

MICROORGANISMS AND METHODS FOR IMPROVED BIOLOGICAL PRODUCTION OF ETHYLENE GLYCOL

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 [0001] This application claims the benefit of U.S. Provisional Patent Application Nos. 63/260,054 filed on August 6, 2021, and 63/261,185 filed on September 14, 2021, the entirety of which is incorporated herein by reference.

FIELD

- 10 [0002] The present disclosure relates to genetically engineered microorganisms and methods for the production of ethylene glycol and ethylene glycol precursors by microbial fermentation, particularly by microbial fermentation of a gaseous substrate.

BACKGROUND

- 15 [0003] Ethylene glycol, also known as monoethylene glycol (MEG), has a current market value of over \$33 billion USD and is an important component of a huge variety of industrial, medical, and consumer products. Ethylene glycol is currently produced using chemical catalysis processes that require large amounts of energy and water, generate a number of undesirable by-products, and rely on petrochemical feedstocks. Demand for sustainable materials has led to some technological advancements, such as the catalytic production of ethylene glycol from sugar-cane derived ethanol.

- 20 [0004] Ethylene glycol precursors are also commercially valuable. For example, glycolate is used in skin care, personal care, dyeing, tanning, and as a cleaning agent. Glyoxylate is an intermediate for vanillin, agricultural chemicals, antibiotics, allantoin, and complexing agents.

- 25 [0005] However, no microorganisms are known to be capable of biologically producing ethylene glycol, and no fully biological route to the production of ethylene glycol has been well-established. Some biological routes to ethylene glycol have been described in the literature from sugars. For example, Alkim *et al.*, *Microb Cell Fact*, 14: 127, 2015 demonstrated ethylene glycol production from (D)-xylose in *E. coli* but noted that aerobic conditions were required to achieve high yields. Similarly, Pereira *et al.*, *Metab Eng*, 34: 80-30 87, 2016 achieved ethylene glycol production from pentoses in *E. coli*. A few studies on ethylene glycol production from pentoses have also been conducted in *S. cerevisiae* but have shown inconsistent results. *See, e.g.*, Uranukul *et al.*, *Metab Eng*, 51: 20-31, 2018.

- [0006] Gas fermentation offers a route to use a wide range of readily available, low cost C1 feedstocks such as industrial waste gases, syngas, or reformed methane into chemicals and

fuels. Since gas fermentation metabolism is significantly different from sugar-fermenting metabolism, use of the above-mentioned routes is not practical, as these routes would require production of sugar precursors from gas *via* gluconeogenesis, an energy negative process. To date, no route to produce ethylene glycol from gaseous substrates is available.

- 5 [0007] In an explorative exercise, Islam *et al.*, *Metab Eng*, 41: 173-181, 2017 predicted hundreds of hypothetical pathways for producing ethylene glycol from syngas in *M. thermoacetica* using cheminformatics tools. However, it is not possible even for a skilled person in the art to incorporate these pathways in a gas fermenting organism, as many of the pathways are infeasible either due to thermodynamic or other constraints. For example,
- 10 nearly 2,000 oxygen or oxygen radical-dependent reactions were included in Islam *et al.*, which would not be feasible in a strictly anaerobic system. The only identified hypothetical pathways by Islam *et al.* that have known reactions require gluconeogenesis or ethanol as an intermediate. Therefore, there remains a need for validated, energetically favorable recombinant production systems that can produce high yields of ethylene glycol and ethylene
- 15 glycol precursors from gaseous substrates.

SUMMARY

[0008] It is against the above background that the present disclosure provides certain advantages and advancements over the prior art.

- [0009] Although this disclosure disclosed herein is not limited to specific advantages or
- 20 functionalities, the disclosure provides a genetically engineered microorganism capable of producing ethylene glycol or a precursor of ethylene glycol from a gaseous substrate.

[0010] In some aspects of the microorganism disclosed herein, the microorganism produces ethylene glycol or the precursor of ethylene glycol through one or more intermediates comprising a disruptive mutation in a gene encoding diol dehydratase.

