulose. Pathway 2 comprises only one gene: XylA that directly converts D-xylose to D-xylulose. In order to increase carbon flux to the desired pathway, two specific genes that could divert carbon flux were identified and deleted: XKS1 gene coding
30 for a xylulokinase (this enzyme can divert carbon flux into the pentose phosphate pathway) and PHO13 gene coding for alkaline phosphatase (can divert carbon from pentose phosphate pathway).

[00116] The first step of the pathway is the conversion of D-xylose into D-xylulose, directly or via the intermediate xylitol. D-xylulose is converted to D-ribulose by the D-

tagatose 3-epimerase enzyme. D-ribulose is then converted to D-Ribulose-1-phosphate by D-ribulokinase. D-Ribulose-1-phosphate is cleaved into glycolaldehyde and DHAP by D-ribulose-phosphate aldolase. DHAP enters the C3 branch for IPA production and glycolaldehyde can be converted to ethylene glycol using glycolaldehyde reductase.

5 The conversion of DHAP to acetyl-CoA (through glyceraldehyde-3-phosphate and pyruvate) is part of the natural *S. cerevisiae* metabolism. One molecule of acetyl-CoA is condensed to another molecule of acetyl-CoA by thiolase, producing acetoacetyl-CoA. The CoA from acetoacetyl-CoA is recycled to a molecule of acetate by acetate:acetoacetyl-CoA transferase or hydrolase, generating one molecule of acetyl-CoA and one of acetoacetate. Acetoacetate is further decarboxylated by acetoacetate decarboxylase to acetone, which is further converted to IPA by a secondary alcohol dehydrogenase enzyme. IPA can further be converted to propene by a dehydratase-isomerase.

[00117] Surprisingly, the main problem of the IPA pathway, excess NADH production, is highly synergistic with a C2-stream for MEG production by complementing the NADH requirement of the C2 branch, while leaving just enough NADH to generate required ATP in an aerobic process, without excess ATP production.

[00118] The described IPA process of US 2010/0311135 and other applications, without carbon fixation, can only achieve 34 wt% versus the energetic maximum yield potential of 47 wt%. Thus, this IPA pathway, even if implemented perfectly, can only achieve 72% of the energetic maximum yield. In the present disclosure, the synergy of coupling IPA with MEG production is such that, without necessity of CO₂ fixation, the combined products' yield potential of 61 wt% is very close (94%) to the energetic (= theoretic, pathway independent) maximum yield potential of 65 wt%.

25 [00119] In a further embodiment, the inventive co-production pathway from xylose is implemented in an organism with natural or added capability to fix CO₂ using excess reducing agents, thereby providing even higher yield potential. Various CO₂ fixation pathways are known and have been implemented in *E. coli* or other hosts. Acetogens, such as *Clostridium ljungdahlii*, can naturally utilize excess NADH generated in the presented xylose fermentation pathway especially efficient to re-capture released CO₂ in the Wood-Ljungdahl pathway to produce the intermediate acetyl-CoA, which can then be used to produce more acetone or related products. CO₂ is released for instance in the pyruvate + CoA + NAD⁺ → acetyl-CoA + CO₂ + 2 NADH or acetoacetone → ace-

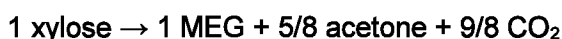
tone + CO₂ reactions. Furthermore, adding a second feedstock, such as hydrogen gas (H₂) or syngas (a composition of H₂, CO, CO₂) or methanol, can provide more reducing agents and even allow acetogens or similarly enabled organisms to re-capture all CO₂ released in the xylose fermentation pathway or CO₂ present in the second feedstock.

- 5 Such a mixotrophic fermentation can thus further increase yield potential. In the case of MEG + acetone from xylose, CO₂ fixation can lead to an increase of 25% relative acetone or 8% total MEG + acetone product yield. With externally added reducing agents, calculated for full capture of all xylose carbon, the yield potential is +100% for acetone which equals +32% total product yield.

- 10 [00120] Yield potentials without CO₂ fixation:



Yield potentials with CO₂ fixation:



- 15 $1 \text{ xylose} \rightarrow 1 \text{ MEG} + 10/18 \text{ IPA} + 4/3 \text{ CO}_2$

Yield potentials with externally added reducing agents, calculated for fixation of CO₂ equivalent to all CO₂ released during xylose fermentation:



- 20 [00121] While this present disclosure is theoretically sound and synergistic, it surprisingly also avoids the biggest metabolic engineering and technical challenges of both MEG and IPA fermentation processes: C3-stream MEG fermentation and carbon fixation for IPA process.

- [00122] In one embodiment, MEG is produced through the conversion of glycolaldehyde in a C-2 branch pathway and acetone is produced through the conversion of DHAP or pyruvate in a C-3 branch pathway. In another embodiment, MEG is produced through the conversion of glycolaldehyde in a C-2 branch pathway and IPA is produced through the conversion of DHAP or pyruvate in a C-3 branch pathway. In a further embodiment, MEG is produced through the conversion of glycolaldehyde in a C-2 branch pathway and propene is produced through the conversion of DHAP or pyruvate in a C-3 branch pathway.
- 25
- 30

[00123] In one embodiment, at least a portion of the excess NADH produced in the C-3 branch is used as a source of reducing equivalents in the C-2 branch. In another

embodiment, at least a portion of the excess NADH produced in the C-3 branch is used to produce ATP.

[00124] In one embodiment, the co-produced MEG and acetone comprise a yield potential greater than 90% of the theoretical maximum yield potential without carbon fixation. In another embodiment, the co-produced MEG and IPA comprise a yield potential greater than 90% of the theoretical maximum yield potential without carbon fixation. In a further embodiment, the co-produced MEG and propene comprise a yield potential greater than 90% of the theoretical maximum yield potential without carbon fixation.

[00125] In one embodiment, excess biomass formation is minimized and production of MEG and acetone is maximized. In another embodiment, excess biomass formation is minimized and production of MEG and IPA is maximized. In a further embodiment, excess biomass formation is minimized and production of MEG and propene is maximized.

Production Compounds

Monoethylene Glycol

[00126] Monoethylene glycol (MEG) is an important raw material for industrial applications. A primary use of MEG is in the manufacture of polyethylene terephthalate (PET) resins, films and fibers. In addition, MEG is important in the production of anti-freezes, coolants, aircraft anti-icer and deicers and solvents. MEG is also known as ethane-1,2-diol or ethylene glycol.

[00127] Ethylene glycol is also used as a medium for convective heat transfer in, for example, automobiles and liquid cooled computers.

[00128] Because of its high boiling point and affinity for water, ethylene glycol is a useful desiccant. Ethylene glycol is widely used to inhibit the formation of natural gas clathrates (hydrates) in long multiphase pipelines that convey natural gas from remote gas fields to a gas processing facility. Ethylene glycol can be recovered from the natural gas and reused as an inhibitor after purification treatment that removes water and inorganic salts.

[00129] Minor uses of ethylene glycol include in the manufacture of capacitors, as a chemical intermediate in the manufacture of 1,4-dioxane, and as an additive to prevent corrosion in liquid cooling systems for personal computers. Ethylene glycol is also used in the manufacture of some vaccines; as a minor ingredient in shoe polish, inks

and dyes; as a rot and fungal treatment for wood; and as a preservative for biological specimens.

Acetone

[00130] Acetone (also known as propanone) is an organic compound with the formula (CH₃)₂CO. It is a colorless, volatile, flammable liquid, and is the simplest ketone.

[00131] Acetone is miscible with water and serves as an important solvent, typically for cleaning purposes in the laboratory. Over 6.7 million tonnes are produced worldwide, mainly for use as a solvent and production of methyl methacrylate and bisphenol A. It is a common building block in organic chemistry. Familiar household uses of acetone are as the active ingredient in nail polish remover and as paint thinner.

Isopropanol

[00132] Isopropyl alcohol (IUPAC name 2-propanol), also called isopropanol, is a compound with the chemical formula C₃H₈O or C₃H₇OH or CH₃CHOHCH₃. It is a colorless, flammable chemical compound with a strong odor. It is the simplest example of a secondary alcohol, where the alcohol carbon atom is attached to two other carbon atoms sometimes shown as (CH₃)₂CHOH. It is a structural isomer of propanol. It has a wide variety of industrial and household uses.

Propene

[00133] Propene, also known as propylene or methyl ethylene, is an unsaturated organic compound having the chemical formula C₃H₆. It has one double bond, and is the second simplest member of the alkene class of hydrocarbons.

[00134] Propene is produced from fossil fuels—petroleum, natural gas, and, to a much lesser extent, coal. Propene is a byproduct of oil refining and natural gas processing.

[00135] In some aspects, the microbes of the present disclosure produce monoethylene glycol (MEG). In some aspects, the microbes of the present disclosure produce MEG and one or more C₃ compounds. In some aspects, the microbes of the present disclosure produce MEG and one or more C₃ compounds. In some aspects, the microbes of the present disclosure produce one or more of the following C₃ compounds: acetone, isopropanol, and propene. In some aspects, the microbes of the present disclosure produce MEG and acetone. In some aspects, the microbes of the present disclosure produce MEG and isopropanol. In some aspects, the microbes of the present disclosure produce MEG and propene. In some aspects, the microbes of the

present disclosure produce MEG, acetone, and isopropanol. In some aspects, the microbes of the present disclosure produce MEG, acetone, and propene. In some aspects, the microbes of the present disclosure produce MEG, isopropanol and propene. In some aspects, the microbes of the present disclosure produce MEG, acetone, isopropanol, and propene.

Generation of Microbial Populations

Isolation of Microbes

[00136] Microbes useful in methods and compositions disclosed herein can be obtained from microbial deposits of microbes, bacteria and/or fungi, that produce or are capable of producing MEG and/or C3 compounds. A method of obtaining microbes may be through the isolation of microbes from any number of environmental samples. Microbes can be obtained from global strain banks.

Genetic Modification

[00137] The genetic modification introduced into one or more microbes of the methods disclosed herein may be a knock-out mutation (e.g. deletion of a promoter, insertion or deletion to produce a premature stop codon, deletion of an entire gene), or it may be elimination or abolishment of activity of a protein domain (e.g. point mutation affecting an active site, or deletion of a portion of a gene encoding the relevant portion of the protein product), or it may alter or abolish a regulatory sequence of a target gene. One or more regulatory sequences may also be inserted, including heterologous regulatory sequences and regulatory sequences found within a genome of a microbial species or genus corresponding to the microbe into which the genetic variation is introduced. Moreover, regulatory sequences may be selected based on the expression level of a gene in a microbial culture. The genetic variation may be a pre-determined genetic variation that is specifically introduced to a target site. The genetic variation may be a random mutation within the target site. The genetic variation may be an insertion or deletion of one or more nucleotides. In some cases, a plurality of different genetic variations (e.g. 2, 3, 4, 5, 10, or more) are introduced into one or more of the isolated bacteria before assessing trait improvement. The plurality of genetic variations can be any of the above types, the same or different types, and in any combination. In some cases, a plurality of different genetic variations are introduced serially, introducing a first genetic variation after a first isolation step, a second genetic variation after a second isolation

step, and so forth so as to accumulate a plurality of desired modifications in the microbes.

[00138] In general, the term "genetic variation" refers to any change introduced into a polynucleotide sequence relative to a reference polynucleotide, such as a reference genome or portion thereof, or reference gene or portion thereof. A genetic variation may be referred to as a "mutation," and a sequence or organism comprising a genetic variation may be referred to as a "genetic variant" or "mutant". Genetic variations can have any number of effects, such as the increase or decrease of some biological activity, including gene expression, metabolism, and cell signaling. Genetic variations can be specifically introduced to a target site, or introduced randomly. A variety of molecular tools and methods are available for introducing genetic variation. For example, genetic variation can be introduced via polymerase chain reaction mutagenesis, oligonucleotide-directed mutagenesis, saturation mutagenesis, fragment shuffling mutagenesis, homologous recombination, recombineering, lambda red mediated recombination, CRISPR/Cas9 systems, chemical mutagenesis, and combinations thereof. Chemical methods of introducing genetic variation include exposure of DNA to a chemical mutagen, e.g., ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), N-nitrosourea (EN U), N-methyl-N-nitro-N'-nitrosoguanidine, 4-nitroquinoline N-oxide, diethylsulfate, benzopyrene, cyclophosphamide, bleomycin, triethylmelamine, acrylamide monomer, nitrogen mustard, vincristine, diepoxyalkanes (for example, diepoxybutane), ICR-170, formaldehyde, procarbazine hydrochloride, ethylene oxide, dimethylnitrosamine, 7,12 dimethylbenz(a) anthracene, chlorambucil, hexamethylphosphoramide, bisulfan, and the like. Radiation mutation-inducing agents include ultraviolet radiation, γ -irradiation, X-rays, and fast neutron bombardment. Genetic variation can also be introduced into a nucleic acid using, e.g., trimethylpsoralen with ultraviolet light. Random or targeted insertion of a mobile DNA element, e.g., a transposable element, is another suitable method for generating genetic variation. Genetic variations can be introduced into a nucleic acid during amplification in a cell-free in vitro system, e.g., using a polymerase chain reaction (PCR) technique such as error-prone PCR. Genetic variations can be introduced into a nucleic acid in vitro using DNA shuffling techniques (e.g., exon shuffling, domain swapping, and the like). Genetic variations can also be introduced into a nucleic acid as a result of a deficiency in a DNA repair enzyme in a cell, e.g., the presence in a cell of a mutant gene encoding a mutant DNA repair enzyme is expected

to generate a high frequency of mutations (i.e., about 1 mutation/100 genes-1 mutation/10,000 genes) in the genome of the cell. Examples of genes encoding DNA repair enzymes include but are not limited to Mut H, Mut S, Mut L, and Mut U, and the homologs thereof in other species (e.g., MSH 1 6, PMS 1 2, MLH 1, GTBP, ERCC-1, and the like). Example descriptions of various methods for introducing genetic variations are provided in e.g., Stemple (2004) *Nature* 5:1-7; Chiang et al. (1993) *PCR Methods Appl* 2(3): 210-217; Stemmer (1994) *Proc. Natl. Acad. Sci. USA* 91:10747-10751; and U.S. Pat. Nos. 6,033,861, and 6,773,900.

[00139] Genetic variations introduced into microbes may be classified as transgenic, cisgenic, intragenomic, intragenetic, intergeneric, synthetic, evolved, rearranged, or SNPs.

[00140] CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats) /CRISPR-associated (Cas) systems can be used to introduce desired mutations. CRISPR/Cas9 provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. The Cas9 protein (or functional equivalent and/or variant thereof, i.e., Cas9-like protein) naturally contains DNA endonuclease activity that depends on the association of the protein with two naturally occurring or synthetic RNA molecules called crRNA and tracrRNA (also called guide RNAs). In some cases, the two molecules are covalently link to form a single molecule (also called a single guide RNA ("sgRNA")). Thus, the Cas9 or Cas9-like protein associates with a DNA-targeting RNA (which term encompasses both the two-molecule guide RNA configuration and the single-molecule guide RNA configuration), which activates the Cas9 or Cas9-like protein and guides the protein to a target nucleic acid sequence. If the Cas9 or Cas9-like protein retains its natural enzymatic function, it will cleave target DNA to create a double-stranded break, which can lead to genome alteration (i.e., editing: deletion, insertion (when a donor polynucleotide is present), replacement, etc.), thereby altering gene expression. Some variants of Cas9 (which variants are encompassed by the term Cas9-like) have been altered such that they have a decreased DNA cleaving activity (in some cases, they cleave a single strand instead of both strands of the target DNA, while in other cases, they have severely reduced to no DNA cleavage activity). Further exemplary descriptions of CRISPR systems for introducing genetic variation can be found in, e.g. US8795965.

[00141] As a cyclic amplification technique, polymerase chain reaction (PCR) mutagenesis uses mutagenic primers to introduce desired mutations. PCR is performed by cycles of denaturation, annealing, and extension. After amplification by PCR, selection of mutated DNA and removal of parental plasmid DNA can be accomplished by: 1) replacement of dCTP by hydroxymethylated-dCTP during PCR, followed by digestion with restriction enzymes to remove non-hydroxymethylated parent DNA only; 2) simultaneous mutagenesis of both an antibiotic resistance gene and the studied gene changing the plasmid to a different antibiotic resistance, the new antibiotic resistance facilitating the selection of the desired mutation thereafter; 3) after introducing a desired mutation, digestion of the parent methylated template DNA by restriction enzyme DpnI which cleaves only methylated DNA, by which the mutagenized unmethylated chains are recovered; or 4) circularization of the mutated PCR products in an additional ligation reaction to increase the transformation efficiency of mutated DNA. Further description of exemplary methods can be found in e.g. US7132265, US6713285, US6673610, US6391548, US5789166, US5780270, US5354670, US5071743, and US20100267147.

[00142] Oligonucleotide-directed mutagenesis, also called site-directed mutagenesis, typically utilizes a synthetic DNA primer. This synthetic primer contains the desired mutation and is complementary to the template DNA around the mutation site so that it can hybridize with the DNA in the gene of interest. The mutation may be a single base change (a point mutation), multiple base changes, deletion, or insertion, or a combination of these. The single-strand primer is then extended using a DNA polymerase, which copies the rest of the gene. The gene thus copied contains the mutated site, and may then be introduced into a host cell as a vector and cloned. Finally, mutants can be selected by DNA sequencing to check that they contain the desired mutation.

[00143] Genetic variations can be introduced using error-prone PCR. In this technique the gene of interest is amplified using a DNA polymerase under conditions that are deficient in the fidelity of replication of sequence. The result is that the amplification products contain at least one error in the sequence. When a gene is amplified and the resulting product(s) of the reaction contain one or more alterations in sequence when compared to the template molecule, the resulting products are mutagenized as compared to the template. Another means of introducing random mutations is exposing cells to a chemical mutagen, such as nitrosoguanidine or ethyl methanesulfonate

(Nestmann, Mutat Res 1975 June; 28(3):323-30), and the vector containing the gene is then isolated from the host.

[00144] Saturation mutagenesis is another form of random mutagenesis, in which one tries to generate all or nearly all possible mutations at a specific site, or narrow region of a gene. In a general sense, saturation mutagenesis is comprised of mutagenizing a complete set of mutagenic cassettes (wherein each cassette is, for example, 1-500 bases in length) in defined polynucleotide sequence to be mutagenized (wherein the sequence to be mutagenized is, for example, from 15 to 100, 000 bases in length). Therefore, a group of mutations (e.g. ranging from 1 to 100 mutations) is introduced into each cassette to be mutagenized. A grouping of mutations to be introduced into one cassette can be different or the same from a second grouping of mutations to be introduced into a second cassette during the application of one round of saturation mutagenesis. Such groupings are exemplified by deletions, additions, groupings of particular codons, and groupings of particular nucleotide cassettes.

[00145] Fragment shuffling mutagenesis, also called DNA shuffling, is a way to rapidly propagate beneficial mutations. In an example of a shuffling process, DNase is used to fragment a set of parent genes into pieces of e.g. about 50-100 bp in length. This is then followed by a polymerase chain reaction (PCR) without primers—DNA fragments with sufficient overlapping homologous sequence will anneal to each other and are then be extended by DNA polymerase. Several rounds of this PCR extension are allowed to occur, after some of the DNA molecules reach the size of the parental genes. These genes can then be amplified with another PCR, this time with the addition of primers that are designed to complement the ends of the strands. The primers may have additional sequences added to their 5' ends, such as sequences for restriction enzyme recognition sites needed for ligation into a cloning vector. Further examples of shuffling techniques are provided in US20050266541.

[00146] Homologous recombination mutagenesis involves recombination between an exogenous DNA fragment and the targeted polynucleotide sequence. After a double-stranded break occurs, sections of DNA around the 5' ends of the break are cut away in a process called resection. In the strand invasion step that follows, an overhanging 3' end of the broken DNA molecule then "invades" a similar or identical DNA molecule that is not broken. The method can be used to delete a gene, remove exons, add a gene, and introduce point mutations. Homologous recombination mutagenesis can be perma-

5 nent or conditional. Typically, a recombination template is also provided. A recombination template may be a component of another vector, contained in a separate vector, or provided as a separate polynucleotide. In some embodiments, a recombination template is designed to serve as a template in homologous recombination, such as within or near a target sequence nicked or cleaved by a site-specific nuclease. A template polynucleotide may be of any suitable length, such as about or more than about 10, 15, 20, 25, 50, 75, 100, 150, 200, 500, 1000, or more nucleotides in length. In some embodiments, the template polynucleotide is complementary to a portion of a polynucleotide comprising the target sequence. When optimally aligned, a template polynucleotide might overlap with one or more nucleotides of a target sequences (e.g. about or more than about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or more nucleotides). In some embodiments, when a template sequence and a polynucleotide comprising a target sequence are optimally aligned, the nearest nucleotide of the template polynucleotide is within about 1, 5, 10, 15, 20, 25, 50, 75, 100, 200, 300, 400, 500, 1000, 5000, 10000, or more nucleotides from the target sequence. Non-limiting examples of site-directed nucleases useful in methods of homologous recombination include zinc finger nucleases, CRISPR nucleases, TALE nucleases, and meganuclease. For a further description of the use of such nucleases, see e.g. US8795965 and US20140301990.

10 [00147] Introducing genetic variation may be an incomplete process, such that some bacteria in a treated population of bacteria carry a desired mutation while others do not. In some cases, it is desirable to apply a selection pressure so as to enrich for bacteria carrying a desired genetic variation. Traditionally, selection for successful genetic variants involved selection for or against some functionality imparted or abolished by the genetic variation, such as in the case of inserting antibiotic resistance gene or abolishing a metabolic activity capable of converting a non-lethal compound into a lethal metabolite. It is also possible to apply a selection pressure based on a polynucleotide sequence itself, such that only a desired genetic variation need be introduced (e.g. without also requiring a selectable marker). In this case, the selection pressure can comprise cleaving genomes lacking the genetic variation introduced to a target site, such that selection is effectively directed against the reference sequence into which the genetic variation is sought to be introduced. Typically, cleavage occurs within 100 nucleotides of the target site (e.g. within 75, 50, 25, 10, or fewer nucleotides from the target site, including cleavage at or within the target site). Cleaving may be directed by a

15 20 25 30

site-specific nuclease selected from the group consisting of a Zinc Finger nuclease, a CRISPR nuclease, a TALE nuclease (TALEN), or a meganuclease. Such a process is similar to processes for enhancing homologous recombination at a target site, except that no template for homologous recombination is provided. As a result, bacteria lacking the desired genetic variation are more likely to undergo cleavage that, left unrepaired, results in cell death. Bacteria surviving selection may then be isolated for assessing conferral of an improved trait.

[00148] A CRISPR nuclease may be used as the site-specific nuclease to direct cleavage to a target site. An improved selection of mutated microbes can be obtained by using Cas9 to kill non-mutated cells. CRISPR nuclease systems employed for selection against non-variants can employ similar elements to those described above with respect to introducing genetic variation, except that no template for homologous recombination is provided. Cleavage directed to the target site thus enhances death of affected cells.

[00149] Other options for specifically inducing cleavage at a target site are available, such as zinc finger nucleases, TALE nuclease (TALEN) systems, and meganuclease. Zinc-finger nucleases (ZFNs) are artificial DNA endonucleases generated by fusing a zinc finger DNA binding domain to a DNA cleavage domain. ZFNs can be engineered to target desired DNA sequences and this enables zinc-finger nucleases to cleave unique target sequences. When introduced into a cell, ZFNs can be used to edit target DNA in the cell (e.g., the cell's genome) by inducing double stranded breaks. Transcription activator-like effector nucleases (TALENs) are artificial DNA endonucleases generated by fusing a TAL (Transcription activator-like) effector DNA binding domain to a DNA cleavage domain. TALENS can be quickly engineered to bind practically any desired DNA sequence and when introduced into a cell, TALENS can be used to edit target DNA in the cell (e.g., the cell's genome) by inducing double strand breaks. Meganucleases (homologous endonuclease) are endodeoxyribonucleases characterized by a large recognition site (double-stranded DNA sequences of 12 to 40 base pairs). Meganucleases can be used to replace, eliminate or modify sequences in a highly targeted way. By modifying their recognition sequence through protein engineering, the targeted sequence can be changed. Meganucleases can be used to modify all genome types, whether bacterial, plant or animal and are commonly grouped into four families: the LAGLIDADG family, the GIY-YIG family, the His-Cyst box family and the HNH family.

Exemplary homing endonucleases include I-SceI, I-CeuI, PI-PspI, PI-Sce, I-SceIV, I-CsmI, I-PanI, I-SceII, I-PpoI, I-SceIII, I-CreI, I-TevI, I-TevII and I-TevIII.

[00150] In some aspects, the disclosure provides for a sequence which shares at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%,
5 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any sequence described herein.

[00151] In some aspects, the disclosure provides for a microbe that comprises a sequence, which shares at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,
10 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any sequence described herein.

[00152] In some aspects, the disclosure provides for a microbe that comprises a nucleic acid sequence, which shares at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%,
15 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any sequence described herein.

[00153] In some aspects, the disclosure provides for a microbe that comprises, or primer that comprises, or probe that comprises, or non-native junction sequence that comprises, a nucleic acid sequence, which shares at least about 70%, 71%, 72%, 73%,
20 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any sequence described herein.

[00154] In some aspects, the disclosure provides for a microbe that comprises a non-native junction sequence that shares at least about 70%, 71%, 72%, 73%, 74%,
25 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any sequence described herein.

[00155] In some aspects, the disclosure provides for a microbe that comprises an amino acid sequence, which shares at least about 70%, 71%, 72%, 73%, 74%, 75%,
30 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any sequence described herein.

Methods of Detecting Genetic Modification

[00156] The present disclosure teaches primers, probes, and assays that are useful for detecting the microbes taught herein. In some aspects, the disclosure provides for methods of detecting the WT parental strains. In other aspects, the disclosure provides for methods of detecting the engineered or modified microbes derived from parent strains or WT strains. In aspects, the present disclosure provides methods of identifying genetic alterations in a microbe.

[00157] In some aspects, the genomic engineering methods of the present disclosure lead to the creation of non-natural nucleotide "junction" sequences in the modified microbes. These non-naturally occurring nucleotide junctions can be used as a type of diagnostic that is indicative of the presence of a particular genetic alteration in a microbe taught herein.

[00158] The present techniques are able to detect these non-naturally occurring nucleotide junctions via the utilization of specialized quantitative PCR methods, including uniquely designed primers and probes. In some aspects, the probes of the disclosure bind to the non-naturally occurring nucleotide junction sequences. In some aspects, traditional PCR is utilized. In other aspects, real-time PCR is utilized. In some aspects, quantitative PCR (qPCR) is utilized. In some aspects, the PCR methods are used to identify heterologous sequences that have been inserted into the genomic DNA or extra-genomic DNA of the microbes.

[00159] Thus, the disclosure can cover the utilization of two common methods for the detection of PCR products in real-time: (1) non-specific fluorescent dyes that intercalate with any double-stranded DNA, and (2) sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary sequence. In some aspects, only the non-naturally occurring nucleotide junction will be amplified via the taught primers, and consequently can be detected via either a non-specific dye, or via the utilization of a specific hybridization probe. In other aspects, the primers of the disclosure are chosen such that the primers flank either side of a junction sequence, such that if an amplification reaction occurs, then said junction sequence is present.

[00160] Aspects of the disclosure involve non-naturally occurring nucleotide junction sequence molecules per se, along with other nucleotide molecules that are capable of binding to said non-naturally occurring nucleotide junction sequences under mild to

stringent hybridization conditions. In some aspects, the nucleotide molecules that are capable of binding to said non-naturally occurring nucleotide junction sequences under mild to stringent hybridization conditions are termed "nucleotide probes."

[00161] In some aspects, genomic DNA can be extracted from samples and used to quantify the presence of microbes of the disclosure by using qPCR. The primers utilized in the qPCR reaction can be primers designed by Primer Blast (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) to amplify unique regions of the wild-type genome or unique regions of the engineered non-intergeneric mutant strains. The qPCR reaction can be carried out using the SYBR GreenER qPCR SuperMix Universal (Thermo Fisher P/N 11762100) kit, using only forward and reverse amplification primers; alternatively, the Kapa Probe Force kit (Kapa Biosystems P/N KK4301) can be used with amplification primers and a TaqMan probe containing a FAM dye label at the 5' end, an internal ZEN quencher, and a minor groove binder and fluorescent quencher at the 3' end (Integrated DNA Technologies).

[00162] Quantitative polymerase chain reaction (qPCR) is a method of quantifying, in real time, the amplification of one or more nucleic acid sequences. The real time quantification of the PCR assay permits determination of the quantity of nucleic acids being generated by the PCR amplification steps by comparing the amplifying nucleic acids of interest and an appropriate control nucleic acid sequence, which may act as a calibration standard.

[00163] TaqMan probes are often utilized in qPCR assays that require an increased specificity for quantifying target nucleic acid sequences. TaqMan probes comprise a oligonucleotide probe with a fluorophore attached to the 5' end and a quencher attached to the 3' end of the probe. When the TaqMan probes remain as is with the 5' and 3' ends of the probe in close contact with each other, the quencher prevents fluorescent signal transmission from the fluorophore. TaqMan probes are designed to anneal within a nucleic acid region amplified by a specific set of primers. As the Taq polymerase extends the primer and synthesizes the nascent strand, the 5' to 3' exonuclease activity of the Taq polymerase degrades the probe that annealed to the template. This probe degradation releases the fluorophore, thus breaking the close proximity to the quencher and allowing fluorescence of the fluorophore. Fluorescence detected in the qPCR assay is directly proportional to the fluorophore released and the amount of DNA template present in the reaction.

[00164] The features of qPCR allow the practitioner to eliminate the labor-intensive post-amplification step of gel electrophoresis preparation, which is generally required for observation of the amplified products of traditional PCR assays. The benefits of qPCR over conventional PCR are considerable, and include increased speed, ease of use, reproducibility, and quantitative ability

Microbes

[00165] As described herein, in some embodiments, the recombinant microorganisms are prokaryotic microorganism. In some embodiments, the prokaryotic microorganisms are bacteria. "Bacteria", or "eubacteria", refers to a domain of prokaryotic organisms. Bacteria include at least eleven distinct groups as follows: (1) Gram-positive (gram+) bacteria, of which there are two major subdivisions: (1) high G+C group (Actinomycetes, Mycobacteria, Micrococcus, others) (2) low G+C group (Bacillus, Clostridia, Lactobacillus, Staphylococci, Streptococci, Mycoplasmas); (2) Proteobacteria, e.g., Purple photosynthetic +non-photosynthetic Gram-negative bacteria (includes most "common" Gram-negative bacteria); (3) Cyanobacteria, e.g., oxygenic phototrophs; (4) Spirochetes and related species; (5) Planctomyces; (6) Bacteroides, Flavobacteria; (7) Chlamydia; (8) Green sulfur bacteria; (9) Green non-sulfur bacteria (also anaerobic phototrophs); (10) Radioresistant micrococci and relatives; (11) Thermotoga and Thermosipho thermophiles.

[00166] "Gram-negative bacteria" include cocci, nonenteric rods, and enteric rods. The genera of Gram-negative bacteria include, for example, Neisseria, Spirillum, Pasteurella, Brucella, Yersinia, Francisella, Haemophilus, Bordetella, Escherichia, Salmonella, Shigella, Klebsiella, Proteus, Vibrio, Pseudomonas, Bacteroides, Acetobacter, Aerobacter, Agrobacterium, Azotobacter, Spirilla, Serratia, Vibrio, Rhizobium, Chlamydia, Rickettsia, Treponema, and Fusobacterium.

[00167] "Gram positive bacteria" include cocci, nonsporulating rods, and sporulating rods. The genera of gram positive bacteria include, for example, Actinomyces, Bacillus, Clostridium, Corynebacterium, Erysipelothrix, Lactobacillus, Listeria, Mycobacterium, Myxococcus, Nocardia, Staphylococcus, Streptococcus, and Streptomyces.

[00168] In some aspects, the microorganisms of the present disclosure are fungi.

[00169] In some aspects, the recombinant microorganism is a eukaryotic microorganism. In some embodiments, the eukaryotic microorganism is a yeast. In exemplary embodiments, the yeast is a member of a genus selected from the group consisting of

Yarrowia, Candida, Saccharomyces, Pichia, Hansenula, Kluyveromyces, Issatchenkia, Zygosaccharomyces, Debaryomyces, Schizosaccharomyces, Pachysolen, Cryptococcus, Trichosporon, Rhodotorula, and Myxozyma.

[00170] In some aspects, the recombinant microorganism is a prokaryotic microorganism. In exemplary embodiments, the prokaryotic microorganism is a member of a genus selected from the group consisting of Escherichia, Clostridium, Zymomonas, Salmonella, Rhodococcus, Pseudomonas, Bacillus, Lactobacillus, Enterococcus, Alcaligenes, Klebsiella, Paenibacillus, Arthrobacter, Corynebacterium, and Brevibacterium.

[00171] In some aspects, microorganism for use in the methods of the present disclosure can be selected from the group consisting of Yarrowia, Candida, Saccharomyces, Pichia, Hansenula, Kluyveromyces, Issatchenkia, Zygosaccharomyces, Debaryomyces, Schizosaccharomyces, Pachysolen, Cryptococcus, Trichosporon, Rhodotorula, Myxozyma, Escherichia, Clostridium, Zymomonas, Salmonella, Rhodococcus, Pseudomonas, Bacillus, Lactobacillus, Enterococcus, Alcaligenes, Klebsiella, Paenibacillus, Arthrobacter, Corynebacterium, and Brevibacterium.

[00172] In some aspects, a microbe resulting from the methods described herein may be a species selected from any of the following genera: Neisseria, Spirillum, Pasteurella, Brucella, Yersinia, Francisella, Haemophilus, Bordetella, Escherichia, Salmonella, Shigella, Klebsiella, Proteus, Vibrio, Pseudomonas, Bacteroides, Acetobacter, Aerobacter, Agrobacterium, Azotobacter, Spirilla, Serratia, Vibrio, Rhizobium, Chlamydia, Rickettsia, Treponema, Fusobacterium, Actinomyces, Bacillus, Clostridium, Corynebacterium, Erysipelothrix, Lactobacillus, Listeria, Mycobacterium, Myxococcus, Nocardia, Staphylococcus, Streptococcus, Streptomyces, Saccharomyces, Pichia, and Aspergillus.

[00173] In some aspects, microorganisms for use in the methods of the present disclosure include Clostridium sp., Clostridium ljungdahlii, Clostridium autoethanogenum, Clostridium ragsdalei, Eubacterium limosum, Butyrivacterium methylotrophicum, Moorella thermoacetica, Clostridium aceticum, Acetobacterium woodii, Alkalibaculum bacchii, Clostridium drakei, Clostridium carboxidivorans, Clostridium formicoaceticum, Clostridium scatologenes, Moorella thermoautotrophica, Acetone nema longum, Blautia producta, Clostridium glycolicum, Clostridium magnum, Clostridium mayombeii, Clostridium methoxybenzovorans, Clostridium acetobutylicum, Clostridium beijerinckii, Oxobacter pfennigii, Thermoanaerobacter kivui, Sporomusa ovata, Thermoacetogeni-

um phaeum, *Acetobacterium carbinolicum*, *Sporomusa termitida*, *Moorella glycerini*, *Eubacterium aggregans*, *Treponema azotonutricium*, *Escherichia coli*, *Saccharomyces cerevisiae*, *Pseudomonas putida*, *Bacillus* sp, *Corynebacterium* sp., *Yarrowia lipolytica*, *Scheffersomyces stipitis*, and *Terrisporobacter glycolicus*.

5 [00174] The term "recombinant microorganism" and "recombinant host cell" are used interchangeably herein and refer to microorganisms that have been genetically modified to express or to overexpress endogenous enzymes, to express heterologous enzymes, such as those included in a vector, in an integration construct, or which have an alteration in expression of an endogenous gene. By "alteration" it is meant that the
10 expression of the gene, or level of a RNA molecule or equivalent RNA molecules encoding one or more polypeptides or polypeptide subunits, or activity of one or more polypeptides or polypeptide subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the absence of the alteration. For example, the term "alter" can mean "inhibit," but the use of the word "alter" is not limited to this definition. It is understood that the terms "recombinant microorganism" and "recombinant host cell" refer not only to the particular recombinant microorganism but to the progeny or potential progeny of such a microorganism. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell,
15 but are still included within the scope of the term as used herein.

[00175] Culturing of the microorganisms used in the methods of the disclosure may be conducted using any number of processes known in the art for culturing and fermenting substrates using the microorganisms. By way of example, those processes generally described in the following articles using gaseous substrates for fermentation
25 may be utilized: (i) K. T. Klasson, et al. (1991). Bioreactors for synthesis gas fermentations resources. *Conservation and Recycling*, 5; 145-165; (ii) K. T. Klasson, et al. (1991). Bioreactor design for synthesis gas fermentations. *Fuel*. 70. 605-614; (iii) K. T. Klasson, et al. (1992). Bioconversion of synthesis gas into liquid or gaseous fuels. *Enzyme and Microbial Technology*. 14; 602-608; (iv) J. L. Vega, et al. (1989). Study of
30 Gaseous Substrate Fermentation: Carbon Monoxide Conversion to Acetate. 2. Continuous Culture. *Biotech. Bioeng.* 34. 6. 785-793; (v) J. L. Vega, et al. (1989). Study of gaseous substrate fermentations: Carbon monoxide conversion to acetate. 1. Batch culture. *Biotechnology and Bioengineering*. 34. 6. 774-784; (vi) J. L. Vega, et al. (1990).

Design of Bioreactors for Coal Synthesis Gas Fermentations. Resources, Conservation and Recycling. 3. 149-160; all of which are incorporated herein by reference.

[00176] The fermentation may be carried out in any suitable bioreactor, such as Continuous Stirred Tank Bioreactor, Bubble Column Bioreactor, Airlift Bioreactor, Fluid-
5 ized Bed Bioreactor, Packed Bed Bioreactor, Photo-Bioreactor, Immobilized Cell Reactor, Trickle Bed Reactor, Moving Bed Biofilm Reactor, Bubble Column, Gas Lift Fermenter, Membrane Reactors such as Hollow Fiber Membrane Bioreactor. Also, in some embodiments, the bioreactor comprises a first, growth reactor in which the microorganisms are cultured, and a second, fermentation reactor, to which fermentation broth from
10 the growth reactor is fed and in which most of the fermentation product (e.g. MEG, acetone, isopropanol, and propene) is produced. In some embodiments, the bioreactor simultaneously accomplishes the culturing of microorganism and the producing the fermentation product (e.g. MEG, acetone, isopropanol, and propene) from carbon sources such substrates and/or feedstocks provided.

15 **Methods of producing a recombinant microorganism that produces or accumulates MEG and one or more three-carbon compounds**

[00177] As discussed above, the present application provides a method of producing a recombinant microorganism that produces or accumulates MEG and one or more three-carbon compounds. In one embodiment, the MEG and one or more three-carbon
20 compounds are co-produced from xylose. In another embodiment, a method of producing a recombinant microorganism that produces or accumulates MEG and one or more three-carbon compounds from exogenous D-xylose comprises introducing into the recombinant microorganism a deletion, insertion, or loss of function mutation in a gene encoding a D-xylulose-5-kinase and/or in a gene encoding a glycoaldehyde dehydrogenase. In some embodiments, the gene encoding the D-xylulose-5-kinase is xylB. In
25 some embodiments, the gene encoding the glycoaldehyde dehydrogenase is aldA.

[00178] In one embodiment, MEG is produced from xylose via ribulose-1-phosphate. In another embodiment, MEG is produced from xylose via xylulose-1-phosphate. In a further embodiment, MEG is produced from xylose via xylonate.

30 [00179] In one embodiment, one or more three-carbon compounds is produced from DHAP or pyruvate. In one embodiment, the one or more three-carbon compounds is acetone. In another embodiment, the one or more three-carbon compounds is iso-

propanol. In a further embodiment, the one or more three-carbon compounds is propene.

[00180] As discussed above, in one aspect, the present disclosure provides a method of producing a recombinant microorganism that produces or accumulates MEG and acetone from exogenous D-xylose, comprising introducing into the recombinant microorganism and/or overexpressing one or more of the following:

[00181] at least one endogenous or exogenous nucleic acid molecule encoding a D-tagatose 3-epimerase that catalyzes the conversion of D-xylulose to D-ribulose;

[00182] at least one endogenous or exogenous nucleic acid molecule encoding a D-ribulokinase that catalyzes the conversion of D-ribulose from (a) to D-ribulose-1-phosphate;

[00183] at least one endogenous or exogenous nucleic acid molecule encoding a D-ribulose-1-phosphate aldolase that catalyzes the conversion of D-ribulose-1-phosphate from (b) to glycolaldehyde and dihydroxyacetonephosphate (DHAP);

[00184] at least one endogenous or exogenous nucleic acid molecule encoding a glycolaldehyde reductase that catalyzes the conversion of glycolaldehyde from (c) to MEG;

[00185] at least one exogenous nucleic acid molecule encoding a thiolase that catalyzes the conversion of acetyl-CoA to acetoacetyl-CoA;

[00186] at least one endogenous or exogenous nucleic acid molecule encoding an acetate:acetoacetyl-CoA transferase or hydrolase that catalyzes the conversion of acetoacetyl-CoA from (e) to acetoacetate; and/or

[00187] at least one endogenous or exogenous nucleic acid molecule encoding an acetoacetate decarboxylase that catalyzes the conversion of acetoacetate from (f) to acetone;

[00188] wherein the produced intermediate DHAP is converted to acetyl-CoA through the endogenous glycolysis pathway in the microorganism, and wherein MEG and acetone are co-produced.

[00189] In one embodiment, the D-tagatose 3-epimerase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Pseudomonas* sp., *Mesorhizobium* sp. and *Rhodobacter* sp. In some embodiments, the D-tagatose 3-epimerase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Pseudomo-*

nas cichorii, *Pseudomonas* sp. ST-24, *Mesorhizobium loti* and *Rhodobacter sphaeroides*. In some embodiments, the one or more nucleic acid molecules is *dte* and/or FJ851309.1, or homolog thereof. In a further embodiment, the D-tagatose 3-epimerase comprises an amino acid sequence selected from the group consisting of
5 SEQ ID NOs: 3 and 5. In yet a further embodiment, the D-tagatose 3-epimerase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2 and 4.

[00190] In one embodiment, the D-ribulokinase is encoded by one or more nucleic acid molecules obtained from *E. coli*. In some embodiments, the one or more nucleic acid molecules is *fucK*, or homolog thereof. In a further embodiment, the D-ribulokinase
10 comprises an amino acid sequence set forth in SEQ ID NO: 8. In yet a further embodiment, the D-ribulokinase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 6 and 7.

[00191] In one embodiment, the D-ribulose-1-phosphate aldolase is encoded by one or more nucleic acid molecules obtained from *E. coli*. In some embodiments, the one or more nucleic acid molecules is *fucA*, or homolog thereof. In a further embodiment, the D-ribulose-1-phosphate aldolase comprises an amino acid sequence set forth in SEQ ID NO: 11. In yet a further embodiment, the D-ribulose-1-phosphate aldolase is
15 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 9 and 10.

[00192] In one embodiment, the method further comprises introducing into the recombinant microorganism and/or overexpressing at least one endogenous or exogenous nucleic acid molecule encoding a secondary alcohol dehydrogenase that catalyzes the conversion of acetone from (g) to isopropanol.

25 [00193] In another embodiment, the method further comprises introducing into the recombinant microorganism and/or overexpressing at least one endogenous or exogenous nucleic acid molecule encoding a dehydratase that catalyzes the conversion of isopropanol to propene.

[00194] In another embodiment, the method further comprises introducing into the
30 recombinant microorganism one or more modifications selected from the group consisting of:

[00195] a deletion, insertion, or loss of function mutation in a gene encoding a D-xylulose-5-kinase that catalyzes the conversion of D-xylulose to D-xylulose-5-phosphate;

5 [00196] a deletion, insertion, or loss of function mutation in a gene encoding a glycolaldehyde dehydrogenase that catalyzes the conversion of glycolaldehyde to glycolic acid; and

[00197] a deletion, insertion, or loss of function mutation in a gene encoding a lactate dehydrogenase that catalyzes the conversion of pyruvate to lactate.

10 [00198] In one embodiment, an endogenous D-xylose isomerase catalyzes the conversion of D-xylose to D-xylulose. In one embodiment, the xylose isomerase is exogenous. In another embodiment, the xylose isomerase is encoded by one or more nucleic acid molecules obtained from *Pyromyces* sp. In another embodiment, the one or more nucleic acid molecules encoding the xylose isomerase is *xylA*, or homolog thereof. In yet another embodiment, the one or more nucleic acid molecules encoding the
15 xylose isomerase comprises an amino acid sequence set forth in SEQ ID NO: 95. In a further embodiment, the one or more nucleic acid molecules encoding the xylose isomerase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 93 and 94.

20 [00199] As discussed above, in another aspect, the present disclosure provides a method of producing a recombinant microorganism that produces or accumulates MEG and acetone from exogenous D-xylose, comprising introducing into the recombinant microorganism and/or overexpressing one or more of the following:

25 [00200] at least one endogenous or exogenous nucleic acid molecule encoding a D-xylulose 1-kinase that catalyzes the conversion of D-xylulose to D-xylulose-1-phosphate;

[00201] at least one endogenous or exogenous nucleic acid molecule encoding a D-xylulose-1-phosphate aldolase that catalyzes the conversion of D-xylulose-1-phosphate from (a) to glycolaldehyde and dihydroxyacetonephosphate (DHAP);

30 [00202] at least one endogenous or exogenous nucleic acid molecule encoding a glycolaldehyde reductase that catalyzes the conversion of glycolaldehyde from (b) to MEG;

[00203] at least one endogenous or exogenous nucleic acid molecule encoding a thiolase that catalyzes the conversion of acetyl-CoA to acetoacetyl-CoA;

[00204] at least one endogenous or exogenous nucleic acid molecule encoding an acetate:acetoacetyl-CoA transferase or hydrolase that catalyzes the conversion of acetoacetyl-CoA from (d) to acetoacetate; and/or

5 [00205] at least one endogenous or exogenous nucleic acid molecule encoding an acetoacetate decarboxylase that catalyzes the conversion of acetoacetate from (e) to acetone;

[00206] wherein the produced intermediate DHAP is converted to acetyl-CoA through the endogenous glycolysis pathway in the microorganism, and wherein MEG and acetone are co-produced.

10 [00207] In one embodiment, the D-xylulose 1-kinase is encoded by one or more nucleic acid molecules obtained from Homo sapiens. In some embodiments, the one or more nucleic acid molecules encoding the D-xylulose 1-kinase is ketohexokinase C (khk-C), or homolog thereof. In another embodiment, the one or more nucleic acid molecules encoding the D-xylulose 1-kinase comprises an amino acid sequence set forth in
15 SEQ ID NO: 55. In a further embodiment, the one or more nucleic acid molecules encoding the D-xylulose 1-kinase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 53 and 54.

[00208] In one embodiment, the D-xylulose-1-phosphate aldolase is encoded by one or more nucleic acid molecules obtained from Homo sapiens. In another embodiment,
20 [00208] the one or more nucleic acid molecules encoding the D-xylulose-1-phosphate aldolase is aldolase B (ALDOB), or homolog thereof. In some embodiments, the one or more nucleic acid molecules encoding the D-xylulose-1-phosphate aldolase comprises an amino acid sequence set forth in SEQ ID NO: 58. In some embodiments, the one or more nucleic acid molecules encoding the D-xylulose-1-phosphate aldolase is encoded
25 by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 56 and 57.

[00209] In one embodiment, the method further comprises introducing into the recombinant microorganism and/or overexpressing at least one endogenous or exogenous nucleic acid molecule encoding a secondary alcohol dehydrogenase that catalyzes the conversion of acetone from (f) to isopropanol.
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[00210] In another embodiment, the method further comprises introducing into the recombinant microorganism and/or overexpressing at least one endogenous or exoge-

nous nucleic acid molecule encoding a dehydratase that catalyzes the conversion of isopropanol to propene.

[00211] In another embodiment, the method further comprises introducing into the recombinant microorganism one or more modifications selected from the group consisting of:

a deletion, insertion, or loss of function mutation in a gene encoding a D-xylulose-5-kinase that catalyzes the conversion of D-xylulose to D-xylulose-5-phosphate;

a deletion, insertion, or loss of function mutation in a gene encoding a glycolaldehyde dehydrogenase that catalyzes the conversion of glycolaldehyde to glycolic acid; and

a deletion, insertion, or loss of function mutation in a gene encoding a lactate dehydrogenase that catalyzes the conversion of pyruvate to lactate.

[00212] In one embodiment, an endogenous D-xylose isomerase catalyzes the conversion of D-xylose to D-xylulose. In one embodiment, the xylose isomerase is exogenous. In another embodiment, the xylose isomerase is encoded by one or more nucleic acid molecules obtained from *Pyromyces* sp. In another embodiment, the one or more nucleic acid molecules encoding the xylose isomerase is *xylA*, or homolog thereof. In yet another embodiment, the one or more nucleic acid molecules encoding the xylose isomerase comprises an amino acid sequence set forth in SEQ ID NO: 95. In a further embodiment, the one or more nucleic acid molecules encoding the xylose isomerase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 93 and 94.

[00213] In some embodiments of any aspect disclosed above, a method of producing a recombinant microorganism that produces or accumulates MEG and one or more three-carbon compounds from exogenous D-xylose comprises introducing into the recombinant microorganism a deletion, insertion, or loss of function mutation in a gene encoding a D-xylulose-5-kinase to prevent the conversion of D-xylulose to D-xylulose-5-phosphate and instead shunt the reaction toward conversion of D-xylulose to D-xylulose-1-phosphate. In some embodiments, the D-xylulose-5-kinase is from *Escherichia coli*. In some embodiments, the D-xylulose-5-kinase is encoded by the *xylB* gene, or homolog thereof.

[00214] In some embodiments of any aspect disclosed above, a method of producing a recombinant microorganism that produces or accumulates MEG and one or more three-carbon compounds from exogenous D-xylose comprises introducing into the recombinant microorganism a deletion, insertion, or loss of function mutation in a gene encoding a glycolaldehyde dehydrogenase to prevent the production of glycolic acid from glycolaldehyde and instead shunt the reaction toward conversion of glycolaldehyde to MEG. In some embodiments, the glycolaldehyde dehydrogenase is from *Escherichia coli*. In some embodiments, the glycolaldehyde dehydrogenase is encoded by the *aldA* gene, or homolog thereof.

[00215] In some embodiments of any aspect disclosed above, a method of producing a recombinant microorganism that produces or accumulates MEG and one or more three-carbon compounds from exogenous D-xylose comprises introducing into the recombinant microorganism a deletion, insertion, or loss of function mutation in a gene encoding a lactate dehydrogenase to prevent the production of lactate from pyruvate and instead shunt the reaction toward production of one or more three-carbon compounds. In some embodiments, the lactate dehydrogenase is from *Escherichia coli*. In some embodiments, the lactate dehydrogenase is encoded by the *ldhA* gene, or homolog thereof.

[00216] As discussed above, in another aspect, the present disclosure provides a method of producing a recombinant microorganism that produces or accumulates MEG and acetone from exogenous D-xylose and glucose, comprising introducing into the recombinant microorganism and/or overexpressing one or more of the following:

[00217] at least one exogenous nucleic acid molecule encoding a xylose reductase or aldose reductase that catalyzes the conversion of D-xylose to xylitol and at least one exogenous nucleic acid molecule encoding a xylitol dehydrogenase that catalyzes the conversion of xylitol to D-xylulose;

[00218] at least one exogenous nucleic acid molecule encoding a D-xylose isomerase that catalyzes the conversion of D-xylose to D-xylulose, and

[00219] wherein the method further comprises introducing into the recombinant microorganism and/or overexpressing one or more of the following:

at least one endogenous or exogenous nucleic acid molecule encoding a D-tagatose 3-epimerase that catalyzes the conversion of D-xylulose from (a) or (b) to D-ribulose;

at least one endogenous or exogenous nucleic acid molecule encoding a D-ribulokinase that catalyzes the conversion of D-ribulose from (c) to D-ribulose-1-phosphate;

5 at least one endogenous or exogenous nucleic acid molecule encoding a D-ribulose-1-phosphate aldolase that catalyzes the conversion of D-ribulose-1-phosphate from (d) to glycolaldehyde and dihydroxyacetonephosphate (DHAP);

at least one endogenous or exogenous nucleic acid molecule encoding a glycolaldehyde reductase or methylglyoxal reductase that catalyzes the conversion of glycolaldehyde from (e) to MEG;

10 at least one endogenous or exogenous nucleic acid molecule encoding a thiolase that catalyzes the conversion of acetyl-CoA to acetoacetyl-CoA;

at least one endogenous or exogenous nucleic acid molecule encoding an acetate:acetoacetyl-CoA transferase or hydrolase that catalyzes the conversion of acetoacetyl-CoA from (g) to acetoacetate; and/or

15 at least one endogenous or exogenous nucleic acid molecule encoding an acetoacetate decarboxylase that catalyzes the conversion of acetoacetate from (h) to acetone;

20 wherein the produced intermediate DHAP is converted to acetyl-CoA through the endogenous glycolysis pathway in the microorganism, and wherein MEG and acetone are co-produced.

[00220] In some embodiments, the xylose reductase or aldose reductase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Hypocrea* sp., *Scheffersomyces* sp., *Saccharomyces* sp., *Pachysolen* sp., *Pichia* sp., *Candida* sp., *Aspergillus* sp., *Neurospora* sp., and *Cryptococcus* sp. In some embodiments, the xylose reductase or aldose reductase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Hypocrea jecorina*, *Scheffersomyces stipitis*, *Saccharomyces cerevisiae*, *Pachysolen tannophilus*, *Pichia stipitis*, *Pichia quercuum*, *Candida shehatae*, *Candida tenuis*, *Candida tropicalis*, *Aspergillus niger*, *Neurospora crassa* and *Cryptococcus lactativorus*. In another embodiment, the one or more nucleic acid molecules encoding the xylose reductase or aldose reductase is *xyl1* and/or *GRE3* or homolog thereof. In some embodiments, the one or more nucleic acid molecules encoding the xylose reductase or aldose reductase comprises an amino acid sequence selected from

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the group consisting of SEQ ID NOs: 84 and 87. In some embodiments, the one or more nucleic acid molecules encoding the xylose reductase or aldose reductase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 82, 83, 85 and 86.

- 5 [00221] In one embodiment of any aspect disclosed above, the xylitol dehydrogenase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Scheffersomyces* sp., *Trichoderma* sp., *Pichia* sp., *Saccharomyces* sp., *Gluconobacter* sp., *Galactocandida* sp., *Neurospora* sp., and *Serratia* sp. In another embodiment, the xylitol dehydrogenase is encoded by one or more
- 10 nucleic acid molecules obtained from a microorganism selected from the group consisting of *Scheffersomyces stipitis*, *Trichoderma reesei*, *Pichia stipitis*, *Saccharomyces cerevisiae*, *Gluconobacter oxydans*, *Galactocandida mastotermitis*, *Neurospora crassa* and *Serratia marcescens*. In another embodiment, the one or more nucleic acid molecules encoding the xylitol dehydrogenase is *xyl2* and/or *xdh1*, or homolog thereof. In
- 15 some embodiments, the one or more nucleic acid molecules encoding the xylitol dehydrogenase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 90 and 92. In some embodiments, the one or more nucleic acid molecules encoding the xylitol dehydrogenase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 88, 89 and 91.
- 20 [00222] In one embodiment, an endogenous D-xylose isomerase catalyzes the conversion of D-xylose to D-xylulose. In one embodiment, the xylose isomerase is exogenous. In another embodiment, the xylose isomerase is encoded by one or more nucleic acid molecules obtained from *Pyromyces* sp. In another embodiment, the one or more nucleic acid molecules encoding the xylose isomerase is *xylA*, or homolog thereof. In yet another embodiment, the one or more nucleic acid molecules encoding the
- 25 xylose isomerase comprises an amino acid sequence set forth in SEQ ID NO: 95. In a further embodiment, the one or more nucleic acid molecules encoding the xylose isomerase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 93 and 94.
- 30 [00223] In one embodiment, the method further comprises introducing into the recombinant microorganism and/or overexpressing at least one endogenous or exogenous nucleic acid molecule encoding a secondary alcohol dehydrogenase that catalyzes the conversion of acetone from (i) to isopropanol.

[00224] In another embodiment, the method further comprises introducing into the recombinant microorganism and/or overexpressing at least one endogenous or exogenous nucleic acid molecule encoding a dehydratase that catalyzes the conversion of isopropanol to propene.

5 [00225] In another embodiment, the method further comprises introducing into the recombinant microorganism one or more modifications selected from the group consisting of:

10 a deletion, insertion, or loss of function mutation in a gene encoding a D-xylulose-5-kinase that catalyzes the conversion of D-xylulose to D-xylulose-5-phosphate; and

a deletion, insertion, or loss of function mutation in a gene encoding an alkaline phosphatase that catalyzes the conversion of D-xylulose-5-phosphate to D-xylulose.

15 [00226] In one embodiment, the enzyme that catalyzes the conversion of D-xylulose to D-xylulose-5-phosphate is a D-xylulose-5-kinase. In some embodiments, the D-xylulose-5-kinase is from *Saccharomyces cerevisiae*. In some embodiments the D-xylulose-5-kinase is encoded by the XKS1 gene, or homolog thereof. In some embodiments, the D-xylulose-5-kinase is from *Pichia stipitis*. In some embodiments the D-xylulose-5-kinase is encoded by the XYL3 gene, or homolog thereof.

20 [00227] In a further embodiment, the microorganism is a fungus.

[00228] As discussed above, in another aspect, the present application provides a method of producing a recombinant microorganism that produces or accumulates MEG and acetone from exogenous D-xylose, comprising introducing into the recombinant microorganism and/or overexpressing one or more of the following:

25 at least one endogenous or exogenous nucleic acid molecule encoding a xylose dehydrogenase that catalyzes the conversion of D-xylose to D-xylonolactone;

at least one endogenous or exogenous nucleic acid molecule encoding a xylonolactonase that catalyzes the conversion of D-xylonolactone from (a) to D-xylonate;

30 at least one endogenous or exogenous nucleic acid molecule encoding a xylonate dehydratase that catalyzes the conversion of D-xylonate from (b) to 2-keto-3-deoxy-xylonate;

at least one endogenous or exogenous nucleic acid molecule encoding a 2-keto-3-deoxy-D-pentolate aldolase that catalyzes the conversion of 2-keto-3-deoxy-xylonate from (c) to glycolaldehyde and pyruvate;

at least one endogenous or exogenous nucleic acid molecule encoding a glycolaldehyde reductase that catalyzes the conversion of glycolaldehyde from (d) to MEG;

at least one exogenous nucleic acid molecule encoding a thiolase that catalyzes the conversion of acetyl-CoA to acetoacetyl-CoA;

at least one endogenous or exogenous nucleic acid molecule encoding an acetate:acetoacetyl-CoA transferase or hydrolase that catalyzes the conversion of acetoacetyl-CoA from (f) to acetoacetate; and/or

at least one exogenous nucleic acid molecule encoding an acetoacetate decarboxylase that catalyzes the conversion of acetoacetate from (g) to acetone;

wherein the produced intermediate pyruvate is converted to acetyl-CoA through the endogenous glycolysis pathway in the microorganism, and wherein MEG and acetone are co-produced.

[00229] In one embodiment, the xylose dehydrogenase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter* sp., *Haloarcula* sp., *Haloferax* sp., *Halorubrum* sp. and *Trichoderma* sp. In another embodiment, the xylose dehydrogenase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter crescentus*, *Haloarcula marismortui*, *Haloferax volcanii*, *Halorubrum lacusprofundi* and *Trichoderma reesei*. In some embodiments, the one or more nucleic acid molecules encoding the xylose dehydrogenase is selected from *xyIB*, *xdh1* (HVO_B0028) and/or *xyd1*, or homolog thereof. In a further embodiment, the one or more nucleic acid molecules encoding the xylose dehydrogenase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 61, 63 and 65. In yet another embodiment, the one or more nucleic acid molecules encoding the xylose dehydrogenase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 59, 60, 62 and 64.

[00230] In one embodiment, the xylonolactonase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from *Caulobacter* sp. and *Haloferax* sp. In another embodiment, the xylonolactonase is encoded by one or more

nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter crescentus*, *Haloferax volcanii* and *Haloferax gibbonsii*. In some embodiments, the one or more nucleic acid molecules encoding the xylonolactonase is *xylC*, or homolog thereof. In a further embodiment, the one or more nucleic acid molecules encoding the xylonolactonase comprises an amino acid sequence set forth in SEQ ID NO: 67. In yet another embodiment, the one or more nucleic acid molecules encoding the xylonolactonase is encoded by a nucleic acid sequence set forth in SEQ ID NO: 66.

[00231] In one embodiment, the xylonate dehydratase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter* sp., *Sulfolobus* sp. and *E. coli*. In another embodiment, the xylonate dehydratase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter crescentus*, *Sulfolobus solfataricus* and *E. coli*. In some embodiments, the one or more nucleic acid molecules encoding the xylonate dehydratase is selected from *xylD*, *yjhG* and/or *yagF*, or homolog thereof. In a further embodiment, the one or more nucleic acid molecules encoding the xylonate dehydratase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 69, 72 and 75. In yet another embodiment, the one or more nucleic acid molecules encoding the xylonate dehydratase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 68, 70, 71, 73 and 74.

[00232] In one embodiment, the 2-keto-3-deoxy-D-pentionate aldolase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from *Pseudomonas* sp. and *E. coli*. In another embodiment, the 2-keto-3-deoxy-D-pentionate aldolase is encoded by one or more nucleic acid molecules obtained from *E. coli*. In some embodiments, the one or more nucleic acid molecules encoding the 2-keto-3-deoxy-D-pentionate aldolase is selected from *yjhH* and/or *yagE*, or homolog thereof. In a further embodiment, the one or more nucleic acid molecules encoding the 2-keto-3-deoxy-D-pentionate aldolase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 78 and 81. In yet another embodiment, the one or more nucleic acid molecules encoding the 2-keto-3-deoxy-D-pentionate aldolase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 76, 77, 79 and 80.

[00233] In one embodiment, the method further comprises introducing into the recombinant microorganism and/or overexpressing at least one endogenous or exogenous nucleic acid molecule encoding a secondary alcohol dehydrogenase that catalyzes the conversion of acetone from (h) to isopropanol.

5 [00234] In another embodiment, the method further comprises introducing into the recombinant microorganism and/or overexpressing at least one endogenous or exogenous nucleic acid molecule encoding a dehydratase that catalyzes the conversion of isopropanol to propene.

[00235] In another embodiment, the method further comprises introducing into the recombinant microorganism one or more modifications selected from the group consisting of:

a deletion, insertion, or loss of function mutation in a gene encoding a D-xylose isomerase that catalyzes the conversion of D-xylose to D-xylulose;

15 a deletion, insertion, or loss of function mutation in a gene encoding a glycolaldehyde dehydrogenase that catalyzes the conversion of glycolaldehyde to glycolic acid; and

a deletion, insertion, or loss of function mutation in a gene encoding a lactate dehydrogenase that catalyzes the conversion of pyruvate to lactate.

[00236] In some embodiments, a method of producing a recombinant microorganism that produces or accumulates MEG and one or more three-carbon compounds from exogenous D-xylose comprises introducing into the recombinant microorganism a deletion, insertion, or loss of function mutation in a gene encoding a D-xylose isomerase to prevent conversion of D-xylose to D-xylulose and instead shunt the reaction toward the conversion of D-xylose to D-xylonate. In one embodiment, the enzyme that catalyzes the conversion of D-xylose to D-xylulose is a D-xylose isomerase. In some embodiments, the D-xylose isomerase is from *Escherichia coli*. In some embodiments, the D-xylose isomerase is encoded by the *xylA* gene, or homolog thereof.

25 [00237] In some embodiments, a method of producing a recombinant microorganism that produces or accumulates MEG and one or more three-carbon compounds from exogenous D-xylose comprises introducing into the recombinant microorganism a deletion, insertion, or loss of function mutation in a gene encoding a glycolaldehyde dehydrogenase to prevent the production of glycolic acid from glycolaldehyde and instead shunt the reaction toward conversion of glycolaldehyde to MEG. In one embodiment,

the glycolaldehyde dehydrogenase is from *Escherichia coli*. In some embodiments, the glycolaldehyde dehydrogenase is encoded by the *aldA* gene, or homolog thereof.

[00238] In some embodiments, a method of producing a recombinant microorganism that produces or accumulates MEG and one or more three-carbon compounds from exogenous D-xylose comprises introducing into the recombinant microorganism a deletion, insertion, or loss of function mutation in a gene encoding a lactate dehydrogenase to prevent the production of lactate from pyruvate and instead shunt the reaction toward production of one or more three-carbon compounds. In one embodiment, the enzyme that catalyzes the conversion of pyruvate to lactate is a lactate dehydrogenase. In particular embodiments, the enzyme converts pyruvate to lactate. In some embodiments, the lactate dehydrogenase is from *Escherichia coli*. In some embodiments, the lactate dehydrogenase is encoded by the *ldhA* gene, or homolog thereof.

[00239] As discussed above, in another aspect, the present application provides a method of producing a recombinant microorganism that produces or accumulates MEG and acetone from exogenous D-xylose, comprising introducing into the recombinant microorganism and/or overexpressing one or more of the following:

at least one endogenous or exogenous nucleic acid molecule encoding a xylose dehydrogenase that catalyzes the conversion of D-xylose to D-xylonate;

at least one endogenous or exogenous nucleic acid molecule encoding a xylonate dehydratase that catalyzes the conversion of D-xylonate from (a) to 2-keto-3-deoxy-xylonate;

at least one endogenous or exogenous nucleic acid molecule encoding a 2-keto-3-deoxy-D-pentonate aldolase that catalyzes the conversion of 2-keto-3-deoxy-xylonate from (b) to glycolaldehyde and pyruvate;

at least one exogenous nucleic acid molecule encoding a glycolaldehyde reductase that catalyzes the conversion of glycolaldehyde from (c) to MEG;

at least one exogenous nucleic acid molecule encoding a thiolase that catalyzes the conversion of acetyl-CoA to acetoacetyl-CoA;

at least one endogenous or exogenous nucleic acid molecule encoding an acetate:acetoacetyl-CoA transferase or hydrolase that catalyzes the conversion of acetoacetyl-CoA from (e) to acetoacetate; and/or

at least one exogenous nucleic acid molecule encoding an acetoacetate decarboxylase that catalyzes the conversion of acetoacetate from (f) to acetone;

wherein the produced intermediate pyruvate is converted to acetyl-CoA through the endogenous glycolysis pathway in the microorganism, and wherein MEG and acetone are co-produced.

[00240] In one embodiment, the xylose dehydrogenase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter* sp., *Haloarcula* sp., *Haloferax* sp., *Halorubrum* sp. and *Trichoderma* sp. In another embodiment, the xylose dehydrogenase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter crescentus*, *Haloarcula marismortui*, *Haloferax volcanii*, *Halorubrum lacusprofundi* and *Trichoderma reesei*. In some embodiments, the one or more nucleic acid molecules encoding the xylose dehydrogenase is selected from *xylB*, *xdh1* (HVO_B0028) and/or *xyd1*, or homolog thereof. In a further embodiment, the one or more nucleic acid molecules encoding the xylose dehydrogenase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 61, 63 and 65. In yet another embodiment, the one or more nucleic acid molecules encoding the xylose dehydrogenase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 59, 60, 62 and 64.

[00241] In one embodiment, the xylonate dehydratase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter* sp., *Sulfolobus* sp. and *E. coli*. In another embodiment, the xylonate dehydratase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter crescentus*, *Sulfolobus solfataricus* and *E. coli*. In some embodiments, the one or more nucleic acid molecules encoding the xylonate dehydratase is selected from *xylD*, *yjhG* and/or *yagF*, or homolog thereof. In a further embodiment, the one or more nucleic acid molecules encoding the xylonate dehydratase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 69, 72 and 75. In yet another embodiment, the one or more nucleic acid molecules encoding the xylonate dehydratase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 68, 70, 71, 73 and 74.

[00242] In one embodiment, the 2-keto-3-deoxy-D-pentonate aldolase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from *Pseudomonas* sp. and *E. coli*. In another embodiment, the 2-keto-3-deoxy-D-pentonate aldolase is encoded by one or more nucleic acid molecules obtained from *E. coli*. In

some embodiments, the one or more nucleic acid molecules encoding the 2-keto-3-deoxy-D-pentolate aldolase is selected from yjhH and/or yagE, or homolog thereof. In a further embodiment, the one or more nucleic acid molecules encoding the 2-keto-3-deoxy-D-pentolate aldolase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 78 and 81. In yet another embodiment, the one or more nucleic acid molecules encoding the 2-keto-3-deoxy-D-pentolate aldolase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 76, 77, 79 and 80.

[00243] In one embodiment, the method further comprises introducing into the recombinant microorganism and/or overexpressing at least one endogenous or exogenous nucleic acid molecule encoding a secondary alcohol dehydrogenase that catalyzes the conversion of acetone from (g) to isopropanol.

[00244] In another embodiment, the method further comprises introducing into the recombinant microorganism and/or overexpressing at least one endogenous or exogenous nucleic acid molecule encoding a dehydratase that catalyzes the conversion of isopropanol to propene.

[00245] In another embodiment, the method further comprises introducing into the recombinant microorganism one or more modifications selected from the group consisting of:

20 a deletion, insertion, or loss of function mutation in a gene encoding a D-xylose isomerase that catalyzes the conversion of D-xylose to D-xylulose;

a deletion, insertion, or loss of function mutation in a gene encoding a glycolaldehyde dehydrogenase that catalyzes the conversion of glycolaldehyde to glycolic acid; and

25 a deletion, insertion, or loss of function mutation in a gene encoding a lactate dehydrogenase that catalyzes the conversion of pyruvate to lactate.

[00246] In some embodiments, a method of producing a recombinant microorganism that produces or accumulates MEG and one or more three-carbon compounds from exogenous D-xylose comprises introducing into the recombinant microorganism a deletion, insertion, or loss of function mutation in a gene encoding a D-xylose isomerase to prevent conversion of D-xylose to D-xylulose and instead shunt the reaction toward the conversion of D-xylose to D-xylonate. In one embodiment, the enzyme that catalyzes the conversion of D-xylose to D-xylulose is a D-xylose isomerase. In some embodi-

ments, the D-xylose isomerase is from *Escherichia coli*. In some embodiments, the D-xylose isomerase is encoded by the *xylA* gene, or homolog thereof.

[00247] In some embodiments, a method of producing a recombinant microorganism that produces or accumulates MEG and one or more three-carbon compounds from exogenous D-xylose comprises introducing into the recombinant microorganism a deletion, insertion, or loss of function mutation in a gene encoding a glycolaldehyde dehydrogenase to prevent the production of glycolic acid from glycolaldehyde and instead shunt the reaction toward conversion of glycolaldehyde to MEG. In one embodiment, the glycolaldehyde dehydrogenase is from *Escherichia coli*. In some embodiments, the glycolaldehyde dehydrogenase is encoded by the *aldA* gene, or homolog thereof.

[00248] In some embodiments, a method of producing a recombinant microorganism that produces or accumulates MEG and one or more three-carbon compounds from exogenous D-xylose comprises introducing into the recombinant microorganism a deletion, insertion, or loss of function mutation in a gene encoding a lactate dehydrogenase to prevent the production of lactate from pyruvate and instead shunt the reaction toward production of one or more three-carbon compounds. In one embodiment, the enzyme that catalyzes the conversion of pyruvate to lactate is a lactate dehydrogenase. In particular embodiments, the enzyme converts pyruvate to lactate. In some embodiments, the lactate dehydrogenase is from *Escherichia coli*. In some embodiments, the lactate dehydrogenase is encoded by the *ldhA* gene, or homolog thereof.

[00249] In one embodiment of any aspect disclosed above, the glycolaldehyde reductase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from *E. coli* and *S. cerevisiae*. In another embodiment, the one or more nucleic acid molecules is selected from *gldA*, *GRE2*, *GRE3*, *yqhD*, *ydjG*, *fucO*, *yafB* (*dkgB*), and/or *yqhE* (*dkgA*), or homolog thereof. In another embodiment, the one or more nucleic acid molecules is *yqhD*. In some embodiments, the *yqhD* comprises a G149E mutation. In a further embodiment, the glycolaldehyde reductase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 15, 17, 20, 23, 25, 28, 30 and 32. In yet a further embodiment, the glycolaldehyde reductase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 12, 14, 16, 18, 19, 21, 22, 24, 26, 27, 29 and 31.

[00250] In one embodiment of any aspect disclosed above, the thiolase or acetyl coenzyme A acetyltransferase is encoded by one or more nucleic acid molecules ob-

tained from a microorganism selected from the group consisting of *Clostridium* sp., *Bacillus* sp., *E. coli*, *Saccharomyces* sp. and *Marinobacter* sp. In some embodiments, the thiolase or acetyl coenzyme A acetyltransferase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Clostridium acetobutylicum*, *Clostridium thermosaccharolyticum*, *Bacillus cereus*, *E. coli*, *Saccharomyces cerevisiae* and *Marinobacter hydrocarbonoclasticus*. In some embodiments, the one or more nucleic acid molecules is *thlA*, *atoB* and/or *ERG10*, or homolog thereof. In a further embodiment, the thiolase or acetyl coenzyme A acetyltransferase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 35, 37 and 40. In yet a further embodiment, the thiolase or acetyl coenzyme A acetyltransferase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 33, 34, 36, 38 and 39.

[00251] In one embodiment of any aspect disclosed above, the acetyl-CoA:acetoacetate-CoA transferase or acetate:acetoacetyl-CoA hydrolase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from *Clostridium* sp. and *E. coli*. In another embodiment, the acetyl-CoA:acetoacetate-CoA transferase or acetate:acetoacetyl-CoA hydrolase is encoded by one or more nucleic acid molecules obtained from *E. coli*. In some embodiments, the one or more nucleic acid molecules encoding the acetyl-CoA:acetoacetate-CoA transferase is *atoA* and/or *atoD*, or homolog thereof. In another embodiment, the acetyl-CoA:acetoacetate-CoA transferase or acetate:acetoacetyl-CoA hydrolase is encoded by one or more nucleic acid molecules obtained from *Clostridium acetobutylicum*. In some embodiments, the one or more nucleic acid molecules encoding the acetate:acetoacetyl-CoA hydrolase is *ctfA* and/or *ctfB*, or homolog thereof. In a further embodiment, the acetyl-CoA:acetoacetate-CoA transferase or acetate:acetoacetyl-CoA hydrolase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 46, 97, 99, 101 and 103. In yet a further embodiment, the acetyl-CoA:acetoacetate-CoA transferase or acetate:acetoacetyl-CoA hydrolase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 41, 42, 44, 45, 96, 98, 100 and 102.

[00252] In one embodiment of any aspect disclosed above, the acetoacetate decarboxylase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Clostridium* sp., *Bacillus* sp., *Chromobacterium* sp. and *Pseudomonas* sp. In another embodiment, the acetoacetate decar-

boxylase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Clostridium acetobutylicum*, *Clostridium beijerinckii*, *Clostridium cellulolyticum*, *Bacillus polymyxa*, *Chromobacterium violaceum* and *Pseudomonas putida*. In some embodiments, the one or more nucleic acid molecules encoding the acetoacetate decarboxylase is *adc*, or homolog thereof. In a further embodiment, the acetoacetate decarboxylase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 49 and 52. In yet another embodiment, the acetoacetate decarboxylase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 47, 48, 50 and 51.

- 10 [00253] In one embodiment of any aspect disclosed above, the enzyme that catalyzes the conversion of acetone to isopropanol is a secondary alcohol dehydrogenase (S-ADH). In another embodiment, the enzyme is a secondary alcohol dehydrogenase that is encoded by a nucleic acid molecule obtained from a microorganism selected from *Burkholderia* sp., *Alcaligenes* sp., *Clostridium* sp., *Thermoanaerobacter* sp., *Phytomonas* sp., *Rhodococcus* sp., *Methanobacterium* sp., *Methanogenium* sp., *Entamoeba* sp., *Trichomonas* sp., and *Tritrichomonas* sp. In some embodiments, the nucleic acid molecule encoding the secondary alcohol dehydrogenase is obtained from a microorganism selected from *Burkholderia* sp. AIU 652, *Alcaligenes eutrophus*, *Clostridium ragsdalei*, *Clostridium beijerinckii*, *Clostridium carboxidivorans*, *Thermoanaerobacter brockii*, *Thermoanaerobacter ethanolicus* (*Clostridium thermohydrosulfuricum*), *Rhodococcus ruber*, *Methanobacterium palustre*, methanogenic archaea *Methanogenium liminatans*, parasitic protist *Entamoeba histolytica*, parasitic protozoan *Tritrichomonas foetus* and human parasite *Trichomonas vaginalis*. In some embodiments, the one or more nucleic acid molecule encoding secondary alcohol dehydrogenase is *adh*, *adhB*,
 25 *EhAdh1*, or homolog thereof. In some embodiments, the S-ADH is predicted from homology and can be from *Thermoanaerobacter mathranii*, *Micrococcus luteus*, *Nocardio-opsis alba*, *Mycobacterium hassiacum*, *Helicobacter suis*, *Candida albicans*, *Candida parapsilosis*, *Candida orthopsilosis*, *Candida metapsilosis*, *Grosmannia clavigera* and *Scheffersomyces stipitis*. In a further embodiment, the alcohol dehydrogenase comprises
 30 an amino acid sequence selected from the group consisting of SEQ ID NOs: 106 and 108. In yet another embodiment, the alcohol dehydrogenase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 104, 105 and 107.

Enzyme Engineering

[00254] The enzymes in the recombinant microorganism can be engineered to improve one or more aspects of the substrate to product conversion. Non-limiting examples of enzymes that can be further engineered for use in methods of the disclosure include an aldolase, an aldehyde reductase, an acetoacetyl coenzyme A hydrolase, a xylose isomerase, a xylitol dehydrogenase and combinations thereof. These enzymes can be engineered for improved catalytic activity, improved selectivity, improved stability, improved tolerance to various fermentation conditions (temperature, pH, etc.), or improved tolerance to various metabolic substrates, products, by-products, intermediates, etc. The term "improved catalytic activity" as used herein with respect to a particular enzymatic activity refers to a higher level of enzymatic activity than that measured relative to a comparable non-engineered enzyme.

[00255] For example, engineering methods have been used to alter the stability, substrate specificity and stereospecificity of aldolases to produce excellent enzymes for biocatalytic processes. The thermostability and solvent tolerance of fructose-1,6-bisphosphate aldolase (FBP-aldolase) was increased using family DNA shuffling of the fda genes from *Escherichia coli* and *Edwardsiella ictaluri*. A fourth generation variant was identified which displayed an average 280-fold higher half-life at 53°C than either parent. The same variant also displayed enhanced activity in various polar and non-polar organic solvents (Hao and Berry 2004 Protein Eng Des Sel 17:689-697).

[00256] As another example, acetoacetyl coenzyme A hydrolase can convert acetoacetyl-CoA to acetoacetate. However, the hydrolase is unspecific in that it also reacts with the same magnitude of order with acetyl-CoA, which is the substrate required for acetoacetyl-CoA formation by the enzyme thiolase. Thus, to create more efficient acetoacetyl-CoA hydrolases, these enzymes have been engineered to have at least 10x higher activity for the acetoacetyl-CoA substrate than for acetyl-CoA substrate by replacing several glutamic acid residues in the enzyme beta subunit that is important for catalysis (WO 2015/042588).

[00257] As another example, the *E. coli* YqhD enzyme is a broad substrate aldehyde reductase with NADPH-dependent reductase activity for more than 10 aldehyde substrates and is a useful enzyme to produce biorenewable fuels and chemicals (Jarboe 2010 Applied Microbiology and Biotechnology 89:249). Though YqhD enzyme activity is beneficial through its scavenging of toxic aldehydes, the enzyme is also NADPH-dependent and contributes to NADPH depletion and growth inhibition of organ-

isms. Error-prone PCR of YqhD was performed in order to improve 1,3-propanediol production from 3-hydroxypropionaldehyde (3-HPA). This directed engineering yielded two mutants, D99QN147H and Q202A, with decreased K_m and increased k_{cat} for certain aldehydes, particularly 3-HPA (Li et al. 2008 Prog. Nat. Sci. 18 (12):1519-1524). The improved catalytic activity of the D99QN147H mutant is consistent with what is known about the structure of YqhD (Sulzenbacher et al. 2004 J. Mol. Biol. 342 (2):489-502), as residues Asp99 and Asn147 both interact with NADPH. Use of the D99QN147H mutant increased 1,3-propanediol production from 3-HPA 2-fold. Mutant YqhD enzymes with increased catalytic efficiency (increased K_{cat}/K_m) toward NADPH have also been described in WO 2011012697 A2, which is herein incorporated in its entirety.

[00258] As another example, xylose isomerase is a metal-dependent enzyme that catalyzes the interconversion of aldose and ketose sugars, primarily between xylose to xylulose and glucose to fructose. It has lower affinity for lyxose, arabinose and mannose sugars. The hydroxyl groups of sugars may define the substrate preference of sugar isomerases. The aspartate at residue 256 of *Thermus thermophilus* xylose isomerase was replaced with arginine (Patel et al. 2012 Protein Engineering, Design & Selection vol. 25 no. 7 pp. 331–336). This mutant xylose isomerase exhibited an increase in specificity for D-lyxose, L-arabinose and D-mannose. The catalytic efficiency of the D256R xylose isomerase mutant was also higher for these 3 substrates compared to the wild type enzyme. It was hypothesized that the arginine at residue 256 in the mutant enzyme may play a role in the catalytic reaction or influence changes in substrate orientation.

[00259] As another example, the enzyme xylitol dehydrogenase plays a role in the utilization of xylose along with xylose reductase. Xylose reductase (XR) reduces xylose to xylitol and then xylitol dehydrogenase (XDH) reoxidizes xylitol to form xylulose. However, since XR prefers NADPH as cosubstrate, while XDH exclusively uses NAD^+ as cosubstrate, a cosubstrate recycling problem is encountered. One solution is to engineer XDH such that its cosubstrate specificity is altered from NAD^+ to $NADP^+$ (Ehrensberger et al. 2006 Structure 14: 567-575). A crystal structure of the *Gluconobacter oxydans* holoenzyme revealed that Asp38 is largely responsible for the NAD^+ specificity of XDH. Asp38 interacts with the hydroxyls of the adenosine ribose, and Met39 stacks under the purine ring and is also located near the 2' hydroxyl. A double mutant (D38S/M39R) XDH was constructed that exclusively used $NADP^+$ without loss of enzyme activity.

Table 1: Description of enzymes

Described Reaction	EC no.	Required enzyme activity	Gene candidate	Source Organism	Natural/ annotated function	Gene Identifier (nt)	SEQ ID NO (nt)	Uniprot ID	SEQ ID NO (AA)
Isomerases that may be used in all xylulose dependent MEG pathways									
D-xylulose + NAD(P)H $\rightleftharpoons$ Xylitol + NAD(P) ⁺	1.1.1.307	xylulose reductase	xyl1	<i>Schefferomyces stipitis</i>	D-xylulose reductase	GeneID: 4839234	82, 83	P31867	84
D-xylulose + NAD(P)H $\rightleftharpoons$ Xylitol + NAD(P) ⁺	1.1.1.307	xylulose reductase	GRE3	<i>Saccharomyces cerevisiae</i>	aldose reductase	Gene-ID:856504	85, 86	P38715	87
Xylitol + NAD ⁺ $\rightleftharpoons$ D-xylulose + NADH	1.1.1.9	xylitol dehydrogenase	xyl2	<i>Schefferomyces stipitis</i>	D-xylulose reductase	GeneID: 4852013	88, 89	P22144	90
Xylitol + NAD ⁺ $\rightleftharpoons$ D-xylulose + NADH	1.1.1.9	xylitol dehydrogenase	xdh1	<i>Trichoderma reesei</i>	Xylitol dehydrogenase	ENA Nr.: AF428150.1	91	Q876R2	92
D-xylulose $\rightleftharpoons$ D-xylulose	5.3.1.5	xylulose isomerase	xylA	<i>Pyromyces</i> sp.	xylulose isomerase	ENA Nr.: CAB76571.1	93, 94	Q9P8C9	95
Glycolaldehyde reductases that may be used in all MEG pathways									

Described Reaction	EC no.	Required enzyme activity	Gene candidate	Source Organism	Natural/ annotated function	Gene Identifier (nt)	SEQ ID NO (nt)	Uniprot ID	SEQ ID NO (AA)
glycolaldehyde + NAD(P)H $\rightleftharpoons$ monoethylene glycol + NAD(P) ⁺	1.1.1.-	glycolaldehyde reductase	gldA	Escherichia coli	glycerol dehydrogenase	Gene-ID:12933659	12	P0A9S5	13
glycolaldehyde + NAD(P)H $\rightleftharpoons$ monoethylene glycol + NAD(P) ⁺	1.1.1.-	glycolaldehyde reductase	GRE2	Saccharomyces cerevisiae	methylglyoxal reductase	Gene-ID:854014	14	Q12068	15
glycolaldehyde + NAD(P)H $\rightleftharpoons$ monoethylene glycol + NAD(P) ⁺	1.1.1.-	glycolaldehyde reductase	GRE3	Saccharomyces cerevisiae	aldose reductase	Gene-ID:856504	16	P38715	17
glycolaldehyde + NAD(P)H $\rightleftharpoons$ monoethylene glycol + NAD(P) ⁺	1.1.1.-	glycolaldehyde reductase	yqhD*	Escherichia coli	Alcohol dehydrogenase	GeneID: 947493	18, 19	Modified version of Q46856 ; G149E	20

Described Reaction	EC no.	Required enzyme activity	Gene candidate	Source Organism	Natural/ annotated function	Gene Identifier (nt)	SEQ ID NO (nt)	Uniprot ID	SEQ ID NO (AA)
glycolaldehyde + NAD(P)H $\rightleftharpoons$ monoethylene glycol + NAD(P) ⁺	1.1.1.-	glycolaldehyde reductase	yqhD	Escherichia coli	Alcohol dehydrogenase	GeneID: 947493	21, 22	Q46856	23
glycolaldehyde + NAD(P)H $\rightleftharpoons$ monoethylene glycol + NAD(P) ⁺	1.1.1.-	glycolaldehyde reductase	ydjg	Escherichia coli	methylglyoxal reductase	GeneID:12930149	24	P77256	25
glycolaldehyde + NAD(P)H $\rightleftharpoons$ monoethylene glycol + NAD(P) ⁺	1.1.1.-	glycolaldehyde reductase	fucO	Escherichia coli	lactaldehyde reductase	GeneID:947273	26, 27	P0A9S1	28
glycolaldehyde + NAD(P)H $\rightleftharpoons$ monoethylene glycol + NAD(P) ⁺	1.1.1.-	glycolaldehyde reductase	yafB (dkgB)	Escherichia coli	methylglyoxal reductase [multi-functional]	545778205	29	P30863	30
glycolaldehyde + NAD(P)H $\rightleftharpoons$ monoethylene glycol + NAD(P) ⁺	1.1.1.-	glycolaldehyde reductase	yqhE (dkgA)	Escherichia coli	2,5-diketo-D-gluconic acid reductase A	GeneID: 947495	31	Q46857	32

Described Reaction	EC no.	Required enzyme activity	Gene candidate	Source Organism	Natural/ annotated function	Gene Identifier (nt)	SEQ ID NO (nt)	Uniprot ID	SEQ ID NO (AA)
Enzymes that may be used in D-ribulose-1-phosphate pathway to MEG									
D-xylulose <=> D-ribulose	5.1.3.-	D-ribulose-3-epimerase	DTE	Pseudomonas cichorii	D-tagatose 3-epimerase	ENA Nr.: BAA24429.1	1, 2	O50580	3
D-xylulose <=> D-ribulose	5.1.3.-	D-ribulose-3-epimerase	C1KKR1	Rhodobacter sphaeroides	D-tagatose 3-epimerase	ENA Nr.: FJ851309.1	4	C1KKR1	5
D-ribulose +ATP <=> D-ribulose-1-phosphate +ADP	2.7.1.-	D-ribulose-1-kinase	fucK	Escherichia coli	L-fuculokinase	GeneID: 946022	6, 7	P11553	8
D-ribulose-1-phosphate <=> glyceraldehyde +dihydroxyacetone phosphate	4.1.2.-	D-ribulose-1-phosphate aldolase	fucA	Escherichia coli	L-fuculose phosphate aldolase	GeneID: 947282	9, 10	P0AB87	11
Enzymes that may be used in D-xylulose-1-phosphate pathway to MEG									
D-xylulose +ATP <=> D-xylulose-1-phosphate +ADP	2.7.1.-	D-xylulose 1-kinase	khk-C (cDNA)	Homo sapiens	ketoheokinase C	GenBank: CR456801.1	53, 54	P50053	55

Described Reaction	EC no.	Required enzyme activity	Gene candidate	Source Organism	Natural/ annotated function	Gene Identifier (nt)	SEQ ID NO (nt)	Uniprot ID	SEQ ID NO (AA)
D-xylulose-1-phosphate $\rightleftharpoons$ glyceraldehyde + dihydroxyacetone phosphate	4.1.2.-	D-xylulose-1-phosphate aldolase	aldoB (cDNA)	Homo sapiens	Fructose-bisphosphate aldolase B	CCDS6756.1	56, 57	P05062	58
Enzymes that may be used in xylonate pathway to MEG									
D-xylulose + NAD ⁺ $\rightleftharpoons$ D-xylonolactone + NADH, or D-xylulose + NAD ⁺ $\rightleftharpoons$ D-xylonate + NADH	1.1.1.175	xylose dehydrogenase	xylB	Caulobacter crescentus	D-xylulose 1-dehydrogenase	GeneID: 7329904	59, 60	B8H1Z0	61
D-xylulose + NADP ⁺ $\rightleftharpoons$ D-xylonolactone + NADPH, or D-xylulose + NADP ⁺ $\rightleftharpoons$ D-xylonate + NADPH	1.1.1.179	xylose dehydrogenase	xdh1, HVO_B0028	Haloferax volcanii	D-xylulose 1-dehydrogenase	GeneID: 8919161	62	D4GP29	63

Described Reaction	EC no.	Required enzyme activity	Gene candidate	Source Organism	Natural/ annotated function	Gene Identifier (nt)	SEQ ID NO (nt)	Uniprot ID	SEQ ID NO (AA)
D-xylose +NADP ⁺ <=> D-xylonolactone +NADPH, or D-xylose +NADP ⁺ <=> D-xylonate +NADPH	1.1.1.179	xylose dehydrogenase	xyd1	Trichoderma reesei	D-xylose 1-dehydrogenase	ENA Nr.: EF136590.1	64	A0A024SMV2	65
D-xylonolactone +H ₂ O <=> D-xylonate	3.1.1.68	xylonolactonase	xylC	Caulobacter crescentus	Xylonolactonase	GeneID: 7329903	66	A0A0H3C6P8	67
D-xylonate <=> 2-keto-3-deoxy-xylonate +H ₂ O	4.2.1.82	xylonate dehydratase	xylD	Caulobacter crescentus	xylonate dehydratase	GeneID: 7329902	68	A0A0H3C6H6	69
D-xylonate <=> 2-keto-3-deoxy-xylonate +H ₂ O	4.2.1.82	xylonate dehydratase	yjhG	Escherichia coli	xylonate dehydratase	GeneID: 946829	70, 71	P39358	72
D-xylonate <=> 2-keto-3-deoxy-xylonate +H ₂ O	4.2.1.82	xylonate dehydratase	yagF	Escherichia coli	xylonate dehydratase	GeneID: 944928	73, 74	P77596	75

Described Reaction	EC no.	Required enzyme activity	Gene candidate	Source Organism	Natural/ annotated function	Gene Identifier (nt)	SEQ ID NO (nt)	Uniprot ID	SEQ ID NO (AA)
2-keto-3-deoxy-xylonate <=> glycolaldehyde + pyruvate	4.1.2.-	2-keto-3-deoxy-D-pentonate aldolase	yjhH	Escherichia coli	Uncharacterized lyase	GeneID: 948825	76, 77	P39359	78
2-keto-3-deoxy-xylonate <=> glycolaldehyde + pyruvate	4.1.2.-	2-keto-3-deoxy-D-pentonate aldolase	yagE	Escherichia coli	Probable 2-keto-3-deoxy-galactonate aldolase	GeneID: 944925	79, 80	P75682	81
Enzymes that may be used in pathway to produce one or more three-carbon compounds									
2 acetyl-CoA -> acetoacetyl-CoA + CoA	2.3.1.9	acetyl co-enzyme A acetyltransferase	thlA	Clostridium acetobutylicum	acetyl co-enzyme A acetyltransferase	3309200	33, 34	P45359	35
2 acetyl-CoA -> acetoacetyl-CoA + CoA	2.3.1.9	acetyl co-enzyme A acetyltransferase	atoB	Escherichia coli	acetyl co-enzyme A acetyltransferase	GeneID: 946727	36	P76461	37

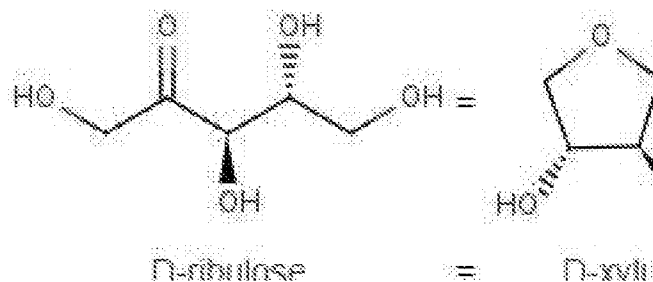
Described Reaction	EC no.	Required enzyme activity	Gene candidate	Source Organism	Natural/ annotated function	Gene Identifier (nt)	SEQ ID NO (nt)	Uniprot ID	SEQ ID NO (AA)
2 acetyl-CoA → acetoacetyl-CoA + CoA	2.3.1.9	acetyl co-enzyme A acetyltransferase	ERG10	Saccharomyces cerevisiae	acetyl co-enzyme A acetyltransferase	856079	38	P41338	39
acetoacetyl-CoA + acetate → acetoacetate + acetyl-CoA	2.8.3.8	Acetyl-CoA:acetoacetate-CoA transferase subunit	atoA	Escherichia coli	Acetyl-CoA:acetoacetate-CoA transferase subunit	48994873	41, 42	P76459	43
acetoacetyl-CoA + acetate → acetoacetate + acetyl-CoA	2.8.3.8	Acetyl-CoA:acetoacetate-CoA transferase subunit	atoD	Escherichia coli	Acetyl-CoA:acetoacetate-CoA transferase subunit	48994873	44, 45	P76458	46
acetoacetate → acetone + CO ₂	4.1.1.4	acetoacetate decarboxylase	adc	Clostridium acetobutylicum	acetoacetate decarboxylase	6466901	47, 48	P23670	49
acetoacetate → acetone + CO ₂	4.1.1.4	acetoacetate decarboxylase	adc	Clostridium beijerinckii	acetoacetate decarboxylase	149901357	50, 51	A6M020	52

Described Reaction	EC no.	Required enzyme activity	Gene candidate	Source Organism	Natural/ annotated function	Gene Identifier (nt)	SEQ ID NO (nt)	Uniprot ID	SEQ ID NO (AA)
acetone + NAD(P)H → 2-propanol + NAD(P) ⁺	1.1.1.2	secondary alcohol dehydrogenase	adh	Clostridium beijerinckii	secondary alcohol dehydrogenase	60592972	104, 105	P25984	106
acetone + NAD(P)H → 2-propanol + NAD(P) ⁺	1.1.1.2	secondary alcohol dehydrogenase	adh	Clostridium carboxidovorans	alcohol dehydrogenase	308066805	107	C6PZV5	108
NADH + NADP ⁺ ↔ NAD ⁺ + NADPH	1.6.1.1.	Soluble pyridine nucleotide transhydrogenase	udhA	Escherichia coli	Soluble pyridine nucleotide transhydrogenase	GeneID: 948461	109	P27306	110
Hydrolases that may be used in pathway to produce one or more three-carbon compounds									
Acetoacetyl-CoA + H ₂ O ⇌ CoA + acetoacetate	3.1.2.11	acetoacetyl-CoA hydrolase	ctfA	Clostridium acetobutylicum	butyrate-acetoacetyl-CoA-transferase, complex A	NCBI- GeneID: 1116168	96	P33752	97

Described Reaction	EC no.	Required enzyme activity	Gene candidate	Source Organism	Natural/ annotated function	Gene Identifier (nt)	SEQ ID NO (nt)	Uniprot ID	SEQ ID NO (AA)
Acetoacetyl-CoA + H ₂ O $\rightleftharpoons$ CoA + acetoacetate	3.1.2.11	acetate:acetoacetyl-CoA hydrolase	ctfB	Clostridium acetobutylicum	butyrate-acetoacetate CoA-transferase, subunit B	NCBI-GenelD: 1116169	98	P23673	99
Acetoacetyl-CoA + H ₂ O $\rightleftharpoons$ CoA + acetoacetate	3.1.2.11	acetate:acetoacetyl-CoA hydrolase	atoA	Escherichia coli (strain K12)	Acetyl-CoA:acetoacetate-CoA transferase subunit	GenelD: 946719	100	P76459	101
Acetoacetyl-CoA + H ₂ O $\rightleftharpoons$ CoA + acetoacetate	3.1.2.11	acetate:acetoacetyl-CoA hydrolase	atoD	Escherichia coli (strain K12)	Acetyl-CoA:acetoacetate-CoA transferase subunit	GenelD: 947525	102	P76458	103

D-tagatose 3-epimerase (EC 5.1.3.31)

[00260] The present disclosure describes enzymes that can catalyze the epimerization of various ketoses at the C-3 position, interconverting D-fructose and D-psicose, D-tagatose and D-sorbose, D-ribulose and D-xylulose, and L-ribulose and L-xylulose. The specificity depends on the species. The enzymes from *Pseudomonas cichorii* and *Rhodobacter sphaeroides* require Mn^{2+} . In one embodiment, the enzyme is D-tagatose 3-epimerase (dte). In another embodiment, the D-tagatose 3-epimerase catalyzes the conversion of D-xylulose to D-ribulose.



[00261] In some embodiments, the D-tagatose 3-epimerase is from *Pseudomonas* spp. In another embodiment, the D-tagatose 3-epimerase is from *Pseudomonas cichorii*. In another embodiment, the D-tagatose 3-epimerase is from *Pseudomonas* sp. ST-24. In another embodiment, the D-tagatose 3-epimerase is from *Mesorhizobium loti*. In another embodiment, the D-tagatose 3-epimerase is from *Rhodobacter sphaeroides* (C1KKR1).

[00262] In one embodiment, the D-tagatose 3-epimerase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Pseudomonas* sp., *Mesorhizobium* sp. and *Rhodobacter* sp. In some embodiments, the D-tagatose 3-epimerase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Pseudomonas cichorii*, *Pseudomonas* sp. ST-24, *Mesorhizobium loti* and *Rhodobacter sphaeroides*. In some embodiments, the one or more nucleic acid molecules is dte and/or FJ851309.1, or homolog thereof. In a further embodiment, the D-tagatose 3-epimerase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 and 5. In yet a further embodiment, the D-tagatose 3-epimerase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2 and 4.

[00263] D-tagatose 3-epimerase may also be known as L-ribulose 3-epimerase or ketose 3-epimerase.

D-ribulokinase (EC 2.7.1.16)

[00264] The present disclosure describes enzymes that can catalyze the following reactions:

[00265] $\text{L-fucose} + \text{ATP} \rightarrow \text{L-fucose 1-phosphate} + \text{ADP} + \text{H}^+$

[00266] $\text{D-ribulose} + \text{ATP} \rightarrow \text{D-ribulose 1-phosphate} + \text{ADP} + \text{H}^+$

[00267] D-ribulokinase may also be known as L-fuculokinase, fuculokinase, ATP: L-fucose 1-phosphotransferase or L-fucose kinase.

[00268] Thus, in some embodiments, the disclosure provides for an enzyme that plays roles in the fucose degradation pathway, the super pathway of fucose and rhamnose degradation and/or the D-arabinose degradation pathway.

[00269] In some embodiments, the enzyme can function as both an L-fuculokinase and a D-ribulokinase, the second enzyme of the L-fucose and D-arabinose degradation pathways, respectively.

[00270] In particular embodiments, the enzyme converts D-ribulose to D-ribulose-1-phosphate. In some embodiments, the D-ribulokinase is from *Escherichia coli*. In some embodiments, the D-ribulokinase is encoded by the *fucK* gene. In one embodiment, the D-ribulokinase is encoded by one or more nucleic acid molecules obtained from *E. coli*. In some embodiments, the one or more nucleic acid molecules is *fucK*, or homolog thereof. In a further embodiment, the D-ribulokinase comprises an amino acid sequence set forth in SEQ ID NO: 8. In yet a further embodiment, the D-ribulokinase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 6 and 7.

D-ribulose-1-phosphate aldolase (EC 4.1.2.17)

[00271] The present disclosure describes enzymes that can catalyze the following reversible reactions:

[00272] $\text{L-fucose 1-phosphate} \rightleftharpoons (\text{S})\text{-lactaldehyde} + \text{dihydroxy acetone phosphate (DHAP)}$

[00273] $\text{D-ribulose 1-phosphate} \rightleftharpoons \text{glycolaldehyde} + \text{dihydroxy acetone phosphate (DHAP)}$

[00274] D-ribulose-1-phosphate aldolase may also be known as L-fuculose-phosphate aldolase, L-fuculose 1-phosphate aldolase or L-fuculose-1-phosphate (S)-lactaldehyde-lyase.

[00275] Thus, in some embodiments, the disclosure provides for an enzyme that plays roles in the fucose degradation pathway, the super pathway of fucose and rhamnose degradation and/or the D-arabinose degradation I pathway. In one embodiment, the enzyme may use Zn^{2+} as a cofactor. In another embodiment, an inhibitor of this enzyme may be phosphoglycolohydroxamate.

[00276] In some embodiments, the enzyme can function as both an L-fuculose-phosphate aldolase and a D-ribulose-phosphate aldolase, the third enzyme of the L-fucose and D-arabinose degradation pathways, respectively.

[00277] The substrate specificity of the enzyme has been tested with a partially purified preparation from an *E. coli* strain.

[00278] Crystal structures of the enzyme and a number of point mutants have been solved. The combination of structural data and enzymatic activity of mutants allowed modelling and refinement of the catalytic mechanism of the enzyme. The enantiomeric selectivity of the enzyme has been studied.

[00279] In particular embodiments, the enzyme converts D-ribulose-1-phosphate to glycolaldehyde and DHAP. In some embodiments, the D-ribulose-1-phosphate aldolase is from *Escherichia coli*. In some embodiments, the D-ribulose-1-phosphate aldolase is encoded by the fucA gene. In one embodiment, the D-ribulose-1-phosphate aldolase is encoded by one or more nucleic acid molecules obtained from *E. coli*. In some embodiments, the one or more nucleic acid molecules is fucA, or homolog thereof. In a further embodiment, the D-ribulose-1-phosphate aldolase comprises an amino acid sequence set forth in SEQ ID NO: 11. In yet a further embodiment, the D-ribulose-1-phosphate aldolase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 9 and 10.

Glycolaldehyde reductase (EC 1.1.1.77)

[00280] The present disclosure describes enzymes that can catalyze the following reversible reactions:

[00281] ethylene glycol + NAD^+ $\rightleftharpoons$ glycolaldehyde + $\text{NADH} + \text{H}^+$

[00282] (S)-propane-1,2-diol + NAD^+ $\rightleftharpoons$ (S)-lactaldehyde + $\text{NADH} + \text{H}^+$

[00283] Glycolaldehyde reductase may also be known as lactaldehyde reductase, propanediol oxidoreductase, (R) [or(S)]-propane-1,2-diol:NAD⁺ oxidoreductase or L-1,2-propanediol oxidoreductase.

[00284] Thus, in some embodiments, the disclosure provides for an enzyme that plays roles in the ethylene glycol degradation pathway, the super pathway of glycol metabolism and degradation, the anaerobic L-lactaldehyde degradation pathway and/or the super pathway of fucose and rhamnose degradation. In one embodiment, the enzyme may use Fe²⁺ as a cofactor.

[00285] L-1,2-propanediol oxidoreductase is an iron-dependent group III dehydrogenase. It anaerobically reduces L-lactaldehyde, a product of both the L-fucose and L-rhamnose catabolic pathways, to L-1,2-propanediol, which is then excreted from the cell.

[00286] Crystal structures of the enzyme have been solved, showing a domain-swapped dimer in which the metal, cofactor and substrate binding sites could be located. An aspartate and three conserved histidine residues are required for Fe²⁺ binding and enzymatic activity.

[00287] *In vitro*, the enzyme can be reactivated by high concentrations of NAD⁺ and efficiently inactivated by a mixture of Fe³⁺ and ascorbate or Fe²⁺ and H₂O₂. Metal-catalyzed oxidation of the conserved His277 residue is proposed to be the cause of the inactivation.

[00288] Expression of FucO enables engineered one-turn reversal of the β -oxidation cycle. FucO activity contributes to the conversion of isobutyraldehyde to isobutanol in an engineered strain.

[00289] In particular embodiments, the enzyme converts glycolaldehyde to MEG. In some embodiments, the glycolaldehyde reductase is from *Escherichia coli*. In some embodiments, the glycolaldehyde reductase is encoded by the fucO gene. In one embodiment, the glycolaldehyde reductase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from *E. coli* and *S. cerevisiae*. In another embodiment, the one or more nucleic acid molecules is selected from gldA, GRE2, GRE3, yqhD, ydjG, fucO, yafB (dkgB), and/or yqhE (dkgA), or homolog thereof. In another embodiment, the one or more nucleic acid molecules is yqhD. In some embodiments, the yqhD comprises a G149E mutation. In a further embodiment, the glycolaldehyde reductase comprises an amino acid sequence selected from the group consisting

of SEQ ID NOs: 13, 15, 17, 20, 23, 25, 28, 30 and 32. In yet a further embodiment, the glycolaldehyde reductase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 12, 14, 16, 18, 19, 21, 22, 24, 26, 27, 29 and 31.

Aldehyde reductases

[00290] A number of aldehyde reductases may be used to convert glycolaldehyde to MEG.

[00291] An NADPH-dependent aldehyde reductase (YqhD) can catalyze the following reactions:

[00292] $\text{acetol} + \text{NADP}^+ \rightleftharpoons \text{methylglyoxal} + \text{NADPH} + \text{H}^+$ (reversible, EC 1.1.1.-)

[00293] $\text{an alcohol} + \text{NADP}^+ \rightleftharpoons \text{an aldehyde} + \text{NADPH} + \text{H}^+$ (reversibility unspecified, EC 1.1.1.2)

[00294] $\text{an aldehyde} + \text{NADP}^+ + \text{H}_2\text{O} \rightarrow \text{a carboxylate} + \text{NADPH} + 2 \text{H}^+$ (EC 1.2.1.4)

[00295] $1,3\text{-propanediol} + \text{NADP}^+ \rightleftharpoons 3\text{-hydroxypropionaldehyde} + \text{NADPH} + \text{H}^+$ (reversibility unspecified, EC 1.1.1.-)

[00296] $\text{D-3,4-dihydroxybutanal} + \text{NADPH} \rightleftharpoons 1,3,4\text{-butanetriol} + \text{NADP}^+$ (reversibility unspecified)

[00297] YqhD is an NADPH-dependent aldehyde reductase that may be involved in glyoxal detoxification and/or be part of a glutathione-independent response to lipid peroxidation.

[00298] It has been reported that various alcohols, aldehydes, amino acids, sugars and α -hydroxy acids have been tested as substrates for YqhD. The purified protein only shows NADP-dependent alcohol dehydrogenase activity, with a preference for alcohols longer than C(3), but with K_m values in the millimolar range, suggesting that they are not the physiological substrates. In contrast, YqhD does exhibit short-chain aldehyde reductase activity with substrates such as propanaldehyde, acetaldehyde, and butanaldehyde, as well as acrolein and malondialdehyde. In a metabolically engineered strain, phenylacetaldehyde and 4-hydroxyphenylacetaldehyde are reduced to 2-phenylethanol and 2-(4-hydroxyphenyl)ethanol by the endogenous aldehyde reductases YqhD, YjgB, and YahK.

[00299] Overexpression of YqhD increases 1,3-propanediol oxidoreductase activity of the cell. *E. coli* has been engineered to express YqhD for the industrial production of 1,3-propanediol. YqhD activity contributes to the production of isobutanol, 1,2-

propanediol, 1,2,4-butanetriol and acetol as well. Mutation of yqhD enables production of butanol by an engineered one-turn reversal of the β -oxidation cycle.

[00300] YqhD has furfural reductase activity, which appears to cause growth inhibition due to depletion of NADPH in metabolically engineered strains that produce alcohol from lignocellulosic biomass.

[00301] The crystal structure of YqhD has been solved at 2 Å resolution. YqhD is an asymmetric dimer of dimers, and the active site contains a Zn^{2+} ion. The NADPH cofactor is modified by hydroxyl groups at positions 5 and 6 in the nicotinamide ring.

[00302] Overexpression of yqhD leads to increased resistance to reactive oxygen-generating compounds such as hydrogen peroxide, paraquat, chromate and potassium tellurite. A yqhD deletion mutant shows increased sensitivity to these compounds and to glyoxal, and contains increased levels of reactive aldehydes that are generated during lipid peroxidation. Conversely, yqhD deletion leads to increased furfural tolerance.

[00303] In particular embodiments, an NADPH-dependent aldehyde reductase converts glycolaldehyde to MEG. In some embodiments, the NADPH-dependent aldehyde reductase is from *Escherichia coli*. In some embodiments, the NADPH-dependent aldehyde reductase is encoded by the yqhD gene.