- 25 [0011] In some aspects of the microorganism disclosed herein, the microorganism produces ethylene glycol or the precursor of ethylene glycol through one or more intermediates selected from the group consisting of 5,10-methylenetetrahydrofolate, oxaloacetate, citrate, malate, and glycine.

- [0012] In some aspects of the microorganism disclosed herein, the microorganism comprises
- 30 one or more of a heterologous enzyme capable of converting oxaloacetate to citrate, a heterologous enzyme capable of converting glycine to glyoxylate, a heterologous enzyme capable of converting iso-citrate to glyoxylate, and a heterologous enzyme capable of converting glycolate to glycolaldehyde.

[0013] In some aspects of the microorganism disclosed herein, the heterologous enzyme capable of converting oxaloacetate to citrate is a citrate [Si]-synthase [2.3.3.1], an ATP citrate synthase [2.3.3.8]; or a citrate (Re)-synthase [2.3.3.3]; the heterologous enzyme capable of converting glycine to glyoxylate is an alanine-glyoxylate transaminase [2.6.1.44], a serine-glyoxylate transaminase [2.6.1.45], a serine-pyruvate transaminase [2.6.1.51], a glycine-oxaloacetate transaminase [2.6.1.35], a glycine transaminase [2.6.1.4], a glycine dehydrogenase [1.4.1.10], an alanine dehydrogenase [1.4.1.1], or a glycine dehydrogenase [1.4.2.1]; the heterologous enzyme capable of converting iso-citrate to glyoxylate is an isocitrate lyase [4.1.3.1]; and/or the heterologous enzyme capable of converting glycolate to glycolaldehyde is a glycolaldehyde dehydrogenase [1.2.1.21], a lactaldehyde dehydrogenase [1.2.1.22], a succinate-semialdehyde dehydrogenase [1.2.1.24], a 2,5-dioxovalerate dehydrogenase [1.2.1.26], an aldehyde dehydrogenase [1.2.1.3/4/5], a betaine-aldehyde dehydrogenase [1.2.1.8], or an aldehyde ferredoxin oxidoreductase [1.2.7.5].

[0014] In some aspects of the microorganism disclosed herein, the heterologous enzymes are derived from a genus selected from the group consisting of *Bacillus*, *Clostridium*, *Escherichia*, *Gluconobacter*, *Hyphomicrobium*, *Lysinibacillus*, *Paenibacillus*, *Pseudomonas*, *Sedimenticola*, *Sporosarcina*, *Streptomyces*, *Thermithiobacillus*, *Thermotoga*, and *Zea*.

[0015] In some aspects of the microorganism disclosed herein, one or more of the heterologous enzymes are codon-optimized for expression in the microorganism.

[0016] In some aspects of the microorganism disclosed herein, the microorganism further comprises one or more of an enzymes capable of converting acetyl-CoA to pyruvate; an enzyme capable of converting pyruvate to oxaloacetate; an enzyme capable of converting pyruvate to malate; an enzyme capable of converting pyruvate to phosphoenolpyruvate; an enzyme capable of converting oxaloacetate to citryl-CoA; an enzyme capable of converting citryl-CoA to citrate; an enzyme capable of converting citrate to aconitate and aconitate to iso-citrate; an enzyme capable of converting phosphoenolpyruvate to oxaloacetate; an enzyme capable of converting phosphoenolpyruvate to 2-phospho-D-glycerate; an enzyme capable of converting 2-phospho-D-glycerate to 3-phospho-D-glycerate; an enzyme capable of converting 3-phospho-D-glycerate to 3-phosphonooxypyruvate; an enzyme capable of converting 3-phosphonooxypyruvate to 3-phospho-L-serine; an enzyme capable of converting 3-phospho-L-serine to serine; an enzyme capable of converting serine to glycine; an enzyme capable of converting 5,10-methylenetetrahydrofolate to glycine; an enzyme capable of converting serine to hydroxypyruvate; an enzyme capable of converting D-glycerate to hydroxypyruvate; an enzyme capable of converting malate to glyoxylate; an

enzyme capable of converting glyoxylate to glycolate; an enzyme capable of converting hydroxypyruvate to glycolaldehyde; and/or an enzyme capable of converting glycolaldehyde to ethylene glycol.