[00304] A multi-functional methylglyoxal reductase (DkgA) can catalyze the following reactions:

[00305] $\text{acetol} + \text{NADP}^+ \rightleftharpoons \text{methylglyoxal} + \text{NADPH} + \text{H}^+$ (the reaction is physiologically favored in the opposite direction, EC 1.1.1.-)

[00306] $\text{isobutanol} + \text{NADP}^+ \rightleftharpoons \text{isobutanal} + \text{NADPH} + \text{H}^+$ (reversibility unspecified, EC 1.1.1.-)

[00307] $\text{ethyl-(2R)-methyl-(3S)-hydroxybutanoate} + \text{NADP}^+ \rightleftharpoons \text{ethyl-2-methylacetate} + \text{NADPH} + \text{H}^+$ (reversibility unspecified, EC 1.1.1.-)

[00308] $2\text{-keto-L-gulonate} + \text{NADP}^+ \leftarrow 2,5\text{-didehydro-D-gluconate} + \text{NADPH} + \text{H}^+$ (the reaction is favored in the opposite direction, EC 1.1.1.346)

[00309] DkgA (YqhE) belongs to the aldo-keto reductase (AKR) family and has been shown to have methylglyoxal reductase and beta-keto ester reductase activity.

[00310] dkgA is reported to encode a 2,5-diketo-D-gluconate reductase (25DKGR) A, one of two 25DKG reductases in *E. coli*. The enzyme uses NADPH as the preferred electron donor and is thought to be involved in ketogluconate metabolism. The specific

activity of the enzyme towards 2,5-diketo-D-gluconate is reported to be almost 1000-fold lower than its activity towards methylglyoxal.

[00311] Due to its low K_m for NADPH, reduction of furans by DkgA may deplete NADPH pools and thereby limit cellular biosynthesis. A broad survey of aldehyde reductases showed that DkgA was one of several endogenous aldehyde reductases that contribute to the degradation of desired aldehyde end products of metabolic engineering.

[00312] A crystal structure of DkgA has been solved at 2.16 Å resolution.

[00313] In particular embodiments, a multi-functional methylglyoxal reductase converts glycolaldehyde to MEG. In some embodiments, the multi-functional methylglyoxal reductase is from *Escherichia coli*. In some embodiments, the multi-functional methylglyoxal reductase is encoded by the *dkgA* gene.

[00314] A multi-functional methylglyoxal reductase (DkgB) can catalyze the following reactions:

[00315] $\text{acetol} + \text{NADP}^+ \rightleftharpoons \text{methylglyoxal} + \text{NADPH} + \text{H}^+$ (the reaction is physiologically favored in the opposite direction, EC 1.1.1.-)

[00316] $4\text{-nitrobenzyl alcohol} + \text{NADP}^+ \rightleftharpoons 4\text{-nitrobenzaldehyde} + \text{NADPH} + \text{H}^+$ (reversibility unspecified, EC 1.1.1.91)

[00317] $2\text{-keto-L-gulonate} + \text{NADP}^+ \leftarrow 2,5\text{-dihydro-D-gluconate} + \text{NADPH} + \text{H}^+$ (the reaction is favored in the opposite direction, EC 1.1.1.346)

[00318] DkgB (YafB) is a member of the aldo-keto reductase (AKR) subfamily 3F. DkgB was shown to have 2,5-diketo-D-gluconate reductase, methylglyoxal reductase and 4-nitrobenzaldehyde reductase activities.

[00319] *dkgB* is reported to encode 2,5-diketo-D-gluconate reductase (25DKGR) B, one of two 25DKG reductases in *E. coli*. The enzyme uses NADPH as the preferred electron donor and is thought to be involved in ketogluconate metabolism. However, the specific activity of the enzyme towards 2,5-diketo-D-gluconate is reported to be almost 1000-fold lower than its activity towards methylglyoxal.

[00320] In particular embodiments, a multi-functional methylglyoxal reductase converts glycolaldehyde to MEG. In some embodiments, the multi-functional methylglyoxal reductase is from *Escherichia coli*. In some embodiments, the multi-functional methylglyoxal reductase is encoded by the *dkgB* gene.

[00321] A methylglyoxal reductase (YeaE) can catalyze the following reaction:

[00322] acetol + NADP⁺ $\rightleftharpoons$ methylglyoxal + NADPH + H⁺ (the reaction is physiologically favored in the opposite direction, EC 1.1.1.-)

[00323] YeaE has been shown to have methylglyoxal reductase activity.

[00324] The subunit structure of YeaE has not been determined, but its amino acid sequence similarity to the aldo-keto reductases DkgA (YqhE) and DkgB (YafB) suggests that it may be monomeric.

[00325] In particular embodiments, a methylglyoxal reductase converts glycolaldehyde to MEG. In some embodiments, the methylglyoxal reductase is from *Escherichia coli*. In some embodiments, the methylglyoxal reductase is encoded by the yeaE gene.

[00326] A L-glyceraldehyde 3-phosphate reductase (yghZ) can catalyze the following reactions:

[00327] L-glyceraldehyde 3-phosphate + NADPH + H⁺ $\rightarrow$ sn-glycerol 3-phosphate + NADP⁺ (EC 1.1.1.-)

[00328] acetol + NADP⁺ $\rightleftharpoons$ methylglyoxal + NADPH + H⁺ (the reaction is physiologically favored in the opposite direction, EC 1.1.1.-)

[00329] YghZ is an L-glyceraldehyde 3-phosphate (L-GAP) reductase. The enzyme is also able to detoxify methylglyoxal at a low rate. YghZ defines the AKR14 (aldo-keto reductase 14) protein family.

[00330] L-GAP is not a natural metabolite and is toxic to *E. coli*. L-GAP is a substrate of both the glycerol-3-phosphate and hexose phosphate transport systems of *E. coli* K-12. It has been postulated that the physiological role of YghZ is the detoxification of L-GAP, which may be formed by non-enzymatic racemization of GAP or by an unknown cellular process.

[00331] The crystal structure of the *E. coli* enzyme has been determined and is suggested to be a tetramer. However, others have found that the protein forms an octamer based on gel filtration and electron microscopy studies.

[00332] In particular embodiments, a L-glyceraldehyde 3-phosphate reductase converts glycolaldehyde to MEG. In some embodiments, the L-glyceraldehyde 3-phosphate reductase is from *Escherichia coli*. In some embodiments, the L-glyceraldehyde 3-phosphate reductase is encoded by the yghZ gene.

[00333] An L-1,2-propanediol dehydrogenase/glycerol dehydrogenase (GldA) can catalyze the following reactions:

[00334] (S)-propane-1,2-diol + NAD⁺ $\rightleftharpoons$ acetol + NADH + H⁺ (reversible reaction)

[00335] aminoacetone + NADH + H⁺ → (R)-1-aminopropan-2-ol + NAD⁺ (EC 1.1.1.75)

[00336] glycerol + NAD⁺ ⇌ dihydroxyacetone + NADH + H⁺ (reversible reaction, EC 1.1.1.6)

[00337] The physiological function of the GldA enzyme has long been unclear. The enzyme was independently isolated as a glycerol dehydrogenase and a D-1-amino-2-propanol:NAD⁺ oxidoreductase. At that time, D-1-amino-2-propanol was thought to be an intermediate for the biosynthesis of vitamin B12, and although *E. coli* is unable to synthesize vitamin B12 *de novo*, enzymes catalyzing the synthesis of this compound were sought. It was later found that GldA was responsible for both activities.

[00338] The primary *in vivo* role of GldA was recently proposed to be the removal of dihydroxyacetone by converting it to glycerol. However, a dual role in the fermentation of glycerol has also recently been established. Glycerol dissimilation in *E. coli* can be accomplished by two different pathways. The glycerol and glycerophosphodiester degradation pathway requires the presence of a terminal electron acceptor and utilizes an ATP-dependent kinase of the Glp system, which phosphorylates glycerol to glycerol-3-phosphate. However, upon inactivation of the kinase and selection for growth on glycerol, it was found that an NAD⁺-linked dehydrogenase, GldA, was able to support glycerol fermentation. Recently, it was shown that GldA was involved in glycerol fermentation both as a glycerol dehydrogenase, producing dihydroxyacetone, and as a 1,2-propanediol dehydrogenase, regenerating NAD⁺ by producing 1,2-propanediol from acetol.

[00339] The enzyme is found in two catalytically active forms, a large form of eight subunits and a small form of two subunits. The large form appears to be the major species.

[00340] In particular embodiments, an L-1,2-propanediol dehydrogenase/glycerol dehydrogenase converts glycolaldehyde to MEG. In some embodiments, the L-1,2-propanediol dehydrogenase/glycerol dehydrogenase is from *Escherichia coli*. In some embodiments, the L-1,2-propanediol dehydrogenase/glycerol dehydrogenase is encoded by the *gldA* gene.

[00341] An NADPH-dependent methylglyoxal reductase (GRE2) from *Saccharomyces cerevisiae* can catalyze the following reactions:

[00342] (S)-lactaldehyde + NADP⁺ ⇌ methylglyoxal + NADPH

[00343] $3\text{-methylbutanol} + \text{NAD(P)}^+ \rightleftharpoons 3\text{-methylbutanal} + \text{NAD(P)H}$

[00344] Gre2 is a versatile enzyme that catalyzes the stereoselective reduction of a broad range of substrates including aliphatic and aromatic ketones, diketones, as well as aldehydes, using NADPH as the cofactor.

[00345] The crystal structures of Gre2 from *S. cerevisiae* in an apo-form at 2.00Å and NADPH-complexed form at 2.40Å resolution have been solved. Gre2 forms a homodimer, each subunit of which contains an N-terminal Rossmann-fold domain and a variable C-terminal domain, which participates in substrate recognition. The induced fit upon binding to the cofactor NADPH makes the two domains shift toward each other, producing an interdomain cleft that better fits the substrate. Computational simulation combined with site-directed mutagenesis and enzymatic activity analysis enabled characterization of a potential substrate-binding pocket that determines the stringent substrate stereoselectivity for catalysis.

[00346] Gre2 catalyzes the irreversible reduction of the cytotoxic compound methylglyoxal (MG) to (S)-lactaldehyde as an alternative to detoxification of MG by glyoxalase I GLO1. MG is synthesized via a bypath of glycolysis from dihydroxyacetone phosphate and is believed to play a role in cell cycle regulation and stress adaptation. GRE2 also catalyzes the reduction of isovaleraldehyde to isoamylalcohol. The enzyme serves to suppress isoamylalcohol-induced filamentation by modulating the levels of isovaleraldehyde, the signal to which cells respond by filamentation. GRE2 is also involved in ergosterol metabolism.

[00347] In particular embodiments, an NADPH-dependent methylglyoxal reductase converts glycolaldehyde to MEG. In some embodiments, the NADPH-dependent methylglyoxal reductase is from *S. cerevisiae*. In some embodiments, the NADPH-dependent methylglyoxal reductase is encoded by the GRE2 gene.

Thiolase/Acetyl coenzyme A acetyltransferase (EC 2.3.1.9)

[00348] The present disclosure describes enzymes that can catalyze the following reaction:

[00349] $2 \text{ acetyl-CoA} \rightleftharpoons \text{acetoacetyl-CoA} + \text{coenzyme A}$ (reversible reaction)

[00350] Thiolase/Acetyl coenzyme A acetyltransferase may also be known as acetyl-CoA-C-acetyltransferase, acetoacetyl-CoA thiolase, acetyl-CoA:acetyl-CoA C-acetyltransferase or thiolase II.

[00351] Thus, in some embodiments, the disclosure provides for an enzyme that plays a role in acetoacetate degradation (to acetyl CoA). In one embodiment, an inhibitor of this enzyme may be acetoacetyl-CoA.

[00352] In particular embodiments, the enzyme converts acetyl-CoA to acetoacetyl-CoA. In some embodiments, the thiolase/acetyl coenzyme A acetyltransferase is from *Clostridium spp.* In some embodiments, the thiolase/acetyl coenzyme A acetyltransferase is from *Clostridium acetobutylicum*. In some embodiments, the thiolase/acetyl coenzyme A acetyltransferase is from *Clostridium thermosaccharolyticum*. In some embodiments, the thiolase/acetyl coenzyme A acetyltransferase is from *Bacillus cereus*. In some embodiments, the thiolase/acetyl coenzyme A acetyltransferase is from *Marinobacter hydrocarbonoclasticus* ATCC 49840. In some embodiments, the thiolase/acetyl coenzyme A acetyltransferase is encoded by the thlA gene. In some embodiments, the thiolase/acetyl coenzyme A acetyltransferase is from *Escherichia coli*. In some embodiments, the thiolase/acetyl coenzyme A acetyltransferase is encoded by the atoB gene.

[00353] In one embodiment, the thiolase or acetyl coenzyme A acetyltransferase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Clostridium sp.*, *Bacillus sp.*, *E. coli*, *Saccharomyces sp.* and *Marinobacter sp.* In some embodiments, the thiolase or acetyl coenzyme A acetyltransferase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Clostridium acetobutylicum*, *Clostridium thermosaccharolyticum*, *Bacillus cereus*, *E. coli*, *Saccharomyces cerevisiae* and *Marinobacter hydrocarbonoclasticus*. In some embodiments, the one or more nucleic acid molecules is thlA, atoB and/or ERG10, or homolog thereof. In a further embodiment, the thiolase or acetyl coenzyme A acetyltransferase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 35, 37 and 40. In yet a further embodiment, the thiolase or acetyl coenzyme A acetyltransferase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 33, 34, 36, 38 and 39.

Acetate:Acetoacetyl-CoA transferase (EC 2.8.3.-)

[00354] The present disclosure describes enzymes that can catalyze the following reaction:

[00355] acetoacetate + acetyl-CoA $\rightleftharpoons$ acetoacetyl-CoA + acetate (reversible reaction, EC 2.8.3.-)

[00356] Acetate:Acetoacetyl-CoA transferase may also be known as acetoacetyl-CoA transferase or acetyl-CoA:acetoacetate-CoA transferase.

[00357] Thus, in some embodiments, the disclosure provides for an enzyme that plays a role in acetoacetate degradation (to acetyl CoA). In one embodiment, inhibitors of this enzyme may include acetyl-CoA and coenzyme A.

[00358] The growth of *E. coli* on short-chain fatty acids (C3-C6) requires the activation of the acids to their respective thioesters. This activation is catalyzed by acetoacetyl-CoA transferase. The reaction takes place in two half-reactions which involves a covalent enzyme-CoA. The enzyme undergoes two detectable conformational changes during the reaction. It is thought likely that the reaction proceeds by a ping-pong mechanism. The enzyme can utilize a variety of short-chain acyl-CoA and carboxylic acid substrates but exhibits maximal activity with normal and 3-keto substrates.

[00359] In particular embodiments, the enzyme converts acetoacetyl-CoA to acetoacetate. In some embodiments, the acetate:acetoacetyl-CoA transferase is from *Clostridium* spp. In some embodiments, the acetate:acetoacetyl-CoA transferase is from *Clostridium acetobutylicum*. In some embodiments, the acetate:acetoacetyl-CoA transferase is from *Escherichia coli*. In some embodiments, the acetate:acetoacetyl-CoA transferase is encoded by the *atoA* and *atoD* genes. In another embodiment, the subunit composition of acetoacetyl-CoA transferase is $[(AtoA)_2][(AtoD)_2]$, with $(AtoA)_2$ being the β complex and $(AtoD)_2$ being the α complex. In one embodiment, the acetate:acetoacetyl-CoA transferase is a fused acetate:acetoacetyl-CoA transferase: α subunit/ β subunit. In another embodiment, the acetate:acetoacetyl-CoA transferase is encoded by the *ydiF* gene.

Acetate:Acetoacetyl-CoA hydrolase (EC 3.1.2.11)

[00360] The present disclosure describes enzymes that can catalyze the following reaction:

[00361] $\text{acetoacetyl-CoA} + \text{H}_2\text{O} \rightleftharpoons \text{CoA} + \text{acetoacetate}$

[00362] Acetoacetyl-CoA hydrolase may also be known as acetoacetyl coenzyme A hydrolase, acetoacetyl CoA deacylase or acetoacetyl coenzyme A deacylase.

[00363] This enzyme belongs to the family of hydrolases, specifically those acting on thioester bonds.

[00364] In particular embodiments, the enzyme converts acetoacetyl-CoA to acetoacetate. In some embodiments, the acetate:acetoacetyl-CoA hydrolase is from

Clostridium spp. In some embodiments, the acetate:acetoacetyl-CoA hydrolase is from *Clostridium acetobutylicum*. In another embodiment, the Acetoacetyl-CoA hydrolase is encoded by the ctfA (subunit A) and/or ctfB (subunit B) genes.

[00365] In a further embodiment, the acetyl-CoA:acetoacetate-CoA transferase or acetate:acetoacetyl-CoA hydrolase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 46, 97, 99, 101 and 103. In yet a further embodiment, the acetyl-CoA:acetoacetate-CoA transferase or acetate:acetoacetyl-CoA hydrolase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 41, 42, 44, 45, 96, 98, 100 and 102.

Acetoacetate decarboxylase (EC 4.1.1.4)

[00366] The present disclosure describes enzymes that can catalyze the following reaction:

[00367] $\text{acetoacetate} + \text{H}^+ \rightarrow \text{acetone} + \text{CO}_2$

[00368] Acetoacetate decarboxylase may also be known as ADC, AADC or acetoacetate carboxy-lyase.

[00369] Thus, in some embodiments, the disclosure provides for an enzyme that plays roles in isopropanol biosynthesis, pyruvate fermentation to acetone, the super pathway of *Clostridium acetobutylicum* acidogenic and solventogenic fermentation and/or the super pathway of *Clostridium acetobutylicum* solventogenic fermentation.

[00370] Acetoacetate decarboxylase (ADC) plays a key role in solvent production in *Clostridium acetobutylicum*. During the acidogenic phase of growth, acids accumulate causing a metabolic shift to solvent production. In this phase acids are re-assimilated and metabolized to produce acetone, butanol and ethanol.

[00371] Preliminary purification and crystallization of the enzyme has revealed that a lysine residue is implicated in the active site. The enzyme is a large complex composed of 12 copies of a single type of subunit.

[00372] The enzyme of *Clostridium acetobutylicum* ATCC 824 has been purified and the adc gene encoding it cloned. The enzyme has also been purified from the related strain *Clostridium acetobutylicum* DSM 792 and the gene cloned and sequenced. The decarboxylation reaction proceeds by the formation of a Schiff base intermediate.

[00373] ADC is a key enzyme in acid uptake, effectively pulling the CoA-transferase reaction in the direction of acetoacetate formation.

[00374] In particular embodiments, the enzyme converts acetoacetate to acetone. In some embodiments, the acetoacetate decarboxylase is from *Clostridium* spp. In some embodiments, the acetoacetate decarboxylase is from *Clostridium acetobutylicum*. In some embodiments, the acetoacetate decarboxylase is from *Clostridium beijerinckii*. In some embodiments, the acetoacetate decarboxylase is from *Clostridium cellulolyticum*. In some embodiments, the acetoacetate decarboxylase is from *Bacillus polymyxa*. In some embodiments, the acetoacetate decarboxylase is from *Chromobacterium violaceum*. In some embodiments, the acetoacetate decarboxylase is from *Pseudomonas putida*. In another embodiment, the acetoacetate decarboxylase is encoded by the *adc* gene.

[00375] In one embodiment, the acetoacetate decarboxylase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Clostridium* sp., *Bacillus* sp., *Chromobacterium* sp. and *Pseudomonas* sp. In another embodiment, the acetoacetate decarboxylase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Clostridium acetobutylicum*, *Clostridium beijerinckii*, *Clostridium cellulolyticum*, *Bacillus polymyxa*, *Chromobacterium violaceum* and *Pseudomonas putida*. In some embodiments, the one or more nucleic acid molecules encoding the acetoacetate decarboxylase is *adc*, or homolog thereof. In a further embodiment, the acetoacetate decarboxylase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 49 and 52. In yet another embodiment, the acetoacetate decarboxylase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 47, 48, 50 and 51.

Alcohol Dehydrogenase (EC 1.1.1.-)

[00376] The present disclosure describes enzymes that can catalyze the reversible oxidation of primary or secondary alcohols to aldehydes or ketones, respectively. In one embodiment, the enzyme is a secondary alcohol dehydrogenase (S-ADH) and catalyzes the reduction of ketones such as acetone into secondary alcohols such as 2-propanol (isopropanol).

[00377] In some embodiments the S-ADH is from *Burkholderia* sp. In some embodiments, the S-ADH is from *Burkholderia* sp. AIU 652. In some embodiments, the S-ADH is from *Alcaligenes* sp. In some embodiments, the S-ADH is from *Alcaligenes eutrophus*. In some embodiments, the S-ADH is from *Clostridium* sp. In some embodi-

ments, the S-ADH is from *Clostridium ragsdalei*. In some embodiments, the S-ADH is from *Clostridium beijerinckii*. In some embodiments, the S-ADH is from *Thermoanaerobacter sp.* In some embodiments, the S-ADH is from *Thermoanaerobacter brockii*. In some embodiments, the S-ADH is from *Thermoanaerobacter ethanolicus* (*Clostridium thermohydrosulfuricum*). In some embodiments, the S-ADH is encoded by the adhB gene. In some embodiments, the S-ADH is from the trypanosomatid *Phytomonas sp.* In some embodiments, the S-ADH is from *Rhodococcus sp.* In some embodiments, the S-ADH is from *Rhodococcus ruber*. In some embodiments, the S-ADH is from *Methanobacterium palustre*. In some embodiments, the S-ADH is from methanogenic archaea *Methanogenium liminatans*. In some embodiments, the S-ADH is from the parasitic protist *Entamoeba histolytica* (EhAdh1). In some embodiments, the S-ADH is from parasitic protozoan *Tritrichomonas foetus*. In some embodiments, the S-ADH is from human parasite *Trichomonas vaginalis*.

[00378] In some embodiments, the S-ADH is predicted from homology and can be from *Thermoanaerobacter mathranii*, *Micrococcus luteus*, *Nocardiopsis alba*, *Mycobacterium hassiacum*, *Helicobacter suis*, *Candida albicans*, *Candida parapsilosis*, *Candida orthopsilosis*, *Candida metapsilosis*, *Grosmannia clavigera* and *Scheffersomyces stipitis*.

[00379] In a further embodiment, the alcohol dehydrogenase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 106 and 108. In yet another embodiment, the alcohol dehydrogenase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 104, 105 and 107.

Dehydratase (EC 4.2.1.-)

[00380] The present disclosure describes enzymes that can catalyze the following reactions:

[00381] isopropanol $\rightleftharpoons$ propene + H₂O

D-xylulose 1-kinase (EC 2.7.1.-)

[00382] The present disclosure describes enzymes that can catalyze the conversion of D-xylulose to D-xylulose-1-phosphate. In some embodiments, the conversion can be catalyzed by a human ketohexokinase C (khk-C), also known as fructokinase.

[00383] Ketohexokinase, or fructokinase, phosphorylates fructose to fructose-1-phosphate. The enzyme is involved in fructose metabolism, which is part of carbohydrate metabolism. It is found in the liver, intestine and kidney cortex.

[00384] In human liver, purified fructokinase, when coupled with aldolase, has been discovered to contribute to an alternative mechanism to produce oxalate from xylitol. In coupled sequence, fructokinase and aldolase produce glycolaldehyde, a precursor to oxalate, from D-xylulose via D-xylulose 1-phosphate.

[00385] In particular embodiments, the enzyme converts D-xylulose to D-xylulose-1-phosphate. In some embodiments, the D-xylulose 1-kinase is a ketohexokinase C. In some embodiments, the ketohexokinase C is from *Homo sapiens*. In some embodiments, the human ketohexokinase C is encoded by the khk-C gene.

[00386] In one embodiment, the D-xylulose 1-kinase is encoded by one or more nucleic acid molecules obtained from *Homo sapiens*. In some embodiments, the one or more nucleic acid molecules encoding the D-xylulose 1-kinase is ketohexokinase C (khk-C), or homolog thereof. In another embodiment, the one or more nucleic acid molecules encoding the D-xylulose 1-kinase comprises an amino acid sequence set forth in SEQ ID NO: 55. In a further embodiment, the one or more nucleic acid molecules encoding the D-xylulose 1-kinase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 53 and 54.

D-xylulose-1-phosphate aldolase (EC 4.1.2.-)

[00387] The present disclosure describes enzymes that can catalyze the conversion of D-xylulose-1-phosphate to glycolaldehyde and DHAP. In some embodiments, the conversion can be catalyzed by a human aldolase B, which is also known as fructose-bisphosphate aldolase B or liver-type aldolase.

[00388] Aldolase B is one of three isoenzymes (A, B, and C) of the class I fructose 1,6-bisphosphate aldolase enzyme (EC 4.1.2.13), and plays a key role in both glycolysis and gluconeogenesis. The generic fructose 1,6-bisphosphate aldolase enzyme catalyzes the reversible cleavage of fructose 1,6-bisphosphate (FBP) into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (DHAP) as well as the reversible cleavage of fructose 1-phosphate (F1P) into glyceraldehyde and dihydroxyacetone phosphate. In mammals, aldolase B is preferentially expressed in the liver, while aldolase A is expressed in muscle and erythrocytes and aldolase C is expressed in the brain. Slight differences in isozyme structure result in different activities for the two substrate molecules: FBP and fructose 1-phosphate. Aldolase B exhibits no preference and thus catalyzes both reactions, while aldolases A and C prefer FBP.

[00389] Aldolase B is a homotetrameric enzyme, composed of four subunits. Each subunit has a molecular weight of 36 kDa and contains an eight-stranded α/β barrel, which encloses lysine 229 (the Schiff-base forming amino acid that is key for catalysis).

[00390] In particular embodiments, the enzyme converts D-xylulose-1-phosphate to glycolaldehyde and DHAP. In some embodiments, the D-xylulose-1-phosphate aldolase is an aldolase B. In some embodiments, the aldolase B is from *Homo sapiens*. In some embodiments, the human aldolase B is encoded by the ALDOB gene.

[00391] In one embodiment, the D-xylulose-1-phosphate aldolase is encoded by one or more nucleic acid molecules obtained from *Homo sapiens*. In another embodiment, the one or more nucleic acid molecules encoding the D-xylulose-1-phosphate aldolase is aldolase B (ALDOB), or homolog thereof. In some embodiments, the one or more nucleic acid molecules encoding the D-xylulose-1-phosphate aldolase comprises an amino acid sequence set forth in SEQ ID NO: 58. In some embodiments, the one or more nucleic acid molecules encoding the D-xylulose-1-phosphate aldolase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 56 and 57.

D-xylose isomerase (EC 5.3.1.5)

[00392] The present disclosure describes enzymes that can catalyze the following reversible reaction:

[00393] D-xylopyranose $\rightleftharpoons$ D-xylulose

[00394] D-xylose isomerase may also be known as xylose isomerase or D-xylose ketol-isomerase.

[00395] Thus, in some embodiments, the disclosure provides for an enzyme that plays a role in xylose degradation.

[00396] Xylose isomerase catalyzes the first reaction in the catabolism of D-xylose.

[00397] Two conserved histidine residues, H101 and H271, were shown to be essential for catalytic activity. The fluorescence of two conserved tryptophan residues, W49 and W188, is quenched during binding of xylose, and W49 was shown to be essential for catalytic activity. The presence of Mg^{2+} , Mn^{2+} or Co^{2+} protects the enzyme from thermal denaturation.

[00398] The subunit composition has not been established experimentally.

[00399] In particular embodiments, the enzyme converts D-xylose to D-xylulose. In some embodiments, the D-xylose isomerase is from *Escherichia coli*. In some embodiments, the D-xylose isomerase is encoded by the xylA gene.

[00400] In some embodiments, a recombinant microorganism producing MEG and a three-carbon compound comprises a deletion, insertion, or loss of function mutation in a gene encoding a D-xylose isomerase to prevent conversion of D-xylose to D-xylulose and instead shunt the reaction toward the conversion of D-xylose to D-xylonate.

[00401] In one embodiment, the recombinant microorganism comprises an endogenous or exogenous xylose isomerase that catalyzes the conversion of D-xylose to D-xylulose. In one embodiment, the xylose isomerase is exogenous. In another embodiment, the xylose isomerase is encoded by one or more nucleic acid molecules obtained from *Pyromyces* sp. In another embodiment, the one or more nucleic acid molecules encoding the xylose isomerase is xylA, or homolog thereof. In yet another embodiment, the one or more nucleic acid molecules encoding the xylose isomerase comprises an amino acid sequence set forth in SEQ ID NO: 95. In a further embodiment, the one or more nucleic acid molecules encoding the xylose isomerase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 93 and 94.

D-xylulose-5-kinase/xylulokinase

[00402] The present disclosure describes enzymes that can catalyze the following reactions:

[00403] $\text{D-xylulose} + \text{ATP} \rightarrow \text{D-xylulose 5-phosphate} + \text{ADP} + \text{H}^+$ (EC 2.7.1.17)

[00404] $\text{ATP} + \text{1-deoxy-D-xylulose} \rightarrow \text{1-deoxy-D-xylulose 5-phosphate} + \text{ADP} + \text{H}^+$ (EC 2.7.1.-)

[00405] D-xylulose-5-kinase may also be known as xylulose kinase or xylulokinase.

[00406] Xylulokinase catalyzes the phosphorylation of D-xylulose, the second step in the xylose degradation pathway, producing D-xylulose-5-phosphate, an intermediate of the pentose phosphate pathway.

[00407] In the absence of substrate, xylulokinase has weak ATPase activity. Xylulokinase can also catalyze the phosphorylation of 1-deoxy-D-xylulose. This would allow a potential salvage pathway for generating 1-deoxy-D-xylulose 5-phosphate for use in the biosynthesis of terpenoids, thiamine and pyridoxal. The rate of phosphorylation of 1-deoxy-D-xylulose is 32-fold lower than the rate of phosphorylation of D-xylulose.

[00408] The kinetic mechanism of the bacterial enzyme has been studied, suggesting a predominantly ordered reaction mechanism. The enzyme undergoes significant conformational changes upon binding of the substrate and of ATP. Two conserved

aspartate residues, D6 and D233, were found to be essential for catalytic activity, and a catalytic mechanism has been proposed.

[00409] Crystal structures of bacterial xylulokinase in the apo form and bound to D-xylulose have been determined at 2.7 and 2.1 Å resolution, respectively.

[00410] In particular embodiments, the enzyme converts D-xylulose to D-xylulose-5-phosphate. In some embodiments, the D-xylulose-5-kinase is from *Escherichia coli*. In some embodiments, the D-xylulose-5-kinase is encoded by the xylB gene. In some embodiments, the D-xylulose-5-kinase is from *Saccharomyces cerevisiae*. In some embodiments the D-xylulose-5-kinase is encoded by the XKS1 gene. In some embodiments, the D-xylulose-5-kinase is from *Pichia stipitis*. In some embodiments the D-xylulose-5-kinase is encoded by the XYL3 gene.

[00411] In some embodiments, a recombinant microorganism producing MEG and a three-carbon compound comprises a deletion, insertion, or loss of function mutation in a gene encoding a D-xylulose-5-kinase to prevent the conversion of D-xylulose to D-xylulose-5-phosphate and instead shunt the reaction toward conversion of D-xylulose to D-xylulose-1-phosphate.

Xylose dehydrogenase (EC 1.1.1.175 or EC 1.1.1.179)

[00412] The present disclosure describes enzymes that can catalyze the following reactions:

[00413] $\text{aldehydo-D-xylose} + \text{NAD}^+ + \text{H}_2\text{O} \rightarrow \text{D-xylonate} + \text{NADH} + 2 \text{H}^+$

[00414] $\alpha\text{-D-xylopyranose} + \text{NAD}^+ \rightleftharpoons \text{D-xylonolactone} + \text{NADH} + \text{H}^+$ (reversibility unspecified, EC 1.1.1.175)

[00415] Xylose dehydrogenase may also be known as D-xylose dehydrogenase, D-xylose 1-dehydrogenase, (NAD⁺)-linked D-xylose dehydrogenase, NAD⁺-D-xylose dehydrogenase, D-xylose:NAD⁺ 1-oxidoreductase

[00416] D-Xylose dehydrogenase catalyzes the NAD⁺-dependent oxidation of D-xylose to D-xylonolactone. This is the first reaction in the oxidative, non-phosphorylative pathway for the degradation of D-xylose in *Caulobacter crescentus*. This pathway is similar to the pathway for L-arabinose degradation in *Azospirillum brasilense*. The amino acid sequence of the *C. crescentus* enzyme is unrelated to that of xylose dehydrogenase from the archaeon *Haloarcula marismortui*, or the L-arabinose 1-dehydrogenase of *Azospirillum brasilense*.

[00417] D-xylose is the preferred substrate for recombinant D-xylose dehydrogenase from *Caulobacter crescentus*. The enzyme can use L-arabinose, but it is a poorer substrate. The K_m for L-arabinose is 166 mM. Other substrates such as D-arabinose, L-xylose, D-ribose, D-galactose, D-glucose and D-glucose-6-phosphate showed little or no activity in the assay, as measured by NADH production. *C. crescentus* D-xylose dehydrogenase can convert D-xylose to D-xylonate directly.

[00418] Partially purified, native D-xylose dehydrogenase from *C. crescentus* had a K_m of 70 μ M for D-xylose. This value was lower than the K_m of 760 μ M for the recombinant, His-tagged enzyme.

[00419] In some embodiments, the D-Xylose dehydrogenase is from the halophilic archaeon *Haloferax volcanii*. The *Haloferax volcanii* D-Xylose dehydrogenase catalyzes the first reaction in the oxidative xylose degradation pathway of the halophilic archaeon *Haloferax volcanii*. The *H. volcanii* D-Xylose dehydrogenase shows 59% amino acid sequence identity to a functionally characterized xylose dehydrogenase from *Haloarcula marismortui* and 56% identity to an ortholog in *Halorubrum lacusprofundi*, but is only 11% identical to the bacterial NAD⁺-dependent xylose dehydrogenase from *Caulobacter crescentus* CB15.

[00420] In particular embodiments, the enzyme converts D-xylose to D-xylonolactone. In some embodiments, the D-Xylose dehydrogenase is from *Caulobacter crescentus*. In some embodiments, the D-Xylose dehydrogenase is encoded by the xylB gene. In some embodiments, the D-Xylose dehydrogenase is from *Haloferax volcanii*. In some embodiments, the D-Xylose dehydrogenase is from *Haloarcula marismortui*. In some embodiments, the D-Xylose dehydrogenase is from *Halorubrum lacusprofundi*. In some embodiments, the D-Xylose dehydrogenase is encoded by the xdh gene.

[00421] In one embodiment, the xylose dehydrogenase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter sp.*, *Haloarcula sp.*, *Haloferax sp.*, *Halorubrum sp.* and *Trichoderma sp.* In another embodiment, the xylose dehydrogenase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter crescentus*, *Haloarcula marismortui*, *Haloferax volcanii*, *Halorubrum lacusprofundi* and *Trichoderma reesei*. In some embodiments, the one or more nucleic acid molecules encoding the xylose dehydrogenase is selected from xylB, xdh1 (HVO_B0028) and/or xyd1, or homolog thereof. In a further embodiment, the one or

more nucleic acid molecules encoding the xylose dehydrogenase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 61, 63 and 65. In yet another embodiment, the one or more nucleic acid molecules encoding the xylose dehydrogenase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 59, 60, 62 and 64.

Xylonolactonase (3.1.1.68)

[00422] The present disclosure describes enzymes that can catalyze the following reaction:

[00423] D-xylono-1,4-lactone + H₂O ↔ D-xylonate

[00424] This enzyme belongs to the family of hydrolases, specifically those acting on carboxylic ester bonds. This enzyme participates in pentose and glucuronate inter-conversions.

[00425] Xylonolactonase may also be known as D-xylonolactonase, xylono-1,4-lactonase, xylono-gamma-lactonase or D-xylono-1,4-lactone lactonohydrolase.

[00426] In particular embodiments, the enzyme converts D-xylonolactone to D-xylonate. In some embodiments, the D-xylonolactonase is from *Haloferax* sp. In some embodiments, the D-xylonolactonase is from *Haloferax volcanii*. In some embodiments, the D-xylonolactonase is from *Haloferax gibbonsii*. In some embodiments, the D-xylonolactonase is from *Caulobacter crescentus*. In some embodiments, the D-xylonolactonase is encoded by the xylC gene.

[00427] In one embodiment, the xylonolactonase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from *Caulobacter* sp. and *Haloferax* sp. In another embodiment, the xylonolactonase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter crescentus*, *Haloferax volcanii* and *Haloferax gibbonsii*. In some embodiments, the one or more nucleic acid molecules encoding the xylonolactonase is xylC, or homolog thereof. In a further embodiment, the one or more nucleic acid molecules encoding the xylonolactonase comprises an amino acid sequence set forth in SEQ ID NO: 67. In yet another embodiment, the one or more nucleic acid molecules encoding the xylonolactonase is encoded by a nucleic acid sequence set forth in SEQ ID NO: 66.

Xylonate dehydratase (EC 4.2.1.82)

[00428] The present disclosure describes enzymes that can catalyze the following reaction:

[00429] D-xylonate $\leftrightarrow$ 2-keto-3-deoxy-D-xylonate + H₂O

[00430] This enzyme belongs to the family of lyases, specifically the hydro-lyases, which cleave carbon-oxygen bonds. This enzyme participates in pentose and glucuronate interconversions.

[00431] Xylonate dehydratase may also be known as D-xylonate hydro-lyase, D-xylonoaldonate dehydratase or D-xylonate dehydratase.

[00432] In particular embodiments, the enzyme converts D-xylonate to 2-keto-3-deoxy-D-xylonate. In some embodiments, the xylonate dehydratase is from *Caulobacter crescentus*. In some embodiments, the xylonate dehydratase is encoded by the xylD gene. In some embodiments, the xylonate dehydratase is from *Escherichia coli*. In some embodiments, the xylonate dehydratase is encoded by the yjhG gene. In some embodiments, the xylonate dehydratase is encoded by the yagF gene. In some embodiments, the xylonate dehydratase is from *Haloferax volcanii*. In some embodiments, the xylonate dehydratase is encoded by the xad gene. In some embodiments, the xylonate dehydratase is from *Sulfolobus solfataricus*.

[00433] In one embodiment, the xylonate dehydratase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter sp.*, *Sulfolobus sp.* and *E. coli*. In another embodiment, the xylonate dehydratase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Caulobacter crescentus*, *Sulfolobus solfataricus* and *E. coli*. In some embodiments, the one or more nucleic acid molecules encoding the xylonate dehydratase is selected from xylD, yjhG and/or yagF, or homolog thereof. In a further embodiment, the one or more nucleic acid molecules encoding the xylonate dehydratase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 69, 72 and 75. In yet another embodiment, the one or more nucleic acid molecules encoding the xylonate dehydratase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 68, 70, 71, 73 and 74.

2-keto-3-deoxy-D-pentionate aldolase (4.1.2.28)

[00434] The present disclosure describes enzymes that can catalyze the following reaction:

[00435] 2-dehydro-3-deoxy-D-pentionate $\rightleftharpoons$ glycolaldehyde + pyruvate (reversibility unspecified)

[00436] This enzyme belongs to the family of lyases, specifically the aldehyde-lyases, which cleave carbon-carbon bonds. This enzyme participates in pentose and glucuronate interconversions.

[00437] 2-keto-3-deoxy-D-pentionate aldolase may also be known as 2-dehydro-3-deoxy-D-pentionate glycolaldehyde-lyase (pyruvate-forming), 2-dehydro-3-deoxy-D-pentionate aldolase, 3-deoxy-D-pentulosonic acid aldolase, and 2-dehydro-3-deoxy-D-pentionate glycolaldehyde-lyase.

[00438] YjhH appears to be a 2-dehydro-3-deoxy-D-pentionate aldolase. Genetic evidence suggests that YagE may also function as a 2-dehydro-3-deoxy-D-pentionate aldolase. yagE is part of the prophage CP4-6.

[00439] A *yjhH yagE* double mutant cannot use D-xylonate as the sole source of carbon, and crude cell extracts do not contain 2-dehydro-3-deoxy-D-pentionate aldolase activity. Both phenotypes are complemented by providing *yjhH* on a plasmid.

[00440] ArcA appears to activate *yjhH* gene expression under anaerobiosis. Two putative ArcA binding sites were identified 211 and 597 bp upstream of this gene, but no promoter upstream of it has been identified.

[00441] The crystal structure of YagE suggests that the protein is a homotetramer. Co-crystal structures of YagE in the presence of pyruvate and 2-keto-3-deoxygalactonate have been solved.

[00442] In particular embodiments, the enzyme converts 2-keto-3-deoxy-xylonate to glycolaldehyde and pyruvate. In some embodiments, the 2-keto-3-deoxy-D-pentionate aldolase is from *Pseudomonas* sp. In some embodiments, the 2-keto-3-deoxy-D-pentionate aldolase is from *Escherichia coli*. In some embodiments, the 2-keto-3-deoxy-D-pentionate aldolase is encoded by the *yjhH* gene. In some embodiments, the 2-keto-3-deoxy-D-pentionate aldolase is encoded by the *yagE* gene.

[00443] In one embodiment, the 2-keto-3-deoxy-D-pentionate aldolase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from *Pseudomonas* sp. and *E. coli*. In another embodiment, the 2-keto-3-deoxy-D-pentionate aldolase is encoded by one or more nucleic acid molecules obtained from *E. coli*. In some embodiments, the one or more nucleic acid molecules encoding the 2-keto-3-deoxy-D-pentionate aldolase is selected from *yjhH* and/or *yagE*, or homolog thereof. In a further embodiment, the one or more nucleic acid molecules encoding the 2-keto-3-deoxy-D-pentionate aldolase comprises an amino acid sequence selected from the

group consisting of SEQ ID NOs: 78 and 81. In yet another embodiment, the one or more nucleic acid molecules encoding the 2-keto-3-deoxy-D-pentonate aldolase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 76, 77, 79 and 80.

Glycolaldehyde dehydrogenase (1.2.1.21)

[00444] The present disclosure describes enzymes that can catalyze the following reaction:

[00445] $\text{glycolaldehyde} + \text{NAD}^+ + \text{H}_2\text{O} \leftrightarrow \text{glycolate} + \text{NADH} + 2 \text{H}^+$

[00446] This enzyme belongs to the family of oxidoreductases, specifically those acting on the aldehyde or oxo group of donor with NAD^+ or NADP^+ as acceptor. This enzyme participates in glyoxylate and dicarboxylate metabolism.

[00447] Glycolaldehyde dehydrogenase may also be known as glycolaldehyde: NAD^+ oxidoreductase or glycol aldehyde dehydrogenase.

[00448] In *E. coli* aldehyde dehydrogenase A (AldA) is an enzyme of relatively broad substrate specificity for small α -hydroxyaldehyde substrates. It is thus utilized in several metabolic pathways.

[00449] L-fucose and L-rhamnose are metabolized through parallel pathways which converge after their corresponding aldolase reactions yielding the same products: dihydroxy-acetone phosphate and L-lactaldehyde. Aerobically, aldehyde dehydrogenase A oxidizes L-lactaldehyde to L-lactate.

[00450] In parallel pathways utilizing the same enzymes, D-arabinose and L-xylose can be metabolized to dihydroxy-acetone phosphate and glycolaldehyde, which is oxidized to glycolate by aldehyde dehydrogenase A.

[00451] Crystal structures of the enzyme alone and in ternary and binary complexes have been solved.

[00452] Aldehyde dehydrogenase A is only present under aerobic conditions and is most highly induced by the presence of fucose, rhamnose or glutamate. The enzyme is inhibited by NADH, which may act as a switch to shift from oxidation of lactaldehyde to its reduction by propanediol oxidoreductase. AldA is upregulated during short-term adaptation to glucose limitation.

[00453] Based on sequence similarity, AldA was predicted to be a succinate-semialdehyde dehydrogenase.

[00454] Regulation of aldA expression has been investigated. The gene is regulated by catabolite repression, repression under anaerobic conditions via ArcA, and induction by the carbon source.

[00455] In particular embodiments, the enzyme converts glycolaldehyde to glycolate. In some embodiments, the glycolaldehyde dehydrogenase is from *Escherichia coli*. In some embodiments, the glycolaldehyde dehydrogenase is encoded by the aldA gene.

[00456] In some embodiments, a recombinant microorganism producing MEG and a three-carbon compound comprises a deletion, insertion, or loss of function mutation in a gene encoding a glycolaldehyde dehydrogenase to prevent the production of glycolic acid from glycolaldehyde and instead shunt the reaction toward conversion of glycolaldehyde to MEG.

Lactate dehydrogenase (1.1.1.28)

[00457] The present disclosure describes enzymes that can catalyze the following reaction:

[00458] (R)-lactate + NAD⁺ $\leftarrow$ pyruvate + NADH + H⁺

[00459] Lactate dehydrogenase (LDH) is an enzyme found in nearly all living cells such as in animals, plants and prokaryotes. LDH catalyzes the conversion of lactate to pyruvic acid and back, as it converts NADH to NAD⁺ and back. A dehydrogenase is an enzyme that transfers a hydride from one molecule to another.

[00460] LDH exist in four distinct enzyme classes. The most common one is NAD(P)-dependent L-lactate dehydrogenase. Other LDHs act on D-lactate and/or are dependent on cytochrome c: D-lactate dehydrogenase (cytochrome) and L-lactate dehydrogenase (cytochrome).

[00461] LDH has been of medical significance because it is found extensively in body tissues, such as blood cells and heart muscle. Because it is released during tissue damage, it is a marker of common injuries and disease such as heart failure.

[00462] Lactate dehydrogenase may also be known as lactic acid dehydrogenase, (R)-lactate:NAD⁺ oxidoreductase or D-lactate dehydrogenase—fermentative.

[00463] In *E. coli*, lactate dehydrogenase (LdhA) is a soluble NAD-linked lactate dehydrogenase (LDH) that is specific for the production of D-lactate. LdhA is a homotetramer and shows positive homotropic cooperativity under higher pH conditions.

[00464] *E. coli* contains two other lactate dehydrogenases: D-lactate dehydrogenase and L-lactate dehydrogenase. Both are membrane-associated flavoproteins required for aerobic growth on lactate.

[00465] LdhA is present under aerobic conditions but is induced when *E. coli* is grown on a variety of sugars under anaerobic conditions at acidic pH. Unlike most of the genes involved in anaerobic respiration, LdhA is not activated by Fnr; rather the ArcAB system and several genes involved in the control of carbohydrate metabolism (csrAB and mlc) appear to regulate expression. The expression of LdhA is negatively affected by the transcriptional regulator ArcA. LdhA belongs to the σ_{32} regulon.

[00466] The LdhA gene is a frequent target for mutations in metabolic engineering, most often to eliminate production of undesirable fermentation side products, but also to specifically produce D-lactate.

[00467] In particular embodiments, the enzyme converts pyruvate to lactate. In some embodiments, the lactate dehydrogenase is from *Escherichia coli*. In some embodiments, the lactate dehydrogenase is encoded by the LdhA gene.

[00468] In some embodiments, a recombinant microorganism producing MEG and a three-carbon compound comprises a deletion, insertion, or loss of function mutation in a gene encoding a lactate dehydrogenase to prevent the production of lactate from pyruvate and instead shunt the reaction toward production of a three-carbon compound.

Xylose reductase or aldose reductase (EC 1.1.1.21)

[00469] The present disclosure describes enzymes that can catalyze the following reactions:

[00470] α -D-xylose + NADPH + H⁺ $\leftrightarrow$ xylitol + NADP

[00471] an alditol + NAD(P)⁺ $\leftrightarrow$ NAD(P)H + aldose

[00472] Aldose reductase may also be known as alditol:NAD(P)⁺ 1-oxidoreductase, polyol dehydrogenase or aldehyde reductase.

[00473] Aldose reductase is a cytosolic oxidoreductase that catalyzes the reduction of a variety of aldehydes and carbonyls, including monosaccharides.

[00474] Aldose reductase may be considered a prototypical enzyme of the aldoketo reductase enzyme superfamily. The enzyme comprises 315 amino acid residues and folds into a β/α -barrel structural motif composed of eight parallel β strands. Adjacent strands are connected by eight peripheral α -helical segments running anti-parallel to the β sheet. The catalytic active site is situated in the barrel core. The NADPH cofac-

tor is situated at the top of the β/α barrel, with the nicotinamide ring projecting down in the center of the barrel and pyrophosphate straddling the barrel lip.

[00475] The reaction mechanism of aldose reductase in the direction of aldehyde reduction follows a sequential ordered path where NADPH binds, followed by the substrate. Binding of NADPH induces a conformational change (Enzyme•NADPH $\rightarrow$ Enzyme*•NADPH) that involves hinge-like movement of a surface loop (residues 213-217) so as to cover a portion of the NADPH in a manner similar to that of a safety belt. The alcohol product is formed via a transfer of the pro-R hydride of NADPH to the face of the substrate's carbonyl carbon. Following release of the alcohol product, another conformational change occurs (E*•NAD(P)⁺ $\rightarrow$ E•NAD(P)⁺) in order to release NADP⁺. Kinetic studies have shown that reorientation of this loop to permit release of NADP⁺ appears to represent the rate-limiting step in the direction of aldehyde reduction. As the rate of coenzyme release limits the catalytic rate, it can be seen that perturbation of interactions that stabilize coenzyme binding can have dramatic effects on the maximum velocity (V_{max}).

[00476] D-xylose-fermenting *Pichia stipitis* and *Candida shehatae* were shown to produce one single aldose reductase (ALR) that is active both with NADPH and NADH. Other yeasts such as *Pachysolen tannophilus* and *C. tropicalis* synthesize multiple forms of ALR with different coenzyme specificities. The significant dual coenzyme specificity distinguishes the *P. stipitis* and the *C. shehatae* enzymes from most other ALRs so far isolated from mammalian or microbial sources. The yeast *Candida tenuis* CBS 4435 produces comparable NADH- and NADPH-linked aldehyde-reducing activities during growth on D-xylose.

[00477] In particular embodiments, the enzyme converts D-xylose to xylitol. In some embodiments, the xylose reductase or aldose reductase is from *Hypocrea jecorina*. In some embodiments, the xylose reductase or aldose reductase is encoded by the xyl1 gene. In some embodiments, the xylose reductase or aldose reductase is from *Saccharomyces cerevisiae*. In some embodiments, the xylose reductase or aldose reductase is encoded by the GRE3 gene. In some embodiments, the xylose reductase or aldose reductase is from *Pachysolen tannophilus*. In some embodiments, the xylose reductase or aldose reductase is from *Pichia sp.* In some embodiments, the xylose reductase or aldose reductase is from *Pichia stipitis*. In some embodiments, the xylose reductase or aldose reductase is from *Pichia quercuum*. In some embodiments, the

xylose reductase or aldose reductase is from *Candida sp.* In some embodiments, the xylose reductase or aldose reductase is from *Candida shehatae*. In some embodiments, the xylose reductase or aldose reductase is from *Candida tenuis*. In some embodiments, the xylose reductase or aldose reductase is from *Candida tropicalis*. In some embodiments, the xylose reductase or aldose reductase is from *Aspergillus niger*. In some embodiments, the xylose reductase or aldose reductase is from *Neurospora crassa*. In some embodiments, the xylose reductase or aldose reductase is from *Cryptococcus lactativorus*.

[00478] In some embodiments, the xylose reductase or aldose reductase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Hypocrea sp.*, *Scheffersomyces sp.*, *Saccharomyces sp.*, *Pachysolen sp.*, *Pichia sp.*, *Candida sp.*, *Aspergillus sp.*, *Neurospora sp.*, and *Cryptococcus sp.* In some embodiments, the xylose reductase or aldose reductase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Hypocrea jecorina*, *Scheffersomyces stipitis*, *Saccharomyces cerevisiae*, *Pachysolen tannophilus*, *Pichia stipitis*, *Pichia quercuum*, *Candida shehatae*, *Candida tenuis*, *Candida tropicalis*, *Aspergillus niger*, *Neurospora crassa* and *Cryptococcus lactativorus*. In another embodiment, the one or more nucleic acid molecules encoding the xylose reductase or aldose reductase is xyl1 and/or GRE3 or homolog thereof. In some embodiments, the one or more nucleic acid molecules encoding the xylose reductase or aldose reductase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 84 and 87. In some embodiments, the one or more nucleic acid molecules encoding the xylose reductase or aldose reductase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 82, 83, 85 and 86.

Xylitol dehydrogenase (1.1.1.9)

[00479] The present disclosure describes enzymes that can catalyze the following reaction:

[00480] $\text{xylitol} + \text{NAD}^+ \leftrightarrow \text{D-xylulose} + \text{NADH} + \text{H}^+$

[00481] Xylitol dehydrogenase may also be known as D-xylulose reductase, NAD⁺-dependent xylitol dehydrogenase, erythritol dehydrogenase, 2,3-cis-polyol(DPN) dehydrogenase (C3-5), pentitol-DPN dehydrogenase, xylitol-2-dehydrogenase or xylitol:NAD⁺ 2-oxidoreductase (D-xylulose-forming).

[00482] Xylitol dehydrogenase (XDH) is one of several enzymes responsible for assimilating xylose into eukaryotic metabolism and is useful for fermentation of xylose contained in agricultural byproducts to produce ethanol. For efficient xylose utilization at high flux rates, cosubstrates should be recycled between the NAD⁺-specific XDH and the NADPH-preferring xylose reductase, another enzyme in the pathway.

[00483] In particular embodiments, the enzyme converts xylitol to D-xylulose. In some embodiments, the xylitol dehydrogenase is from yeast. In some embodiments, the xylitol dehydrogenase is from *Pichia* sp., *Saccharomyces* sp., *Gluconobacter* sp., *Galactocandida* sp., *Neurospora* sp. or *Serratia* sp. In some embodiments, the xylitol dehydrogenase is from *Pichia stipitis*, *S. cerevisiae*, *Gluconobacter oxydans*, *Galactocandida mastotermitis*, *Neurospora crassa* or *Serratia marcescens*. In some embodiments, the xylitol dehydrogenase is encoded by xyl2 or xdh1.

[00484] In one embodiment, the xylitol dehydrogenase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Scheffersomyces* sp., *Trichoderma* sp., *Pichia* sp., *Saccharomyces* sp., *Gluconobacter* sp., *Galactocandida* sp., *Neurospora* sp., and *Serratia* sp. In another embodiment, the xylitol dehydrogenase is encoded by one or more nucleic acid molecules obtained from a microorganism selected from the group consisting of *Scheffersomyces stipitis*, *Trichoderma reesei*, *Pichia stipitis*, *Saccharomyces cerevisiae*, *Gluconobacter oxydans*, *Galactocandida mastotermitis*, *Neurospora crassa* and *Serratia marcescens*. In another embodiment, the one or more nucleic acid molecules encoding the xylitol dehydrogenase is xyl2 and/or xdh1, or homolog thereof. In some embodiments, the one or more nucleic acid molecules encoding the xylitol dehydrogenase comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 90 and 92. In some embodiments, the one or more nucleic acid molecules encoding the xylitol dehydrogenase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 88, 89 and 91.

Alkaline phosphatase (EC 3.1.3.1)

[00485] Alkaline phosphatase is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. It is sometimes used synonymously as basic phosphatase.

[00486] The *S. cerevisiae* Pho13 alkaline phosphatase enzyme is a monomeric protein with molecular mass of 60 kDa and hydrolyzes p-nitrophenyl phosphate with maximal activity at pH 8.2 with strong dependence on Mg^{2+} ions and an apparent K_m of 3.6×10^{-5} M. No other substrates tested except phosphorylated histone II-A and casein were hydrolyzed at any significant rate. These data suggest that the physiological role of the p-nitrophenyl phosphate-specific phosphatase may involve participation in reversible protein phosphorylation.