[0017] In some aspects of the microorganism disclosed herein, the microorganism
5 overexpresses the heterologous enzyme capable of converting oxaloacetate to citrate, the heterologous enzyme capable of converting glycine to glyoxylate, and/or the heterologous enzyme capable of converting glycolate to glycolaldehyde.

[0018] In some aspects of the microorganism disclosed herein, the microorganism
overexpresses the enzyme capable of converting pyruvate to oxaloacetate, the enzyme
10 capable of converting citrate to aconitate and aconitate to iso-citrate, the enzyme capable of converting phosphoenolpyruvate to oxaloacetate, the enzyme capable of converting serine to glycine, the enzyme capable of converting 5,10-methylenetetrahydrofolate to glycine, the enzyme capable of converting glyoxylate to glycolate; and/or the enzyme capable of converting glycolaldehyde to ethylene glycol.

15 [0019] In some aspects of the microorganism disclosed herein, the microorganism comprises a disruptive mutation in one or more enzymes selected from the group consisting of isocitrate dehydrogenase, glycerate dehydrogenase, glycolate dehydrogenase, glycerate dehydrogenase, glycolate dehydrogenase, aldehyde ferredoxin oxidoreductase, and aldehyde dehydrogenase

[0020] In some aspects of the microorganism disclosed herein, the microorganism is a
20 member of a genus selected from the group consisting of *Acetobacterium*, *Alkalibaculum*, *Blautia*, *Butyribacterium*, *Clostridium*, *Cupriavidus*, *Eubacterium*, *Moorella*, *Oxobacter*, *Sporomusa*, and *Thermoanaerobacter*.

[0021] In some aspects of the microorganism disclosed herein, the microorganism is derived from a parental microorganism selected from the group consisting of *Acetobacterium woodii*,
25 *Alkalibaculum bacchii*, *Blautia producta*, *Butyribacterium methylotrophicum*, *Clostridium aceticum*, *Clostridium autoethanogenum*, *Clostridium carboxidivorans*, *Clostridium coskatii*, *Clostridium drakei*, *Clostridium formicoaceticum*, *Clostridium ljungdahlii*, *Clostridium magnum*, *Clostridium ragsdalei*, *Clostridium scatologenes*, *Cupriavidus necator*, *Eubacterium limosum*, *Moorella thermautotrophica*, *Moorella thermoacetica*, *Oxobacter pfennigii*, *Sporomusa ovata*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, and
30 *Thermoanaerobacter kiuvi*.

[0022] In some aspects of the microorganism disclosed herein, the microorganism is derived from a parental bacterium selected from the group consisting of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, and *Clostridium ragsdalei*.

[0023] In some aspects of the microorganism disclosed herein, the microorganism comprises a native or heterologous Wood-Ljungdahl pathway.

[0024] In some aspects of the microorganism disclosed herein, the microorganism produces glyoxylate or glycolate as a precursor of ethylene glycol.

5 [0025] The disclosure further provides a method of producing ethylene glycol or a precursor of ethylene glycol comprising culturing the microorganism disclosed herein in a nutrient medium and in the presence of a substrate, whereby the microorganism produces ethylene glycol or the precursor of ethylene glycol.

10 [0026] In some aspects of the method disclosed herein, the substrate comprises one or more of CO, CO₂, and H₂.

[0027] In some aspects of the method disclosed herein, at least a portion of the substrate is industrial waste gas, industrial off gas, or syngas.

[0028] In some aspects of the method disclosed herein, the microorganism produces glyoxylate or glycolate as precursors of ethylene glycol.

15 [0029] In some aspects of the method disclosed herein, the method further comprises separating the ethylene glycol or the ethylene glycol precursor from the nutrient medium.

[0030] In some aspects of the method disclosed herein, the microorganism further produces one or more of ethanol, 2,3-butanediol, and succinate.

20 [0031] The disclosure further provides a composition comprising ethylene glycol produced by the method described herein. In some aspects, the composition is an antifreeze, a preservative, a dehydrating agent, or a drilling fluid.