[00487] In particular embodiments, the enzyme converts D-xylulose-5-phosphate to D-xylulose. In some embodiments, the alkaline phosphatase is from yeast. In some embodiments, the alkaline phosphatase is from *Saccharomyces* sp. In some embodiments, the alkaline phosphatase is from *S. cerevisiae*. In some embodiments, the alkaline phosphatase is encoded by the PHO13 gene.

[00488] In some embodiments, a recombinant microorganism producing MEG and a three-carbon compound comprises a deletion, insertion, or loss of function mutation in a gene encoding an alkaline phosphatase to prevent the conversion of D-xylulose-5-phosphate to D-xylulose.

Soluble pyridine nucleotide transhydrogenase (EC 1.6.1.1.)

[00489] The present disclosure describes enzymes that can catalyze the following reaction:



[00491] Soluble pyridine nucleotide transhydrogenase may also be known as NAD(P)⁺ transhydrogenase (B-specific), STH, pyridine nucleotide transhydrogenase, or transhydrogenase.

[00492] *E. coli* contains both a soluble and a membrane-bound pyridine nucleotide transhydrogenase. The soluble pyridine nucleotide transhydrogenase is the *sthA* or *udhA* gene product; its primary physiological role appears to be the reoxidation of NADPH (Canonaco F. et al. (2001) Metabolic flux response to phosphoglucose isomerase knock-out in *Escherichia coli* and impact of overexpression of the soluble transhydrogenase UdhA. FEMS Microbiol Lett 204(2): 247-252; Sauer U. et al. (2004) The soluble and membrane-bound transhydrogenases UdhA and PntAB have divergent functions in NADPH metabolism of *Escherichia coli*. J Biol Chem 279(8): 6613-6619). The membrane-bound proton-translocating transhydrogenase is the *pntAB* gene product; PntAB is a major source of NADPH (Sauer et al. 2004).

[00493] UdhA contains noncovalently bound FAD and is present in a form consisting of seven or eight monomers (Boonstra B. et al. (1999) The *udhA* gene of *Escherichia coli* encodes a soluble pyridine nucleotide transhydrogenase. J Bacteriol 181(3): 1030-1034).

[00494] Moderate overexpression of UdhA (SthA) allows an increased maximal growth rate of a phosphoglucose isomerase mutant (Canonaco et al. 2001), and a *pgi sthA* double mutant is not viable (Sauer et al. 2004). These phenotypes may be due to the ability of UdhA to restore the cellular redox balance under conditions of excess NADPH formation (Canonaco et al. 2001; Sauer et al. 2004). Mutations in *sthA* appear during adaptation of a *pgi* mutant strain to growth on glucose minimal medium (Charusanti P. et al. (2010) Genetic basis of growth adaptation of *Escherichia coli* after deletion of *pgi*, a major metabolic gene." PLoS Genet 6(11): e1001186).

[00495] Transcription of *sthA* is downregulated by growth on glycerol (Sauer et al. 2004).

[00496] In some embodiments, expression of a transhydrogenase can increase activity of a NADPH-dependent alcohol dehydrogenase, leading to improved acetone to 2-propanol conversion. In one embodiment, the soluble pyridine nucleotide transhydrogenase is encoded by one or more nucleic acid molecules obtained from *E. coli*. In another embodiment, the one or more nucleic acid molecules encoding the soluble pyridine nucleotide transhydrogenase is *udhA*, or homolog thereof. In some embodiments, the one or more nucleic acid molecules encoding the soluble pyridine nucleotide transhydrogenase comprises an amino acid sequence set forth in SEQ ID NO: 110. In some embodiments, the one or more nucleic acid molecules encoding the soluble pyridine nucleotide transhydrogenase is encoded by a nucleic acid sequence set forth in SEQ ID NO: 109.

Enzyme overexpression or enzyme downregulation/deletion for Increased Pathway Flux

[00497] In various embodiments described herein, the exogenous and endogenous enzymes in the recombinant microorganism participating in the biosynthesis pathways described herein may be overexpressed.

[00498] The terms "overexpressed" or "overexpression" refers to an elevated level (e.g., aberrant level) of mRNAs encoding for a protein(s), and/or to elevated levels of protein(s) in cells as compared to similar corresponding unmodified cells expressing

basal levels of mRNAs or having basal levels of proteins. In particular embodiments, mRNA(s) or protein(s) may be overexpressed by at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 8-fold, 10-fold, 12-fold, 15-fold or more in microorganisms engineered to exhibit increased gene mRNA, protein, and/or activity.

[00499] In some embodiments, a recombinant microorganism of the disclosure is generated from a host that contains the enzymatic capability to synthesize substrates such as D-xylulose, D-ribulose, D-ribulose-1-phosphate, D-xylulose-1-phosphate, D-xylonolactone, D-xylonate, 2-keto-3-deoxy-xylonate, glycolaldehyde, DHAP, pyruvate, acetoacetyl-CoA or acetoacetate. In some embodiments, it can be useful to increase the synthesis or accumulation of, for example, D-xylulose, D-ribulose, D-ribulose-1-phosphate, D-xylulose-1-phosphate, D-xylonolactone, D-xylonate, 2-keto-3-deoxy-xylonate, glycolaldehyde, DHAP, pyruvate, acetoacetyl-CoA or acetoacetate, to increase the production of MEG and one or more three-carbon compounds.

[00500] In some embodiments, it may be useful to increase the expression of endogenous or exogenous enzymes involved in the MEG and three-carbon compound biosynthesis pathways to increase flux from, for example, D-xylulose, D-ribulose, D-ribulose-1-phosphate, D-xylulose-1-phosphate, D-xylonolactone, D-xylonate, 2-keto-3-deoxy-xylonate, glycolaldehyde, DHAP, pyruvate, acetoacetyl-CoA or acetoacetate, thereby resulting in increased synthesis or accumulation of MEG and one or more three-carbon compounds.

[00501] Increased synthesis or accumulation can be accomplished by, for example, overexpression of nucleic acids encoding one or more of the above-described MEG and three-carbon compound biosynthesis pathway enzymes. Overexpression of a MEG and three-carbon compound biosynthesis pathway enzyme or enzymes can occur, for example, through increased expression of an endogenous gene or genes, or through the expression, or increased expression, of an exogenous gene or genes. Therefore, naturally occurring organisms can be readily modified to generate non-natural, MEG and three-carbon compound producing microorganisms through overexpression of one or more nucleic acid molecules encoding a MEG and three-carbon compound biosynthesis pathway enzyme. In addition, a non-naturally occurring organism can be generated by mutagenesis of an endogenous gene that results in an increase in activity of an enzyme in the MEG and three-carbon compound biosynthesis pathways.

[00502] Equipped with the present disclosure, the skilled artisan will be able to readily construct the recombinant microorganisms described herein, as the recombinant microorganisms of the disclosure can be constructed using methods well known in the art as exemplified above to exogenously express at least one nucleic acid encoding a MEG and three-carbon compound biosynthesis pathway enzyme in sufficient amounts to produce MEG and one or more three-carbon compounds.

[00503] Methods for constructing and testing the expression levels of a non-naturally occurring MEG and three-carbon compound-producing host can be performed, for example, by recombinant and detection methods well known in the art. Such methods can be found described in, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Third Ed., Cold Spring Harbor Laboratory, New York (2001); Ausubo et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Md. (1999).

[00504] A variety of mechanisms known in the art can be used to express, or over-express, exogenous or endogenous genes. For example, an expression vector or vectors can be constructed to harbor one or more MEG and three-carbon compound biosynthesis pathway enzymes encoding nucleic acids as exemplified herein operably linked to expression control sequences functional in the host organism. Expression vectors applicable for use in the microbial host organisms of the invention include, for example, plasmids, phage vectors, viral vectors, episomes and artificial chromosomes, including vectors and selection sequences or markers operable for stable integration into a host chromosome. Selectable marker genes also can be included that, for example, provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply critical nutrients not in the culture media. Expression control sequences can include constitutive and inducible promoters, transcription enhancers, transcription terminators, and the like which are well known in the art. When two or more exogenous encoding nucleic acids are to be co-expressed, both nucleic acids can be inserted, for example, into a single expression vector or in separate expression vectors. For single vector expression, the encoding nucleic acids can be operationally linked to one common expression control sequence or linked to different expression control sequences, such as one inducible promoter and one constitutive promoter. The transformation of exogenous nucleic acid sequences involved in a metabolic or synthetic pathway can be confirmed using methods well known in the art.

[00505] As will be understood by those of skill in the art, it can be advantageous to modify a coding sequence to enhance its expression in a particular host. The genetic code is redundant with 64 possible codons, but most organisms typically use a subset of these codons. The codons that are utilized most often in a species are called optimal codons, and those not utilized very often are classified as rare or low-usage codons. Codons can be substituted to reflect the preferred codon usage of the host, a process sometimes called "codon optimization" or "controlling for species codon bias."

[00506] Optimized coding sequences containing codons preferred by a particular prokaryotic or eukaryotic host (see also, Murray et al. (1989) Nucl. Acids Res. 17:477-508) can be prepared, for example, to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced from a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, typical stop codons for *S. cerevisiae* and mammals are UAA and UGA, respectively. The typical stop codon for monocotyledonous plants is UGA, whereas insects and *E. coli* commonly use UAA as the stop codon (Dalphin et al. (1996) Nucl. Acids Res. 24: 216-218).

[00507] Those of skill in the art will recognize that, due to the degenerate nature of the genetic code, a variety of nucleic acid sequences can be used to encode a given enzyme of the disclosure. The nucleic acid sequences encoding the biosynthetic enzymes are referenced herein merely to illustrate an embodiment of the disclosure, and the disclosure includes any nucleic acid sequences that encode the amino acid sequences of the polypeptides and proteins of the enzymes of the present disclosure. In similar fashion, a polypeptide can typically tolerate one or more amino acid substitutions, deletions, and insertions in its amino acid sequence without loss or significant loss of a desired activity. The disclosure includes such polypeptides with different amino acid sequences than the specific proteins described herein so long as the modified or variant polypeptides have the enzymatic anabolic or catabolic activity of the reference polypeptide. Furthermore, the amino acid sequences encoded by the nucleic acid sequences shown herein merely illustrate embodiments of the disclosure.

[00508] Expression control sequences are known in the art and include, for example, promoters, enhancers, polyadenylation signals, transcription terminators, internal ribosome entry sites (IRES), and the like, that provide for the expression of the polynucleotide sequence in a host cell. Expression control sequences interact specifically with

cellular proteins involved in transcription (Maniatis et al., Science, 236: 1237-1245 (1987)). Exemplary expression control sequences are described in, for example, Goeddel, Gene Expression Technology: Methods in Enzymology, Vol. 185, Academic Press, San Diego, Calif. (1990).

[00509] In various embodiments, an expression control sequence may be operably linked to a polynucleotide sequence. By "operably linked" is meant that a polynucleotide sequence and an expression control sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the expression control sequence(s). Operably linked promoters are located upstream of the selected polynucleotide sequence in terms of the direction of transcription and translation. Operably linked enhancers can be located upstream, within, or downstream of the selected polynucleotide.

[00510] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of MEG and one or more three-carbon compounds.

[00511] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes the conversion of D-xylulose to D-xylulose-5-phosphate. In some such embodiments, the enzyme that catalyzes the conversion of D-xylulose to D-xylulose-5-phosphate is a D-xylulose-5-kinase. In some embodiments, the D-xylulose-5-kinase is from *Escherichia coli*. In some embodiments, the D-xylulose-5-kinase is encoded by the *xylB* gene or homologs thereof. In some embodiments, the manipulation prevents the conversion of D-xylulose to D-xylulose-5-phosphate and instead shunts the reaction toward conversion of D-xylulose to D-xylulose-1-phosphate.

[00512] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes the conversion of glycolaldehyde to glycolic acid. In some such embodiments, the enzyme that catalyzes the conversion of glycolaldehyde to glycolic acid is a glycolaldehyde dehydrogenase. In some embodiments, the glycolaldehyde dehydrogenase is from *Escherichia coli*. In some embodiments, the glycolaldehyde dehydrogenase is encoded by the *aldA* gene or homologs thereof. In some embodiments, the

manipulation prevents the production of glycolic acid from glycolaldehyde and instead shunts the reaction toward conversion of glycolaldehyde to MEG.

[00513] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes the conversion of pyruvate to lactate. In some such embodiments, the enzyme that catalyzes the conversion of pyruvate to lactate is a lactate dehydrogenase. In some embodiments, the lactate dehydrogenase is from *Escherichia coli*. In some embodiments, the lactate dehydrogenase is encoded by the *ldhA* gene or homologs thereof. In some embodiments, the manipulation prevents the production of lactate from pyruvate and instead shunts the reaction toward production of a three-carbon compound.

[00514] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes the conversion of D-xylulose to D-xylulose-5-phosphate. In some such embodiments, the enzyme that catalyzes the conversion of D-xylulose to D-xylulose-5-phosphate is a D-xylulose-5-kinase. In some embodiments, the D-xylulose-5-kinase is from *Saccharomyces cerevisiae*. In some embodiments the D-xylulose-5-kinase is encoded by the *XKS1* gene or homologs thereof. In some embodiments, the D-xylulose-5-kinase is from *Pichia stipitis*. In some embodiments the D-xylulose-5-kinase is encoded by the *XYL3* gene or homologs thereof. In some embodiments, the manipulation prevents the conversion of D-xylulose to D-xylulose-5-phosphate and instead shunts the reaction toward conversion of D-xylulose to D-xylulose-1-phosphate.

[00515] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes the conversion of D-xylose to D-xylulose. In some such embodiments, the enzyme that catalyzes the conversion of D-xylose to D-xylulose is a D-xylose isomerase. In some embodiments, the D-xylose isomerase is from *E. coli*. In some embodiments, the D-xylose isomerase is encoded by the *xylA* gene or homologs thereof. In some embodiments, the manipulation prevents conversion of D-xylose to D-xylulose and instead shunts the reaction toward the conversion of D-xylose to D-xylonate.

[00516] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes the conversion of D-xylulose-5-phosphate to D-xylulose. In some such

embodiments, the enzyme that catalyzes the conversion of D-xylulose-5-phosphate to D-xylulose is an alkaline phosphatase. In some embodiments, the alkaline phosphatase is from *S. cerevisiae*. In some embodiments, the alkaline phosphatase is encoded by the PHO13 gene or homologs thereof. In some embodiments, the manipulation prevents the conversion of D-xylulose-5-phosphate to D-xylulose.

[00517] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes the conversion of D-xylose to D-xylulose. In some such embodiments, the enzyme that catalyzes the conversion of D-xylose to D-xylulose is a D-xylose isomerase. In some embodiments, the D-xylose isomerase is from *E. coli*. In some embodiments, the D-xylose isomerase is encoded by the *xylA* gene or homologs thereof. In some embodiments, the manipulation prevents conversion of D-xylose to D-xylulose and instead shunts the reaction toward the conversion of D-xylose to D-xylonate.

Modified Microbes and Compositions Thereof

Microbial Compositions

[00518] In some aspects, the microbes of the disclosure are combined into microbial compositions.

[00519] In some aspects, the microbial compositions of the present disclosure are solid. Where solid compositions are used, it may be desired to include one or more carrier materials including, but not limited to: mineral earths such as silicas, talc, kaolin, limestone, chalk, clay, dolomite, diatomaceous earth; calcium sulfate; magnesium sulfate; magnesium oxide; zeolites, calcium carbonate; magnesium carbonate; trehalose; chitosan; shellac; albumins; starch; skim milk powder; sweet whey powder; maltodextrin; lactose; inulin; dextrose; and products of vegetable origin such as cereal meals, tree bark meal, wood meal, and nutshell meal.

[00520] In some aspects, the microbial compositions of the present disclosure are liquid. In further embodiments, the liquid comprises a solvent that may include water or an alcohol or a saline or carbohydrate solution. In some embodiments, the microbial compositions of the present disclosure include binders such as polymers, carboxymethylcellulose, starch, polyvinyl alcohol, and the like.

[00521] In some aspects, microbial compositions of the present disclosure comprise saccharides (e.g., monosaccharides, disaccharides, trisaccharides, polysaccharides, oligosaccharides, and the like), polymeric saccharides, lipids, polymeric lipids,

lipopolysaccharides, proteins, polymeric proteins, lipoproteins, nucleic acids, nucleic acid polymers, silica, inorganic salts and combinations thereof. In a further embodiment, microbial compositions comprise polymers of agar, agarose, gelrite, gellan gum, and the like. In some aspects, microbial compositions comprise plastic capsules, emulsions (e.g., water and oil), membranes, and artificial membranes. In some embodiments, emulsions or linked polymer solutions may comprise microbial compositions of the present disclosure. See Harel and Bennett (US Patent 8,460,726 B2).

[00522] In some aspects, microbial compositions of the present disclosure occur in a solid form (e.g., dispersed lyophilized spores) or a liquid form (microbes interspersed in a storage medium). In some embodiments, microbial compositions of the present disclosure are added in dry form to a liquid to form a suspension immediately prior to use.

[00523] In some aspects, the microbial composition of the present disclosure possesses a water activity (a_w) of less than 0.750, 0.700, 0.650, 0.600, 0.550, 0.500, 0.475, 0.450, 0.425, 0.400, 0.375, 0.350, 0.325, 0.300, 0.275, 0.250, 0.225, 0.200, 0.190, 0.180, 0.170, 0.160, 0.150, 0.140, 0.130, 0.120, 0.110, 0.100, 0.095, 0.090, 0.085, 0.080, 0.075, 0.070, 0.065, 0.060, 0.055, 0.050, 0.045, 0.040, 0.035, 0.030, 0.025, 0.020, 0.015, 0.010, or 0.005.

[00524] In some aspects, the microbial composition of the present disclosure possesses a water activity (a_w) of less than about 0.750, about 0.700, about 0.650, about 0.600, about 0.550, about 0.500, about 0.475, about 0.450, about 0.425, about 0.400, about 0.375, about 0.350, about 0.325, about 0.300, about 0.275, about 0.250, about 0.225, about 0.200, about 0.190, about 0.180, about 0.170, about 0.160, about 0.150, about 0.140, about 0.130, about 0.120, about 0.110, about 0.100, about 0.095, about 0.090, about 0.085, about 0.080, about 0.075, about 0.070, about 0.065, about 0.060, about 0.055, about 0.050, about 0.045, about 0.040, about 0.035, about 0.030, about 0.025, about 0.020, about 0.015, about 0.010, or about 0.005.

[00525] The water activity values are determined by the method of Saturated Aqueous Solutions (Multon, "Techniques d'Analyse E De Controle Dans Les Industries Agroalimentaires" APRIA (1981)) or by direct measurement using a viable Robotronic BT hygrometer or other hygrometer or hygroscope.

Feedstock

[00526] In some aspects, the disclosure is drawn to a method of producing MEG and/or one or more C3 products in a culture medium containing a feedstock providing a

carbon source such that the MEG and/or one or more C3 products are produced and recovered/collected/isolated. The recovery/collection/isolation can be by methods known in the art, such as distillation, membrane-based separation gas stripping, solvent extraction, and expanded bed adsorption.

[00527] In some aspects, the feedstock comprises a carbon source. In some aspects, the carbon source may be selected from sugars, glycerol, alcohols, organic acids, alkanes, fatty acids, lignocellulose, proteins, carbon dioxide, and carbon monoxide. In one aspect, the carbon source is a sugar. In one aspect, the sugar is glucose or oligomers of glucose thereof. In one aspect, the oligomers of glucose are selected from fructose, sucrose, starch, cellobiose, maltose, lactose and cellulose. In one aspect, the sugar is a five carbon sugar. In one aspect, the sugar is a six carbon sugar. In some aspects, the feedstock comprises one or more five carbon sugars and/or one or more six carbon sugars. In some aspects, the feedstock comprises one or more of xylose, glucose, arabinose, galactose, maltose, fructose, mannose, sucrose, and/or combinations thereof. In some aspects, the feedstock comprises one or more of xylose and/or glucose. In some aspects, the feedstock comprises one or more of arabinose, galactose, maltose, fructose, mannose, sucrose, and/or combinations thereof.

[00528] In some aspects, the microbes utilize one or more five carbon sugars (pentoses) and/or one or more six carbon sugars (hexoses). In some aspects, the microbes utilize one or more of xylose and/or glucose. In some aspects, the microbes utilize one or more of arabinose, galactose, maltose, fructose, mannose, sucrose, and/or combinations thereof. In some aspects, the microbes utilize one or more of xylose, glucose, arabinose, galactose, maltose, fructose, mannose, sucrose, and/or combinations thereof.

[00529] In some aspects, hexoses may be selected from D-allose, D-altrose, D-glucose, D-mannose, D-gulose, D-idose, D-galactose, D-talose, D-tagatose, D-sorbose, D-fructose, D-psicose, and other hexoses known in the art. In some aspects, pentoses may be selected from D-xylose, D-ribose, D-arabinose, D-lyxose, D-xylulose, D-ribulose, and other pentoses known in the art. In some embodiments, the hexoses and pentoses may be selected from the levorotary or dextrorotary enantiomer of any of the hexoses and pentoses disclosed herein.

[00530] In some aspects, total amount of C5 and/or C6 carbohydrates fed to a bioreactor / growth medium during the growth phase is at least 5 kg carbohydrate/m³,

at least 10 kg carbohydrate/m³, at least 20 kg carbohydrate/m³, at least 30 kg carbohydrate/m³, at least 40 kg carbohydrate/m³, at least 50 kg carbohydrate/m³, at least 60 kg carbohydrate/m³, at least 70 kg carbohydrate/m³, at least 80 kg carbohydrate/m³, at least 90 kg carbohydrate/m³, at least 100 kg carbohydrate/m³, at least 150 kg carbohydrate/m³, at least 200 kg carbohydrate/m³, at least 250 kg carbohydrate/m³, at least 300 kg carbohydrate/m³, at least 400 kg carbohydrate/m³ at least 500 kg carbohydrate/m³, at least 600 kg carbohydrate/m³, at least 700 kg carbohydrate/m³, up to 800 kg carbohydrate /m³. In some embodiments, total amount of C5 and/or C6 carbohydrates fed to the bioreactor / growth medium during the growth phase ranges from about 10 kg carbohydrate/m³ up to 500 kg carbohydrate/m³.

[00531] In some aspects, time required for the growth phase varies between 1 to 200 hours. In further embodiments, the time of the growth phase is between 5 to 50 hours. The time is dependent on carbohydrate feeds and/or feedstocks.

[00532] In some aspects, the total amount of C5 and/or C6 carbohydrates fed to the bioreactor / growth medium during the production phase is at least 50 kg carbohydrate/m³, at least 60 kg carbohydrate/m³, at least 70 kg carbohydrate/m³, at least 80 kg carbohydrate/m³, at least 90 kg carbohydrate/m³, at least 100 kg carbohydrate/m³, at least 150 kg carbohydrate/m³, at least 200 kg carbohydrate/m³, at least 250 kg carbohydrate/m³, at least 300 kg carbohydrate/m³, at least 400 kg carbohydrate/m³, at least 500 kg carbohydrate/m³, at least 600 kg carbohydrate/m³, at least 700 kg carbohydrate/m³, at least 800 kg carbohydrate/m³, at least 900 kg carbohydrate/m³ up to 1000 kg carbohydrate /m³. In some embodiments, total amount of C5 and/or C6 carbohydrates fed to the bioreactor / growth medium during the production phase ranges from about 100 kg carbohydrate/m³ up to 800 kg carbohydrate /m³.

[00533] In some aspects, time required for the production phase varies between 5 to 500 hours. In further embodiments, the time for the production phase varies from 10 to 300 hours for batch and fed-batch operations. In other embodiments, the time of the production phase is up to 300 hours with continuous fermentation.

[00534] In some aspects, the total amount of C5 and/or C6 carbohydrates fed to the bioreactor / growth medium for one-phase process is at least 50 kg carbohydrate/m³, at least 60 kg carbohydrate/m³, at least 70 kg carbohydrate/m³, at least 80 kg carbohydrate/m³, at least 90 kg carbohydrate/m³, at least 100 kg carbohydrate/m³, at least 150 kg carbohydrate/m³, at least 200 kg carbohydrate/m³, at least 250 kg car-

bohydrate/m³, at least 300 kg carbohydrate/m³, at least 400 kg carbohydrate/m³, at least 500 kg carbohydrate/m³, at least 600 kg carbohydrate/m³, at least 700 kg carbohydrate/m³, at least 800 kg carbohydrate/m³, at least 900 kg carbohydrate/m³ up to 1000 kg carbohydrate /m³. In some aspects, total amount of C5 and/or C6 carbohydrates fed to the bioreactor / growth medium during the production phase ranges from about 100 kg carbohydrate/m³ up to 800 kg carbohydrate /m³.

[00535] In some aspects, time required for the production phase in the one-phase process varies between 5 to 500 hours. In further aspects, the time required for production phase in the one-phase process varies between 5 to 300 hours.

[00536] In some aspects, the one-phase or multi-phase production processes take about 5, about 10, about 25, about 50, about 75, about 100, about 125, about 150, about 175, about 200, about 225, about 250, about 275, about 300 about 325, about 350, about 375, about 400, about 425, about 450, about 475, or about 500 hours.

[00537] In some aspects, the one-phase or multi-phase production processes take 5, 10, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300 325, 350, 375, 400, 425, 450, 475, or 500 hours.

Improvement of Traits

[00538] Methods of the present disclosure may be employed to introduce or improve one or more of a variety of desirable traits. Examples of traits that may be introduced or improved include: increased rate of production of MEG and/or one or more C3 compounds, increased rate of production of MEG, increased rate of production of one or more C3 compounds, increased rate of production of MEG and one or more C3 compounds, increased yield of MEG and/or one or more C3 compounds, increased yield of MEG, increased yield of one or more C3 compounds, increased yield of MEG and one or more C3 compounds, and other traits described herein.

[00539] In some aspects, a microbe resulting from the methods described herein exhibits a difference in the trait that is at least about 1% greater, for example at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 9%, at least about 9%, at least about 10%, at least about 11%, at least about 12%, at least about 13%, at least about 14%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, or at least 100%, at least about 200%, at

least about 300%, at least about 400% or greater than a reference under control conditions. In additional examples, a microbe resulting from the methods described herein exhibits a difference in the trait that is at least about 5% greater, for example at least about 5%, at least about 8%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 75%, at least about 80%, at least about 80%, at least about 90%, or at least 100%, at least about 200%, at least about 300%, at least about 400% or greater than a reference unmodified microbe or base strain.

[00540] In some aspects, the increase or decrease of any one or more of the traits of the present disclosure is an increase of about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% relative to an unmodified microbe or a base strain.

[00541] In some aspects, the increase or decrease of any one or more of the traits of the present disclosure is an increase of at least 0.1%, at least 0.2%, at least 0.3%, at least 0.4%, at least 0.5%, at least 0.6%, at least 0.7%, at least 0.8%, at least 0.9%, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26%, at

least 27%, at least 28%, at least 29%, at least 30%, at least 31%, at least 32%, at least 33%, at least 34%, at least 35%, at least 36%, at least 37%, at least 38%, at least 39%, at least 40%, at least 41%, at least 42%, at least 43%, at least 44%, at least 45%, at least 46%, at least 47%, at least 48%, at least 49%, at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% relative an unmodified microbe or a base strain.

[00542] In some aspects, a microbe resulting from the methods described herein exhibits an increase in MEG yield by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe or a base strain.

[00543] In some aspects, a microbe resulting from the methods described herein exhibits an increase in the yield of one or more C3 compounds by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe or a base strain.

[00544] In some aspects, a microbe resulting from the methods described herein exhibits an increase in MEG yield by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe or a base strain; and an increase in one or more C3 compounds yield by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe or a base strain.

[00545] In some aspects, a microbe resulting from the methods described herein exhibits an increase in the rate of MEG production by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe or a base strain.

[00546] In some aspects, a microbe resulting from the methods described herein exhibits an increase in the rate of production of one or more C3 compounds by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe or a base strain.

[00547] In some aspects, a microbe resulting from the methods described herein exhibits an increase in the rate of MEG production by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe or a base strain; and an increase in the rate of production of one or more C3 compounds by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe or a base strain.

[00548] In some aspects, a microbe resulting from the methods described herein exhibits (1) an increase in the rate of MEG production by at least 2%, (2) an increase in the rate of production of one or more C3 compounds by at least 2%, (3) an increase in MEG yield by at least 2%, and (4) an increase in the yield of one or more C3 compounds by at least 2%.

[00549] In some aspects, a microbe resulting from the methods described herein exhibits (1) an increase in the rate of MEG production by at least 5%, 10%, or 15%, (2) an increase in the rate of production of one or more C3 compounds by at least 5%, 10%, or 15%, (3) an increase in MEG yield by at least 5%, 10%, or 15%, and (4) an increase in the yield of one or more C3 compounds by at least 5%, 10%, or 15%.

[00550] In some aspects, a microbe resulting from the methods described herein exhibits (1) an increase in the rate of MEG production by at least 10%, 15%, 20%, 25%,

or 30%, (2) an increase in the rate of production of one or more C3 compounds by at least 10%, 15%, 20%, 25%, or 30%, (3) an increase in MEG yield by at least 10%, 15%, 20%, 25%, or 30%, and (4) an increase in the yield of one or more C3 compounds by at least 10%, 15%, 20%, 25%, or 30%.

[00551] In some aspects, MEG is produced at least 0.5 kg/m³ h, 1 kg/m³ h, at least 2 kg/m³ h, at least 3 kg/m³ h, at least 4 kg/m³ h, at least 5 kg/m³ h, 6 kg/m³ h, at least 7 kg/m³ h, at least 8 kg/m³ h, at least 9 kg/m³ h, at least 10 kg/m³ h, at least 15 kg/m³ h, or at least 20 kg/m³ h.

[00552] In some aspects, acetone is produced at least 0.2 kg/m³ h, 0.5 kg/m³ h, at least 1 kg/m³ h, at least 2 kg/m³ h, at least 3 kg/m³ h, at least 4 kg/m³ h, at least 5 kg/m³ h, 6 kg/m³ h, at least 7 kg/m³ h, at least 8 kg/m³ h, at least 9 kg/m³ h, at least 10 kg/m³ h, at least 15 kg/m³ h, or at least 20 kg/m³ h.

[00553] In some aspects, isopropanol is produced at least at least 0.2 kg/m³ h, 0.5 kg/m³ h, 1 kg/m³ h, at least 2 kg/m³ h, at least 3 kg/m³ h, at least 4 kg/m³ h, at least 5 kg/m³ h, 6 kg/m³ h, at least 7 kg/m³ h, at least 8 kg/m³ h, at least 9 kg/m³ h, at least 10 kg/m³ h, at least 15 kg/m³ h, or at least 20 kg/m³ h.

[00554] In some aspects, isopropanol is produced at least at least 0.5 kg/m³ h, 1 kg/m³ h, at least 2 kg/m³ h, at least 3 kg/m³ h, at least 4 kg/m³ h, at least 5 kg/m³ h, 6 kg/m³ h, at least 7 kg/m³ h, at least 8 kg/m³ h, at least 9 kg/m³ h, at least 10 kg/m³ h, at least 15 kg/m³ h, or at least 20 kg/m³ h.

[00555] In some aspects, propene is produced at least at least 0.5 kg/m³ h, 1 kg/m³ h, at least 2 kg/m³ h, at least 3 kg/m³ h, at least 4 kg/m³ h, at least 5 kg/m³ h, 6 kg/m³ h, at least 7 kg/m³ h, at least 8 kg/m³ h, at least 9 kg/m³ h, at least 10 kg/m³ h, at least 15 kg/m³ h, or at least 20 kg/m³ h.

[00556] In some aspects, the combined products of the biological processes of the present disclosure result in a production of at least 0.5 kg/m³ h, 1 kg/m³ h, at least 2 kg/m³ h, at least 3 kg/m³ h, at least 4 kg/m³ h, at least 5 kg/m³ h, 6 kg/m³ h, at least 7 kg/m³ h, at least 8 kg/m³ h, at least 9 kg/m³ h, at least 10 kg/m³ h, at least 15 kg/m³ h, or at least 20 kg/m³ h of MEG, acetone, isopropanol, propene, precursors thereof, and/or mixtures thereof.

Metabolic Engineering to Improve Flux through the C3 Pathway

[00557] C3 compounds are produced from Acetyl-CoA, which is a key metabolite in synthetic and oxidative pathways. The production of C3 has to compete for Acetyl-

CoA with natural reactions of the cell. Irreversible and strongly pushed reactions towards the C3 production are essential for improving C3 compounds yield, titer and/or productivity. Acetoacetyl CoA synthase and/or hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase are enzymes capable of pulling the flux through the C3 pathway.

[00558] The utilization of both strategies for expression of these enzymes to improve yield, titer and/or productivity of MEG pathway is new. MEG improvement is due to the higher flux of carbon through the pathway, pulled by the higher production of C3 from acetic acid. More acetic acid is produced to be converted to C3, accelerating the overall carbon through MEG pathway and decreasing leakages.

[00559] Acetoacetyl CoA synthase (nphT7) – Malonyl-CoA bypass with or without acetoacetyl-CoA thiolase (thlA) deletion. Acetoacetyl CoA synthase (NphT7 - EC:2.3.1.19) catalyzes the condensation of acetyl-CoA and malonyl-CoA to form acetoacetyl-CoA and CoA. The synthesis of acetoacetyl-CoA in *E. coli* is a reversible reaction catalyzed by acetoacetyl-CoA thiolase (EC 2.3.1.9) from two molecules of acetyl-CoA. Although acetoacetyl-CoA thiolase produces acetoacetyl-CoA, this enzyme prefers acetoacetyl-CoA thiolysis to acetoacetyl-CoA synthesis. The expression of nphT7 gene can be used to significantly increase the concentration of acetoacetyl-CoA in cells since the reaction is not reversible and has a strong pull due to the use of one ATP. It is expected that the expression of nphT7 improve yield, titer and/or productivity for C3 pathways due to a higher concentration of acetoacetyl-CoA that is converted to acetone, isopropanol or propene. However, the improvement of flux through the C3 pathway has a synergetic effect on xylose assimilation and conversion to MEG, improving yield, titer and/or productivity in the C2 pathway.

[00560] HMG-CoA bypass - hydroxymethylglutaryl-CoA synthase (ERG13) and hydroxymethylglutaryl-CoA lyase (YngG) with or without acetoacetyl-CoA transferase (AtoDA) deletion. HMG-CoA bypass is composed by two steps: condensation of Acetyl-CoA and acetoacetyl-CoA to form (S)-3-hydroxy-3-methylglutaryl-CoA and CoA by the Hydroxymethylglutaryl-CoA synthase (ERG13 - EC:2.3.3.10) and conversion of (S)-3-hydroxy-3-methylglutaryl-CoA to acetyl-CoA and acetoacetate by the Hydroxymethylglutaryl-CoA lyase (YngG - EC:4.1.3.4). Acetoacetate is the direct precursor of the C3 pathways for acetone, propanol and propene and can be produced by the Acetate CoA-transferase (AtoDA) native from *E. coli*. The expression of ERG13 and YngG can be

used to significantly increase the concentration of acetoacetate compared to the reaction performed by the Acetate CoA-transferase (AtoDA), since the transferase is reversible and dependent on the acetate concentration. HMG-CoA bypass poses an alternative that is essentially an energy-favored reaction and not dependent on acetate concentration and regulation. It is expected that the expression of HGM-CoA bypass improve yield, titer and/or productivity for C3 pathways due to a higher concentration of acetoacetate that is converted to C3 products. However, as already mentioned, the improvement of flux through the C3 pathway has a synergetic effect on xylose assimilation and conversion to MEG, improving yield, titer and/or productivity in the C2 pathway.

Metabolic Engineering of the Xylonate Pathway

[00561] The optimization of gene expression of the entire xylonate pathway will avoid carbon loss to side reactions, avoid intermediate accumulation and generate strains with better performance regarding yields, titer and productivity to both ethylene glycol and C3 compounds. In some aspects, the described optimizations are focused on the first and last step. In some aspects, different enzyme sources are considered for steps 1 to 4.

[00562] In some aspects, optimization is conducted not only aiming ethylene glycol production but also with attention to benefits/prejudice on C3 co-production.

[00563] The production of ethylene glycol through the xylonate pathway consists of 5 enzymatic steps. The optimized pathway for the co-production of ethylene glycol and acetone is described below:

[00564] D-xylonolactone is produced by the oxidation of D-xylose (EC 1.1.1.175 or 1.1.1.179).

[00565] Sources: *Caulobacter crescentus*, *Burkholderia xenovorans*, *Haloferax volcanii*, *Halomonas elongata*, *Pseudomonas fluorescens*, *Trichoderma reesei*, *Sus crofa*, *Pseudomonas putida*, *Sphingomonas elodea*

[00566] xylonolactone is hydrolyzed to yield D-xylonate (EC 3.1.1.68)

[00567] Sources: *Caulobacter crescentus*, *Burkholderia xenovorans*, *Haloferax volcanii*, *Halomonas elongata*, *Sphingomonas elodea*

[00568] D-xylonate is dehydrated to 2-keto-3-deoxy pentanoic acid (EC 4.2.1.82)

[00569] Sources: *Escherichia coli*, *Caulobacter crescentus*, *Burkholderia xenovorans*, *Haloferax volcanii*, *Halomonas elongata*, *Sphingomonas elodea*, *Pseudomonas sp.*, *Achromobacter xylosoxidans*, *Mesorhizobium sp.*, *Zymomonas mobilis*, *Agrobacte-*

rium tumefaciens, Herbaspirillum seropedicae, Actinoplanes missouriensis, Aspergillus oryzae

[00570] 2-keto-3-deoxy-pentanoic acid is converted to glycolaldehyde and pyruvate (EC 4.1.2.20).

[00571] Sources: Escherichia coli, Sulfolobus sp., Paraburkholderia phytofirmans, Sphingomonas wittichii, Pseudomonas sp., Azotobacter vinelandii, Scheffersomyces stipites, Picrophilus torridus. Trichoderma reesei.

[00572] Glycoaldehyde is reduced to ethylene glycol (EC 1.1.1.77)

[00573] Sources: Escherichia coli

[00574] The variations on the xylonate pathway, particularly in steps 1-3 that are responsible for initiating the flux of xylose through the pathway, can have a large impact on ethylene glycol productivity even though the overall yield might not be improved. C3 pathway is also positively affected, on both yield, titer and productivity. This aspect is probably related to the decrease of xylonic acid accumulation that in certain levels can be toxic to the host cell and decreases the carbon flux to pyruvate, decreasing the pool of acetyl-CoA for C3 production.

[00575] In some aspects, a modified microbe of the present disclosure comprises an overexpressed heterologous xylose dehydrogenase.

[00576] In some aspects, a modified microbe of the present disclosure comprises an overexpressed heterologous xylonolactonase.

[00577] In some aspects, a modified microbe of the present disclosure comprises a overexpressed homologous xylonate dehydratase or overexpression or expression of a heterologous xylonate dehydratase.

[00578] In some aspects, a modified microbe of the present disclosure comprises a overexpressed homologous 3-deoxy-D-glycerol pentanone sugar acid aldolase or overexpression or expression of a heterologous 3-deoxy-D-glycerol pentanone sugar acid aldolase.

[00579] In some aspects, a modified microbe of the present disclosure comprises a overexpressed homologous glycoaldehyde reductase.

[00580] In some aspects, a modified microbe of the present disclosure comprises a overexpressed homologous glycoaldehyde reductase.

[00581] In some aspects, a modified microbe of the present disclosure comprises (1) an overexpressed heterologous xylose dehydrogenase, (2) an overexpressed het-

erologous xylonolactonase, (3) a overexpressed homologous xylonate dehydratase or overexpression or expression of a heterologous xylonate dehydratase, (4) a overexpressed homologous glycoaldehyde reductase, and/or (5) overexpression of a homologous glycoaldehyde reductase.

[00582] In some aspects, the overexpressed, or expressed sequences are such due to being placed under the control of a non-native control sequences. In some aspects, the expression of heterologous xylose dehydrogenase in a modified microbe of the present disclosure is increased by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 200%, 250%, 300%, 400%, or 500% relative to an unmodified microbe.

[00583] In some aspects, the expression of a heterologous xylonolactonase in a modified microbe of the present disclosure is increased by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 200%, 250%, 300%, 400%, or 500% relative to an unmodified microbe.

[00584] In some aspects, the expression of a heterologous or homologous xylonate dehydratase in a modified microbe of the present disclosure is increased by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 200%, 250%, 300%, 400%, or 500% relative to an unmodified microbe.

[00585] In some aspects, the expression of a homologous or heterologous 3-deoxy-D-glycerol pentanone sugar acid aldolase in a modified microbe of the present disclosure is increased by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 200%, 250%, 300%, 400%, or 500% relative to an unmodified microbe.

[00586] In some aspects, the expression of a homologous glycoaldehyde reductase in a modified microbe of the present disclosure is increased by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%,

19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 200%, 250%, 300%, 400%, or 500% relative to an unmodified microbe.

Metabolic Engineering of the Acetate Pathway

[00587] In some aspects, the alteration of key enzymes of acetate metabolism can change the activity of the central pathways of *E. coli*. Deletions of genes *pta*, *ackA*, or *poxB*, or overexpression of *acs* can alter the co-regulation of the acetate metabolism, glyoxylate shunt, and the anaplerotic/gluconeogenic pathways, affecting the efficient assimilation of the carbon sources. The deletion of genes *pta*, *ackA*, *poxB*, or overexpression of acetyl-CoA synthetase improves xylulose assimilation and thus increase flux through the entire pathway, resulting in higher yields and productivity of ethylene glycol and consequently, C3 compounds. In addition, a higher expression of acetyl-CoA synthetase can enhance the ability of strains to use the acetate already present in the substrate as a carbon source.

[00588] The acetate pathway is composed by four enzymes: a phosphate acetyltransferase, an acetate kinase, a pyruvate oxidase and an acetyl-CoA synthetase. The phosphate acetyltransferase is codified by *pta* gene and catalyzes the reversible reaction: acetyl-CoA + phosphate $\leftrightarrow$ acetyl phosphate + coenzyme A (EC Number: 2.3.1.8). The acetate kinase is codified by *ackA* gene and catalyzes the reversible reaction: acetate + ATP $\leftrightarrow$ acetyl phosphate + ADP (EC Number: 2.7.2.1), being involved in the generation of most of the ATP formed catabolically during anaerobic growth (reaction 22, 23 and 24 Figure 1). The pyruvate oxidase is codified by *poxB* gene and catalyzes the reaction: pyruvate + a ubiquinone [inner membrane] + H₂O $\rightarrow$ CO₂ + acetate + an ubiquinol [inner membrane] (EC Number: 1.2.5.1), being the main pathway for acetate production in stationary phase. The acetyl-CoA synthetase is codified by *acs* gene and catalyzes the irreversible reaction: acetate + ATP + coenzyme A $\rightarrow$ acetyl-CoA + AMP + diphosphate (EC Number: 6.2.1.1), having a mainly anabolic role, scavenging acetate present in the extracellular medium.

[00589] The deletion of *pta*, *ackA* or *poxB* genes can alter the flux of carbon through the pathway, increasing not only the pool of acetyl-CoA available for C3 production but also the uptake and assimilation of xylose through MEG pathway. The disruption of the acetate futile cycle discharges more acetyl-CoA that is rapidly converted to C3 compounds through C3 synthetic pathway. In order to avoid a shortage of acetyl-

CoA, more pyruvate has to be produced through the conversion of xylose to MEG and DHAP or pyruvate, increasing the carbon flux through the pathway and leading to higher yields and productivity.

[00590] The pool of acetyl-CoA can also be increased by over-expressing *acs* gene (acetyl-CoA synthetase) or by increasing the amount of active Acs. The enzyme Acs is regulated by the Pat/CobB system, where the protein lysine acetyltransferase (Pka) inactivates Acs by acetylation, while the NAD⁺-dependent regulator protein deacetylase CobB releases Acs from repression by deacetylating it. Therefore, deletion of *patZ* gene, also known as *pka*, or overexpression of *cobB* gene can guarantee higher amounts of active Acs. Another way to increase Acs amount is by *arcA* gene deletion, a regulator of TCA genes expression, whose deletion takes to higher expression of *acs* gene.

[00591] In some aspects, a modified microbe of the present disclosure comprises a disrupted or deleted phosphate acetyltransferase (*pta*) nucleic acid sequence.

[00592] In some aspects, a modified microbe of the present disclosure comprises a disrupted or deleted acetate kinase (*ackA*) nucleic acid sequence.

[00593] In some aspects, a modified microbe of the present disclosure comprises a disrupted or deleted pyruvate oxidase (*poxB*) nucleic acid sequence.

[00594] In some aspects, a modified microbe of the present disclosure comprises a disrupted or deleted *arcA* regulator nucleic acid sequence.

[00595] In some aspects, a modified microbe of the present disclosure comprises a disrupted or deleted lysine acetyltransferase (*pka*) nucleic acid sequence.

[00596] In some aspects, a modified microbe of the present disclosure comprises a disrupted or deleted phosphate acetyltransferase (*pta*) nucleic acid sequence, acetate kinase (*ackA*) nucleic acid sequence, pyruvate oxidase (*poxB*) nucleic acid sequence, *arcA* regulator nucleic acid sequence, and/or lysine acetyltransferase (*pka*) nucleic acid sequence.

[00597] In some aspects, a modified microbe of the present disclosure comprises an overexpressed CobB regulator.

[00598] In some aspects, a modified microbe of the present disclosure comprises an overexpressed *acs* (acetyl-CoA synthetase).

[00599] In some aspects, a modified microbe of the present disclosure comprises an overexpressed CobB regulator and/or an overexpressed *acs* (acetyl-CoA synthetase).

[00600] In some aspects, a modified microbe of the present disclosure comprises an overexpressed CobB regulator, overexpressed *acs* (acetyl-CoA synthetase), a disrupted or deleted phosphate acetyltransferase (*pta*) nucleic acid sequence, acetate kinase (*ackA*) nucleic acid sequence, pyruvate oxidase (*poxB*) nucleic acid sequence, *arcA* regulator nucleic acid sequence, and/or lysine acetyltransferase (*pka*) nucleic acid sequence.

[00601] In some aspects, the overexpressed *acs* and/or CobB regulator are overexpressed due to being placed under the control of a non-native control sequences. In some aspects, the control sequence is an operator. In some aspects, the control sequence is a promoter. In some aspects, the control sequence is a constitutive promoter. In some aspects, the native control sequences are modified to cause the overexpression.

[00602] In some aspects, a modified microbe of the present disclosure comprises one or more mutations in one or more phosphate acetyltransferase nucleic acid sequences, in one or more acetate kinase nucleic acid sequences, in one or more pyruvate oxidase nucleic acid sequences, in one or more *arcA* regulator nucleic acid sequences, in one or more lysine acetyltransferase nucleic acid sequences.

[00603] In some aspects, the translation of one or more nucleic acid sequences encoding a phosphate acetyltransferase (*pta*) in a modified microbe of the present disclosure is reduced by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe.

[00604] In some aspects, the translation of one or more nucleic acid sequences encoding a acetate kinase (*ackA*) in a modified microbe of the present disclosure is reduced by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe.

[00605] In some aspects, the translation of one or more nucleic acid sequences encoding a pyruvate oxidase (*poxB*) in a modified microbe of the present disclosure is

reduced by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe.

[00606] In some aspects, the translation of one or more nucleic acid sequences encoding an *arcA* regulator in a modified microbe of the present disclosure is reduced by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe.

[00607] In some aspects, the translation of one or more nucleic acid sequences encoding a lysine acetyltransferase (*pka*) in a modified microbe of the present disclosure is reduced by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe.

[00608] In some aspects, the expression of *CobB* regulator in a modified microbe of the present disclosure is increased by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 200%, 250%, 300%, 400%, or 500% relative to an unmodified microbe.

[00609] In some aspects, the expression of *acs* (acetyl-CoA synthetase) in a modified microbe of the present disclosure is increased by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 200%, 250%, 300%, 400%, or 500% relative to an unmodified microbe.

Deletion of Competing Pathways To Improve Carbon Flux Through MEG and C3 Pathways

[00610] In some aspects, the deletion of key enzymes of competing pathways such as methylglyoxal synthase and glyoxylate carboligase will avoid carbon loss to side reactions redirecting the flux of carbon through the MEG and C3 pathways.

[00611] In some aspects, the modifications described herein are performed in microbes that have already been modified to coproduce MEG and one or more C3 compound such that the carbon flux is modulated in the MEG and/or C3 pathways in such a way as to allow for more efficient production of MEG and/or C3 compounds.

[00612] Methylglyoxal synthase (mgsA - EC:4.2.3.3) converts DHAP to methylglyoxal + Pi in *E. coli*. Methylglyoxal synthase can be further converted to pyruvate through D-lactate. This sequence provides a by-pass of the normal glycolytic reactions for the conversion of DHAP to pyruvate. Although methylglyoxal synthase is present in *E. coli* at a reasonable activity, it is possible that the normal intracellular concentrations of Pi and DHAP may prevent it being fully active. However, any factor which raised the DHAP concentration or decreased the Pi concentration would tend to de-inhibit the enzyme. DHAP can accumulate depending on the flux of carbon through glycolysis. The synthetic pathway for production of MEG from xylose has DHAP as an intermediate (Xylulose and Ribulose-1P pathways, for xylonate pathway methylglyoxal can be formed from pyruvate) and since the flux to the Pentose Phosphate Pathway is blocked, all the xylose has to pass through MEG synthetic pathway. This can generate an overflow of carbon through the synthetic pathway leading to accumulation of DHAP, which does not happen on WT uptake of xylose. Accumulation of DHAP de-inhibits mgsA that converts the DHAP to methylglyoxal. Deletion of mgsA can force the flux through MEG and C3 pathways improving MEG and C3 production and decreasing accumulation of intermediates.

[00613] Glyoxylate carboligase (gcl - EC:4.1.1.47) condenses two molecules of glyoxylate to form tartronate semialdehyde and carbon dioxide in *E. coli*. Glyoxylate carboligase can be formed from TCA or glycolate. Glycolate can be produced from glycolaldehyde decreasing the yield of MEG. The deletion of gcl can improve the overall yield of MEG and C3 by preventing the loss of glycolaldehyde (C2 branch) and glyoxylate through side reactions. The deletion of gcl can also maintain the carbon within the TCA cycle or converted it to pyruvate. The conversion of carbon from the TCA to pyruvate can increase the concentration of acetyl-CoA increasing the yield of C3 pathway.

[00614] Surprisingly, the deletion or disruption of mgsA and gcl has not only an effect on MEG production, but also increases the overall yield and/or productivity (rate of production) and or titer of compounds of the C3 pathway. This improvement is due to

an optimization of the flux through the pathway, modifying the acetic acid production profile and increasing or accelerating acetone production.

[00615] In some aspects, a modified microbe of the present disclosure comprises a disrupted methylglyoxal synthase (mgsA) nucleic acid sequence. In some aspects, a modified microbe of the present disclosure comprises a disrupted glyoxylate carboligase (gcl) nucleic acid sequence. In some aspects, a modified microbe of the present disclosure comprises (1) a disrupted methylglyoxal synthase (mgsA) nucleic acid sequence and (2) a disrupted glyoxylate carboligase (gcl) nucleic acid sequence.

[00616] In some aspects, a modified microbe of the present disclosure comprises one or more mutations in one or more methylglyoxal synthase nucleic acid sequences and/or one or more mutations in one or more glyoxylate carboligase nucleic acid sequences.

[00617] In some aspects, the translation of one or more nucleic acid sequences encoding a methylglyoxal synthase in a modified microbe of the present disclosure is reduced by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe.

[00618] In some aspects, the translation of one or more nucleic acid sequences encoding a glyoxylate carboligase in a modified microbe of the present disclosure is reduced by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe.

[00619] In some aspects, the translation of one or more nucleic acid sequences encoding (1) a glyoxylate carboligase and (2) a methylglyoxal synthase in a modified microbe of the present disclosure is reduced by at least about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% relative to an unmodified microbe.

[00620] In some aspects, the translation of one or more nucleic acid sequences encoding (1) a glyoxylate carboligase and (2) a methylglyoxal synthase in a modified microbe of the present disclosure is reduced by at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% relative to an unmodified microbe.