[0032] The disclosure further provides a polymer comprising ethylene glycol produced by the method described herein. In some aspects, the polymer is a homopolymer or a copolymer. In some aspects, the polymer is polyethylene glycol (PEG) or polyethylene terephthalate (PET).

25 [0033] The disclosure further provides a method of producing a polyethylene terephthalate (PET) product from a gaseous substrate comprising 1) forming at least one PET component, wherein the at least one PET component is selected from monoethylene glycol, a terephthalic acid (PTA), or any combinations thereof; 2) processing the at least one PET component into PET; 3) polymerizing the PET to form a PET resin; and 4) processing the PET resin into a PET product. The disclosure provides that the PTA may be derived from fossil sources, or either directly or indirectly from gas fermentation. Examples of PET products may be those disclosed in US 2020/0048665A1, hereby incorporated by reference in its entirety.

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[0034] The disclosure provides a method for producing a PET polymer from a gaseous substrate comprising 1) providing at least one diacid compound, comprising a terephthalate compound; 2) providing at least one diol compound, comprising monoethylene glycol, 3) copolymerizing a mixture of the diacid compound and the diol compound to obtain a PET polymer comprising diacid components and diol components.

[0035] The disclosure further provides a composition comprising the polymer described herein. In some aspects, the composition is a fiber, a resin, a film, or a plastic.

[0036] These and other features and advantages of the present disclosure will be more fully understood from the following detailed description taken together with the accompanying claims. It is noted that the scope of the claims is defined by the recitations therein and not by the specific discussion of features and advantages set forth in the present description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] The following detailed description of the embodiments of the present disclosure can be best understood when read in conjunction with the following drawings, where like structure is indicated with like reference numerals and in which:

[0038] Figure 1 is a schematic showing pathways for the production of ethylene glycol, glycolate, and glyoxylate from a gaseous substrate comprising CO, CO₂, and/or H₂.

[0039] Figures 2A-2E are maps of plasmids used in Examples 1-4. Figure 2A is a map of expression shuttle vector, pIPL12, as described in Example 1. Figure 2B is a map of plasmid pMEG042, which comprises *B. subtilis* citrate synthase, *E. coli* isocitrate lyase, and *G. oxydans* glycolaldehyde dehydrogenase, as described in Example 1. Figure 2C is a map of plasmid pMEG058, which comprises *S. thiotaurini* alanine-glyoxylate aminotransferase and *P. fluorescens* aldehyde dehydrogenase, as described in Example 2. Figure 2D is a map of plasmid pMEG059, which comprises *S. thiotaurini* alanine-glyoxylate aminotransferase and *G. oxydans* aldehyde dehydrogenase, as described in Example 3. Figure 2E is a map of plasmid pMEG061, which comprises *C. acidurici* class V aminotransferase and *P. fluorescens* aldehyde dehydrogenase, as described in Example 4.

[0040] Figure 3A shows biomass levels (g dry cell weight/L) of *C. autoethanogenum* expressing pMEG042 (clones 1-3) or *C. autoethanogenum* wild-type (Neg Ctrl 1 or Neg Ctrl 2). Figure 3B shows ethylene glycol produced over time in *C. autoethanogenum* growing autotrophically and carrying expression vector pMEG042, as compared to the wild-type (Neg Ctrl 1 or Neg Ctrl 2). Figure 3C shows glycolate produced over time in *C. autoethanogenum* growing autotrophically and carrying expression vector pMEG042. See Example 1.

[0041] Figure 4A shows biomass levels (g dry cell weight/L) of *C. autoethanogenum* expressing pMEG058 (clones 1-2) or *C. autoethanogenum* wild-type (Neg Ctrl 1). Figure 4B shows ethylene glycol produced over time in *C. autoethanogenum* growing autotrophically and carrying expression vector pMEG058, as compared to the wild-type (Neg Ctrl 1). See Example 2.