Table 3: Description of Sequences

SEQ ID NO: 1	<i>Pseudomonas cichorii</i> D-tagatose 3-epimerase DTE NT sequence
SEQ ID NO: 2	<i>Pseudomonas cichorii</i> D-tagatose 3-epimerase DTE codon optimized NT sequence
SEQ ID NO: 3	<i>Pseudomonas cichorii</i> D-tagatose 3-epimerase DTE AA sequence
SEQ ID NO: 4	<i>Rhodobacter sphaeroides</i> D-tagatose 3-epimerase FJ851309.1 NT sequence
SEQ ID NO: 5	<i>Rhodobacter sphaeroides</i> D-tagatose 3-epimerase FJ851309.1 AA sequence
SEQ ID NO: 6	<i>Escherichia coli</i> L-fuculokinase FucK NT sequence
SEQ ID NO: 7	<i>Escherichia coli</i> L-fuculokinase FucK codon optimized NT sequence
SEQ ID NO: 8	<i>Escherichia coli</i> L-fuculokinase fucK AA sequence
SEQ ID NO: 9	<i>Escherichia coli</i> L-fuculose phosphate aldolase fucA NT sequence
SEQ ID NO: 10	<i>Escherichia coli</i> L-fuculose phosphate aldolase fucA codon optimized NT sequence
SEQ ID NO: 11	<i>Escherichia coli</i> L-fuculose phosphate aldolase fucA AA sequence
SEQ ID NO: 12	<i>Escherichia coli</i> glycerol dehydrogenase gldA NT sequence
SEQ ID NO: 13	<i>Escherichia coli</i> glycerol dehydrogenase gldA AA sequence
SEQ ID NO: 14	<i>Saccharomyces cerevisiae</i> methylglyoxal reductase GRE2 NT sequence
SEQ ID NO: 15	<i>Saccharomyces cerevisiae</i> methylglyoxal reductase GRE2 AA sequence
SEQ ID NO: 16	<i>Saccharomyces cerevisiae</i> aldose reductase GRE3 NT sequence
SEQ ID NO: 17	<i>Saccharomyces cerevisiae</i> aldose reductase GRE3 AA sequence
SEQ ID NO: 18	<i>Escherichia coli</i> alcohol dehydrogenase yqhD* NT sequence
SEQ ID NO: 19	<i>Escherichia coli</i> alcohol dehydrogenase yqhD* codon optimized NT sequence
SEQ ID NO: 20	<i>Escherichia coli</i> alcohol dehydrogenase yqhD* AA sequence
SEQ ID NO: 21	<i>Escherichia coli</i> alcohol dehydrogenase yqhD NT sequence
SEQ ID NO: 22	<i>Escherichia coli</i> alcohol dehydrogenase yqhD codon optimized

	NT sequence
SEQ ID NO: 23	Escherichia coli alcohol dehydrogenase yqhD AA sequence
SEQ ID NO: 24	Escherichia coli methylglyoxal reductase ydjG NT sequence
SEQ ID NO: 25	Escherichia coli methylglyoxal reductase ydjG AA sequence
SEQ ID NO: 26	Escherichia coli lactaldehyde reductase fucO NT sequence
SEQ ID NO: 27	Escherichia coli lactaldehyde reductase fucO codon optimized NT sequence
SEQ ID NO: 28	Escherichia coli lactaldehyde reductase fucO AA sequence
SEQ ID NO: 29	Escherichia coli methylglyoxal reductase yafB (dkgB) [multifunctional] NT sequence
SEQ ID NO: 30	Escherichia coli methylglyoxal reductase yafB (dkgB) [multifunctional] AA sequence
SEQ ID NO: 31	Escherichia coli 2,5-diketo-D-gluconic acid reductase A yqhE (dkgA) NT sequence
SEQ ID NO: 32	Escherichia coli 2,5-diketo-D-gluconic acid reductase A yqhE (dkgA) AA sequence
SEQ ID NO: 33	Clostridium acetobutylicum acetyl coenzyme A acetyltransferase thlA NT sequence
SEQ ID NO: 34	Clostridium acetobutylicum acetyl coenzyme A acetyltransferase thlA codon optimized NT sequence
SEQ ID NO: 35	Clostridium acetobutylicum acetyl coenzyme A acetyltransferase thlA AA sequence
SEQ ID NO: 36	Escherichia coli acetyl coenzyme A acetyltransferase atoB NT sequence
SEQ ID NO: 37	Escherichia coli acetyl coenzyme A acetyltransferase atoB AA sequence
SEQ ID NO: 38	Saccharomyces cerevisiae acetyl coenzyme A acetyltransferase ERG10 NT sequence
SEQ ID NO: 39	Saccharomyces cerevisiae acetyl coenzyme A acetyltransferase ERG10 codon optimized NT sequence
SEQ ID NO: 40	Saccharomyces cerevisiae acetyl coenzyme A acetyltransferase ERG10 AA sequence
SEQ ID NO: 41	Escherichia coli Acetyl-CoA:acetoacetate-CoA transferase subunit atoA NT sequence
SEQ ID NO: 42	Escherichia coli Acetyl-CoA:acetoacetate-CoA transferase subunit atoA codon optimized NT sequence
SEQ ID NO: 43	Escherichia coli Acetyl-CoA:acetoacetate-CoA transferase subunit atoA AA sequence
SEQ ID NO: 44	Escherichia coli Acetyl-CoA:acetoacetate-CoA transferase subunit

	atoD NT sequence
SEQ ID NO: 45	Escherichia coli Acetyl-CoA:acetoacetate-CoA transferase subunit atoD codon optimized NT sequence
SEQ ID NO: 46	Escherichia coli Acetyl-CoA:acetoacetate-CoA transferase subunit atoD AA sequence
SEQ ID NO: 47	Clostridium acetobutylicum acetoacetate decarboxylase adc NT sequence
SEQ ID NO: 48	Clostridium acetobutylicum acetoacetate decarboxylase adc co- don optimized NT sequence
SEQ ID NO: 49	Clostridium acetobutylicum acetoacetate decarboxylase adc AA sequence
SEQ ID NO: 50	Clostridium beijerinckii acetoacetate decarboxylase adc NT se- quence
SEQ ID NO: 51	Clostridium beijerinckii acetoacetate decarboxylase adc codon optimized NT sequence
SEQ ID NO: 52	Clostridium beijerinckii acetoacetate decarboxylase adc AA se- quence
SEQ ID NO: 53	Homo sapiens ketohexokinase C khk-C cDNA sequence
SEQ ID NO: 54	Homo sapiens ketohexokinase C khk-C codon optimized cDNA sequence
SEQ ID NO: 55	Homo sapiens ketohexokinase C khk-C AA sequence
SEQ ID NO: 56	Homo sapiens Fructose-bisphosphate aldolase B aldolase B cDNA sequence
SEQ ID NO: 57	Homo sapiens Fructose-bisphosphate aldolase B aldolase B codon optimized cDNA sequence
SEQ ID NO: 58	Homo sapiens Fructose-bisphosphate aldolase B aldolase B AA se- quence
SEQ ID NO: 59	Caulobacter crescentus D-xylose 1-dehydrogenase xylB NT se- quence
SEQ ID NO: 60	Caulobacter crescentus D-xylose 1-dehydrogenase xylB codon optimized NT sequence
SEQ ID NO: 61	Caulobacter crescentus D-xylose 1-dehydrogenase xylB AA se- quence
SEQ ID NO: 62	Haloferax volcanii D-xylose 1-dehydrogenase xdh1, HVO_B0028 NT sequence
SEQ ID NO: 63	Haloferax volcanii D-xylose 1-dehydrogenase xdh1, HVO_B0028 AA sequence
SEQ ID NO: 64	Trichoderma reesei D-xylose 1-dehydrogenase xyd1 NT se- quence

SEQ ID NO: 65	Trichoderma reesei D-xylose 1-dehydrogenase xyd1 AA sequence
SEQ ID NO: 66	Caulobacter crescentus Xylonolactonase xylC NT sequence
SEQ ID NO: 67	Caulobacter crescentus Xylonolactonase xylC AA sequence
SEQ ID NO: 68	Caulobacter crescentus xylonate dehydratase xylD NT sequence
SEQ ID NO: 69	Caulobacter crescentus xylonate dehydratase xylD AA sequence
SEQ ID NO: 70	Escherichia coli xylonate dehydratase yjhG NT sequence
SEQ ID NO: 71	Escherichia coli xylonate dehydratase yjhG codon optimized NT sequence
SEQ ID NO: 72	Escherichia coli xylonate dehydratase yjhG AA sequence
SEQ ID NO: 73	Escherichia coli xylonate dehydratase yagF NT sequence
SEQ ID NO: 74	Escherichia coli xylonate dehydratase yagF codon optimized NT sequence
SEQ ID NO: 75	Escherichia coli xylonate dehydratase yagF AA sequence
SEQ ID NO: 76	Escherichia coli Uncharacterized lyase yjhH NT sequence
SEQ ID NO: 77	Escherichia coli Uncharacterized lyase yjhH codon optimized NT sequence
SEQ ID NO: 78	Escherichia coli Uncharacterized lyase yjhH AA sequence
SEQ ID NO: 79	Escherichia coli Probable 2-keto-3-deoxy-galactonate aldolase yagE NT sequence
SEQ ID NO: 80	Escherichia coli Probable 2-keto-3-deoxy-galactonate aldolase yagE codon optimized NT sequence
SEQ ID NO: 81	Escherichia coli Probable 2-keto-3-deoxy-galactonate aldolase yagE AA sequence
SEQ ID NO: 82	Scheffersomyces stipitis D-xylose reductase xyl1 NT sequence
SEQ ID NO: 83	Scheffersomyces stipitis D-xylose reductase xyl1 codon optimized NT sequence
SEQ ID NO: 84	Scheffersomyces stipitis D-xylose reductase xyl1 AA sequence
SEQ ID NO: 85	Saccharomyces cerevisiae aldose reductase GRE3 NT sequence
SEQ ID NO: 86	Saccharomyces cerevisiae aldose reductase GRE3 codon optimized NT sequence
SEQ ID NO: 87	Saccharomyces cerevisiae aldose reductase GRE3 AA sequence
SEQ ID NO: 88	Scheffersomyces stipitis D-xylulose reductase xyl2 NT sequence
SEQ ID NO: 89	Scheffersomyces stipitis D-xylulose reductase xyl2 codon optimized NT sequence
SEQ ID NO: 90	Scheffersomyces stipitis D-xylulose reductase xyl2 AA sequence
SEQ ID NO: 91	Trichoderma reesei Xylitol dehydrogenase xdh1 NT sequence
SEQ ID NO: 92	Trichoderma reesei Xylitol dehydrogenase xdh1 AA sequence
SEQ ID NO: 93	Pyromyces sp. xylose isomerase xylA NT sequence

SEQ ID NO: 94	Pyromyces sp. xylose isomerase xylA codon optimized NT sequence
SEQ ID NO: 95	Pyromyces sp. xylose isomerase xylA AA sequence
SEQ ID NO: 96	Clostridium acetobutylicum butyrate-acetoacetate CoA-transferase, complex A ctfA NT sequence
SEQ ID NO: 97	Clostridium acetobutylicum butyrate-acetoacetate CoA-transferase, complex A ctfA AA sequence
SEQ ID NO: 98	Clostridium acetobutylicum butyrate-acetoacetate CoA-transferase, subunit B ctfB NT sequence
SEQ ID NO: 99	Clostridium acetobutylicum butyrate-acetoacetate CoA-transferase, subunit B ctfB AA sequence
SEQ ID NO: 100	Escherichia coli (strain K12) Acetyl-CoA:acetoacetate-CoA transferase subunit atoA NT sequence
SEQ ID NO: 101	Escherichia coli (strain K12) Acetyl-CoA:acetoacetate-CoA transferase subunit atoA AA sequence
SEQ ID NO: 102	Escherichia coli (strain K12) Acetyl-CoA:acetoacetate-CoA transferase subunit atoD NT sequence
SEQ ID NO: 103	Escherichia coli (strain K12) Acetyl-CoA:acetoacetate-CoA transferase subunit atoD AA sequence
SEQ ID NO: 104	Clostridium beijerinckii secondary alcohol dehydrogenase adh NT sequence
SEQ ID NO: 105	Clostridium beijerinckii secondary alcohol dehydrogenase adh codon optimized NT sequence
SEQ ID NO: 106	Clostridium beijerinckii secondary alcohol dehydrogenase adh AA sequence
SEQ ID NO: 107	Clostridium carboxidivorans alcohol dehydrogenase adh NT sequence
SEQ ID NO: 108	Clostridium carboxidivorans alcohol dehydrogenase adh AA sequence
SEQ ID NO: 109	Escherichia coli soluble pyridine nucleotide transhydrogenase NT sequence
SEQ ID NO: 110	Escherichia coli soluble pyridine nucleotide transhydrogenase AA sequence
SEQ ID NO: 111	Forward primer to amplify fucA and fucO
SEQ ID NO: 112	Reverse primer to amplify fucA and fucO
SEQ ID NO: 113	Forward primer to amplify fucK

SEQ ID NO: 114	Reverse primer to amplify fucK
SEQ ID NO: 115	Forward primer to amplify thl
SEQ ID NO: 116	Reverse primer to amplify thl
SEQ ID NO: 117	Forward primer to amplify fucO
SEQ ID NO: 118	Reverse primer to amplify fucO
SEQ ID NO: 119	Forward primer to amplify atoA/D
SEQ ID NO: 120	Reverse primer to amplify atoA/D

EXAMPLES**Example 1. Co-production of ethylene glycol (MEG), acetone and isopropanol (IPA) in E. coli using xylulose-1-phosphate pathway**

[00621] E. coli K12 strain MG1655 was used as host for the expression of MEG+IPA pathways. Two genes that could divert the carbon flux from MEG+IPA pathway were identified as target for deletion: aldA and xylB genes. A MEG pathway was integrated at xylB locus, enabling a stable integration concomitantly with xylB deletion. Production of MEG through xylulose-1-phosphate pathway requires the expression of three genes: khkC (D-xylulose-1-kinase enzyme), aldoB (D-xylulose -1-phosphate aldolase enzyme) and fucO (aldehyde reductase enzyme). khkC (KhkC amino acid sequence set forth in SEQ ID NO: 55) and aldoB (AldoB amino acid sequence set forth in SEQ ID NO: 58) genes were codon optimized for E. coli and synthesized. FucO gene is native from E. coli and was PCR amplified (Forward Primer: ATGGCTAACAGAATGATTCTG (SEQ ID NO: 117) and Reverse Primer: TTACCAGGCGGTATGGTAAAGCT (SEQ ID NO: 118)).

[00622] A MEG integration cassette was composed of an operon containing khkC (D-xylulose-1-kinase enzyme), aldoB (D-xylulose-1-phosphate aldolase enzyme), fucO (aldehyde reductase enzyme) genes and rplM terminator under the control of proD promoter (constitutive promoter) flanked by regions homologous to upstream and downstream of xylB gene. For each gene a specific RBS sequence was utilized. An antibiotic marker was also added to the cassette for the selection of transformants. The cassette was constructed using In-fusion commercial kit, confirmed by sequencing and

transformed in *E. coli* K12 MG1655 strain. The proper integration of a MEG pathway at *xylB* locus, yielding a deleted *xylB* strain with a MEG pathway integrated, was confirmed by sequencing.

[00623] The strain harboring a MEG pathway at *xylB* locus was used as host for integration of an IPA pathway at *aldA* locus, enabling a stable integration concomitantly with *aldA* deletion. Production of isopropanol requires the expression of five genes: *thl* (thiolase), *atoA/D* (acetate:acetoacetyl-CoA transferase), *adc* (acetoacetate decarboxylase) and *adh* (secondary alcohol dehydrogenase). *atoA/D* gene is native from *E. coli* and was PCR amplified (Forward Primer: CTGTTGTTATATTGTAATGATGTATGCAA-GAGGGATAAA (SEQ ID NO: 119) and Reverse Primer: TATATCTCCTTCTTAAAGTTCATAAATCACCCCGTTGC (SEQ ID NO: 120)). *thl* (*Thl* amino acid sequence set forth in SEQ ID NO: 35), *adc* (*Adc* amino acid sequence set forth in SEQ ID NO: 49) and *adh* (*Adh* amino acid sequence set forth in SEQ ID NO: 106) were codon optimized for *E. coli* and synthesized.

[00624] An IPA integration cassette was composed of an operon containing *thl* (thiolase), *adh* (secondary alcohol dehydrogenase), *adc* (acetoacetate decarboxylase), *atoA/D* (acetate:acetoacetyl-CoA transferase) genes and T1 terminator under the control of a medium strength constitutive promoter (modified from *RecA*) flanked by regions homologous to upstream and downstream of *aldA* gene. For each gene a specific RBS sequence was utilized. An antibiotic marker was included into the cassette for the selection of transformants. The cassette was constructed using In-fusion commercial kit, confirmed by sequencing and transformed in *E. coli* K12 MG1655 strain. The proper integration of an IPA pathway at *aldA* locus, yielding a deleted *aldA* strain with an IPA pathway integrated, was confirmed by sequencing.

[00625] The *xylB aldA* deleted strain with MEG and IPA pathways integrated in the genome was inoculated in 3mL of TB media for pre-culture. After 16 hours of cultivation, 100% of the pre-culture was transferred to 100 mL of TB media containing 15g/L of xylose. The flasks were incubated at 37°C, 250 rpm until complete consumption of xylose. The initial OD of the cultivation was 0.3.

[00626] Xylose was fully consumed after 30 hours of cultivation (FIG. 4). Ethylene glycol, acetone and isopropanol reached a maximum titer of 3.5 g/L, 70 mg/L and 400 mg/L respectively.

[00627] The overall yield of co-production was calculated considering the amount of ethylene glycol, isopropanol and acetone produced per gram of xylose consumed. MEG is the product with the highest yield, 0.237 g/g, followed by isopropanol, 0.029 g/g and acetone, 0.006 g/g (FIG. 7). The best co-production yield, obtained after 48 hours of fermentation, was 0.27 g products/g xylose (44% of maximum theoretical yield).

Example 2. Co-production of ethylene glycol (MEG), acetone and isopropanol (IPA) in *E. coli* using xylonate pathway

[00628] *E. coli* K12 strain MG1655 was used as host for the expression of MEG+IPA pathways. Two genes that could divert the carbon flux from MEG+IPA pathway were identified as target for deletion: *aldA* and *xylA* genes. A MEG pathway was integrated at *xylA* locus, enabling a stable integration concomitantly with *xylA* deletion. Production of MEG through a xylonate pathway requires the expression of two genes: *xdh* (*Xdh* amino acid sequence set forth in SEQ ID NO: 61) from *Caulobacter crescentus* was codon optimized for *E. coli* and synthesized. *FucO* gene is native from *E. coli* and was PCR amplified (Forward Primer: ATGGCTAACAGAATGATTCTG (SEQ ID NO: 117) and Reverse Primer: TTACCAGGCGGTATGGTAAAGCT (SEQ ID NO: 118)). Two other native enzymes could be overexpressed to improve MEG production through a xylonate pathway: D-xylonate dehydratase (*yjhG*, *yagF*, or homologs thereof) and aldolase (*yjhH*, *yagE*, or homologs thereof).

[00629] A MEG integration cassette was composed of an operon containing *xdh* (D-xylose dehydrogenase), *fucO* (aldehyde reductase enzyme) genes and *rnpB* terminator under the control of *proD* promoter (constitutive promoter) flanked by regions homologous to upstream and downstream of *xylA* gene. For each gene a specific RBS sequence was utilized. An antibiotic marker was also added to the cassette for the selection of transformants. The cassette was constructed using In-fusion commercial kit, confirmed by sequencing and transformed in *E. coli* K12 MG1655 strain. The proper integration of a MEG pathway at *xylA* locus, yielding a deleted *xylA* strain with a MEG pathway integrated, was confirmed by sequencing.

[00630] The strain harboring a MEG pathway at *xylA* locus was used as host for integration of an IPA pathway at *aldA* locus, enabling a stable integration concomitantly with *aldA* deletion. Production of isopropanol requires the expression of five genes: *thl* (thiolase), *atoA/D* (acetate:acetoacetyl-CoA transferase), *adc* (acetoacetate decarboxylase) and *adh* (secondary alcohol dehydrogenase). *AtoA/D* gene is native from *E. coli*

and was PCR amplified (Forward Primer: CTGTTGTTATATTGTAATGATGTATGCAAGAGGGATAAA (SEQ ID NO: 119) and Reverse Primer: TATATCTCCTTCTTA AAGTTCATAAATCACCCCGTTGC (SEQ ID NO: 120)). thl (Thl amino acid sequence set forth in SEQ ID NO: 35), adc (Adc amino acid sequence set forth in SEQ ID NO: 49) and adh (Adh amino acid sequence set forth in SEQ ID NO: 106) were codon optimized for *E. coli* and synthesized.

[00631] An IPA integration cassette was composed of an operon containing thl (thiolase), adh (secondary alcohol dehydrogenase), adc (acetoacetate decarboxylase), atoA/D (acetate:acetoacetyl-CoA transferase) genes and T1 terminator under the control of a medium strength constitutive promoter (modified from RecA) flanked by regions homologous to upstream and downstream of aldA gene. For each gene a specific RBS sequence was utilized. An antibiotic marker was included into the cassette for the selection of transformants. The cassette was constructed using In-fusion commercial kit, confirmed by sequencing and transformed in *E. coli* K12 MG1655 strain. The proper integration of an IPA pathway at aldA locus, yielding a deleted aldA strain with an IPA pathway integrated, was confirmed by sequencing.

[00632] The xylA aldA deleted strain with MEG and IPA pathways integrated in the genome was inoculated in 3mL of TB media for pre-culture. After 16 hours of cultivation, 100% of the pre-culture was transferred to 100 mL of TB media containing 15g/L of xylose. The flasks were incubated at 37°C, 250 rpm until complete consumption of xylose. The initial OD of the cultivation was 0.3.

[00633] Xylose was fully consumed before 24 hours of cultivation (FIG. 8). Ethylene glycol, acetone and isopropanol reached a maximum titer of 5 g/L, 170 mg/L and 420 mg/L respectively.

[00634] The overall yield of co-production was calculated considering the amount of ethylene glycol, isopropanol and acetone produced per gram of xylose consumed. MEG is the product with the highest yield, 0.339 g/g, followed by isopropanol, 0.028 g/g and acetone, 0.008 g/g (FIG. 9). The best co-production yield, obtained after 48 hours of fermentation, was 0.375 g products/g xylose (61% of maximum theoretical yield).

Example 3. Direct production of propylene from glucose

[00635] Vectors pZs*13 containing an IPA pathway in an operon under pLacO promoter and pET28a containing LinD gene were co-transformed into BL21Star (DE3) using electroporation. Production of isopropanol requires the expression of five genes:

thl (thiolase), atoA/D (acetate:acetoacetyl-CoA transferase), adc (acetoacetate decarboxylase) and adh (secondary alcohol dehydrogenase). atoA/D gene is native from *E. coli* and was PCR amplified (Forward Primer: CTGTTGTTATATTGTAATGATGTATGCAAGAGGGATAAA (SEQ ID NO: 119) and Reverse Primer: TATATCTCCTTCTTAAAGTTCATAAATCACCCCGTTGC (SEQ ID NO: 120)). thl (Thl amino acid sequence set forth in SEQ ID NO: 35), adc (Adc amino acid sequence set forth in SEQ ID NO: 49) and adh (Adh amino acid sequence set forth in SEQ ID NO: 106) were codon optimized for *E. coli* and synthesized. An operon containing thl (thiolase), adh (secondary alcohol dehydrogenase), adc (acetoacetate decarboxylase), atoA/D (acetate:acetoacetyl-CoA transferase) genes and T1 terminator under the control of the inducible promoter pLLacO was constructed in a pZs*13 backbone. The candidate selection was done using kanamycin and ampicillin in LB medium. The strain herein was referred to as IPA+LinD. This combination of plasmids provides a strain capable of producing isopropanol from glucose and also expressing linalool isomerase dehydratase enzyme.

[00636] One single colony of IPA+LinD, pZs*13_IPA and pET28a_LinD was inoculated in TB medium containing 10g/L glycerol supplemented with kanamycin (50 µg/mL) and ampicillin (100 µg/mL) at 37°C, 220rpm. After 20 hours, a new inoculation was done using optical density of 0.2 in TB medium containing 1.5g/L glycerol supplemented with appropriate antibiotics at 37°C, 220rpm. After 3 hours, the OD achieved 1.0 at 600nm and IPTG was added to a final concentration of 1mM. The flasks were incubated at 18°C, 220rpm.

[00637] After 16 hours, the OD was measured and the cultures were concentrated to reach OD 20 using the following media as described for each assay:

- (a) pZs*13_IPA in TB 20g/L glucose (control for isopropanol production),
- (b) IPA+LinD in TB 10g/L glycerol and 3g/L isopropanol (control for propylene production),
- (c) IPA+LinD in TB 20g/L glucose and 3g/L isopropanol (control for propylene production),
- (d) IPA+LinD in TB 20g/L glucose (candidate 1 for propylene production),
- (e) IPA+LinD in TB 20g/L glucose (candidate 2 for propylene production)

[00638] One aliquot of all cultures were lysate for expression analysis and the cells were collected by centrifugation at 5000 rpm for 20 min and 4°C. The pellet was kept in -80°C for 1 hour then it was thawed on ice and resuspended in 10% of original volume

in Tris-HCl 50mM pH 7.5. The lysis was done by sonication (3-5 cycles, 10/10 minutes, 25% amplitude) on ice after that to separate the soluble fraction it was centrifuged at 5000 rpm for 30 min at 4°C. The samples were heated at 95°C for 10 minutes and analyzed in SDS-PAGE (FIG. 10).

[00639] 1.0 mL aliquots of each culture were placed in 2mL headspace vials in triplicate and incubated at 37°C, 225 rpm. At the end of 116 hours of incubation the vials were removed from the shaking incubator and the propylene and isopropanol concentration was analyzed in GC-MS. A control containing only TB medium 20g/L glucose was done in order to verify contamination in the end of incubation period. 1.0 mL of the headspace phase was injected in gas chromatograph (Focus GC - Thermo) equipped with electron impact mass spectrometer detector (ISQ – Thermo). Helium was used as a carrier gas with a flow rate of 1.5 mL/min, the split rate used was 10 with a split flow of 15 mL/min. The volatile compounds were separated in a HP-Plot/Q column (Agilent) with initial temperature held at 90°C for 1.0 min followed by a first ramp at 13.3°C/min to 130°C and a second one at 45°C/min to 200°C held for 1 min. The retention time of propylene under these conditions was 1.51 min and of isopropanol was 4.3 min. The product reaction was identified both by comparison with propylene and isopropanol standards and by comparison with a data base of mass fragmentation.

[00640] The production of isopropanol in assays (a), (d) and (e) were 0.5 g/L and in (b) and (c) 3.0g/L as expected. The production of 4 10⁻⁵ mM of propylene was observed in the assay (b) positive control for propylene and a significant production was observed in the assays (d) and (e), candidates with IPA+LinD co-transformed (FIG. 11). No amount of propylene was observed in the control reaction that contained only TB medium.

Example 4: Expression of malonyl-CoA bypass in MEG + acetone co-producing strain – via xylonate pathway

[00641] The E. coli K12 strain MG1655 was used as the host for the deletion of two genes that could divert the carbon flux from MEG+Acetone pathway: aldA and xylA. The genes were successfully deleted and the deletions were confirmed by PCR and sequencing.

[00642] The next step was the integration of the MEG pathway. An operon expressed under control of the proD promoter containing xdh gene (xylose dehydrogenase) and fucO gene (glycoaldehyde reductase), encoding respectively for the first and

last enzymes of the xylonate pathway, was integrated in the E.coli genome and an additional copy of xdh gene also under control of proD promoter was integrated in a different loci.

[00643] The last step was the integration of the acetone pathway. An operon expressed under control of OXB11 promoter containing thIA gene (acetoacetyl-CoA thiolase); atoAD genes (acetate:acetoacetyl-CoA transferase) and adc gene (acetoacetate decarboxylase) was integrated in the E.coli genome, generating the base strain. All the integrations were confirmed by PCR and sequencing. nphT7 gene (acetoacetyl CoA synthase) was expressed under the control of the GAPDH promoter in a pZS* vector backbone. The plasmid was constructed using an In-fusion commercial kit and confirmed by sequencing. The confirmed plasmid was transformed in the base strain. Colonies from transformations were inoculated in 5 mL of mineral media containing 12.85 g/L of xylose and 2.15 g/L of glucose for pre-culture. After 16 hours of cultivation 5% of the pre-culture was transferred to 100 mL of fresh media. The flasks were incubated at 37°C, 250 rpm until complete consumption of glucose and xylose. The initial OD of the cultivation was 0.1. For all strains, xylose was fully consumed after 48 hours of cultivation.

[00644] After 24 hours of cultivation approximately 1.3 g/L of MEG could be detected in the parental strain while 1.7 g/L could be detected at the same time in the strain with nphT7 expressed (FIG. 12A). The strains produced approximately 4 g/L of MEG in 48h of cultivation while the total amount of acetone was increased 60% (FIG. 12B), probably related to the higher production of acetic acid (FIG. 12C). The peak production of xylonic acid was decreased 2.9 times (FIG. 12D). The expression of nphT7 gene provided an improvement at velocity of co-production in relation with its parental strain.

Example 5: Expression HMG-CoA in MEG + acetone co-producing strain – via xylulose pathway

[00645] The E. coli K12 strain MG1655 was used as the host for the deletion of two genes that could divert the carbon flux from MEG + Acetone pathway: aldA and xylB. The genes were successfully deleted and the deletions were confirmed by PCR and sequencing.

[00646] The next step was the integration of the MEG pathway. An operon expressed under control of the proD promoter containing khk-C gene (ketohexokinase),

aldoB gene (fructose-1,6-bisphosphate aldolase) and fucO gene (glycoaldehyde reductase) was integrated in the E.coli genome and an additional copy of khk-C and aldoB genes also under control of proD promoter was integrated in a different loci.

[00647] The last step was the integration of acetone pathway. An operon expressed under control of OXB11 promoter containing thIA gene (acetoacetyl-CoA thiolase); atoAD genes (acetate:acetoacetyl-CoA transferase) and adc gene (acetoacetate decarboxylase) was integrated in the E.coli genome, generating the base strain. All the integrations were confirmed by PCR and sequencing. ERG13 gene (hydroxymethylglutaryl-CoA synthase) and yngG gene (hydroxymethylglutaryl-CoA lyase) were expressed in operon and under the control of the Tac promoter in a pZA vector backbone. The plasmid was constructed using an In-fusion commercial kit and confirmed by sequencing. The confirmed plasmid was transformed in the base strain. Colonies from transformations were inoculated in 5 mL of mineral media containing 12.85 g/L of xylose and 2.15 g/L of glucose for pre-culture. After 16 hours of cultivation 5% of the pre-culture was transferred to 100 mL of fresh media. The flasks were incubated at 37°C, 250 rpm until complete consumption of glucose and xylose. The initial OD of the cultivation was 0.1. For all strains, xylose was fully consumed after 55 hours of cultivation.

[00648] After 32 hours of cultivation approximately 1.2 g/L of MEG could be detected in the parental strain while 1.9 g/L could be detected at the same time in the strain with HMG-CoA expressed (**FIG. 13A**). The strains produced approximately 4 g/L of MEG in 55h of cultivation while the total amount of acetone was increased 41% (**FIG. 13B**), with little effect on acetic acid production (**FIG. 13C**) and xylulose accumulation (**FIG. 13D**). The expression of HMG-CoA by-pass guaranteed an improvement at velocity of co-production in relation with its parental strain.

Example 6: Replacement of exogenous atoDA by ERG13 and yngG in acetone operon and deletion of endogenous atoDA in a MEG + Acetone co-producing strain via xylulose pathway with deletion of pta gene

[00649] The E. coli K12 strain MG1655 was used as host for the deletion of two genes that could divert the carbon flux from the MEG + Acetone pathway: aldA and xylB. The genes were successfully deleted and the deletions were confirmed by PCR and sequencing.

[00650] The next step was the integration of the MEG pathway. An operon expressed under the control of the proD promoter containing khk-C gene (ketoheko-

nase), *aldoB* gene (fructose-1,6-bisphosphate aldolase), and *fucO* gene (glycoaldehyde reductase) was integrated in the *E. coli* genome and an additional copy of *khk-C* and *aldoB* genes also under the control of the OXB20 promoter were integrated in a different locus.

[00651] The next step was the integration of the acetone pathway via an operon in the *E. coli* genome. An operon expressed under control of OXB11 promoter containing *thlA* gene (acetoacetyl-CoA thiolase); *atoAD* genes (acetate:acetoacetyl-CoA transferase) and *adc* gene (acetoacetate decarboxylase) was integrated into the *E. coli* genome, generating the base strain. All the integrations were confirmed by PCR and sequencing.

[00652] The next step was the deletion of *pta* gene. This gene was successfully deleted and the deletion was confirmed by PCR and sequencing. The base strain was used as the host strain for the deletion of exogenous *atoDA* present at acetone operon with integration of *ERG13* and *yngG* genes and deletion of endogenous *atoDA*. The *ERG13* and *yngG* genes were successfully integrated, *atoDA* gene was successfully deleted, and both modifications were confirmed by PCR and sequencing.

[00653] Colonies of the modified strains were inoculated in 5 mL of mineral media for pre-culture. After 16 hours of cultivation, the pre-culture was transferred to 100 mL of mineral media containing 12.85 g/L of xylose and 2.15 g/L of glucose. The flasks were incubated at 37°C, 250 rpm until complete consumption of xylose. The initial OD of the cultivation was 0.2.

[00654] Higher amounts of MEG were detected for *atoDA::ERG13,yngG ΔatoDA* (FIG. 14B) strain in relation with the parental strain. Compare with FIG. 14A. The replacement of exogenous *atoDA* gene by *ERG13* and *yngG* gene coupled with the deletion of endogenous *atoDA* resulted in an improved rate and amount of MEG production compared to the parental strain.

Example 7: Expression of xylonate dehydratase *yagF* and *pta* deletion in a MEG + Acetone co-producing strain via xylonate pathway

[00655] The *E. coli* K12 strain MG1655 was used as host for the deletion of two genes that could divert the carbon flux from the MEG+Acetone pathway: *aldA* and *xylA*. The genes were successfully deleted and the deletions were confirmed by PCR and sequencing.

[00656] The next step was the integration of the MEG pathway. An operon expressed under control of the proD promoter containing xdh gene (xylose dehydrogenase) and fucO gene (glycoaldehyde reductase), encoding respectively for the first and last enzymes of the xylonate pathway, was integrated in the E.coli genome and an additional copy of xdh gene also under control of proD promoter was integrated in a different loci.

[00657] The next step was the integration of the acetone pathway via an operon in the E.coli genome. An operon expressed under control of OXB11 promoter containing thlA gene (acetoacetyl-CoA thiolase); atoAD genes (acetate:acetoacetyl-CoA transferase) and adc gene (acetoacetate decarboxylase) was integrated into the E. coli genome, generating the base strain. All the integrations were confirmed by PCR and sequencing.

[00658] The last step was the deletion of pta gene. This gene was successfully deleted and the deletion was confirmed by PCR and sequencing. Plasmid containing xylonate dehydratase yagF sequence was expressed under the control of the OXB11 promoter in a pZS* vector backbone. The plasmid was constructed using an In-fusion commercial kit and confirmed by sequencing. The confirmed plasmid was transformed in the base strain.

[00659] Colonies from transformations were inoculated in 5 mL of mineral media for pre-culture. After 16 hours of cultivation, the pre-culture was transferred to 100 ml of mineral media containing 12.85 g/L of xylose and 2.15 g/L of glucose. The flasks were incubated at 37°C, 250 rpm until complete consumption of xylose. The initial OD of the cultivation was 0.2.

[00660] Higher amounts of MEG and acetone were detected for Δ pta + yagF overexpression (FIG. 15) strain in relation with the Δ pta strain. Expression of yagF resulted in an improvement at amount of MEG and acetone production compared to the parental strain.

Example 8: Deletion of mgsA in a MEG + Acetone co-producing strain via xylonate pathway

[00661] The *E. coli* K12 strain MG1655 was used as host for the deletion of two genes that could divert the carbon flux from the MEG + C3 compound pathway: aldA and xylA. The deletions were confirmed by PCR and sequencing.

[00662] The next step was the integration of the MEG pathway. An operon expressed under the control of the proD promoter containing xdh gene (xylose dehydrogenase) and fucO gene (glycoaldehyde reductase), encoding respectively the first and last enzymes of the xylonate pathway, were integrated in a different loci.

[00663] The last step was the integration of the acetone pathway via an operon in the *E.coli* genome. An operon expressed under control of OXB11 promoter containing thlA gene (acetoacetyl-CoA thiolase); atoAD genes (acetate:acetoacetyl-CoA transferase) and adc gene (acetoacetate decarboxylase) was integrated into the *E. coli* genome, generating the base strain. All the integrations were confirmed by PCR and sequencing. The mgsA gene was deleted in the base strain and the deletion was confirmed by PCR and sequencing.

[00664] Colonies were inoculated in 5 mL of mineral media containing 12.85 g/L of xylose and 2.15 g/L of glucose for pre-culture. After 16 hours of cultivation, 5% of the pre-culture was transferred to 100 mL of fresh media. The flasks were incubated at 37°C, 250 rpm until complete consumption of glucose and xylose. The initial OD of the cultivation was 0.1. For all strains, xylose was fully consumed after 48 hours of cultivation.

[00665] After 24 hours of cultivation approximately 1.1 g/L of MEG was detected in the parental strain while 1.7 g/L was detected at the same time point in the strain with mgsA deleted (**FIG. 16A**). The strains produced approximately 4 g/L of MEG in 48h of cultivation while the total amount of acetone was increased 1.7 times (**FIG. 16B**), that was related to the higher production of acetic acid (**FIG. 16C**). The peak production of xylonic acid was decreased by 41% (**FIG. 16D**). The deletion of mgsA provided an improvement at velocity of co-production in relation with the parental strain.

Example 9: Deletion of mgsA in a MEG + Acetone co-producing strain via xylulose pathway

[00666] The *E. coli* K12 strain MG1655 was used as host for the deletion of two genes that could divert the carbon flux from MEG + IPA pathway: aldA and xylB. The genes were successfully deleted and deletion confirmed by PCR and sequencing.

[00667] The next step was the integration of the MEG pathway. An operon expressed under control of the proD promoter containing khk-C gene (ketohexokinase), aldolB gene (fructose-1,6-bisphosphate aldolase) and fucO gene (glycoaldehyde reduc-

tase) was integrated in E.coli genome and an additional copy of khk-C and aldB genes also under control of proD promoter was integrated in a different loci.

[00668] The last step was the integration of acetone pathway. An operon expressed under control of OXB11 promoter containing thIA gene (acetoacetyl-CoA thiolase); atoAD genes (acetate:acetoacetyl-CoA transferase) and adc gene (acetoacetate decarboxylase) was integrated in E.coli genome, generating the base strain. All the integrations were confirmed by PCR and sequencing. The mgsA gene were deleted in the base strain and the deletion was confirmed by PCR and sequencing.

[00669] Colonies were inoculated in 5 mL of mineral media containing 12.85 g/L of xylose and 2.15 g/L of glucose for pre-culture. After 16 hours of cultivation 5% of the pre-culture was transferred to 100 mL of fresh media. The flasks were incubated at 37°C, 250 rpm until complete consumption of glucose and xylose. The initial OD of the cultivation was 0.1. For all strains, xylose was fully consumed after 55 hours of cultivation.

[00670] The strains produced approximately 4 g/L of MEG in 55h of cultivation (FIG. 17A) while the total amount of acetone was increased 31% (FIG. 17B) with little effect on acetic acid production (FIG. 17C) and xylulose accumulation (FIG. 17D). The deletion of mgsA provided an improvement at velocity of co-production in relation with its parental strain.

Example 10: Deletion of gcl in a MEG + Acetone co-producing strain – via xylonate pathway

[00671] The E. coli K12 strain MG1655 was used as host for the deletion of two genes that could divert the carbon flux from MEG + IPA pathway: aldA and xylA. The genes were successfully deleted and the deletions were confirmed by PCR and sequencing.

[00672] The next step was the integration of the MEG pathway. An operon expressed under control of the proD promoter containing xdh gene (xylose dehydrogenase) and fucO gene (glycoaldehyde reductase), encoding respectively for the first and last enzymes of the xylonate pathway, was integrated in E.coli genome and an additional copy of xdh gene also under control of proD promoter was integrated in a different loci.

[00673] The last step was the integration of acetone pathway. An operon expressed under control of OXB11 promoter containing thIA gene (acetoacetyl-CoA thio-

lase); *atoAD* genes (acetate:acetoacetyl-CoA transferase) and *adc* gene (acetoacetate decarboxylase) was integrated in *E. coli* genome, generating the base strain. All the integrations were confirmed by PCR and sequencing. The *gcl* gene were deleted in the base strain and the deletion was confirmed by PCR and sequencing.

[00674] Colonies were inoculated in 5 mL of mineral media containing 12.85 g/L of xylose and 2.15 g/L of glucose for pre-culture. After 16 hours of cultivation 5% of the pre-culture was transferred to 100 mL of fresh media. The flasks were incubated at 37°C, 250 rpm until complete consumption of glucose and xylose. The initial OD of the cultivation was 0.1. For all strains, xylose was fully consumed after 48 hours of cultivation.

[00675] After 24 hours of cultivation approximately 1.7 g/L of MEG could be detected in the parental strain while 2.1 g/L could be detected at the same time point in the strain with *gcl* deleted (FIG. 18A). The strains produced approximately 4 g/L of MEG in 48h of cultivation while the total amount of acetone was increased by 15% (FIG. 18B), probably related to the higher production of acetic acid (FIG. 18C). The peak production of xylonic acid was decreased in 15% (FIG. 18D). The deletion of *gcl* provided an improvement at velocity of co-production in relation with its parental strain.

Example 11: Deletion of *ackA* in a MEG + Acetone co-producing strain via xylulose pathway

[00676] The *E. coli* K12 strain MG1655 was used as host for the deletion of two genes that could divert the carbon flux from the MEG + Acetone pathway: *aldA* and *xylB*. The genes were successfully deleted and the deletions were confirmed by PCR and sequencing.

[00677] The next step was the integration of the MEG pathway. An operon expressed under the control of the *proD* promoter containing *khk-C* gene (ketohehexokinase), *aldoB* gene (fructose-1,6-bisphosphate aldolase), and *fucO* gene (glycoaldehyde reductase) was integrated in the *E. coli* genome and an additional copy of *khk-C* and *aldoB* genes also under the control of the *proD* promoter were integrated in a different locus.

[00678] The last step was the integration of the acetone pathway via an operon in the *E. coli* genome. An operon expressed under control of OXB11 promoter containing *thlA* gene (acetoacetyl-CoA thiolase); *atoAD* genes (acetate:acetoacetyl-CoA transferase) and *adc* gene (acetoacetate decarboxylase) was integrated into the *E. coli* ge-

nome, generating the base strain. All the integrations were confirmed by PCR and sequencing. The base strain was used as the host strain for the deletion of two genes related to the acetate pathway: *pta* and *ackA*. The genes were successfully deleted and the deletion was confirmed by sequencing.

[00679] Colonies of the deleted strains were inoculated in 5 mL of mineral media for pre-culture. After 16 hours of cultivation, the pre-culture was transferred to 100 ml of mineral media containing 12.85 g/L of xylose and 2.15 g/L of glucose. The flasks were incubated at 37°C, 250 rpm until complete consumption of xylose. The initial OD of the cultivation was 0.2.

[00680] Higher amounts of MEG were detected for Δ *pta* (FIG. 19A) and Δ *ackA* (FIG. 19B) strains in relation with the parental strain. The deletion of *pta* or *ackA* resulted in an improvement at velocity of MEG production in relation with the parental strain.

Example 12: Deletion of *arcA* in a MEG + Acetone co-producing strain via xylose pathway

[00681] The *E. coli* K12 strain MG1655 was used as host for the deletion of two genes that could divert the carbon flux from the MEG + Acetone pathway: *aldA* and *xylB*. The genes were successfully deleted and the deletions were confirmed by PCR and sequencing.

[00682] The next step was the integration of the MEG pathway. An operon expressed under the control of the *proD* promoter containing *khk-C* gene (ketohexokinase), *aldoB* gene (fructose-1,6-bisphosphate aldolase), and *fucO* gene (glycoaldehyde reductase) was integrated in the *E. coli* genome and an additional copy of *khk-C* and *aldoB* genes also under the control of the *proD* promoter were integrated in a different locus.

[00683] The last step was the integration of the acetone pathway via an operon in the *E. coli* genome. An operon expressed under control of OXB11 promoter containing *thlA* gene (acetoacetyl-CoA thiolase); *atoAD* genes (acetate:acetoacetyl-CoA transferase) and *adc* gene (acetoacetate decarboxylase) was integrated into the *E. coli* genome, generating the base strain. All the integrations were confirmed by PCR and sequencing. The base strain was used as the host strain for the deletion of *arcA* gene, which deletion is related to induction of TCA cycle genes and higher expression of *acs* gene when compared to WT. This gene was successfully deleted and the deletion was confirmed by PCR and sequencing.

[00684] Colonies of the deleted strains were inoculated in 5 mL of mineral media for pre-culture. After 16 hours of cultivation, the pre-culture was transferred to 100 ml of mineral media containing 12.85 g/L of xylose and 2.15 g/L of glucose. The flasks were incubated at 37°C, 250 rpm until complete consumption of xylose. The initial OD of the cultivation was 0.2.

[00685] Higher productivity of MEG (FIG. 20A) and higher productivity and titer of acetone (FIG. 20B) were detected for Δ arcA strain in relation with the parental strain. The deletion of arcA resulted in an improvement at velocity of MEG production and improvement at velocity and amount of acetone production in relation with the parental strain.

Example 13: Deletion of arcA and pka in a MEG + Acetone co-producing strain via xylonate pathway and with deletion of pta

[00686] The *E. coli* K12 strain MG1655 was used as host for the deletion of two genes that could divert the carbon flux from the MEG + Acetone pathway: aldA and xylA. The genes were successfully deleted and the deletions were confirmed by PCR and sequencing.

[00687] The next step was the integration of the MEG pathway. An operon expressed under control of the proD promoter containing xdh gene (xylose dehydrogenase) and fucO gene (glycoaldehyde reductase), encoding respectively for the first and last enzymes of the xylonate pathway, was integrated in the *E. coli* genome and an additional copy of xdh gene also under control of proD promoter was integrated in a different loci.

[00688] The next step was the integration of the acetone pathway via an operon in the *E. coli* genome. An operon expressed under control of OXB11 promoter containing thlA gene (acetoacetyl-CoA thiolase); atoAD genes (acetate:acetoacetyl-CoA transferase) and adc gene (acetoacetate decarboxylase) was integrated into the *E. coli* genome, generating the base strain. All the integrations were confirmed by PCR and sequencing.

[00689] The last step was the deletion of pta gene. This gene was successfully deleted and the deletion was confirmed by PCR and sequencing. The base strain was used as the host strain for the deletion of two genes related to the acetate pathway: pka and arcA. The genes were successfully deleted and the deletion was confirmed by sequencing.

[00690] Colonies of the deleted strains were inoculated in 5 mL of mineral media for pre-culture. After 16 hours of cultivation, the pre-culture was transferred to 100 ml of mineral media containing 12.85 g/L of xylose and 2.15 g/L of glucose. The flasks were incubated at 37°C, 250 rpm until complete consumption of xylose. The initial OD of the cultivation was 0.2.

[00691] Higher amounts of MEG (FIG. 21A) and acetone (FIG. 21B) were detected for Δ pta Δ arcA and Δ pta Δ pka strain in relation with the Δ pta strain. The deletion of arcA and pka resulted in an improvement at velocity and amount of MEG and acetone production in relation with the parental strain.

Example 14: Expression of heterologous xylolactonase in MEG + acetone co-producing strain – via xylonate pathway

[00692] The E. coli K12 strain MG1655 was used as the host for the deletion of two genes that could divert the carbon flux from MEG + Acetone pathway: aldA and xylA. The genes were successfully deleted and the deletions were confirmed by PCR and sequencing.

[00693] The next step was the integration of the MEG pathway. An operon expressed under control of the proD promoter containing xdh gene (xylose dehydrogenase) and fucO gene (glycoaldehyde reductase), encoding respectively for the first and last enzymes of the xylonate pathway, was integrated in the E.coli genome and an additional copy of xdh gene also under control of proD promoter was integrated in a different loci.

[00694] The last step was the integration of the acetone pathway. An operon expressed under control of OXB11 promoter containing thlA gene (acetoacetyl-CoA thiolase); atoAD genes (acetate:acetoacetyl-CoA transferase) and adc gene (acetoacetate decarboxylase) was integrated in E.coli genome, generating the base strain. All the integrations were confirmed by PCR and sequencing. Plasmids containing different sequences encoding the second enzyme of the xylonate pathway (xylolactonase), were expressed under the control of the OXB11 promoter in a pZS* vector backbone. The plasmids were constructed using an In-fusion commercial kit and confirmed by sequencing. The confirmed plasmids were transformed in the base strain. Colonies from transformations were inoculated in 5 mL of mineral media containing 12.85 g/L of xylose and 2.15 g/L of glucose for pre-culture. After 16 hours of cultivation 5% of the pre-culture was transferred to 100 mL of fresh media. The flasks were incubated at 37°C,

250 rpm until complete consumption of glucose and xylose. The initial OD of the cultivation was 0.1. For all strains, xylose was fully consumed after 48 hours of cultivation.

[00695] After 24 hours of cultivation, approximately 1.3 g/L of MEG could be detected in the parental strain while 2.1 up to 2.7 g/L could be detected at the same time in strains harboring xylolactonase expressed in plasmids (**FIG. 22A**). All the strains produced approximately 4 g/L of MEG in 48h of cultivation while the total amount of acetone was increased 2.1 to 2.6 times (**FIG. 22B**), probably related to the higher production of acetic acid (**FIG. 22C**). The peak production of xylonic acid was decreased up to 4.7 times (**FIG. 22D**). The expression of xylolactonase provided an improvement at velocity of co-production in relation with its parental strain.

Example 15: Expression of heterologous xylonate dehydratase in MEG + acetone co-producing strain – via xylonate pathway

[00696] The *E. coli* K12 strain MG1655 was used as the host for the deletion of two genes that could divert the carbon flux from MEG + Acetone pathway: *aldA* and *xylA*. The genes were successfully deleted and the deletions were confirmed by PCR and sequencing.

[00697] The next step was the integration of the MEG pathway. An operon expressed under control of the *proD* promoter containing *xdh* gene (xylose dehydrogenase) and *fucO* gene (glycoaldehyde reductase), encoding respectively for the first and last enzymes of the xylonate pathway, was integrated in the *E. coli* genome and an additional copy of *xdh* gene also under control of *proD* promoter was integrated in a different loci.

[00698] The last step was the integration of acetone pathway. An operon expressed under control of OXB11 promoter containing *thlA* gene (acetoacetyl-CoA thiolase); *atoAD* genes (acetate:acetoacetyl-CoA transferase) and *adc* gene (acetoacetate decarboxylase) was integrated in the *E. coli* genome, generating the base strain. All the integrations were confirmed by PCR and sequencing. Plasmids containing different sequences encoding the third enzyme of the xylonate pathway (xylonate dehydratase), were expressed under the control of the OXB11 promoter in a pZS* vector backbone. The plasmids were constructed using an In-fusion commercial kit and confirmed by sequencing. The confirmed plasmids were transformed in the base strain. Colonies from transformations were inoculated in 5 mL of mineral media containing 12.85 g/L of xylose and 2.15 g/L of glucose for pre-culture. After 16 hours of cultivation 5% of the pre-

culture was transferred to 100 mL of fresh media. The flasks were incubated at 37°C, 250 rpm until complete consumption of glucose and xylose. The initial OD of the cultivation was 0.1. For all strains, xylose was fully consumed after 48 hours of cultivation.

[00699] After 24 hours of cultivation approximately 0.8 g/L of MEG could be detected in the parental strain while 1.3 up to 1.8 g/L could be detected at the same time in strains harboring xylonate dehydratase expressed in plasmids (FIG. 23A). All the strains produced approximately 4 g/L of MEG in 48h of cultivation while the total amount of acetone was increased 1.5 to 2.2 times (FIG. 23B), probably related to the higher production of acetic acid (FIG. 23C). The peak production of xylonic acid was decreased up to 70% (FIG. 23D). The expression of xylonate dehydratase provided an improvement at velocity of co-production in relation with its parental strain.

ENUMERATED EMBODIMENTS

[00700] Embodiment 1. A method of modulating the flux of carbon through the monoethylene glycol (MEG) biosynthesis pathway and one or more C3 compound biosynthesis pathways, the method comprising:

modifying a microbe coproducing MEG and one or more C3 compounds by:

i. disrupting one or more nucleic acid sequences encoding methylglyoxal synthase (mgsA), and/or

ii disrupting one or more nucleic acid sequences encoding glyoxylate carboligase (gcl);

wherein the MEG and/or the one or more C3 compounds is produced at a faster rate or exhibits an increased yield and/or titer; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding methylglyoxal synthases and/or glyoxylate carboligases.

[00701] Embodiment 2. The method of Embodiment 1, wherein the C3 compounds are selected from acetone, isopropanol, and propene.

[00702] Embodiment 3. The method of Embodiment 1, wherein the disrupting is selected from a deletion, a point mutation, a substitution, an insertion, or a frameshift.

[00703] Embodiment 4. The method of Embodiment 3, wherein the deletion comprises the deletion of the one or more nucleic acid sequences.

[00704] Embodiment 5. The method of Embodiment 1, wherein translation of the one or more nucleic acid sequences encoding methylglyoxal synthase and/or the one or more nucleic acid sequences encoding glyoxylate carboligase is reduced by at least 50%.

[00705] Embodiment 6. The method of Embodiment 1, wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and an increased yield or titer.

[00706] Embodiment 7. The method of Embodiment 1, wherein the one or more nucleic acid sequences encoding methylglyoxal synthase and the one or more nucleic acid sequences encoding glyoxylate carboligase are disrupted.

[00707] Embodiment 8. The method of Embodiment 1, wherein the microbe is a bacterium or a fungus.

[00708] Embodiment 9. The method of Embodiment 8, wherein the microbe is selected from one of the following genera: *Escherichia*, *Corynebacterium*, *Saccharomyces*, *Lactobacillus*, *Bacillus*, *Clostridium*, *Pichia*, and *Aspergillus*.

[00709] Embodiment 10. The method of Embodiment 8, wherein the bacterium is an *Escherichia coli*.

[00710] Embodiment 11. The method of Embodiment 1, wherein the MEG exhibits an increased yield or titer.

[00711] Embodiment 12. The method of Embodiment 11, wherein the increased yield or titer is an increase of at least 2%.

[00712] Embodiment 13. The method of Embodiment 11, wherein the increased yield or titer is an increase of at least 15%.

[00713] Embodiment 14. The method of Embodiment 1, wherein the MEG is produced at a faster rate.

[00714] Embodiment 15. The method of Embodiment 14, wherein the faster rate is an increase of at least 2%.

[00715] Embodiment 16. The method of Embodiment 14, wherein the faster rate is an increase of at least 15%.

[00716] Embodiment 17. The method of Embodiment 1, wherein the one or more C3 compounds is acetone.

[00717] Embodiment 18. The method of Embodiment 17, wherein the acetone exhibits an increased yield or titer.

[00718] Embodiment 19. The method of Embodiment 18, wherein the increased yield or titer is an increase of at least 2%.

[00719] Embodiment 20. The method of Embodiment 18, wherein the increased yield or titer is an increase of at least 15%.

[00720] Embodiment 21. The method of Embodiment 17, wherein the acetone is produced at a faster rate.

[00721] Embodiment 22. The method of Embodiment 21, wherein the faster rate is an increase of at least 2%.

[00722] Embodiment 23. The method of Embodiment 21, wherein the faster rate is an increase of at least 15%.

[00723] Embodiment 24. The method of Embodiment 1, wherein
(i) the MEG exhibits an increased yield or titer of at least 2%, and
(ii) the one or more C3 compounds exhibits an increased yield or titer of at least 2%.

[00724] Embodiment 25. The method of Embodiment 1, wherein
(i) the MEG exhibits an increased yield or titer of at least 15%, and
(ii) the one or more C3 compounds exhibits an increased yield or titer of at least 15%.

[00725] Embodiment 26. The method of Embodiment 1, wherein
(i) the rate of MEG production exhibits an increase of at least 2%, and
(ii) the rate of the one or more C3 compound production exhibits an increase of at least 2%.

[00726] Embodiment 27. The method of Embodiment 1, wherein
(i) the rate of MEG production exhibits an increase of at least 15%, and
(ii) the rate of the one or more C3 compound production exhibits an increase of at least 15%.

[00727] Embodiment 28. The method of Embodiment 1, wherein the microbe utilizes xylose, glucose and/or a mixture of xylose and glucose in the coproduction of the MEG and the one or more C3 compounds.

[00728] Embodiment 29. The method of Embodiment 1, wherein the microbe utilizes arabinose, galactose, maltose, fructose, mannose, sucrose, and/or combinations thereof in the coproduction of the MEG and the one or more C3 compounds.

[00729] Embodiment 30. A recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

(i) disrupting one or more nucleic acid sequences encoding methylglyoxal synthase

(mgsA), and/or

(ii) disrupting one or more nucleic acid sequences encoding glyoxylate carboligase (gcl);

wherein the MEG and/or the one or more C3 compounds is produced at a faster rate or exhibits an increased yield or titer; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding methylglyoxal synthases and/or glyoxylate carboligases.

[00730] Embodiment 31. The recombinant microbe of Embodiment 30, wherein the C3 compounds are selected from acetone, isopropanol, and propene.

[00731] Embodiment 32. The recombinant microbe of Embodiment 31, wherein the disrupting is selected from a deletion, a point mutation, a substitution, an insertion, or a frameshift.

[00732] Embodiment 33. The recombinant microbe of Embodiment 32, wherein the deletion comprises the deletion of the one or more nucleic acid sequences.

[00733] Embodiment 34. The recombinant microbe of Embodiment 30, wherein the translation of the one or more nucleic acid sequences encoding methylglyoxal synthase and/or the one or more nucleic acid sequences encoding glyoxylate carboligase is reduced by at least 50%.

[00734] Embodiment 35. The recombinant microbe of Embodiment 30, wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and an increased yield or titer.

[00735] Embodiment 36. The recombinant microbe of Embodiment 30, wherein the one or more nucleic acid sequences encoding methylglyoxal synthase and the one or more nucleic acid sequences encoding glyoxylate carboligase are disrupted.

[00736] Embodiment 37. The recombinant microbe of Embodiment 30, wherein the microbe is a bacterium or a fungus.

[00737] Embodiment 38. The recombinant microbe of Embodiment 37, wherein the microbe is selected from one of the following genera: *Escherichia*, *Corynebacterium*, *Saccharomyces*, *Lactobacillus*, *Bacillus*, *Clostridium*, *Pichia*, and *Aspergillus*.

[00738] Embodiment 39. The recombinant microbe of Embodiment 38, wherein the bacterium is an *Escherichia coli*.

[00739] Embodiment 40. The recombinant microbe of Embodiment 30, wherein the MEG exhibits an increased yield or titer.

[00740] Embodiment 41. The recombinant microbe of Embodiment 40, wherein the increased yield or titer is an increase of at least 2%.

[00741] Embodiment 42. The recombinant microbe of Embodiment 40, wherein the increased yield or titer is an increase of at least 15%.

[00742] Embodiment 43. The method of Embodiment 30, wherein the MEG is produced at a faster rate.

[00743] Embodiment 44. The method of Embodiment 43, wherein the faster rate is an increase of at least 2%.

[00744] Embodiment 45. The method of Embodiment 43, wherein the faster rate is an increase of at least 15%.

[00745] Embodiment 46. The recombinant microbe of Embodiment 30, wherein the one or more C3 compounds is acetone.

[00746] Embodiment 47. The recombinant microbe of Embodiment 46, wherein the acetone exhibits an increased yield or titer.

[00747] Embodiment 48. The recombinant microbe of Embodiment 47, wherein the increased yield or titer is an increase of at least 2%.

[00748] Embodiment 49. The recombinant microbe of Embodiment 47, wherein the increased yield or titer is an increase of at least 15%.

[00749] Embodiment 50. The method of Embodiment 46, wherein the acetone is produced at a faster rate.

[00750] Embodiment 51. The method of Embodiment 50, wherein the faster rate is an increase of at least 2%.

[00751] Embodiment 52. The method of Embodiment 50, wherein the faster rate is an increase of at least 15%.

[00752] Embodiment 53. The method of Embodiment 30, wherein
(i) the MEG exhibits an increased yield or titer of at least 2%, and
(ii) the one or more C3 compounds exhibits an increased yield or titer of at least 2%.

[00753] Embodiment 54. The method of Embodiment 30, wherein
(i) the MEG exhibits an increased yield or titer of at least 15%, and

(ii) the one or more C3 compounds exhibits an increased yield or titer of at least 15%.

[00754] Embodiment 55. The method of Embodiment 30, wherein

(i) the rate of MEG production exhibits an increase of at least 2%, and
(ii) the rate of the one or more C3 compound production exhibits an increase of at least 2%.

[00755] Embodiment 56. The method of Embodiment 30, wherein

(i) the rate of MEG production exhibits an increase of at least 15%, and
(ii) the rate of the one or more C3 compound production exhibits an increase of at least 15%.

[00756] Embodiment 57. The recombinant microbe of Embodiment 30, wherein the microbe utilizes xylose, glucose and/or a mixture of xylose and glucose in the coproduction of the MEG and the one or more C3 compounds.

[00757] Embodiment 58. The recombinant microbe of Embodiment 30, wherein the microbe utilizes arabinose, galactose, maltose, fructose, mannose, sucrose, and/or combinations thereof in the coproduction of the MEG and the one or more C3 compounds.

[00758] Embodiment 59. A method of modulating the flux of carbon through the monoethylene glycol (MEG) biosynthesis pathway and one or more C3 compound biosynthesis pathways, the method comprising:

modifying a microbe coproducing MEG and one or more C3 compounds by performing one or more of the following:

i. disrupting one or more polynucleotide sequences encoding a phosphate acetyltransferase,

ii. disrupting one or more polynucleotide sequences encoding an acetate kinase,

iii. disrupting one or more polynucleotide sequences encoding a pyruvate oxidase,

iv. disrupting one or more polynucleotide sequences encoding an ArcA regulator,

v. disrupting one or more polynucleotide sequences encoding a lysine acetyltransferase,

vi. overexpressing one or more endogenous or exogenous polynucleotide sequences encoding a CobB regulator, and

vii. overexpressing one or more endogenous or exogenous polynucleotide sequences encoding an acetyl-CoA synthetase;

wherein the MEG and/or the one or more C3 compounds are produced at a faster rate or exhibit an increased yield or titer; as compared to a microbe lacking the disruption and/or the overexpression of the endogenous or exogenous polynucleotides of any one or more of i-vii.

[00759] Embodiment 60. The method of Embodiment 59, wherein the disrupting is selected from a deletion, a point mutation, a substitution, an insertion, or a frameshift.

[00760] Embodiment 61. The method of Embodiment 60, wherein the deletion comprises the deletion of the one or more nucleic acid sequences.

[00761] Embodiment 62. The method of Embodiment 59, wherein the translation of the one or more polypeptides in i-v is reduced by at least 50%

[00762] Embodiment 63. The method of Embodiment 59, wherein the one or more polynucleotide sequences encoding at least two of the following polypeptides are disrupted: phosphate acetyltransferase, acetate kinase, pyruvate oxidase, ArcA regulator, and lysine acetyltransferase.

[00763] Embodiment 64. The method of Embodiment 59, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences in vi and/or vii yields an increase of at least 5% of the polypeptide encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00764] Embodiment 65. The method of Embodiment 59, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences in vi and/or vii yields an increase of at least 30% of the polypeptide encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00765] Embodiment 66. The method of Embodiment 59, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences in vi and/or vii yields an increase of at least 70% of the polypeptide encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00766] Embodiment 67. The method of Embodiment 59, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences yields an

increase of at least 300% of the polypeptide encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00767] Embodiment 68. The method of Embodiment 59, wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or an increased yield or titer.

[00768] Embodiment 69. The method of Embodiment 59, wherein the microbe is a bacterium or a fungus.

[00769] Embodiment 70. The method of Embodiment 69, wherein the microbe is selected from one of the following genera: *Escherichia*, *Corynebacterium*, *Saccharomyces*, *Lactobacillus*, *Bacillus*, *Clostridium*, *Pichia*, and *Aspergillus*.

[00770] Embodiment 71. The method of Embodiment 59, wherein the MEG exhibits an increased yield or titer.

[00771] Embodiment 72. The method of Embodiment 71, wherein the increased yield or titer is an increase of at least 2%.

[00772] Embodiment 73. The method of Embodiment 71, wherein the increased yield or titer is an increase of at least 15%.

[00773] Embodiment 74. The method of Embodiment 59, wherein the MEG is produced at a faster rate.

[00774] Embodiment 75. The method of Embodiment 74, wherein the faster rate is an increase of at least 2%.

[00775] Embodiment 76. The method of Embodiment 74, wherein the faster rate is an increase of at least 15%.

[00776] Embodiment 77. The method of Embodiment 59, wherein the one or more C3 compounds is acetone.

[00777] Embodiment 78. The method of Embodiment 77, wherein the acetone exhibits an increased yield or titer.

[00778] Embodiment 79. The method of Embodiment 78, wherein the increased yield or titer is an increase of at least 2%.

[00779] Embodiment 80. The method of Embodiment 78, wherein the increased yield or titer is an increase of at least 15%.

[00780] Embodiment 81. The method of Embodiment 77, wherein the acetone is produced at a faster rate.

[00781] Embodiment 82. The method of Embodiment 81, wherein the faster rate is an increase of at least 2%.

[00782] Embodiment 83. The method of Embodiment 81, wherein the faster rate is an increase of at least 15%.

[00783] Embodiment 84. The method of Embodiment 59, wherein
(i) the MEG exhibits an increased yield or titer of at least 2%, and
(ii) the one or more C3 compounds exhibits an increased yield or titer of at least 2%.

[00784] Embodiment 85. The method of Embodiment 59, wherein
(i) the MEG exhibits an increased yield or titer of at least 15%, and
(ii) the one or more C3 compounds exhibits an increased yield or titer of at least 15%.

[00785] Embodiment 86. The method of Embodiment 59, wherein
(i) the rate of MEG production exhibits an increase of at least 2%, and
(ii) the rate of the one or more C3 compound production exhibits an increase of at least 2%.

[00786] Embodiment 87. The method of Embodiment 59, wherein
(i) the rate of MEG production exhibits an increase of at least 15%, and
(ii) the rate of the one or more C3 compound production exhibits an increase of at least 15%.

[00787] Embodiment 88. The method of Embodiment 59, wherein the microbe utilizes xylose, cellobiose, arabinose, mannose, and/or glucose in the coproduction of the MEG and the one or more C3 compounds.

[00788] Embodiment 89. The method of Embodiment 59, wherein the C3 compounds are selected from acetone, isopropanol, and propene.

[00789] Embodiment 90. A recombinant microbe capable of coproducing MEG and one or more C3 compounds, wherein the microbe coproducing MEG and one or more C3 compounds by performing one or more of the following:

- i. disrupting one or more polynucleotide sequences encoding a phosphate acetyltransferase,
- ii. disrupting one or more polynucleotide sequences encoding an acetate kinase,
- iii. disrupting one or more polynucleotide sequences encoding a pyruvate oxidase,

iv. disrupting one or more polynucleotide sequences encoding an ArcA regulator,

v. disrupting one or more polynucleotide sequences encoding a lysine acetyltransferase,

vi. overexpressing one or more endogenous or exogenous polynucleotide sequences encoding a CobB regulator, and

vii. overexpressing one or more endogenous or exogenous polynucleotide sequences encoding an acetyl-CoA synthetase;

wherein the MEG and/or the one or more C3 compounds are produced at a faster rate or exhibit an increased yield or titer; as compared to a microbe lacking the disruption and/or the overexpression of the endogenous or exogenous polynucleotides of any one or more of i-vii.

[00790] Embodiment 91. The recombinant microbe of Embodiment 90, wherein the disrupting is selected from a deletion, a point mutation, a substitution, an insertion, or a frameshift.

[00791] Embodiment 92. The recombinant microbe of Embodiment 91, wherein the deletion comprises the deletion of the one or more nucleic acid sequences.

[00792] Embodiment 93. The recombinant microbe of Embodiment 90, wherein the translation of the one or more polypeptides in i-v is reduced by at least 50%

[00793] Embodiment 94. The recombinant microbe of Embodiment 92, wherein the one or more polynucleotide sequences encoding at least two of the following polypeptides are disrupted: phosphate acetyltransferase, acetate kinase, pyruvate oxidase, ArcA regulator, and lysine acetyltransferase.

[00794] Embodiment 95. The recombinant microbe of Embodiment 90, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences in vi and/or vii yields an increase of at least 5% of the polypeptide encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00795] Embodiment 96. The recombinant microbe of Embodiment 90, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences in vi and/or vii yields an increase of at least 30% of the polypeptide encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00796] Embodiment 97. The recombinant microbe of Embodiment 90, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequenc-

es in vi and/or vii yields an increase of at least 70% of the polypeptide encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00797] Embodiment 98. The recombinant microbe of Embodiment 90, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences yields an increase of at least 30% of the enzyme encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00798] Embodiment 99. The recombinant microbe of Embodiment 90, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences yields an increase of at least 70% of the enzyme encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00799] Embodiment 100. The recombinant microbe of Embodiment 90, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences yields an increase of at least 300% of the polypeptide encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00800] Embodiment 101. The recombinant microbe of Embodiment 90, wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or an increased yield or titer.

[00801] Embodiment 102. The recombinant microbe of Embodiment 90, wherein the microbe is a bacterium or a fungus.

[00802] Embodiment 103. The recombinant microbe of Embodiment 102, wherein the microbe is selected from one of the following genera: *Escherichia*, *Corynebacterium*, *Saccharomyces*, *Lactobacillus*, *Bacillus*, *Clostridium*, *Pichia*, and *Aspergillus*.

[00803] Embodiment 104. The recombinant microbe of Embodiment 90, wherein the MEG exhibits an increased yield or titer.

[00804] Embodiment 105. The recombinant microbe of Embodiment 104, wherein the increased yield or titer is an increase of at least 2%.

[00805] Embodiment 106. The recombinant microbe of Embodiment 104, wherein the increased yield or titer is an increase of at least 15%.

[00806] Embodiment 107. The method of Embodiment 90, wherein the MEG is produced at a faster rate.

[00807] Embodiment 108. The method of Embodiment 107, wherein the faster rate is an increase of at least 2%.

[00808] Embodiment 109. The method of Embodiment 107, wherein the faster rate is an increase of at least 15%.

[00809] Embodiment 110. The recombinant microbe of Embodiment 90, wherein the one or more C3 compounds is acetone.

[00810] Embodiment 111. The recombinant microbe of Embodiment 110, wherein the acetone exhibits an increased yield or titer.

[00811] Embodiment 112. The recombinant microbe of Embodiment 111, wherein the increased yield or titer is an increase of at least 2%.

[00812] Embodiment 113. The recombinant microbe of Embodiment 111, wherein the increased yield or titer is an increase of at least 15%.

[00813] Embodiment 114. The method of Embodiment 110, wherein the acetone is produced at a faster rate.

[00814] Embodiment 115. The method of Embodiment 114, wherein the faster rate is an increase of at least 2%.

[00815] Embodiment 116. The method of Embodiment 114, wherein the faster rate is an increase of at least 15%.

[00816] Embodiment 117. The method of Embodiment 90, wherein
(i) the MEG exhibits an increased yield or titer of at least 2%, and
(ii) the one or more C3 compounds exhibits an increased yield or titer of at least 2%.

[00817] Embodiment 118. The method of Embodiment 90, wherein
(i) the MEG exhibits an increased yield or titer of at least 15%, and
(ii) the one or more C3 compounds exhibits an increased yield or titer of at least 15%.

[00818] Embodiment 119. The method of Embodiment 90, wherein
(i) the rate of MEG production exhibits an increase of at least 2%, and
(ii) the rate of the one or more C3 compound production exhibits an increase of at least 2%.

[00819] Embodiment 120. The method of Embodiment 90, wherein
(i) the rate of MEG production exhibits an increase of at least 15%, and
(ii) the rate of the one or more C3 compound production exhibits an increase of at least 15%.

[00820] Embodiment 121. The method of Embodiment 90, wherein the microbe utilizes xylose, cellobiose, arabinose, mannose, and/or glucose in the coproduction of the MEG and the one or more C3 compounds.

[00821] Embodiment 122. The method of Embodiment 90, wherein the C3 compounds are selected from acetone, isopropanol, and propene.

[00822] Embodiment 123. A method of modulating the flux of carbon through the monoethylene glycol (MEG) biosynthesis pathway and one or more C3 compound biosynthesis pathways, the method comprising:

modifying a microbe coproducing MEG and one or more C3 compounds by performing one or more of the following:

i. introducing one or more exogenous polynucleotide sequences encoding a xylose dehydrogenase,

ii. introducing one or more endogenous or exogenous polynucleotide sequences encoding a xylonolactonase,

iii. introducing one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase,

iv. introducing one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase,

v. overexpressing one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase,

vi. overexpressing one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase, and

vii. overexpressing one or more endogenous or exogenous polynucleotide sequences encoding a glycoaldehyde reductase;

wherein the MEG and/or the one or more C3 compounds are produced at a faster rate or exhibit an increased yield or titer; as compared to a microbe lacking the endogenous or exogenous introduced enzymes and/or the overexpression of the endogenous or exogenous enzymes of any one or more of i-vii.

[00823] Embodiment 124. The method of Embodiment 123, wherein the microbe comprises one or more exogenous polynucleotide sequences encoding a xylose dehydrogenase.

[00824] Embodiment 125. The method of Embodiment 123, wherein the microbe comprises one or more endogenous or exogenous polynucleotide sequences encoding a xylonolactonase.

[00825] Embodiment 126. The method of Embodiment 123, wherein the microbe comprises one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase.

[00826] Embodiment 127. The method of Embodiment 123, wherein the microbe comprises one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase.

[00827] Embodiment 128. The method of Embodiment 123, wherein the microbe overexpresses one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase.

[00828] Embodiment 129. The method of Embodiment 123, wherein the microbe overexpresses one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase.

[00829] Embodiment 130. The method of Embodiment 123, wherein the microbe overexpresses one or more endogenous or exogenous polynucleotide sequences encoding a glycoaldehyde reductase.

[00830] Embodiment 131. The method of Embodiment 123, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences yields an increase of at least 5% of the enzyme encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00831] Embodiment 132. The method of Embodiment 123, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences yields an increase of at least 30% of the enzyme encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00832] Embodiment 133. The method of Embodiment 123, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences yields an increase of at least 70% of the enzyme encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00833] Embodiment 134. The method of Embodiment 123, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences

yields an increase of at least 300% of the enzyme encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00834] Embodiment 135. The method of Embodiment 123, wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or an increased yield or titer.

[00835] Embodiment 136. The method of Embodiment 123, wherein the microbe is a bacterium or a fungus.

[00836] Embodiment 137. The method of Embodiment 136, wherein the microbe is selected from one of the following genera: *Escherichia*, *Corynebacterium*, *Saccharomyces*, *Lactobacillus*, *Bacillus*, *Clostridium*, *Pichia*, and *Aspergillus*.

[00837] Embodiment 138. The method of Embodiment 123, wherein the MEG exhibits an increased yield or titer.

[00838] Embodiment 139. The method of Embodiment 138, wherein the increased yield or titer is an increase of at least 2%.

[00839] Embodiment 140. The method of Embodiment 138, wherein the increased yield or titer is an increase of at least 15%.

[00840] Embodiment 141. The method of Embodiment 123, wherein the MEG is produced at a faster rate.

[00841] Embodiment 142. The method of Embodiment 138, wherein the faster rate is an increase of at least 2%.

[00842] Embodiment 143. The method of Embodiment 138, wherein the faster rate is an increase of at least 15%.

[00843] Embodiment 144. The method of Embodiment 123, wherein the one or more C3 compounds is acetone.

[00844] Embodiment 145. The method of Embodiment 144, wherein the acetone exhibits an increased yield or titer.

[00845] Embodiment 146. The method of Embodiment 145, wherein the increased yield or titer is an increase of at least 2%.

[00846] Embodiment 147. The method of Embodiment 145, wherein the increased yield or titer is an increase of at least 15%.

[00847] Embodiment 148. The method of Embodiment 144, wherein the acetone is produced at a faster rate.

[00848] Embodiment 149. The method of Embodiment 148, wherein the faster rate is an increase of at least 2%.

[00849] Embodiment 150. The method of Embodiment 148, wherein the faster rate is an increase of at least 15%.

[00850] Embodiment 151. The method of Embodiment 123, wherein
(i) the MEG exhibits an increased yield or titer of at least 2%, and/or
(ii) the one or more C3 compounds exhibits an increased yield or titer of at least 2%.

[00851] Embodiment 152. The method of Embodiment 123, wherein
(i) the MEG exhibits an increased yield or titer of at least 15%, and
(ii) the one or more C3 compounds exhibits an increased yield or titer of at least 15%.

[00852] Embodiment 153. The method of Embodiment 123, wherein
(i) the rate of MEG production exhibits an increase of at least 2%, and
(ii) the rate of the one or more C3 compound production exhibits an increase of at least 2%.

[00853] Embodiment 154. The method of Embodiment 123, wherein
(i) the rate of MEG production exhibits an increase of at least 15%, and
(ii) the rate of the one or more C3 compound production exhibits an increase of at least 15%.

[00854] Embodiment 155. The method of Embodiment 123, wherein the microbe utilizes xylose, cellobiose, arabinose, mannose, and/or glucose in the coproduction of the MEG and the one or more C3 compounds.

[00855] Embodiment 156. The method of Embodiment 123, wherein the C3 compounds are selected from acetone, isopropanol, and propene.

[00856] Embodiment 157. A recombinant microbe capable of coproducing MEG and one or more C3 compounds, wherein the microbe coproducing MEG and one or more C3 compounds comprises one or more of the following:

- i. one or more exogenous polynucleotide sequences encoding a xylose dehydrogenase,
- ii. one or more endogenous or exogenous polynucleotide sequences encoding a xylonolactonase,

iii. one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase,

iv. one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase,

v. one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase,

vi. one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase, and

vii. one or more endogenous or exogenous polynucleotide sequences encoding a glycoaldehyde reductase;

wherein the enzymes of v-vii are overexpressed as compared to their native expression; and

wherein the MEG and/or the one or more C3 compounds are produced at a faster rate or exhibit an increased yield or titer; as compared to a microbe lacking the endogenous or exogenous introduced enzymes and/or lacking the overexpression of the endogenous or exogenous enzymes of any one or more of i-vii.

[00857] Embodiment 158. The recombinant microbe of Embodiment 157, wherein the microbe comprises one or more exogenous polynucleotide sequences encoding a xylose dehydrogenase.

[00858] Embodiment 159. The recombinant microbe of Embodiment 157, wherein the microbe comprises one or more endogenous or exogenous polynucleotide sequences encoding a xylonolactonase.

[00859] Embodiment 160. The recombinant microbe of Embodiment 157, wherein the microbe comprises one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase.

[00860] Embodiment 161. The recombinant microbe of Embodiment 157, wherein the microbe comprises one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase.

[00861] Embodiment 162. The recombinant microbe of Embodiment 157, wherein the microbe overexpresses one or more endogenous or exogenous polynucleotide sequences encoding a xylonate dehydratase.

[00862] Embodiment 163. The recombinant microbe of Embodiment 157, wherein the microbe overexpresses one or more endogenous or exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase.

[00863] Embodiment 164. The recombinant microbe of Embodiment 157, wherein the microbe overexpresses one or more endogenous or exogenous polynucleotide sequences encoding a glycoaldehyde reductase.

[00864] Embodiment 165. The recombinant microbe of Embodiment 157, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences yields an increase of at least 5% of the enzyme encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00865] Embodiment 166. The recombinant microbe of Embodiment 157, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences yields an increase of at least 30% of the enzyme encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00866] Embodiment 167. The recombinant microbe of Embodiment 157, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences yields an increase of at least 70% of the enzyme encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00867] Embodiment 168. The recombinant microbe of Embodiment 157, wherein the overexpression of the one or more endogenous or exogenous polynucleotide sequences yields an increase of at least 300% of the enzyme encoded by the one or more endogenous or exogenous polynucleotide sequences.

[00868] Embodiment 169. The recombinant microbe of Embodiment 157, wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or an increased yield or titer.

[00869] Embodiment 170. The recombinant microbe of Embodiment 157, wherein the microbe is a bacterium or a fungus.

[00870] Embodiment 171. The recombinant microbe of Embodiment 170, wherein the microbe is selected from one of the following genera: *Escherichia*, *Corynebacterium*, *Saccharomyces*, *Lactobacillus*, *Bacillus*, *Clostridium*, *Pichia*, and *Aspergillus*.

[00871] Embodiment 172. The recombinant microbe of Embodiment 157, wherein the MEG exhibits an increased yield or titer.

[00872] Embodiment 173. The recombinant microbe of Embodiment 172, wherein the increased yield or titer is an increase of at least 2%.

[00873] Embodiment 174. The recombinant microbe of Embodiment 172, wherein the increased yield or titer is an increase of at least 15%.

[00874] Embodiment 175. The method of Embodiment 157, wherein the MEG is produced at a faster rate.

[00875] Embodiment 176. The method of Embodiment 175, wherein the faster rate is an increase of at least 2%.

[00876] Embodiment 177. The method of Embodiment 175, wherein the faster rate is an increase of at least 15%.

[00877] Embodiment 178. The recombinant microbe of Embodiment 157, wherein the one or more C3 compounds is acetone.

[00878] Embodiment 179. The recombinant microbe of Embodiment 178, wherein the acetone exhibits an increased yield or titer.

[00879] Embodiment 180. The recombinant microbe of Embodiment 179, wherein the increased yield or titer is an increase of at least 5%.

[00880] Embodiment 181. The recombinant microbe of Embodiment 179, wherein the increased yield or titer is an increase of at least 30%.

[00881] Embodiment 182. The method of Embodiment 178, wherein the acetone is produced at a faster rate.

[00882] Embodiment 183. The method of Embodiment 182, wherein the faster rate is an increase of at least 2%.

[00883] Embodiment 184. The method of Embodiment 182, wherein the faster rate is an increase of at least 15%.

[00884] Embodiment 185. The method of Embodiment 157, wherein
(i) the MEG exhibits an increased yield or titer of at least 2%, and
(ii) the one or more C3 compounds exhibits an increased yield or titer of at least 2%.

[00885] Embodiment 186. The method of Embodiment 157, wherein
(i) the MEG exhibits an increased yield or titer of at least 15%, and
(ii) the one or more C3 compounds exhibits an increased yield or titer of at least 15%.

[00886] Embodiment 187. The method of Embodiment 157, wherein

(i) the rate of MEG production exhibits an increase of at least 2%, and
(ii) the rate of the one or more C3 compound production exhibits an increase of at least 2%.

[00887] Embodiment 188. The method of Embodiment 157, wherein

(i) the rate of MEG production exhibits an increase of at least 15%, and
(ii) the rate of the one or more C3 compound production exhibits an increase of at least 15%.

[00888] Embodiment 189. The recombinant microbe of Embodiment 157, wherein the microbe utilizes xylose, cellobiose, arabinose, mannose, and/or glucose in the coproduction of the MEG and the one or more C3 compounds.

[00889] Embodiment 190. The recombinant microbe of Embodiment 157, wherein the C3 compounds are selected from acetone, isopropanol, and propene.

[00890] Embodiment 191. A method of modulating the flux of carbon through the monoethylene glycol (MEG) biosynthesis pathway and one or more C3 compound biosynthesis pathways, the method comprising:

modifying a microbe coproducing MEG and one or more C3 compounds by:

i. introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

ii. introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe not having been introduced an acetoacetyl CoA, hydroxymethylglutaryl-CoA synthase, and/or hydroxymethylglutaryl-CoA lyase.

[00891] Embodiment 192. The method of Embodiment 191, wherein the microbe comprises a deletion of one or more polynucleotide sequences encoding acetoacetyl-CoA thiolase.

[00892] Embodiment 193. The method of Embodiment 191, wherein the microbe lacks a functional acetoacetyl-CoA thiolase.

[00893] Embodiment 194. The method of Embodiment 191, wherein the microbe comprises a functional acetoacetyl-CoA thiolase.

[00894] Embodiment 195. The method of Embodiment 191, wherein the microbe comprises a deletion of one or more polynucleotide sequences encoding acetoacetyl-CoA transferase (AtoDA).

[00895] Embodiment 196. The method of Embodiment 191, wherein the microbe comprises a functional acetoacetyl-CoA transferase (AtoDA).

[00896] Embodiment 197. The method of Embodiment 192 or Embodiment 195 wherein the deletion comprises the deletion of the one or more polynucleotide sequences.

[00897] Embodiment 198. The method of Embodiment 191, wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or an increased yield or titer.

[00898] Embodiment 199. The method of Embodiment 191, wherein the microbe is a bacterium or a fungus.

[00899] Embodiment 200. The method of Embodiment 199, wherein the bacterium is an *Escherichia coli*.

[00900] Embodiment 201. The method of Embodiment 191, wherein the MEG exhibits an increased yield or titer.

[00901] Embodiment 202. The method of Embodiment 201, wherein the increased yield or titer is an increase of at least 2%.

[00902] Embodiment 203. The method of Embodiment 202, wherein the increased yield or titer is an increase of at least 15%.

[00903] Embodiment 204. The method of Embodiment 191, wherein the one or more C3 compounds is acetone.

[00904] Embodiment 205. The method of Embodiment 204, wherein the acetone exhibits an increased yield or titer.

[00905] Embodiment 206. The method of Embodiment 205, wherein the increased yield or titer is an increase of at least 2%.

[00906] Embodiment 207. The method of Embodiment 206, wherein the increased yield or titer is an increase of at least 15%.

[00907] Embodiment 208. The method of Embodiment 191, wherein the microbe utilizes xylose, cellobiose, arabinose, mannose, and/or glucose in the coproduction of the MEG and the one or more C3 compounds.

[00908] Embodiment 209. The method of Embodiment 191, wherein the C3 compounds are selected from acetone, isopropanol, and propene.

[00909] Embodiment 210. A recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by:

i. introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

ii. introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or exhibits an increased yield or titer; as compared to a microbe not having been introduced an acetoacetyl CoA, hydroxymethylglutaryl-CoA synthase, and/or hydroxymethylglutaryl-CoA lyase.

[00910] Embodiment 211. The recombinant microbe of Embodiment 210, wherein the microbe comprises a deletion of one or more polynucleotide sequences encoding acetoacetyl-CoA thiolase.

[00911] Embodiment 212. The recombinant microbe of Embodiment 211, wherein the microbe lacks a functional acetoacetyl-CoA thiolase.

[00912] Embodiment 213. The recombinant microbe of Embodiment 211, wherein the microbe comprises a functional acetoacetyl-CoA thiolase.

[00913] Embodiment 214. The recombinant microbe of Embodiment 211, wherein the microbe comprises a deletion of one or more polynucleotide sequences encoding acetoacetyl-CoA transferase (AtoDA).

[00914] Embodiment 215. The recombinant microbe of Embodiment 211, wherein the microbe comprises a functional acetoacetyl-CoA transferase (AtoDA).

[00915] Embodiment 216. The recombinant microbe of Embodiment 212 or Embodiment 215, wherein the deletion comprises the deletion of the one or more polynucleotide sequences.

[00916] Embodiment 217. The recombinant microbe of Embodiment 211, wherein the C3 compounds are selected from acetone, isopropanol, and propene.

[00917] Embodiment 218. The recombinant microbe of Embodiment 211, wherein the MEG and/or the one or more C3 compounds is produced at a faster rate and/or an increased yield or titer.

[00918] Embodiment 219. The recombinant microbe of Embodiment 211, wherein the microbe is a bacterium or a fungus.

[00919] Embodiment 220. The recombinant microbe of Embodiment 219, wherein the bacterium is an *Escherichia coli*.

[00920] Embodiment 221. The recombinant microbe of Embodiment 211, wherein the MEG exhibits an increased yield or titer.

[00921] Embodiment 222. The recombinant microbe of Embodiment 221, wherein the increased yield or titer is an increase of at least 5%.

[00922] Embodiment 223. The recombinant microbe of Embodiment 221, wherein the increased yield or titer is an increase of at least 30%.

[00923] Embodiment 224. The recombinant microbe of Embodiment 211, wherein the one or more C3 compounds is acetone.

[00924] Embodiment 225. The recombinant microbe of Embodiment 224, wherein the acetone exhibits an increased yield or titer.

[00925] Embodiment 226. The recombinant microbe of Embodiment 225, wherein the increased yield or titer is an increase of at least 2%.

[00926] Embodiment 227. The recombinant microbe of Embodiment 225, wherein the increased yield or titer is an increase of at least 15%.

[00927] Embodiment 228. The recombinant microbe of Embodiment 211, wherein the microbe utilizes xylose, cellobiose, arabinose, mannose, and/or glucose in the coproduction of the MEG and the one or more C3 compounds.

INCORPORATION BY REFERENCE

[00928] All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as, an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world. Further, the following references are hereby incorporated by reference.

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CLAIMS**What is claimed is:**

1. A recombinant microbe capable of coproducing MEG and one or more C3 compounds, wherein the microbe comprises

(i) a disruption of one or more nucleic acid sequences encoding methylglyoxal synthase (mgsA), and/or

(ii) a disruption of one or more nucleic acid sequences encoding glyoxylate carboligase (gcl);

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding methylglyoxal synthases and/or glyoxylate carboligases.

2. A recombinant microbe capable of coproducing MEG and one or more C3 compounds, wherein the microbe comprises one or more of the following

(i) a disruption of one or more exogenous polynucleotide sequences encoding a phosphate acetyltransferase,

(ii) a disruption of one or more endogenous polynucleotide sequences encoding an acetate kinase,

(iii) a disruption of one or more endogenous polynucleotide sequences encoding a pyruvate oxidase,

(iv) a disruption of one or more endogenous polynucleotide sequences encoding an ArcA regulator,

(v) a disruption of one or more endogenous polynucleotide sequences encoding a lysine acetyltransferase,

(vi) one or more overexpressed endogenous polynucleotide sequences encoding a CobB regulator, and

(vii) one or more overexpressed endogenous polynucleotide sequences encoding an acetyl-CoA synthetase;

wherein the MEG and/or the one or more C3 compounds are produced at a faster titer, rate or exhibit an increased yield; as compared to a microbe lacking the disruption and/or the overexpression of the endogenous polynucleotides of any one or more of i-vii.

3. A recombinant microbe capable of coproducing MEG and one or more C3 compounds, wherein the microbe comprises one or more of the following

(i) one or more exogenous polynucleotide sequences encoding a xylose dehydrogenase,

(i) one or more exogenous polynucleotide sequences encoding a xylonolactonase,

(iii) one or more exogenous polynucleotide sequences encoding a xylonate dehydratase,

(iv) one or more exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase,

(v) one or more overexpressed endogenous polynucleotide sequences encoding a xylonate dehydratase,

(vi) one or more overexpressed endogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase, and

(vii) one or more overexpressed endogenous polynucleotide sequences encoding a glycoaldehyde reductase;

wherein the MEG and/or the one or more C3 compounds are produced at a faster titer, rate or exhibit an increased yield; as compared to a microbe lacking the exogenous introduced enzymes and/or the overexpression of the endogenous enzymes of any one or more of i-vii.

4. A recombinant microbe capable of coproducing MEG and one or more C3 compounds, wherein the microbe comprises:

(i) one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

(ii) one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe not having been introduced an acetoacetyl CoA, hydroxymethylglutaryl-CoA synthase, or hydroxymethylglutaryl-CoA lyase.

5. The recombinant microbe of claim 1, wherein the microbe further comprises one or more modifications of claim 2.

6. The recombinant microbe of claim 1, wherein the microbe further comprises one or more modifications of claim 3.

7. The recombinant microbe of claim 1, wherein the microbe further comprises one or more modifications of claim 4.

8. The recombinant microbe of claim 2, wherein the microbe further comprises one or more modifications of claim 3.

9. The recombinant microbe of claim 2, wherein the microbe further comprises one or more modifications of claim 4.

10. The recombinant microbe of claim 3, wherein the microbe further comprises modification of claim 4.

11. The recombinant microbe of claim 1, wherein the microbe further comprises one or more modification of claims 2 and 3.

12. The recombinant microbe of claim 1, wherein the microbe further comprises one or more modification of claims 2 and 4.

13. The recombinant microbe of claim 1, wherein the microbe further comprises one or more modifications of claims 3 and 4.

14. The recombinant microbe of claim 2, wherein the microbe further comprises one or more modifications of claims 3 and 4.

15. The recombinant microbe of claim 1, wherein the microbe further comprises one or more modifications of claims 2, 3, and 4.

16. A recombinant microbe capable of coproducing MEG and one or more C3 compounds wherein the microbe comprises one or more of the following:

(i) one or more disrupted nucleic acid sequences encoding methylglyoxal synthase (mgsA),

(ii) one or more disrupted nucleic acid sequences encoding glyoxylate carboligase (gcl),

(iii) one or more disrupted polynucleotide sequences encoding a phosphate acetyltransferase,

(iv) one or more disrupted polynucleotide sequences encoding an acetate kinase,

(v) one or more disrupted polynucleotide sequences encoding a pyruvate oxidase,

(vi) one or more disrupted endogenous polynucleotide sequences encoding an ArcA regulator,

(vii) one or more disrupted endogenous polynucleotide sequences encoding a lysine acetyltransferase,

(viii) one or more overexpressed endogenous polynucleotide sequences encoding a CobB regulator,

(ix) one or more overexpressed endogenous polynucleotide sequences encoding an acetyl-CoA synthetase,

(x) one or more exogenous polynucleotide sequences encoding a xylose dehydrogenase,

(xi) one or more exogenous polynucleotide sequences encoding a xylonolactonase,

(xii) one or more exogenous polynucleotide sequences encoding a xylonate dehydratase,

(xiii) one or more exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase,

(xiv) one or more overexpressed endogenous polynucleotide sequences encoding a xylonate dehydratase,

(xv) one or more overexpressed endogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase,

(xvi) one or more overexpressed endogenous polynucleotide sequences encoding a glycoaldehyde reductase,

(xvii) one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and

(xviii) one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking the modification.

17. A recombinant microbe capable of coproducing MEG and one or more C3 compounds, wherein the microbe comprises one or more of the following:

i. one or more disrupted nucleic acid sequences encoding methylglyoxal synthase (mgsA), and

ii. one or more disrupted nucleic acid sequences encoding glyoxylate carboligase (gcl) and/or;

iii. one or more disrupted polynucleotide sequences encoding a phosphate acetyltransferase, and/or

iv. one or more disrupted polynucleotide sequences encoding an acetate kinase, and/or

v. one or more exogenous polynucleotide sequences encoding a xylonolactonase, and/or

vi. one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase, and/or

vii. one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

viii. one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding methylglyoxal synthases and any of the modifications above.

18. A recombinant microbe capable of coproducing MEG and one or more C3 compounds, the microbe comprising one or more of the following:

i. one or more disrupted nucleic acid sequences encoding glyoxylate carboligase (gcl); and

i. one or more disrupted polynucleotide sequences encoding a phosphate acetyltransferase, and/or

ii. one or more disrupted polynucleotide sequences encoding an acetate kinase, and/or

iii. one or more exogenous polynucleotide sequences encoding a xylonolactonase, and/or

iv. one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase, and/or

v. one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

vi. one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding glyoxylate carboligase (gcl) and any of the modifications above.

19. A recombinant microbe capable of coproducing MEG and one or more C3 compounds, the microbe comprising one or more of the following:

i. one or more disrupted polynucleotide sequences encoding a phosphate acetyltransferase and

ii. disrupting one or more endogenous polynucleotide sequences encoding an acetate kinase, and/or

iii. introducing one or more exogenous polynucleotide sequences encoding a xylonolactonase, and/or

iv. introducing one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase, and/or

v. introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

vi. introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase, and/or;

vii. disrupting one or more endogenous polynucleotide sequences encoding an ArcA regulator, and/or,

viii. disrupting one or more endogenous polynucleotide sequences encoding a lysine acetyltransferase, and/or

ix. overexpressing one or more endogenous polynucleotide sequences encoding an acetyl-CoA synthetase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking a

disruption of one or more nucleic acid sequences encoding a phosphate acetyltransferase and any of the modifications above.

20. A recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by:

- i. disrupting one or more endogenous polynucleotide sequences encoding an acetate kinase and
- ii. introducing one or more exogenous polynucleotide sequences encoding a xylonolactonase, and/or
- iii. one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase, and/or
- iv. one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or
- v. one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding acetate kinase and any of the modifications above.

21. A recombinant microbe capable of coproducing MEG and one or more C3 compounds, the microbe comprising one or more of the following:

- i. one or more exogenous polynucleotide sequences encoding a xylonolactonase and
- ii. one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase, and/or
- iii. one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or
- iv. one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking the exogenous introduced or endogenous overexpressed xylonolactonase, and any of the modifications above.

22. A recombinant microbe capable of coproducing MEG and one or more C3 compounds, the microbe comprising one or more of the following:

- i. one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase and
- ii. one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or
- iii. one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking the exogenous introduced or endogenous overexpressed xylonate dehydratase, any of the modifications above.

23. A recombinant microbe capable of coproducing MEG and one or more C3 compounds, the microbe comprising one or more of the following:

- i. one or more overexpressed endogenous polynucleotide sequences encoding an acetyl-CoA synthetase;
- and
- ii. one or more disrupted nucleic acid sequences encoding methylglyoxal synthase (mgsA),
- iii. one or more disrupted nucleic acid sequences encoding glyoxylate carboligase (gcl) and/or;
- iv. one or more disrupted polynucleotide sequences encoding an acetate kinase, and/or
- v. one or more exogenous polynucleotide sequences encoding a xylonolactonase, and/or

vi. one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase, and/or

vii. one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

viii. one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

ix. one or more disrupted polynucleotide sequences encoding an ArcA regulator, and/or,

x. one or more disrupted polynucleotide sequences encoding a lysine acetyltransferase, and/or

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking the endogenous overexpressed acetyl-CoA synthetase and any of the modifications above.

24. A method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by:

(i) disrupting one or more nucleic acid sequences encoding methylglyoxal synthase (mgsA), and/or

(ii) disrupting one or more nucleic acid sequences encoding glyoxylate carboligase (gcl);

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding methylglyoxal synthases and/or glyoxylate carboligases.

25. A method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by performing one or more of the following:

(i) disrupting one or more exogenous polynucleotide sequences encoding a phosphate acetyltransferase,

(ii) disrupting one or more endogenous polynucleotide sequences encoding an acetate kinase,

(iii) disrupting one or more endogenous polynucleotide sequences encoding a pyruvate oxidase,

(iv) disrupting one or more endogenous polynucleotide sequences encoding an ArcA regulator,

(v) disrupting one or more endogenous polynucleotide sequences encoding a lysine acetyltransferase,

(vi) overexpressing one or more endogenous polynucleotide sequences encoding a CobB regulator, and

(vii) overexpressing one or more endogenous polynucleotide sequences encoding an acetyl-CoA synthetase;

wherein the MEG and/or the one or more C3 compounds are produced at a faster titer, rate or exhibit an increased yield; as compared to a microbe lacking the disruption and/or the overexpression of the endogenous polynucleotides of any one or more of i-vii.

26. A method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by performing one or more of the following:

(i) introducing one or more exogenous polynucleotide sequences encoding a xylose dehydrogenase,

(ii) introducing one or more exogenous polynucleotide sequences encoding a xylonolactonase,

(iii) introducing one or more exogenous polynucleotide sequences encoding a xylonate dehydratase,

(iv) introducing one or more exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase,

(v) overexpressing one or more endogenous polynucleotide sequences encoding a xylonate dehydratase,

(vi) overexpressing one or more endogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase, and

(vii) overexpressing one or more endogenous polynucleotide sequences encoding a glycoaldehyde reductase;

wherein the MEG and/or the one or more C3 compounds are produced at a faster titer, rate or exhibit an increased yield; as compared to a microbe lacking the exogenous introduced enzymes and/or the overexpression of the endogenous enzymes of any one or more of i-vii.

27. A method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by:

(i) introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

(ii) introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe not having been introduced an acetoacetyl CoA, hydroxymethylglutaryl-CoA synthase, or hydroxymethylglutaryl-CoA lyase.

28. The method of claim 24, wherein the microbe comprises one or more modifications of claim 25.

29. The method of claim 24, wherein the microbe comprises one or more modifications of claim 26.

30. The method of claim 24, wherein the microbe comprises one or more modifications of claim 27.

31. The method of claim 25, wherein the microbe comprises one or more modifications of claim 26.

32. The method of claim 25, wherein the microbe comprises one or more modifications of claim 27.

33. The method of claim 26, wherein the microbe comprises modification of claim 27.

34. The method of claim 24, wherein the microbe comprises one or more modification of claims 25 and 26.

35. The method of claim 24, wherein the microbe comprises one or more modification of claims 25 and 27.

36. The method of claim 24, wherein the microbe comprises one or more modifications of claims 26 and 27.

37. The method of claim 25, wherein the microbe comprises one or more modifications of claims 26 and 27.

38. The method of claim 24, wherein the microbe comprises one or more modifications of claims 25, 26, and 27.

39. A method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by performing one or more of the following:

(i) disrupting one or more nucleic acid sequences encoding methylglyoxal synthase (mgsA),

(ii) disrupting one or more nucleic acid sequences encoding glyoxylate carboligase (gcl),

(iii) disrupting one or more exogenous polynucleotide sequences encoding a phosphate acetyltransferase,

(iv) disrupting one or more endogenous polynucleotide sequences encoding an acetate kinase,

(v) disrupting one or more endogenous polynucleotide sequences encoding a pyruvate oxidase,

(vi) disrupting one or more endogenous polynucleotide sequences encoding an ArcA regulator,

(vii) disrupting one or more endogenous polynucleotide sequences encoding a lysine acetyltransferase,

(viii) overexpressing one or more endogenous polynucleotide sequences encoding a CobB regulator,

(ix) overexpressing one or more endogenous polynucleotide sequences encoding an acetyl-CoA synthetase,

(x) introducing one or more exogenous polynucleotide sequences encoding a xylose dehydrogenase,

(xi) introducing one or more exogenous polynucleotide sequences encoding a xylonolactonase,

(xii) introducing one or more exogenous polynucleotide sequences encoding a xylonate dehydratase,

(xiii) introducing one or more exogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase,

(xiv) overexpressing one or more endogenous polynucleotide sequences encoding a xylonate dehydratase,

(xv) overexpressing one or more endogenous polynucleotide sequences encoding a 3-deoxy-D-glycerol pentanone sugar acid aldolase,

(xvi) overexpressing one or more endogenous polynucleotide sequences encoding a glycoaldehyde reductase,

(xvii) introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and

(xviii) introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking the modification.

40. A method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by:

ix. disrupting one or more nucleic acid sequences encoding methylglyoxal synthase (mgsA), and

x. disrupting one or more nucleic acid sequences encoding glyoxylate carboligase (gcl) and/or;

xi. disrupting one or more exogenous polynucleotide sequences encoding a phosphate acetyltransferase, and/or

xii. disrupting one or more endogenous polynucleotide sequences encoding an acetate kinase, and/or

xiii. introducing one or more exogenous polynucleotide sequences encoding a xylonolactonase, and/or

xiv. introducing one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase, and/or

xv. introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

xvi. introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding methylglyoxal synthases and any of the modifications above.

41. A method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by:

ii. disrupting one or more nucleic acid sequences encoding glyoxylate carboligase (gcl); and

vii. disrupting one or more exogenous polynucleotide sequences encoding a phosphate acetyltransferase, and/or

viii. disrupting one or more endogenous polynucleotide sequences encoding an acetate kinase, and/or

ix. introducing one or more exogenous polynucleotide sequences encoding a xylonolactonase, and/or

x. introducing one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase, and/or

xi. introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

xii. introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding glyoxylate carboligase (gcl) and any of the modifications above.

42. A method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by:

x. disrupting one or more exogenous polynucleotide sequences encoding a phosphate acetyltransferase and

xi. disrupting one or more endogenous polynucleotide sequences encoding an acetate kinase, and/or

xii. introducing one or more exogenous polynucleotide sequences encoding a xylonolactonase, and/or

xiii. introducing one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase, and/or

xiv. introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

xv. introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase, and/or;

xvi. disrupting one or more endogenous polynucleotide sequences encoding an ArcA regulator, and/or,

xvii. disrupting one or more endogenous polynucleotide sequences encoding a lysine acetyltransferase, and/or

xviii. overexpressing one or more endogenous polynucleotide sequences encoding an acetyl-CoA synthetase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding a phosphate acetyltransferase and any of the modifications above.

43. A method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by:

vi. disrupting one or more endogenous polynucleotide sequences encoding an acetate kinase and

vii. introducing one or more exogenous polynucleotide sequences encoding a xylonolactonase, and/or

viii. introducing one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase, and/or

ix. introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

x. introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking a disruption of one or more nucleic acid sequences encoding acetate kinase and any of the modifications above.

44. A method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by:

v. introducing one or more exogenous polynucleotide sequences encoding a xylonolactonase and

vi. introducing one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase, and/or

vii. introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

viii. introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking the exogenous introduced or endogenous overexpressed xylonolactonase, and any of the modifications above.

45. A method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by:

iv. introducing one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase

and

v. introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

vi. introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking the exogenous introduced or endogenous overexpressed xylonate dehydratase, any of the modifications above.

46. A method of making a recombinant microbe capable of coproducing MEG and one or more C3 compounds by:

modifying a microbe coproducing MEG and one or more C3 compounds by:

xi. overexpressing one or more endogenous polynucleotide sequences encoding an acetyl-CoA synthetase;

and

xii. disrupting one or more nucleic acid sequences encoding methylglyoxal synthase (mgsA),

xiii. disrupting one or more nucleic acid sequences encoding glyoxylate carboligase (gcl) and/or;

xiv. disrupting one or more endogenous polynucleotide sequences encoding an acetate kinase, and/or

xv. introducing one or more exogenous polynucleotide sequences encoding a xylonolactonase, and/or

xvi. introducing one or more exogenous polynucleotide or sequences or overexpressing an endogenous polynucleotide or sequences encoding a xylonate dehydratase, and/or

xvii. introducing one or more polynucleotide sequences encoding acetoacetyl CoA synthase, and/or

xviii. introducing one or more polynucleotide sequences encoding hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA lyase;

xix. disrupting one or more endogenous polynucleotide sequences encoding an ArcA regulator, and/or,

xx. disrupting one or more endogenous polynucleotide sequences encoding a lysine acetyltransferase, and/or

wherein the MEG and/or the one or more C3 compounds is produced at a faster titer, rate or exhibits an increased yield; as compared to a microbe lacking the endogenous overexpressed acetyl-CoA synthetase and any of the modifications above.

47. The method of any one of claims 24-46, wherein the microbe is a bacterium or a fungus.

48. The method of claim 47, wherein the bacterium is an *Escherichia coli*.

49. The method of any one of claims 24-46, wherein the MEG exhibits an increased yield or titer.

50. The method of claim 49, wherein the increased yield or titer is an increase of at least 2%.

51. The method of claim 49, wherein the increased yield or titer is an increase of at least 15%.

52. The method of any one of claims 24-46, wherein the one or more C3 compounds is acetone.

53. The method of claim 52, wherein the acetone exhibits an increased yield or titer.

54. The method of claim 53, wherein the increased yield or titer is an increase of at least 2%.

55. The method of claim 53, wherein the increased yield or titer is an increase of at least 15%.

56. The method of any one of claims 24-46, wherein the microbe utilizes xylose, cellobiose, arabinose, mannose, and/or glucose in the coproduction of the MEG and the one or more C3 compounds.

57. The method of any one of claims 24-46, wherein the C3 compounds are selected from acetone, isopropanol, and propene.

58. The recombinant microbe of any one of claims 1-23, wherein the microbe is a bacterium or a fungus.

59. The recombinant microbe of claim 58, wherein the bacterium is an *Escherichia coli*.

60. The recombinant microbe of any one of claims 1-23, wherein the MEG exhibits an increased yield or titer.

61. The recombinant microbe of claim 60, wherein the increased yield or titer is an increase of at least 2%.

62. The recombinant microbe of claim 60, wherein the increased yield or titer is an increase of at least 15%.

63. The recombinant microbe of any one of claims 1-23, wherein the one or more C3 compounds is acetone.

64. The recombinant microbe of claim 63, wherein the acetone exhibits an increased yield or titer.

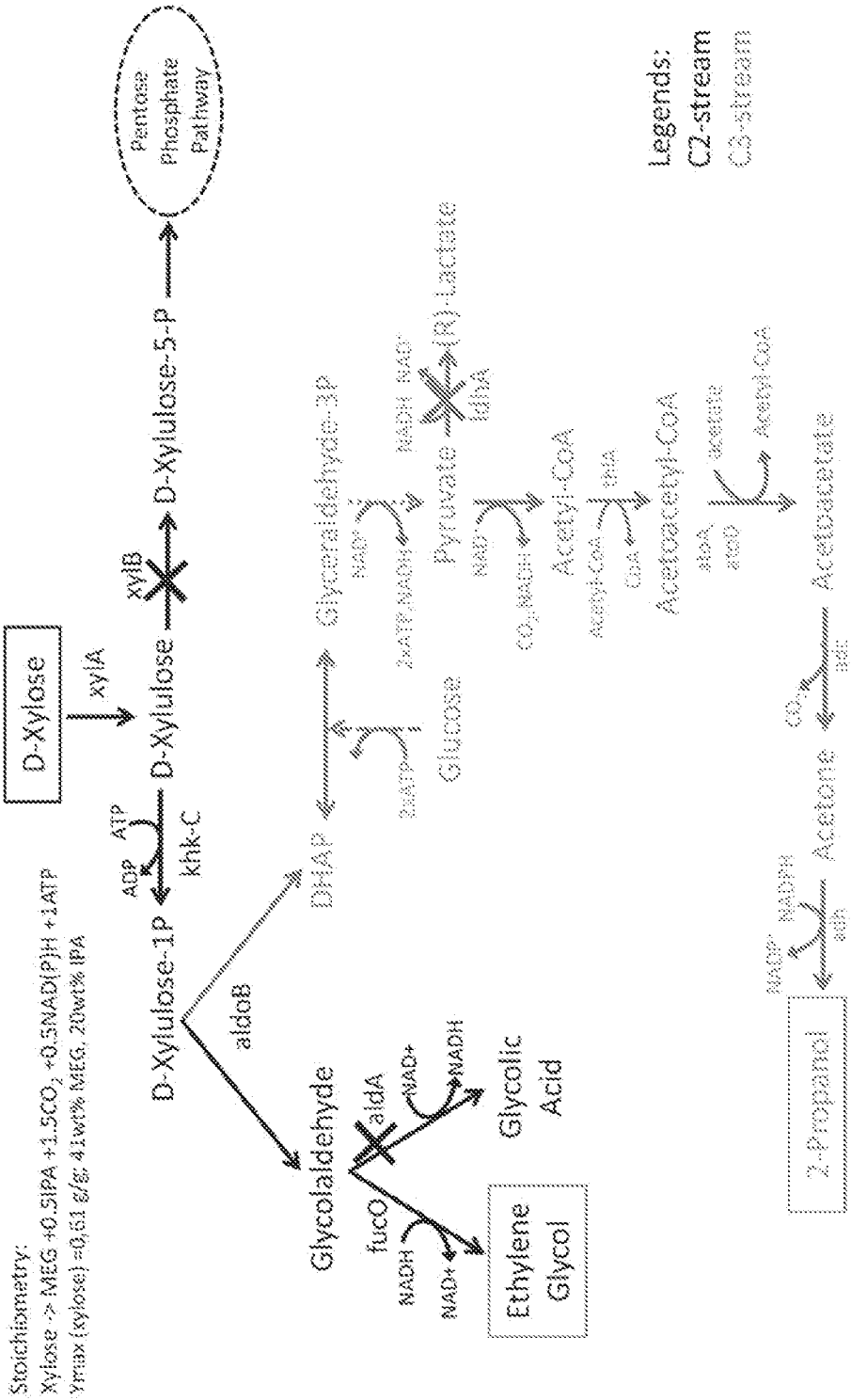
65. The recombinant microbe of claim 64, wherein the increased yield or titer is an increase of at least 2%.

66. The recombinant microbe of claim 64, wherein the increased yield or titer is an increase of at least 15%.

67. The recombinant microbe of any one of claims 1-23, wherein the microbe utilizes xylose, cellobiose, arabinose, mannose, and/or glucose in the coproduction of the MEG and the one or more C3 compounds.

68. The recombinant microbe of any one of claims 1-23, wherein the C3 compounds are selected from acetone, isopropanol, and propene.

FIG. 2



4/30

FIG. 4

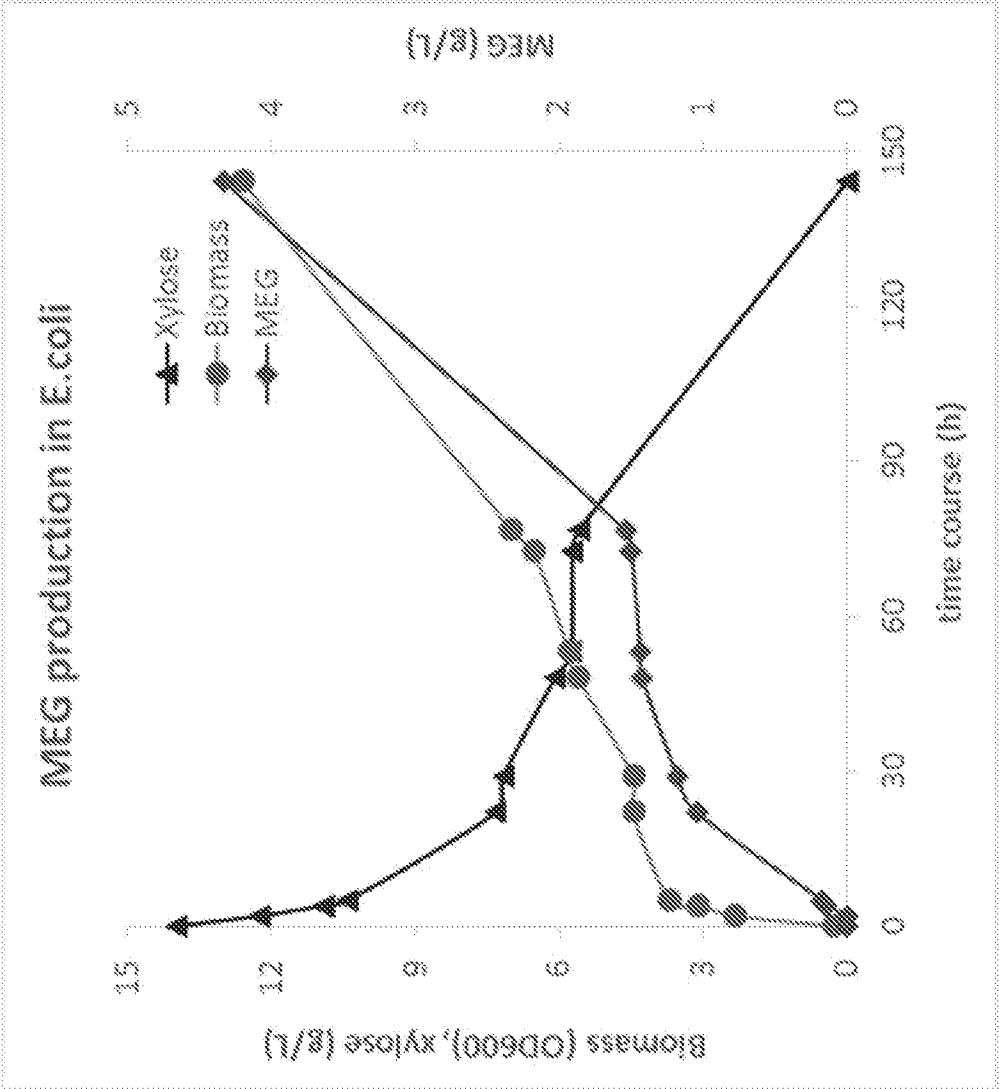
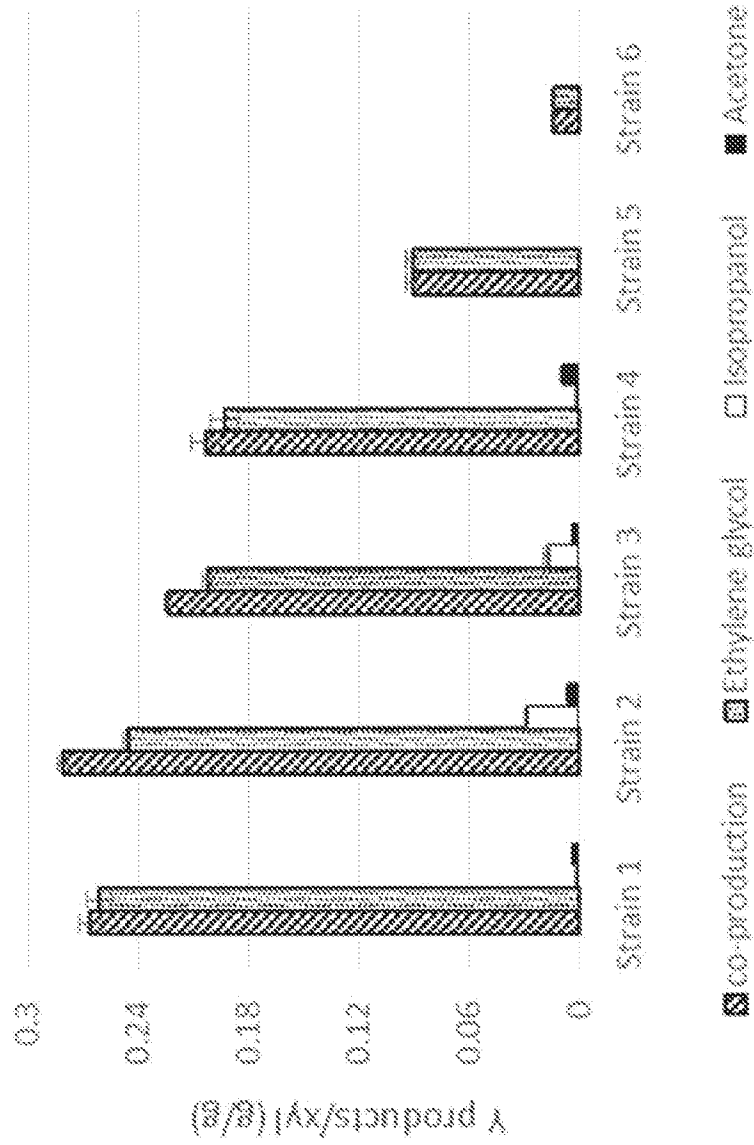


FIG. 5



6/30

FIG. 6

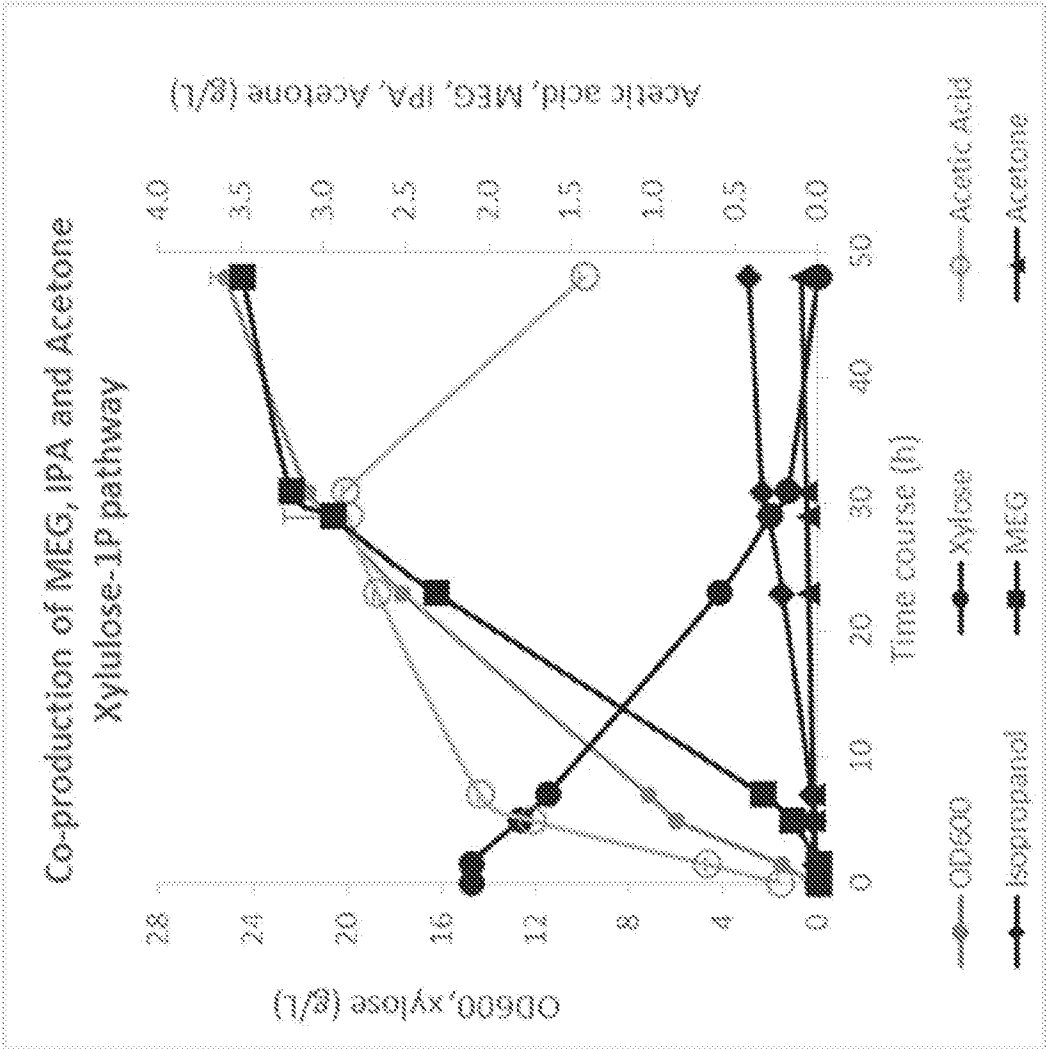
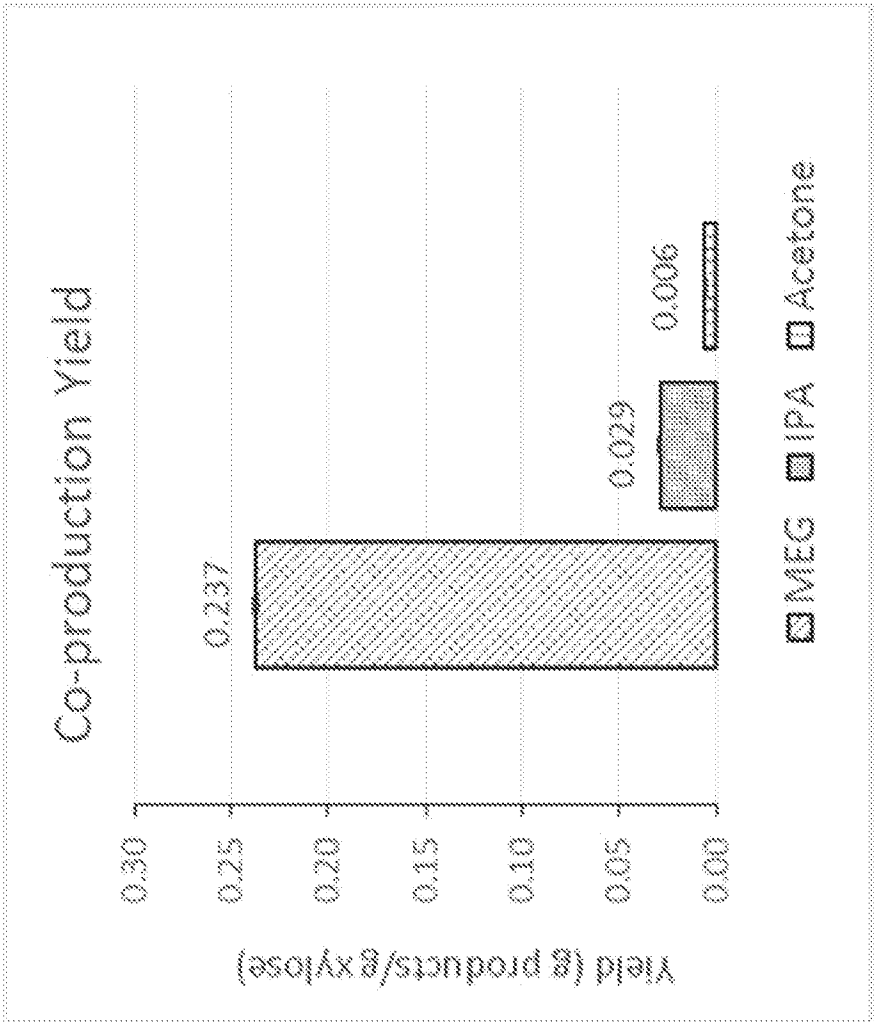
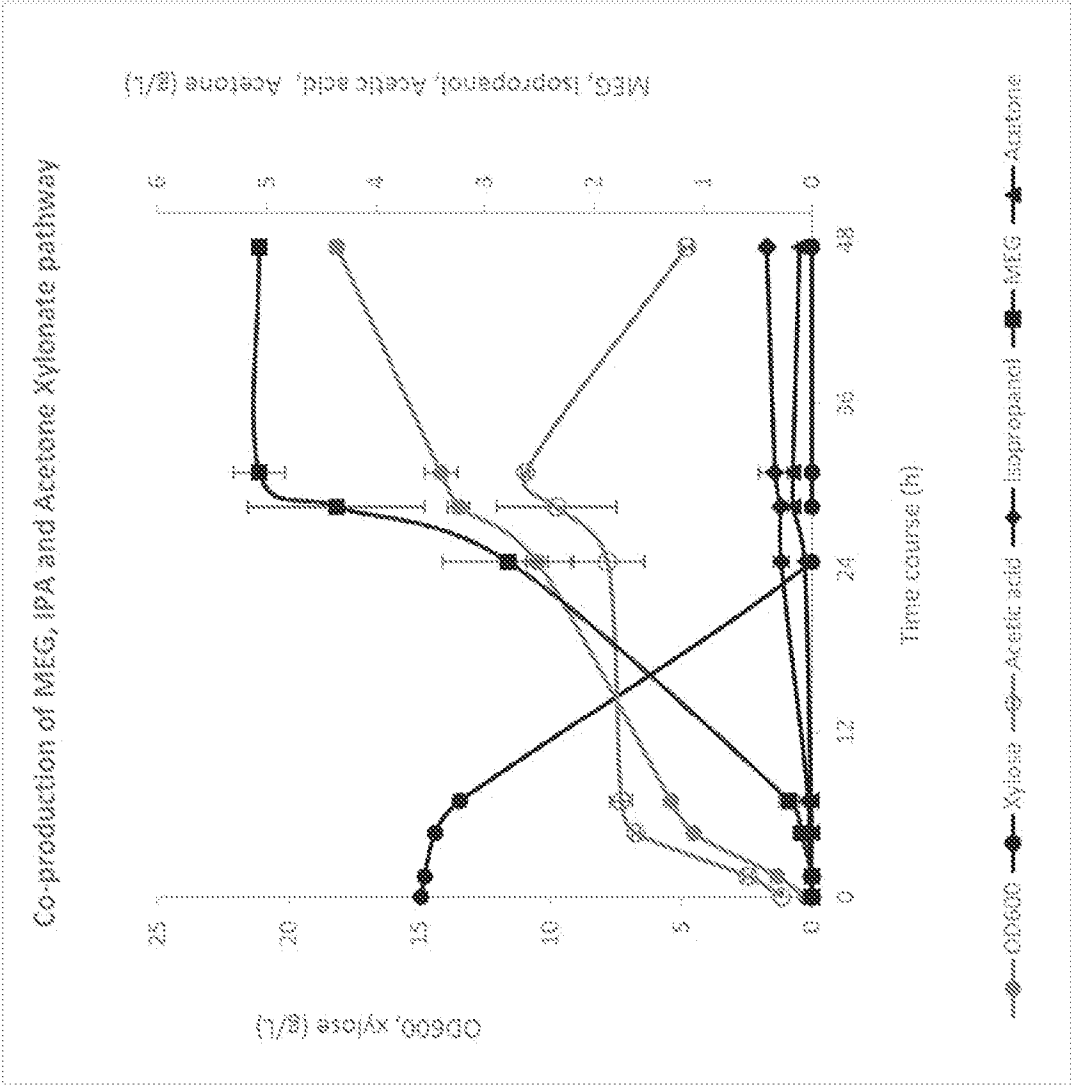


FIG. 7



8/30

FIG. 8



9/30

FIG. 9

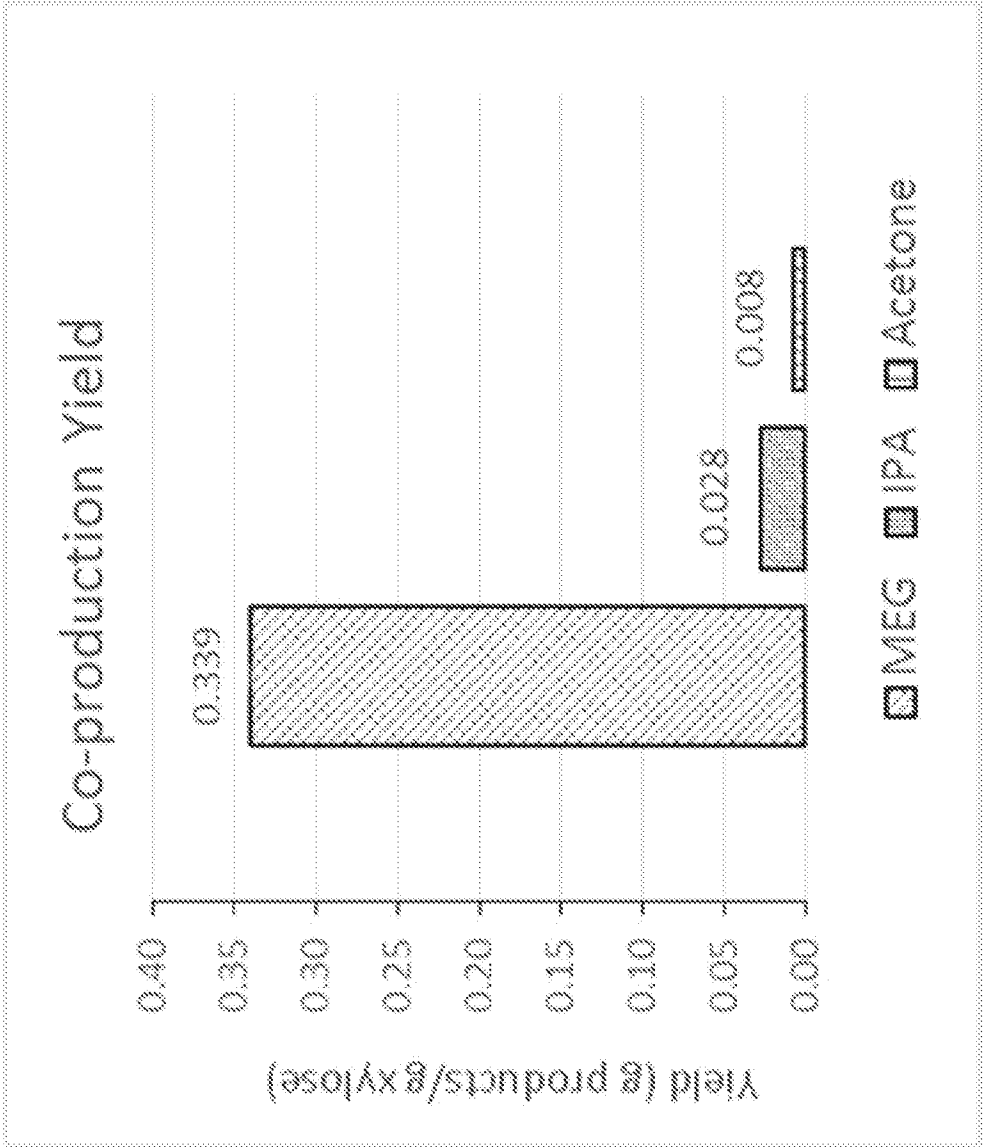


FIG. 10

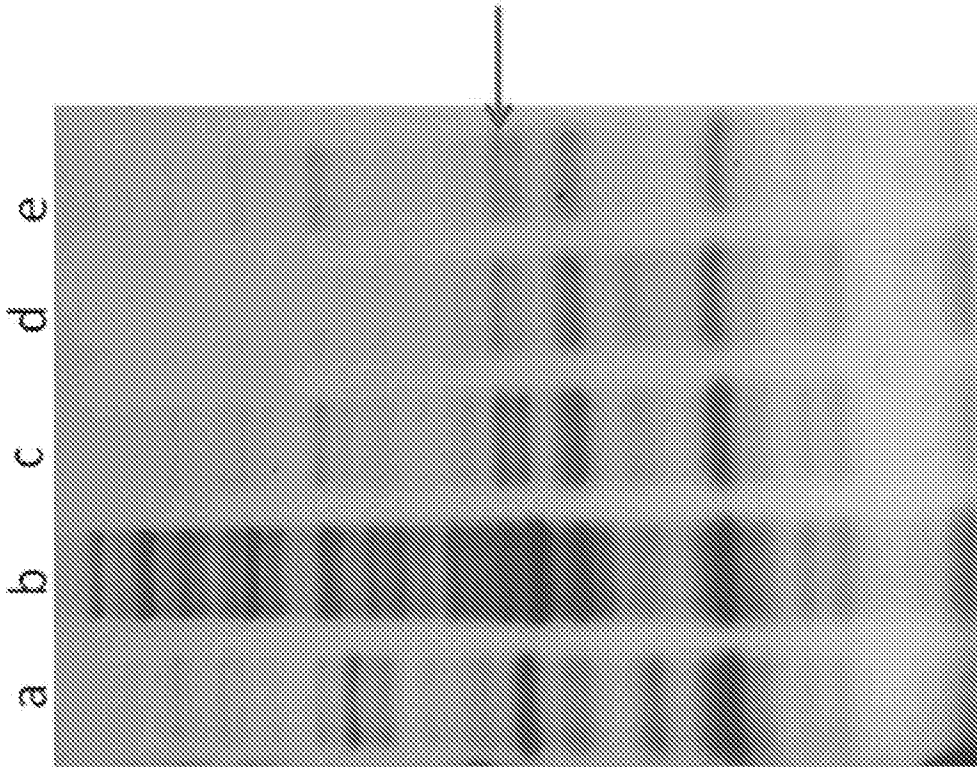
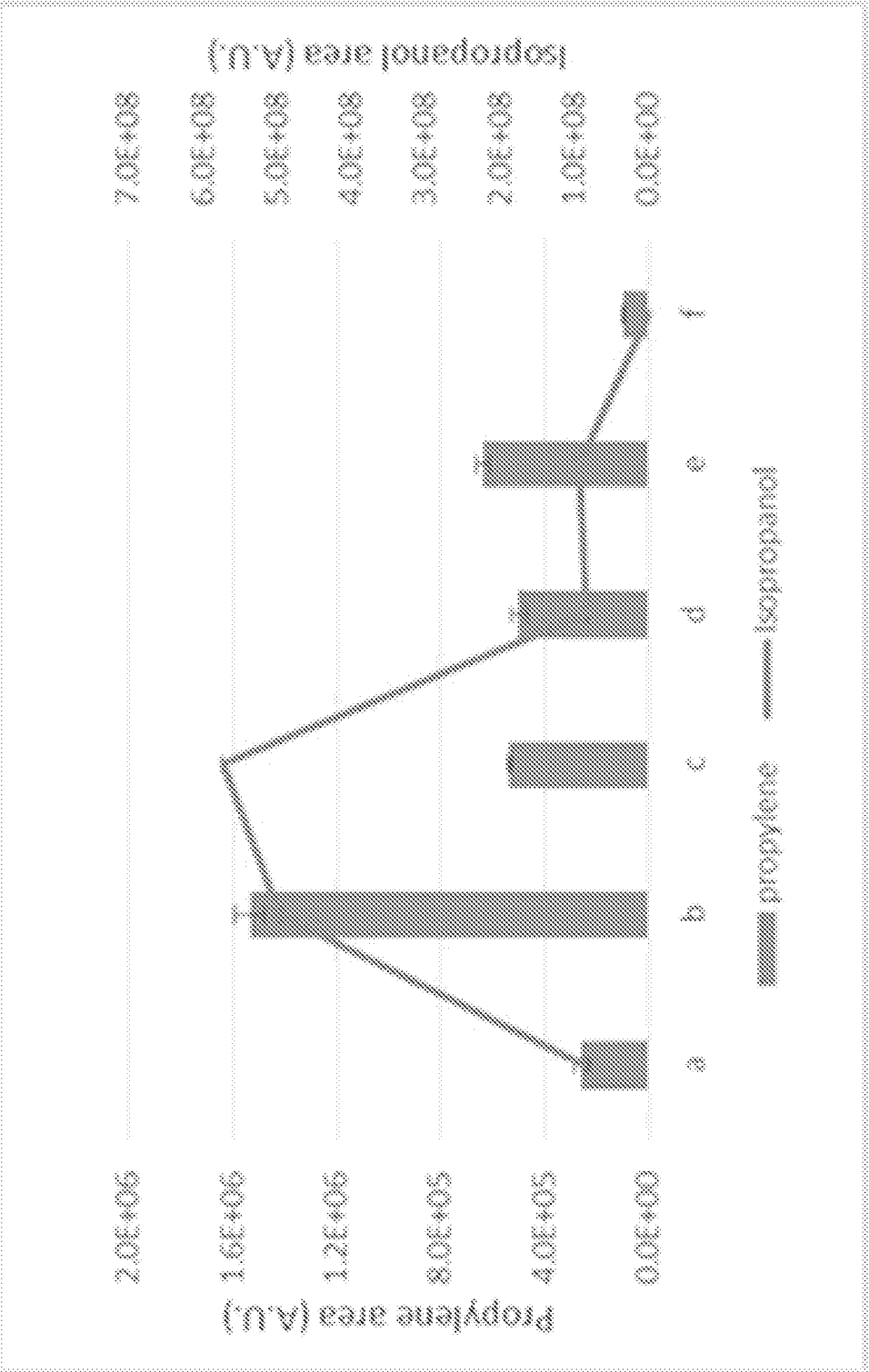


FIG. 11



12/30

FIG. 12A

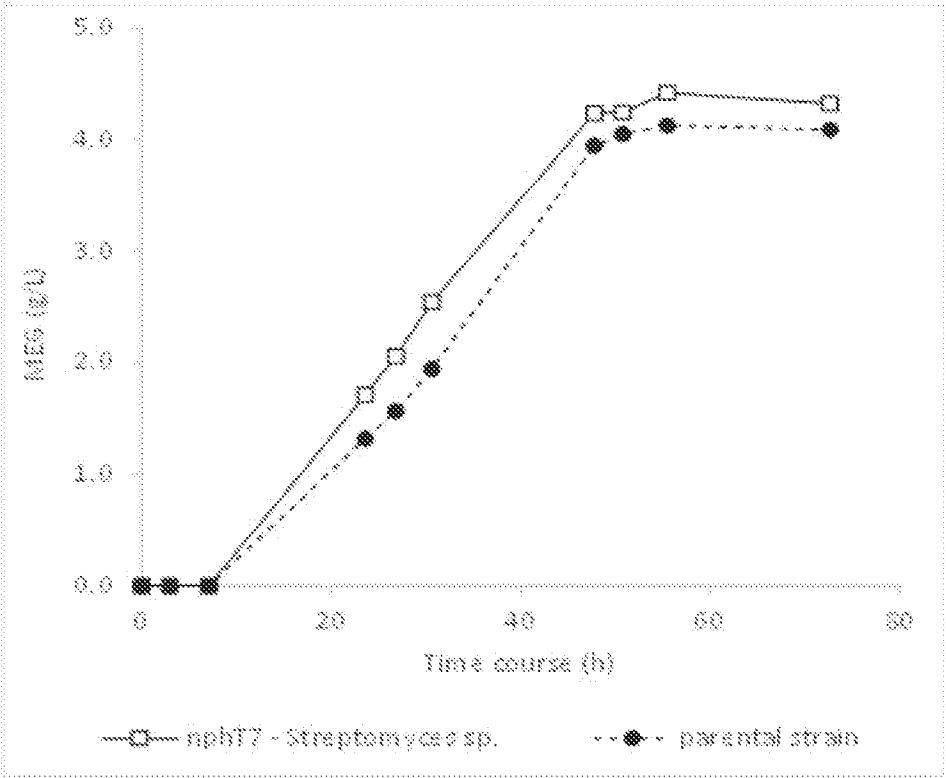


FIG. 12B

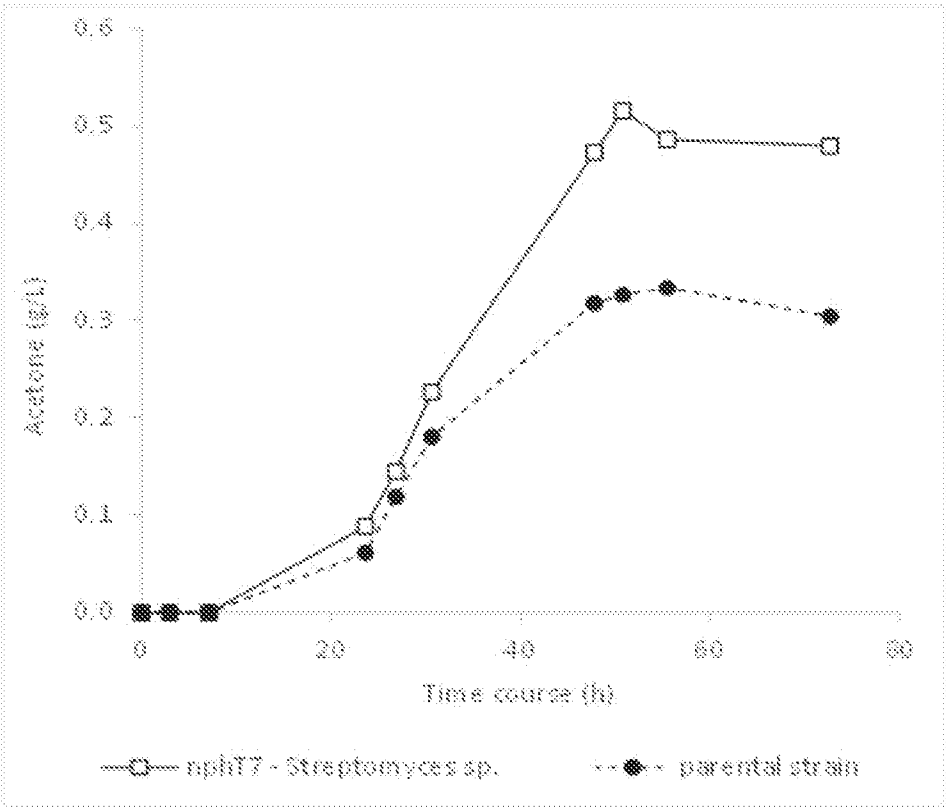


FIG. 12C

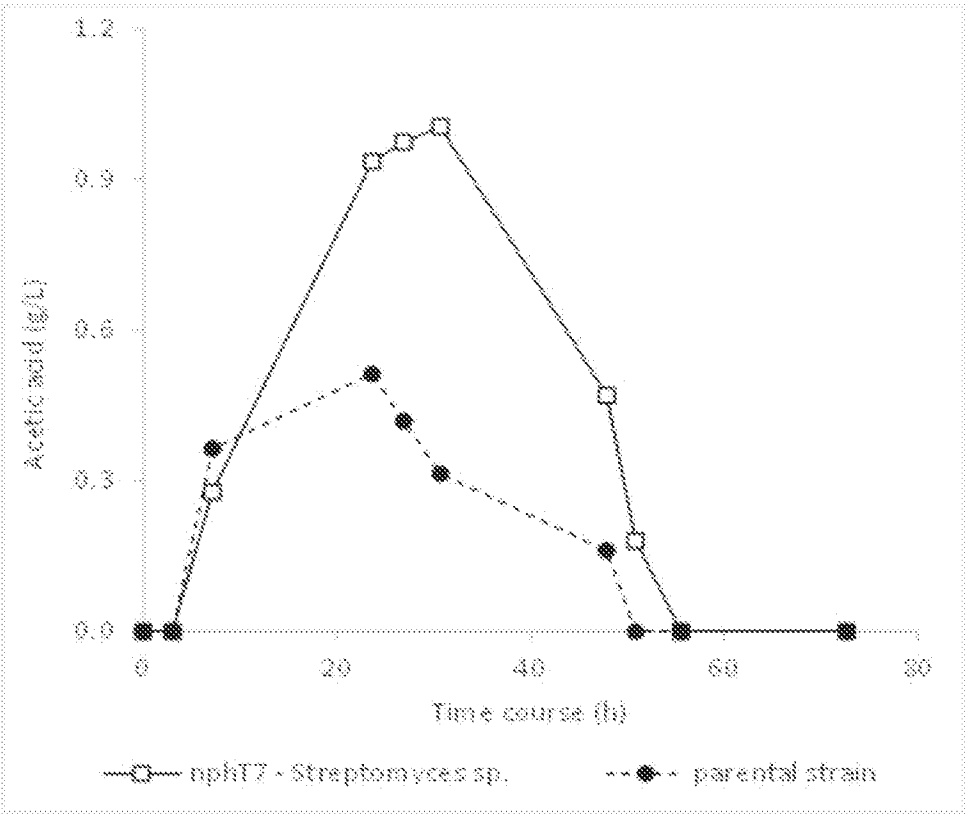


FIG. 12D

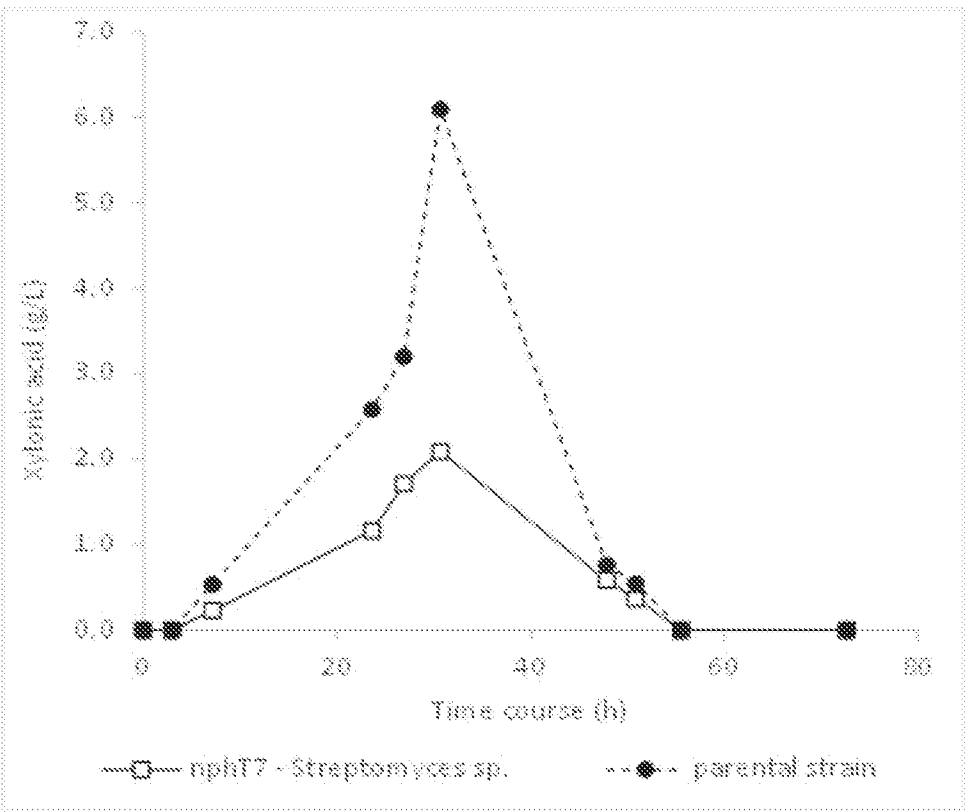


FIG. 13A

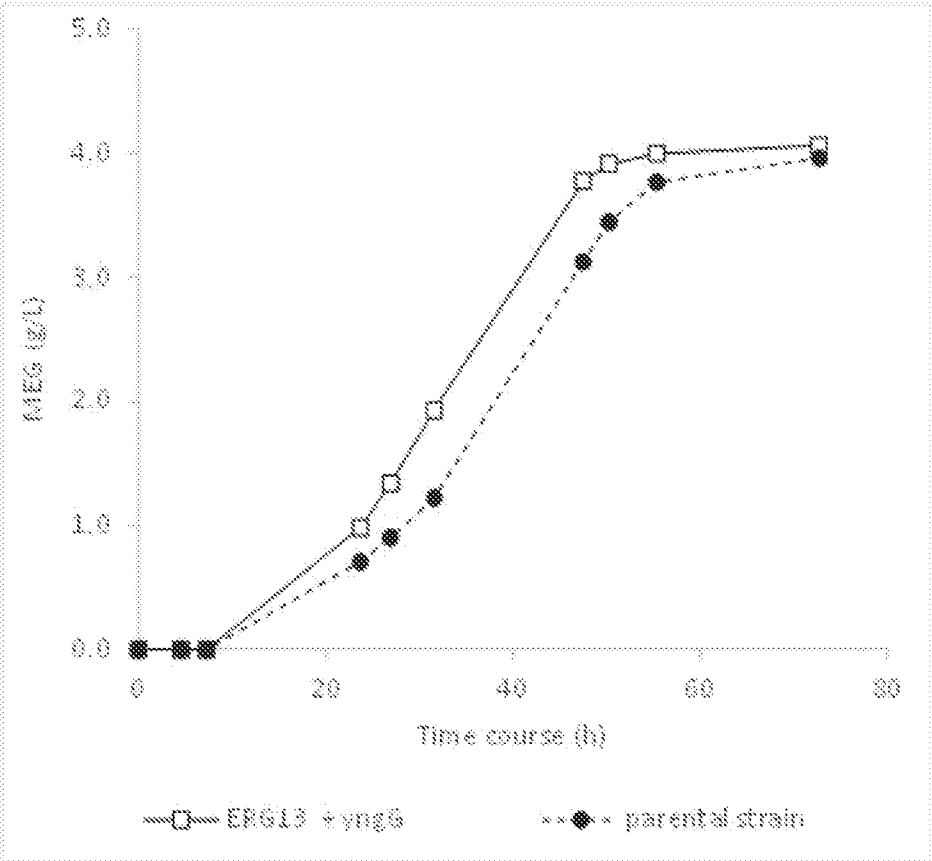
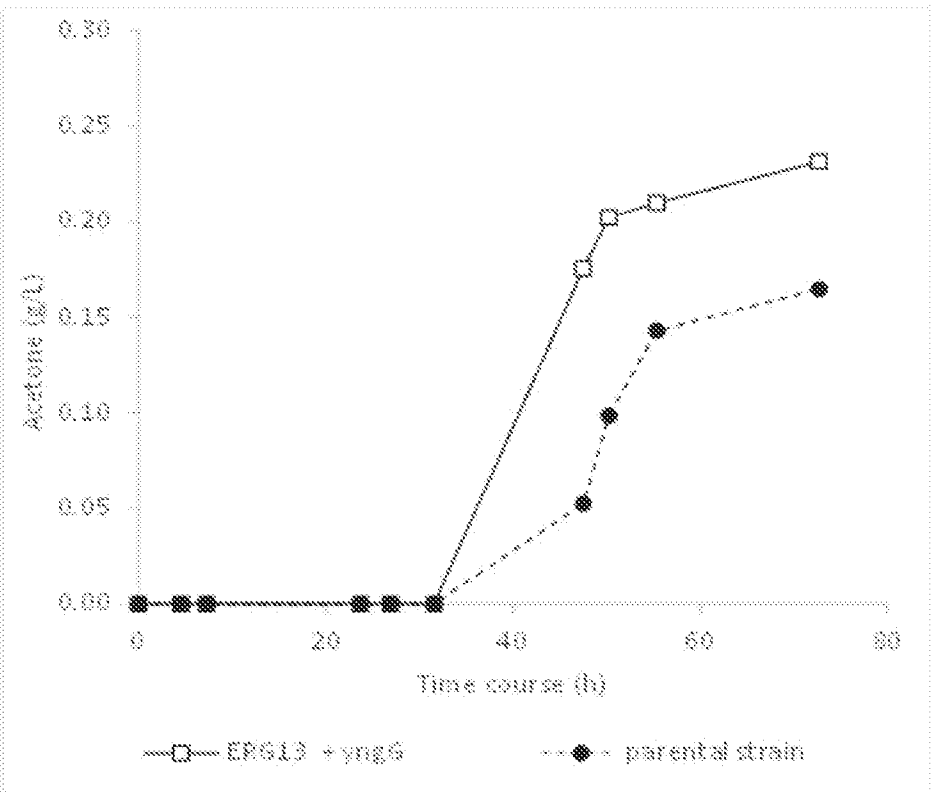


FIG. 13B



15/30

FIG. 13C

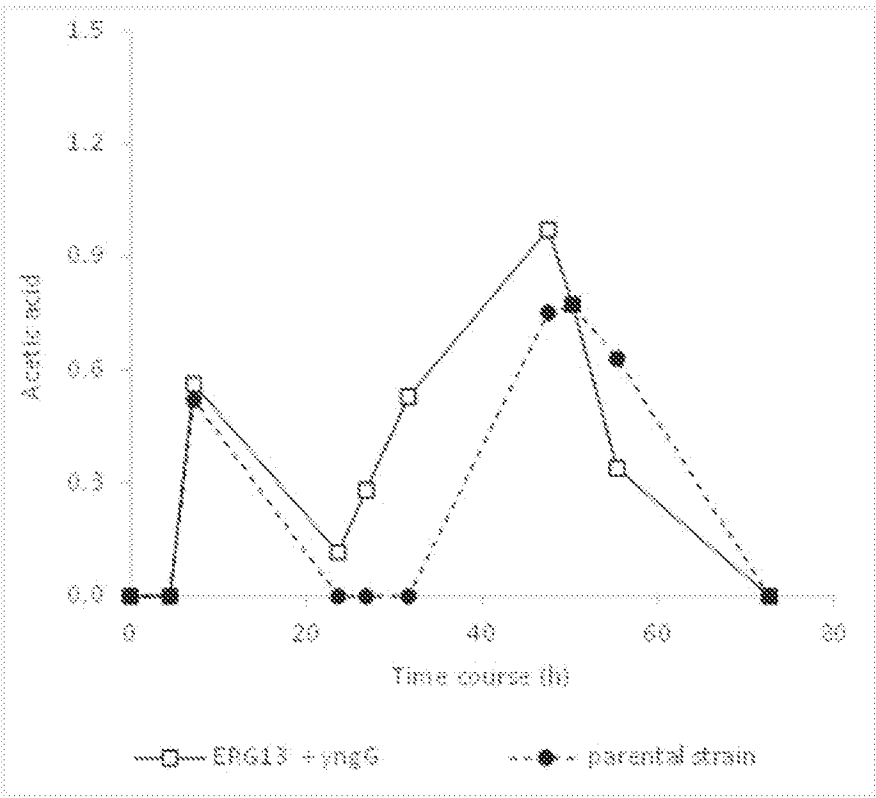
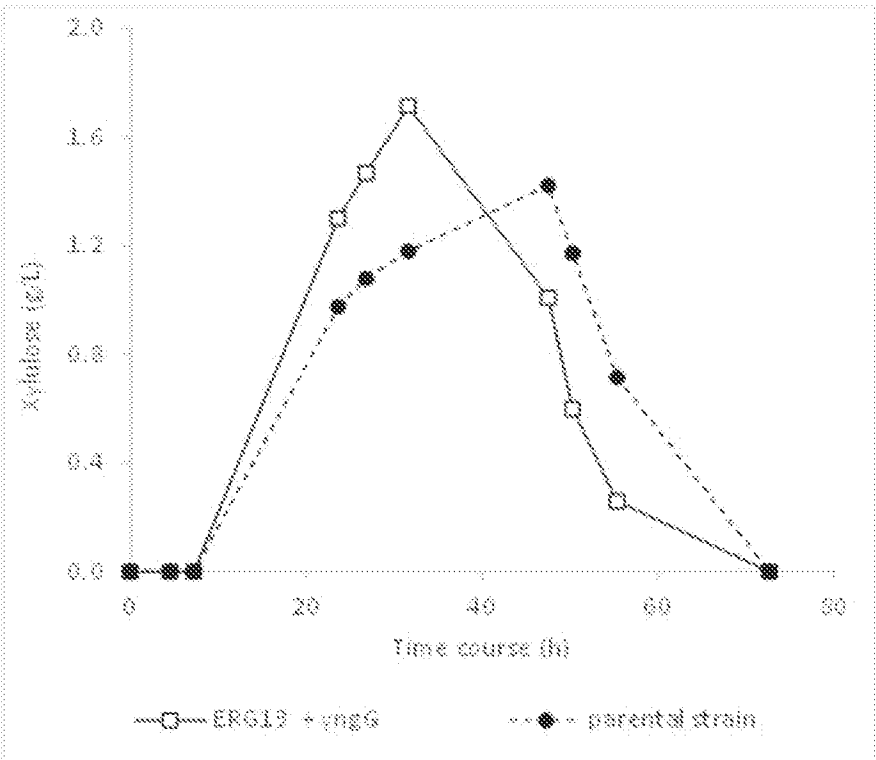


FIG. 13D



16/30

FIG. 14A

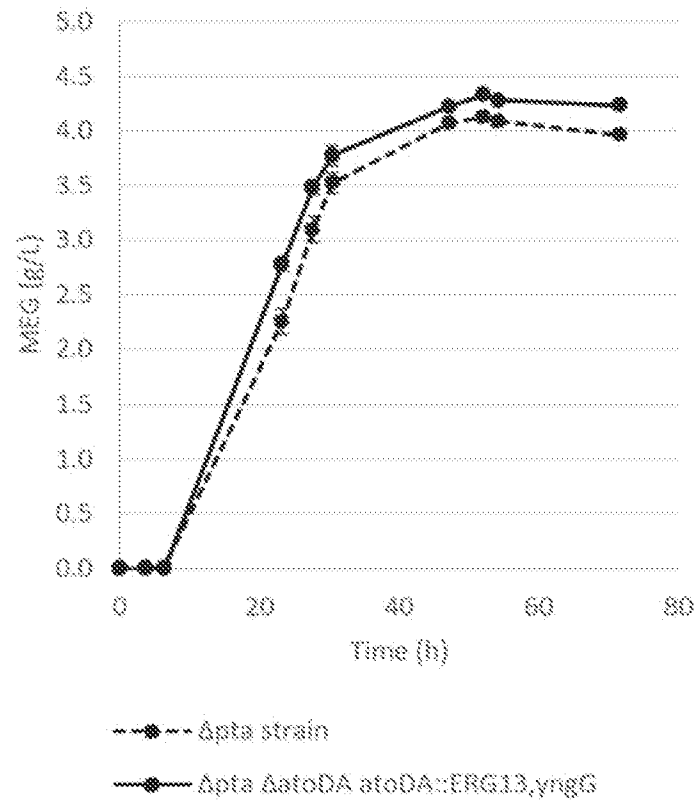


FIG. 14B

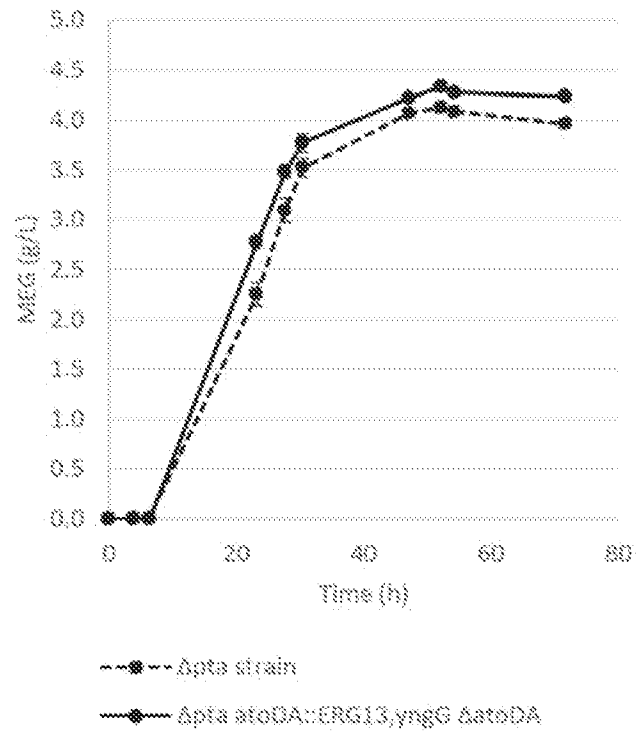
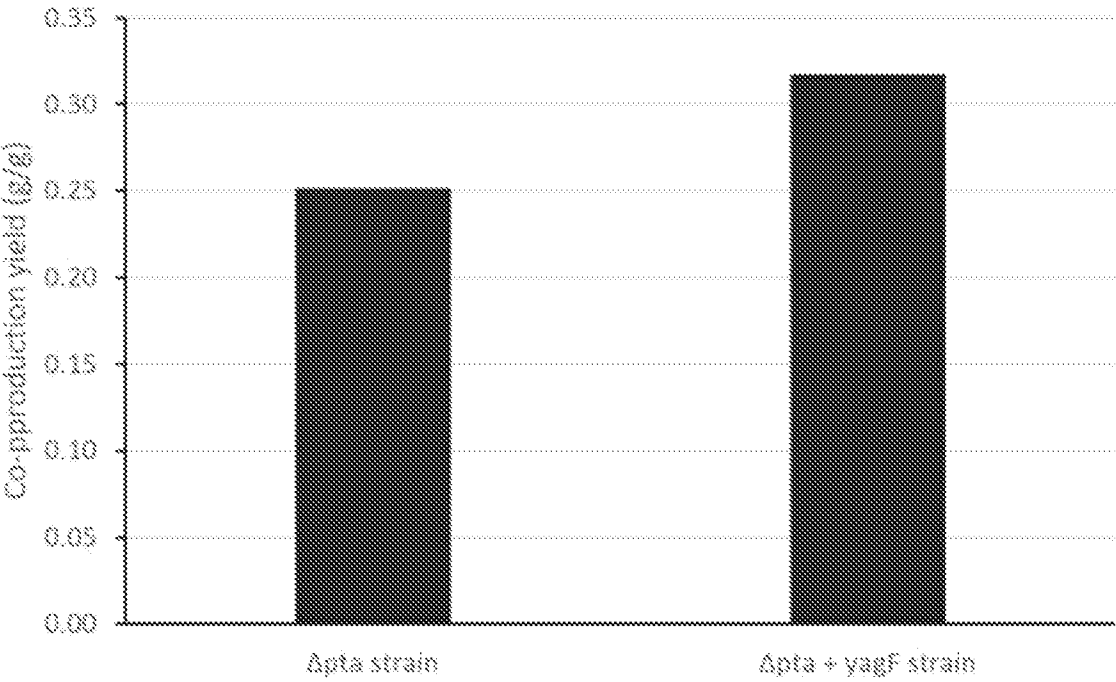


FIG. 15



18/30
FIG. 16A

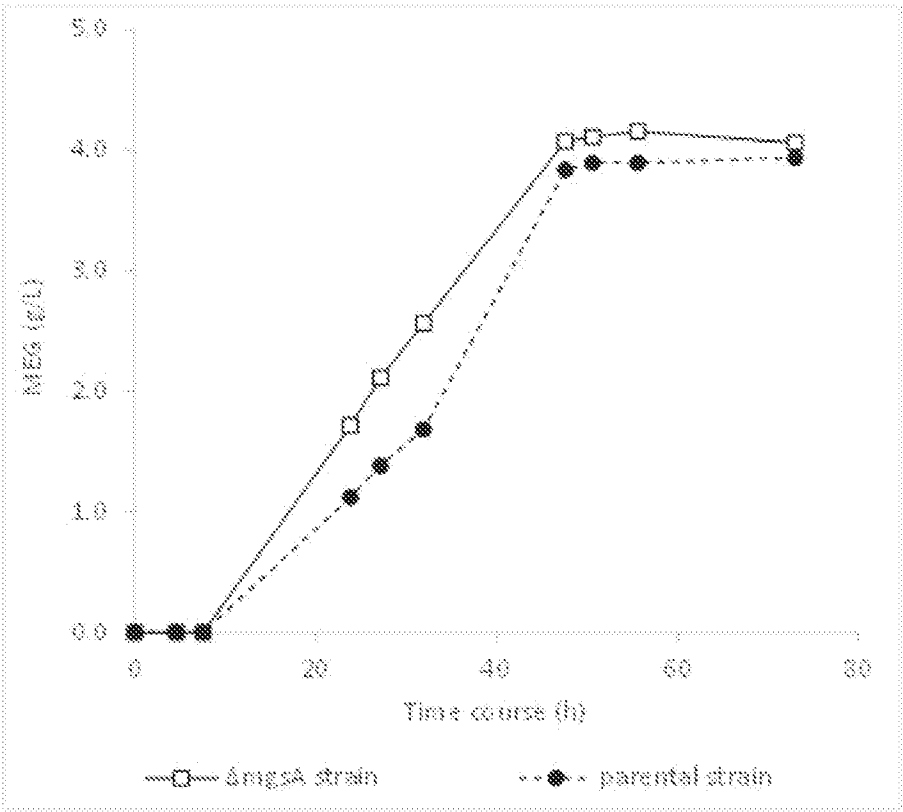
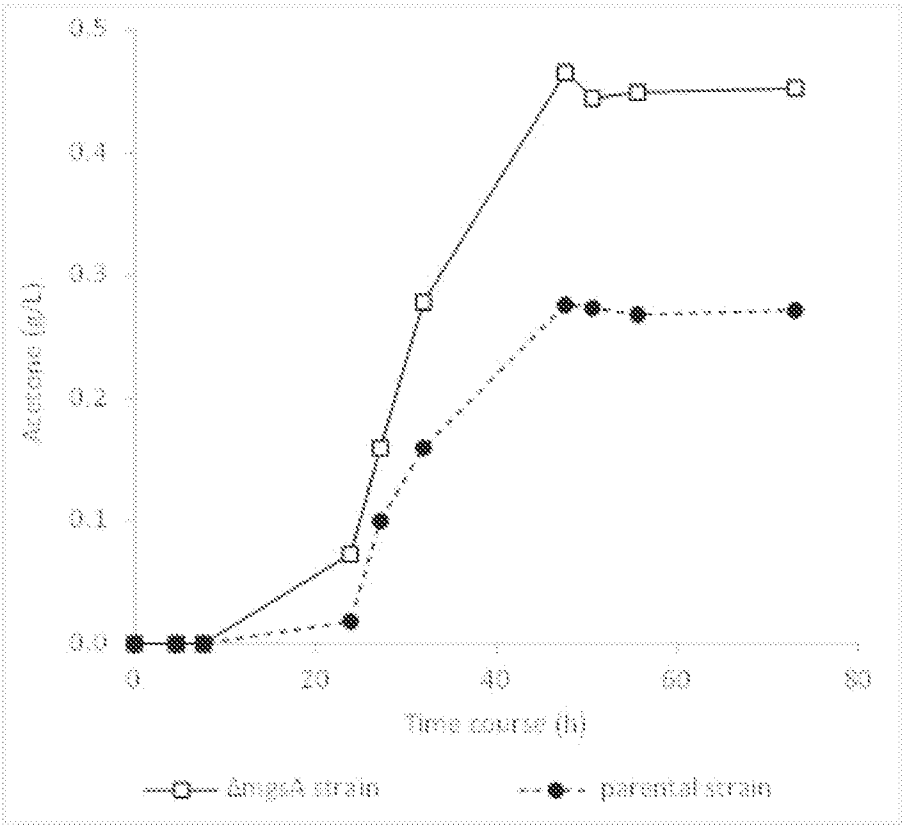


FIG. 16B



19/30

FIG. 16C

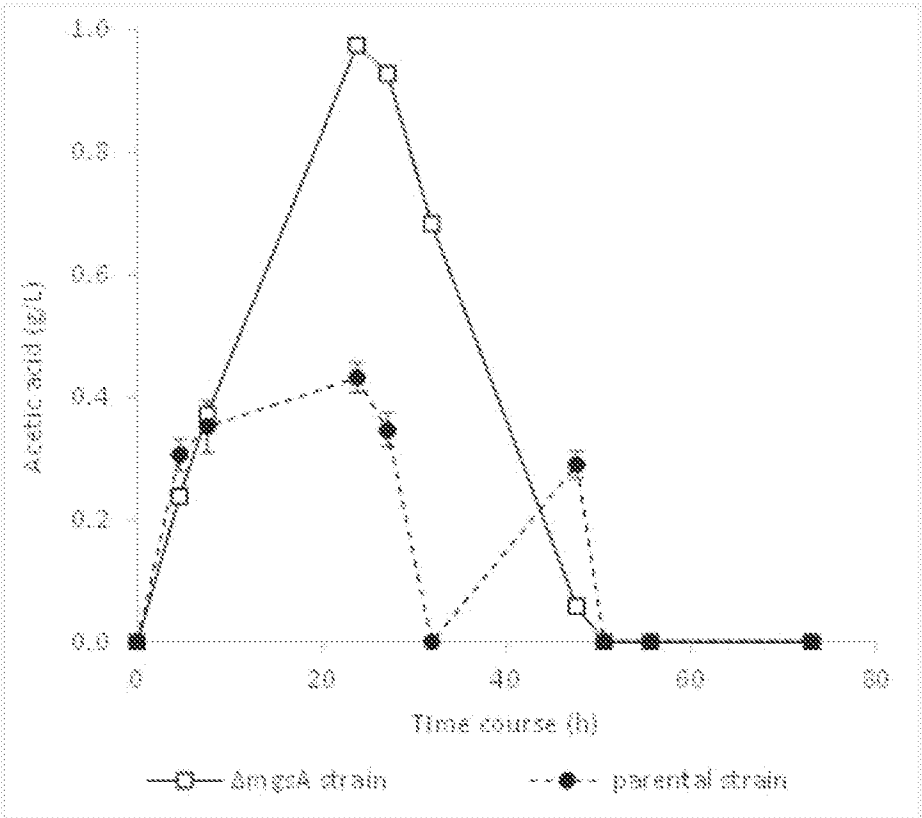
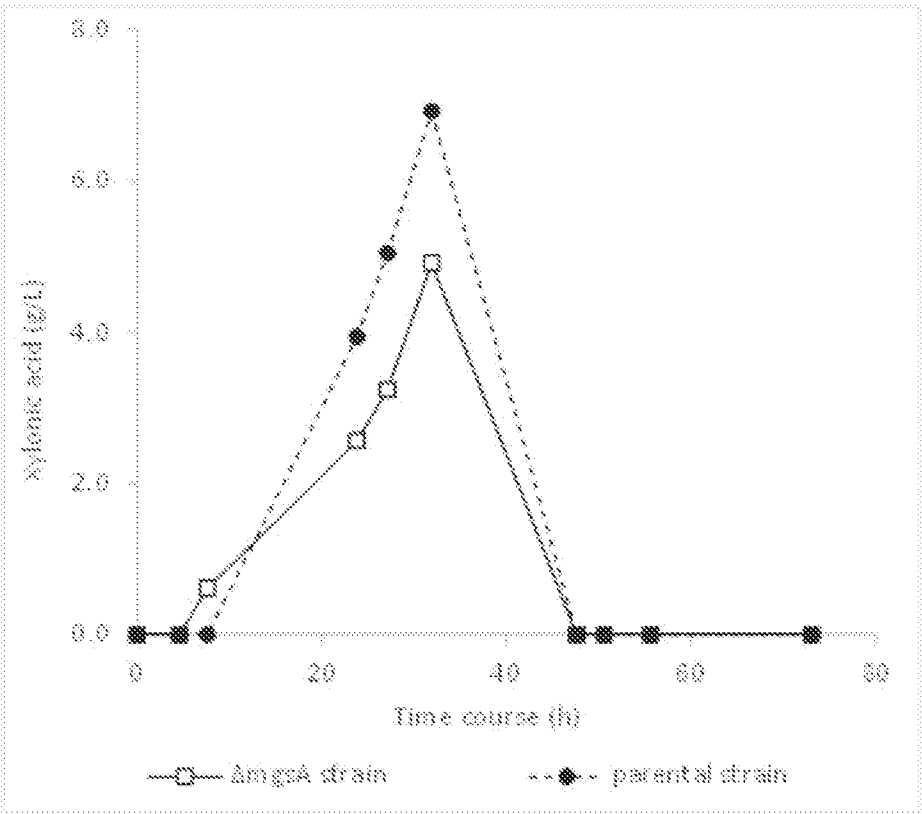


FIG. 16D



20/30

FIG. 17A

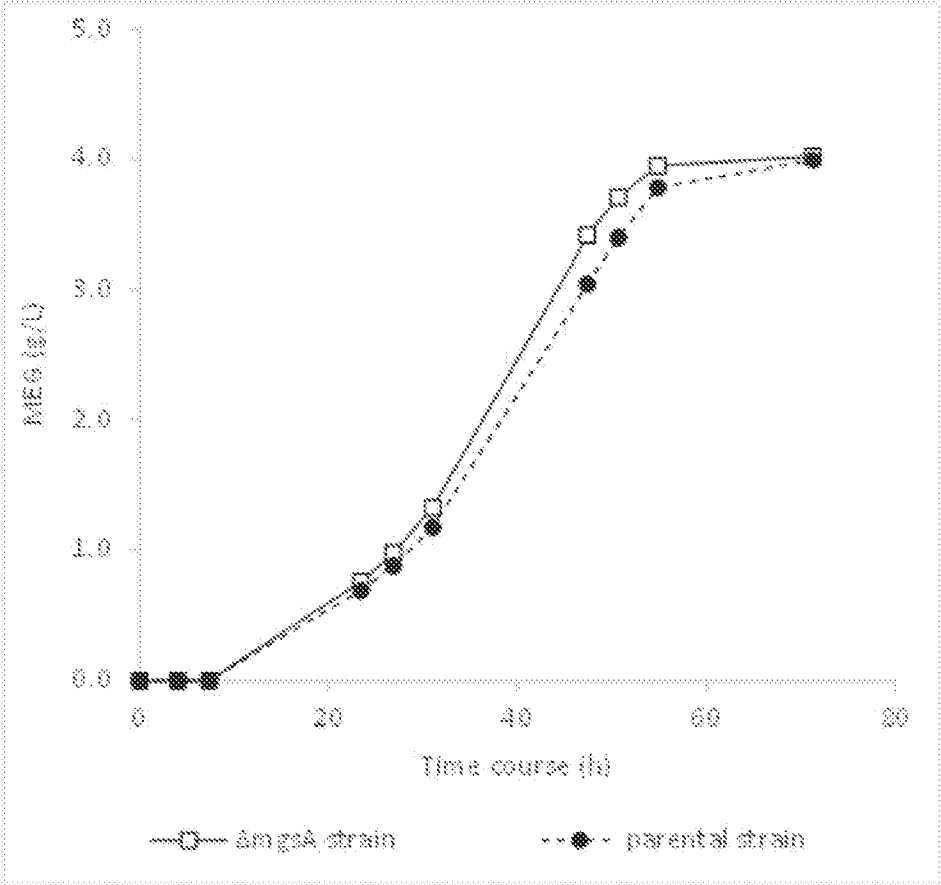
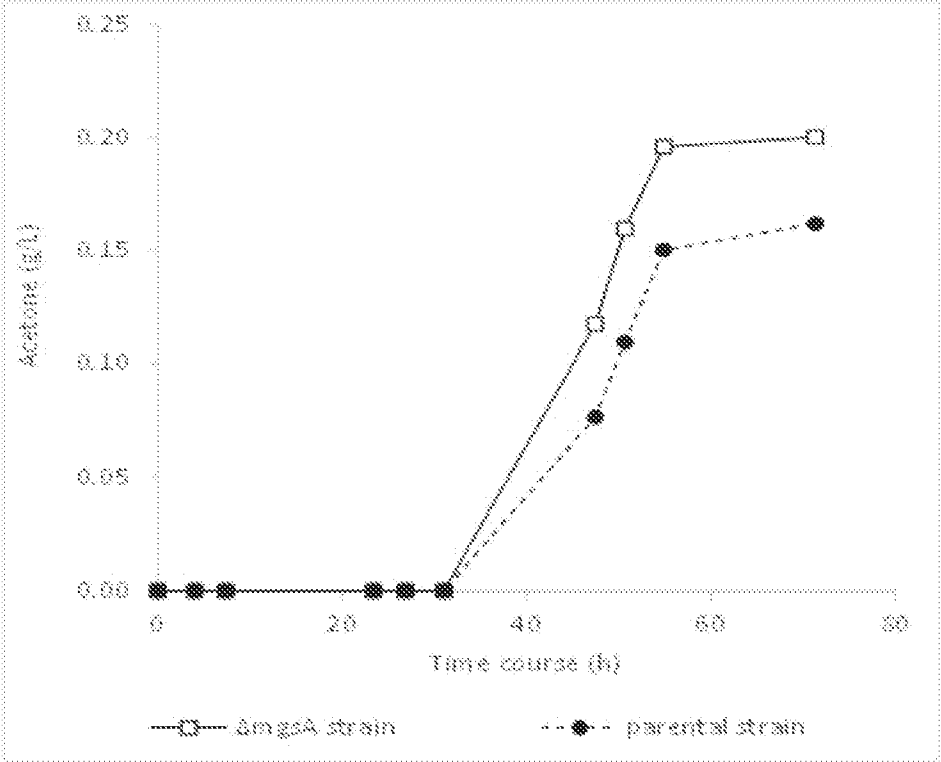


FIG. 17B



21/30

FIG. 17C

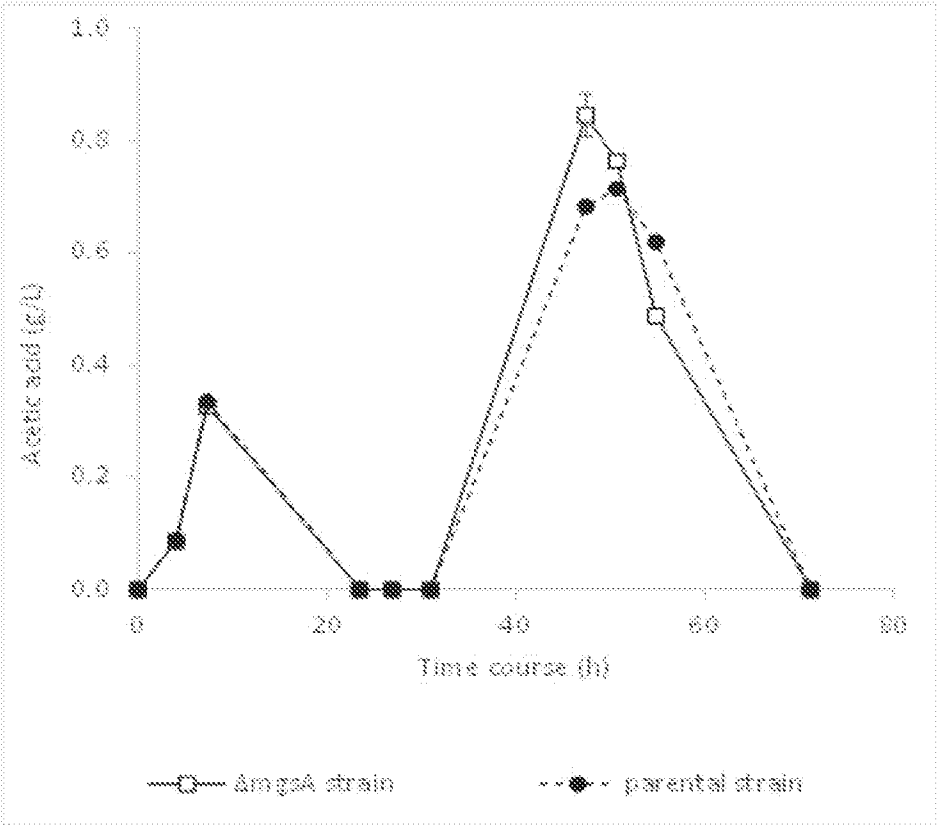
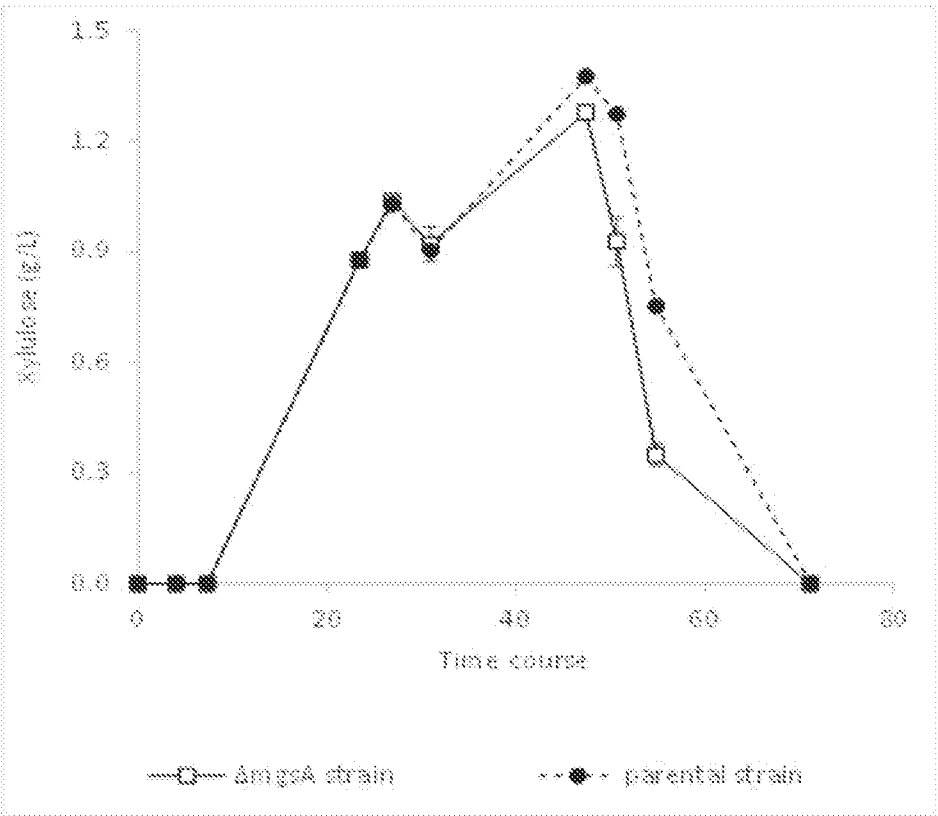


FIG. 17D



22/30
FIG. 18A

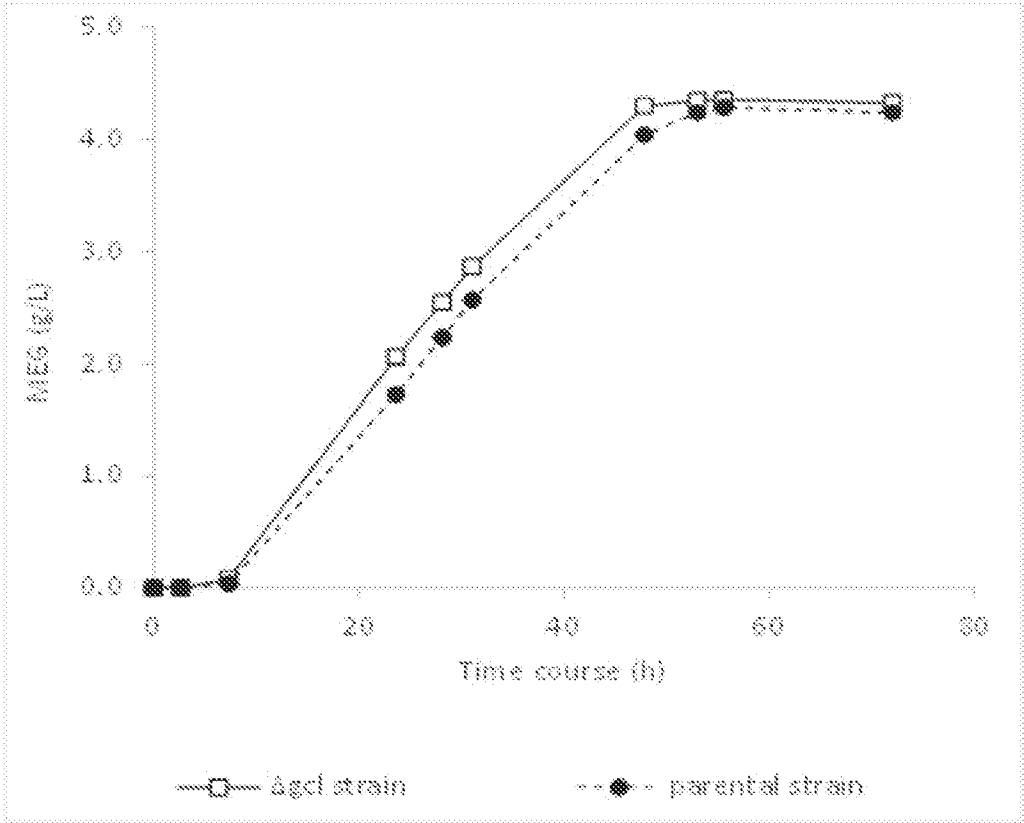
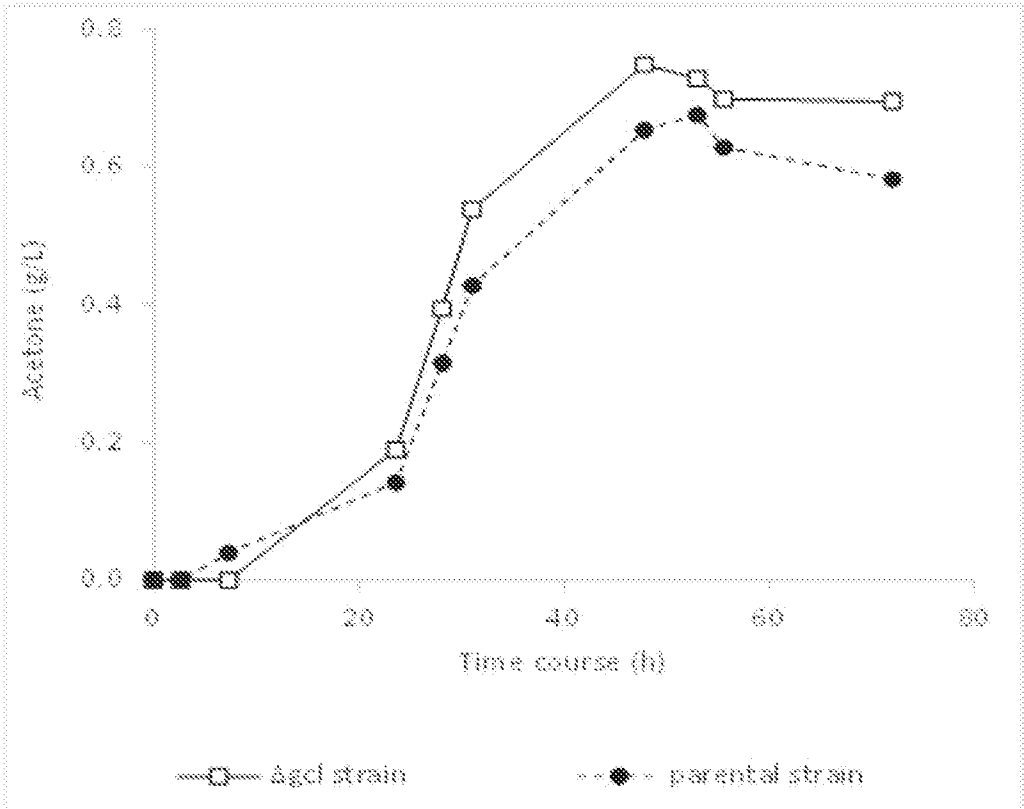


FIG. 18B



23/30

FIG. 18C

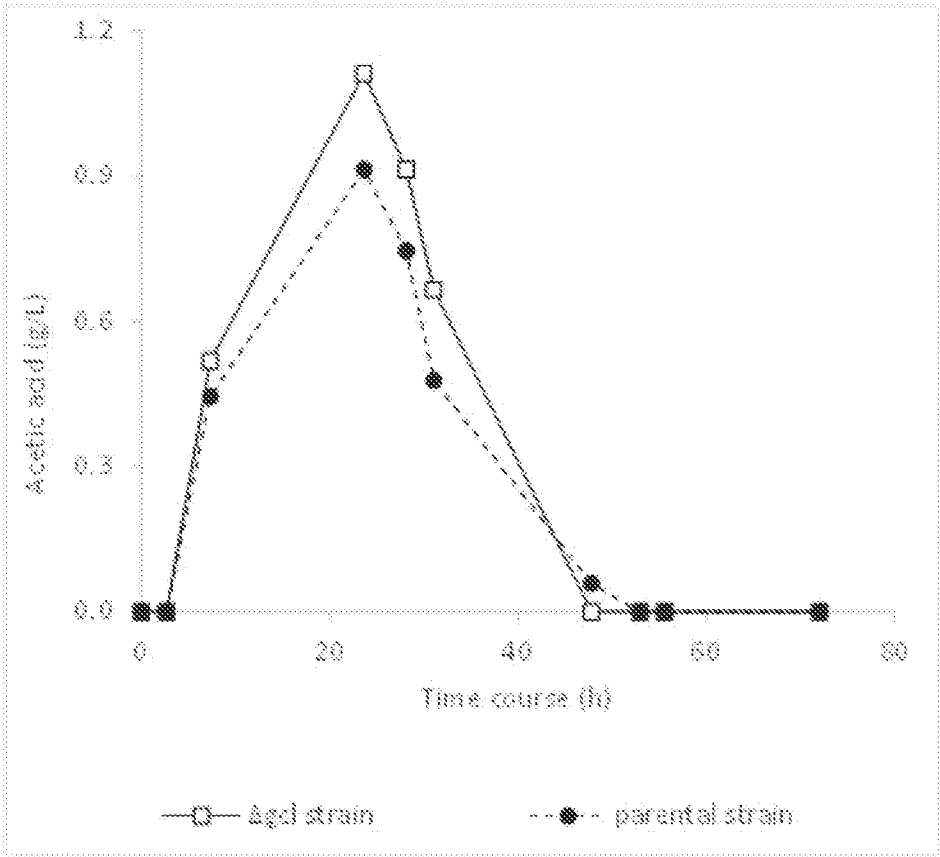
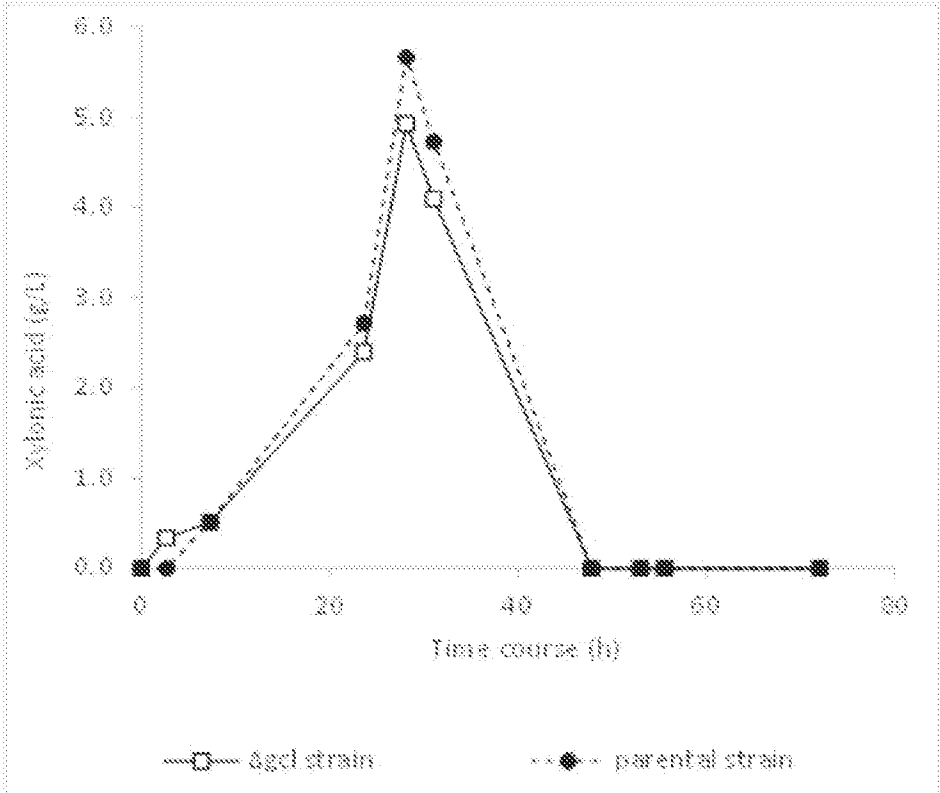


FIG. 18D



24/30
FIG. 19A

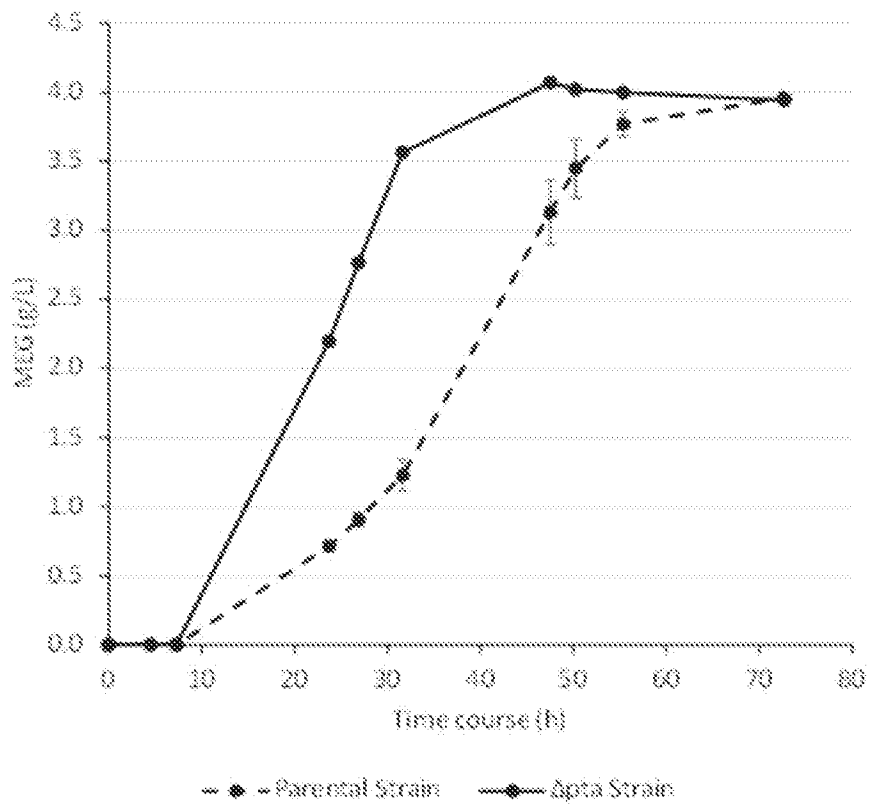
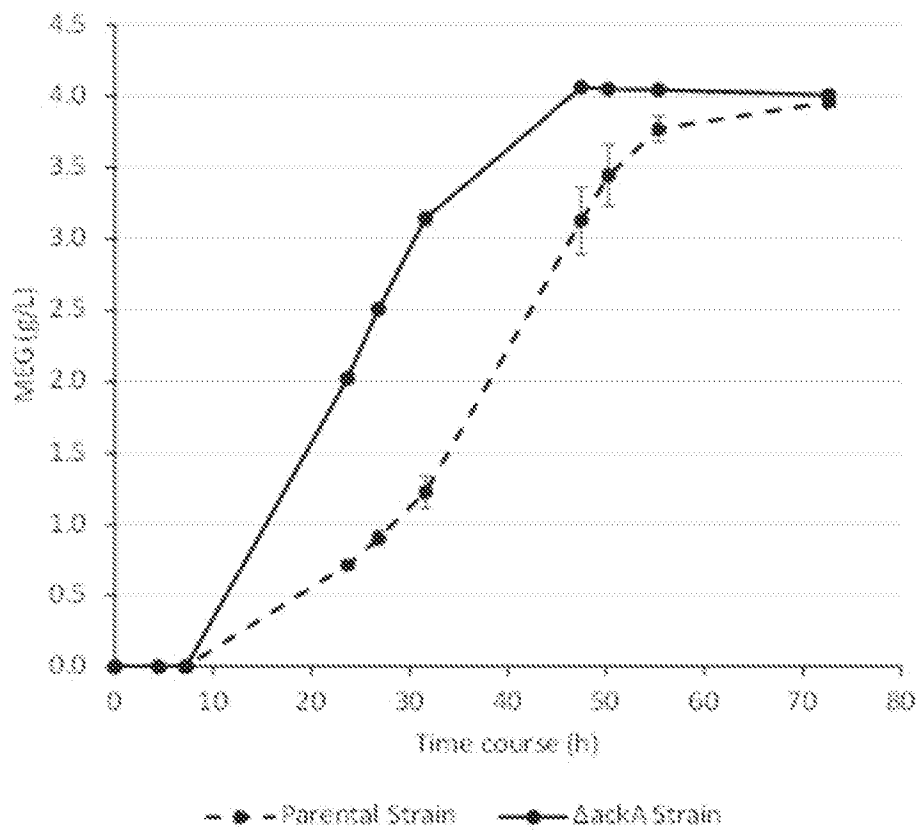


FIG. 19B



25/30

FIG. 20A

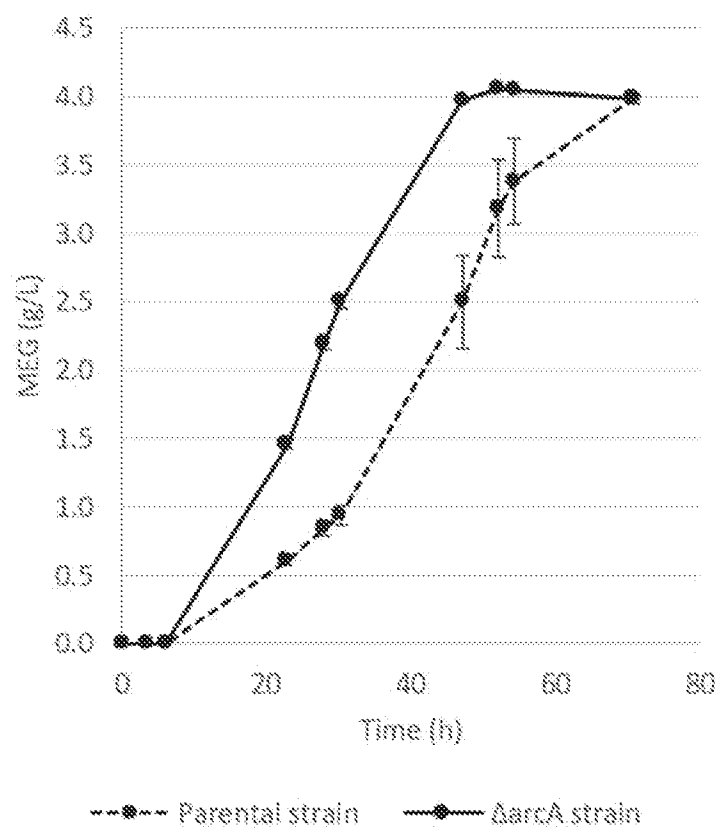
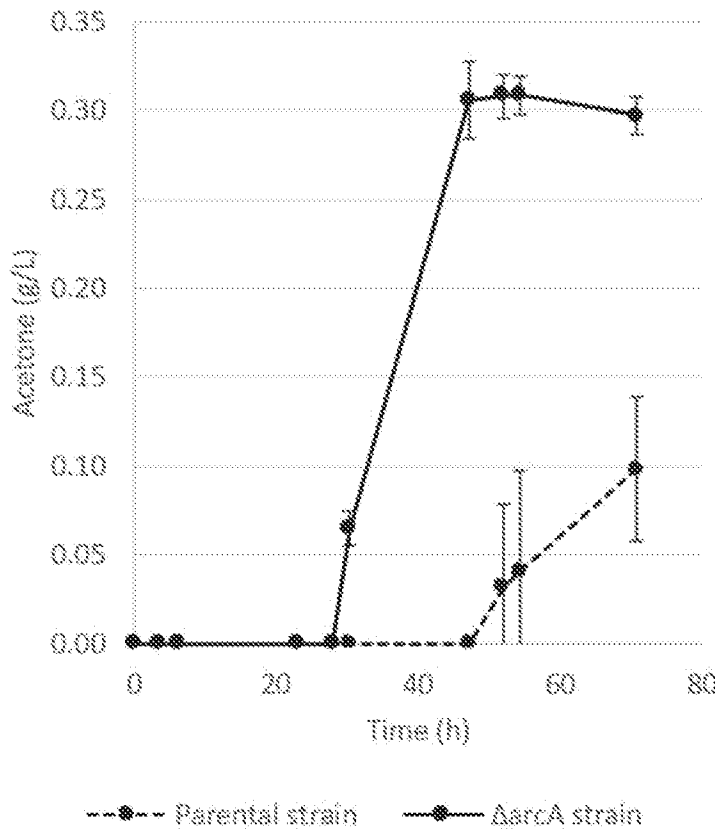


FIG. 20B



26/30

FIG. 21A

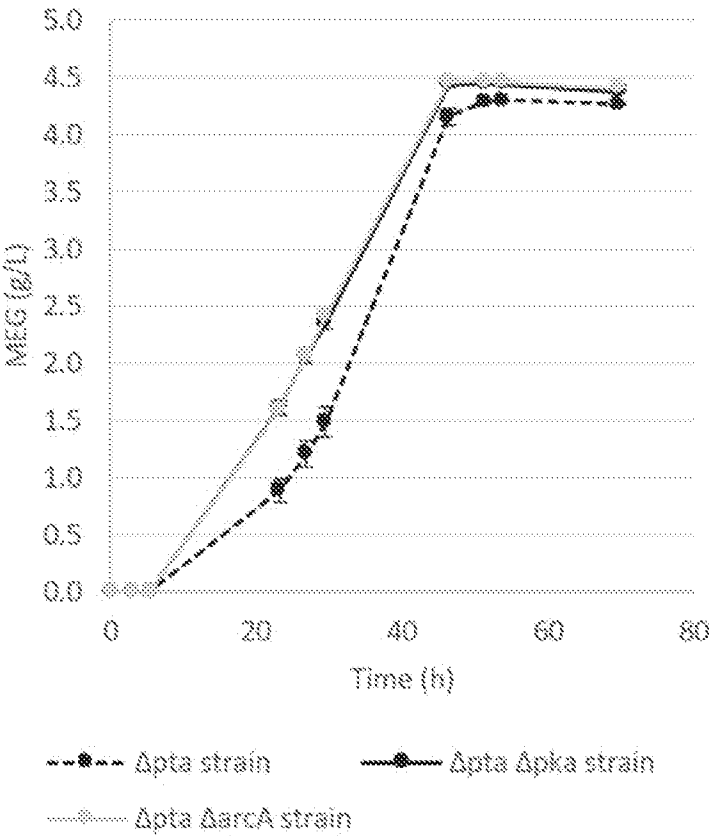
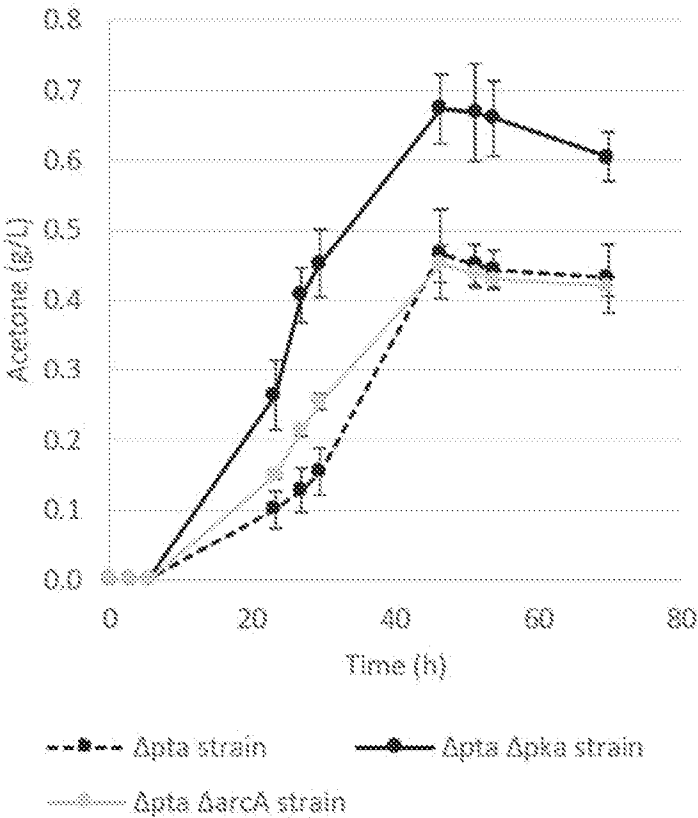


FIG. 21B



27/30

FIG 22A

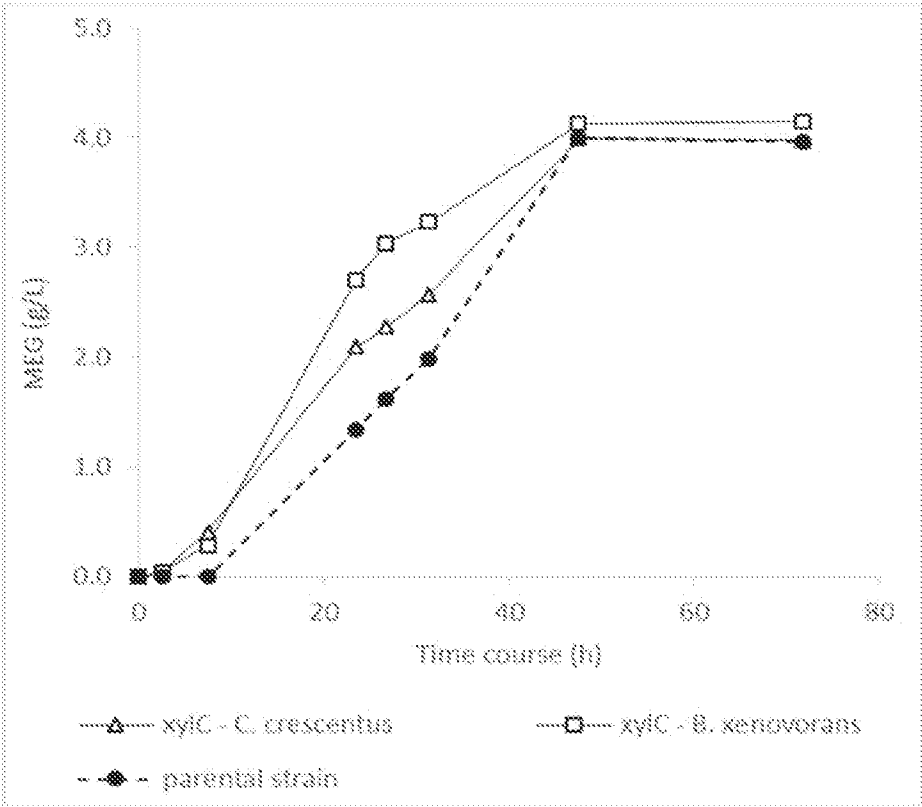
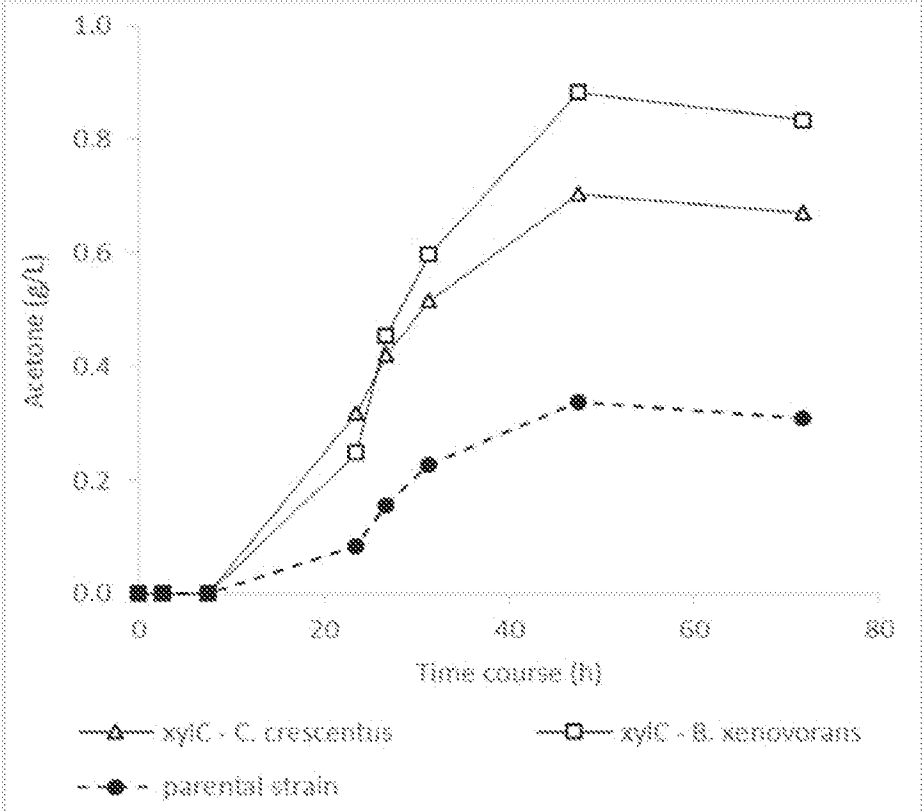


FIG 22B



28/30

FIG 22C

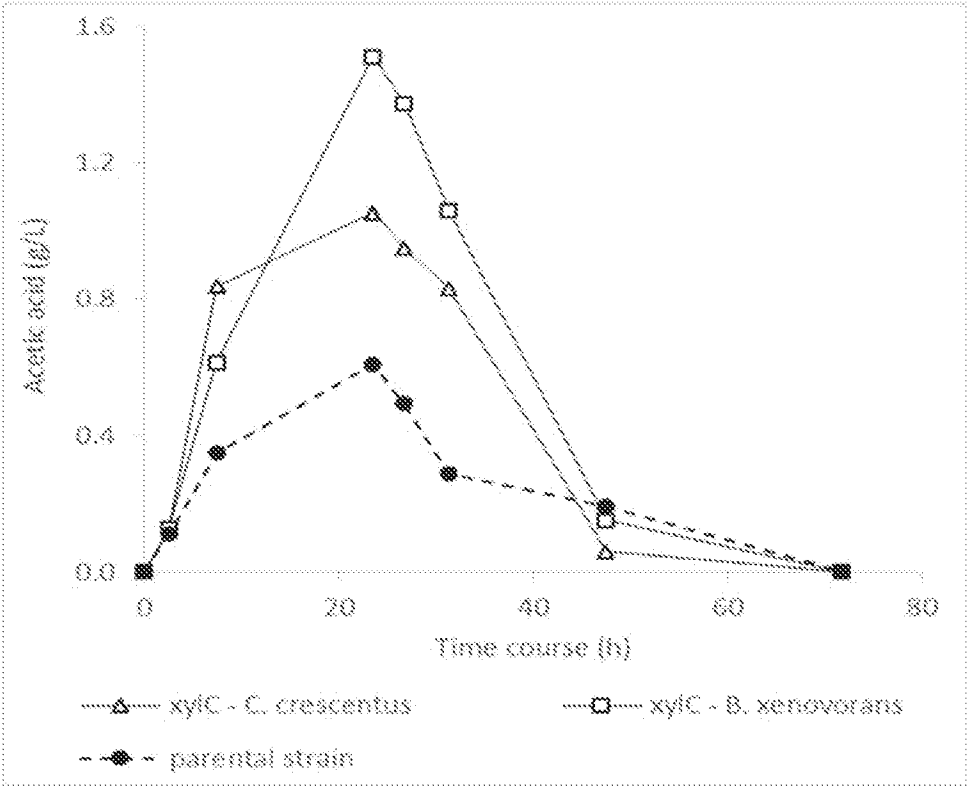
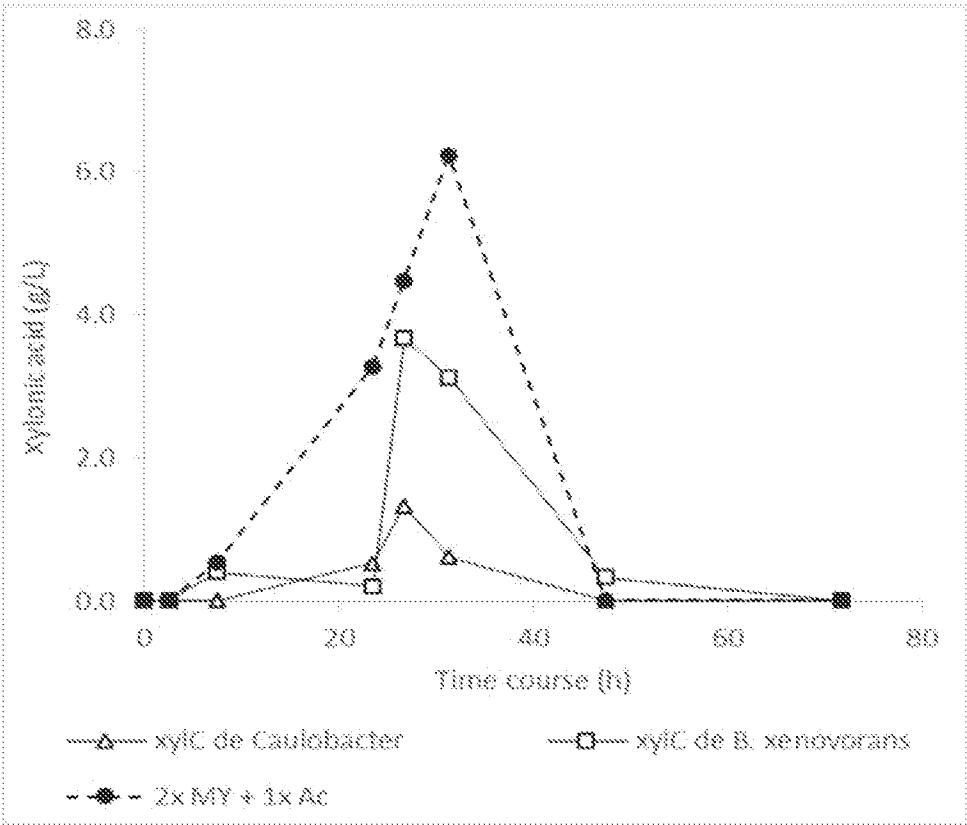


FIG 22D



29/30

FIG. 23A

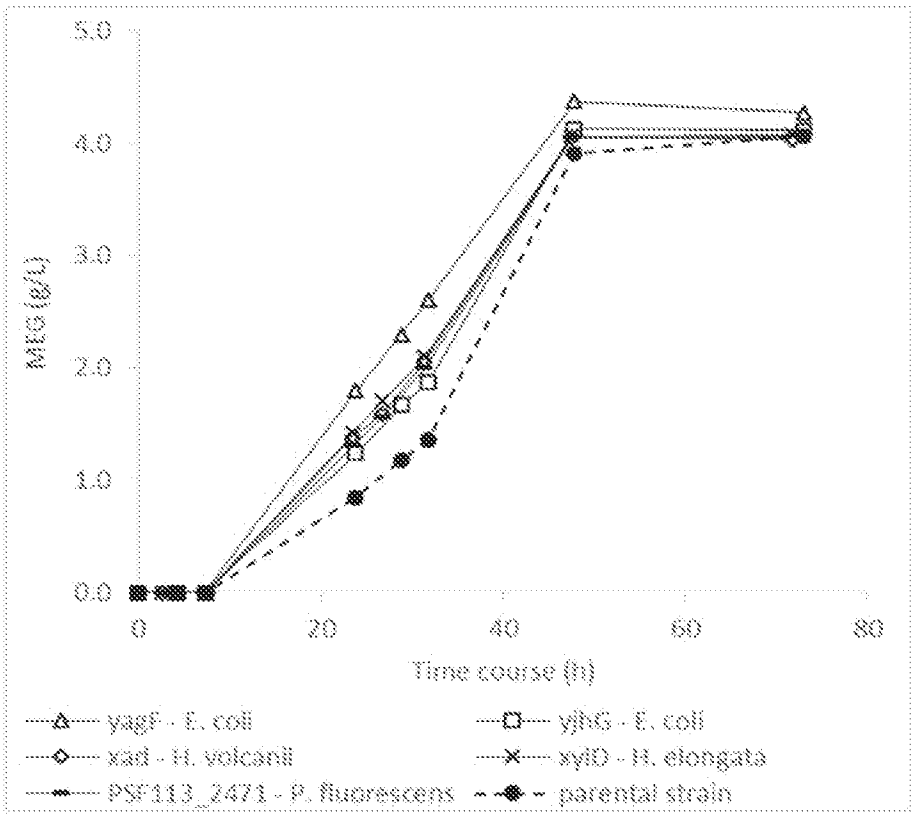
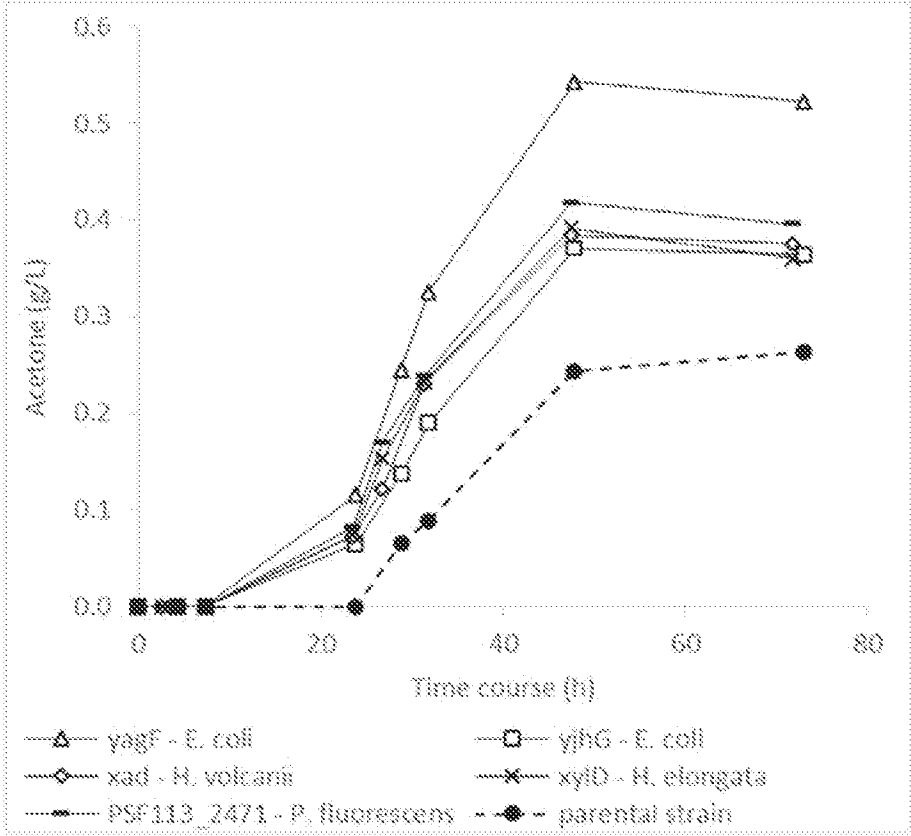


FIG. 23B



30/30

FIG. 23C

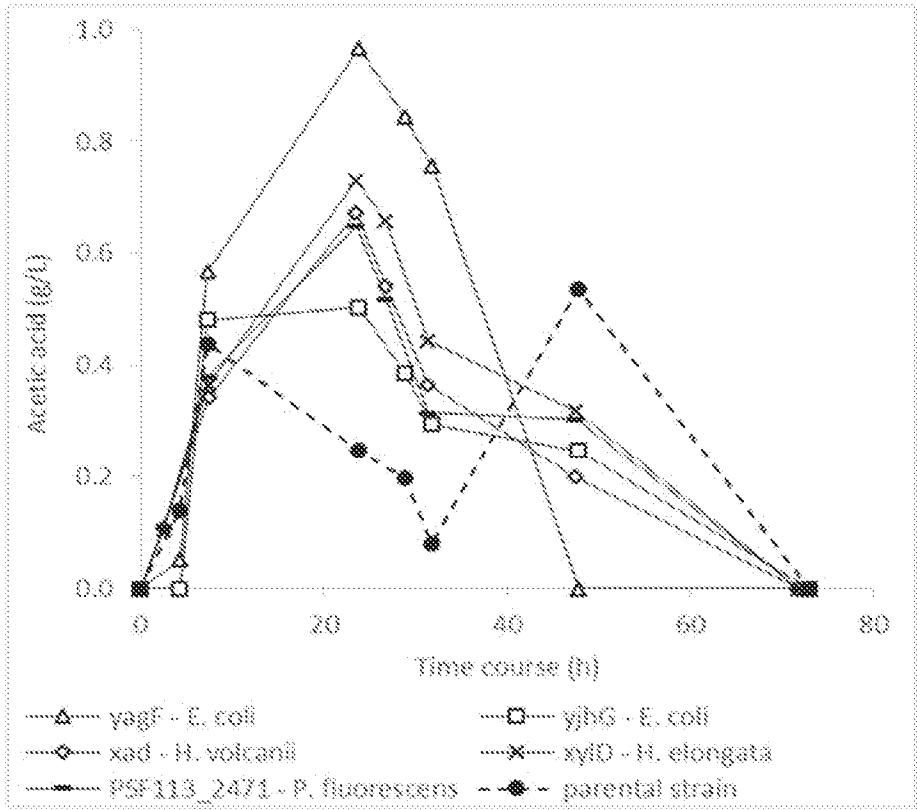


FIG. 23D