[0042] Figure 5A shows biomass levels (g dry cell weight/L) of *C. autoethanogenum* expressing pMEG059 (clones 1-3) or *C. autoethanogenum* wild-type (Neg Ctrl). Figure 5B shows ethylene glycol produced over time in *C. autoethanogenum* growing autotrophically and carrying expression vector pMEG059, as compared to the wild-type (Neg Ctrl). See Example 3.

[0043] Figure 6A shows biomass levels (g dry cell weight/L) of *C. autoethanogenum* expressing pMEG061 (clones 1) or *C. autoethanogenum* wild-type (Neg Ctrl 1). Figure 6B shows ethylene glycol produced over time in *C. autoethanogenum* growing autotrophically and carrying expression vector pMEG061, as compared to the wild-type (Neg Ctrl 1). See Example 4.

[0044] Figure 7 shows biomass levels (g dry cell weight/L) of two different genotypes of *C. autoethanogenum*: one containing the identified native diol dehydratase (Base), and one with the diol dehydratase gene deleted (KO). Each strain has two variants, one carrying the pMEG042 expression vector, and one carrying no vector (negative control). Values shown are calculated from the average of 3 technical replicates. Error bars show standard deviation.

[0045] Figure 8A shows MEG (mg/L) produced over time in two different genotypes of *C. autoethanogenum*: one containing the identified native diol dehydratase (Base), and one with the diol dehydratase gene deleted (KO). Each strain has two variants, one carrying the pMEG042 expression vector, and one carrying no vector (negative control). Values shown are calculated from the average of 3 technical replicates. Error bars show standard deviation. Figure 8B shows MEG (mg/g dry cell weight) produced over time in different genotypes of *C. autoethanogenum*: one containing the identified native diol dehydratase (Base), and one with the diol dehydratase gene deleted (KO). Each strain has two variants, one carrying the pMEG042 expression vector, and one carrying no vector (negative control). Values shown are calculated from the average of 3 technical replicates. Error bars show standard deviation.

DETAILED DESCRIPTION

[0046] The following description of embodiments is given in general terms. The disclosure is further elucidated from the disclosure given under the heading “Examples” herein below,

which provides experimental data supporting the disclosure, specific examples of various aspects of the disclosure, and means of performing the disclosure.

[0047] The inventors have surprisingly been able to engineer a carboxydutrophic acetogenic microorganism to produce ethylene glycol or a precursor of ethylene glycol by fermentation of a substrate comprising CO and/or CO₂ comprising a disruptive mutation in a gene encoding diol dehydratase.

[0048] Unless otherwise defined, the following terms as used throughout this specification are defined as follows:

[0049] The disclosure provides microorganisms for the biological production of ethylene glycol. A “microorganism” is a microscopic organism, especially a bacterium, archaeon, virus, or fungus. In a preferred embodiment, the microorganism of the disclosure is a bacterium.

[0050] The term “non-naturally occurring” when used in reference to a microorganism is intended to mean that the microorganism has at least one genetic modification not found in a naturally occurring strain of the referenced species, including wild-type strains of the referenced species. Non-naturally occurring microorganisms are typically developed in a laboratory or research facility. The microorganisms of the disclosure are non-naturally occurring.

[0051] The terms “genetic modification,” “genetic alteration,” or “genetic engineering” broadly refer to manipulation of the genome or nucleic acids of a microorganism by the hand of man. Likewise, the terms “genetically modified,” “genetically altered,” or “genetically engineered” refers to a microorganism containing such a genetic modification, genetic alteration, or genetic engineering. These terms may be used to differentiate a lab-generated microorganism from a naturally-occurring microorganism. Methods of genetic modification of include, for example, heterologous gene expression, gene or promoter insertion or deletion, nucleic acid mutation, altered gene expression or inactivation, enzyme engineering, directed evolution, knowledge-based design, random mutagenesis methods, gene shuffling, and codon optimization. The microorganisms of the disclosure are genetically engineered.

[0052] “Recombinant” indicates that a nucleic acid, protein, or microorganism is the product of genetic modification, engineering, or recombination. Generally, the term “recombinant” refers to a nucleic acid, protein, or microorganism that contains or is encoded by genetic material derived from multiple sources, such as two or more different strains or species of microorganisms. The microorganisms of the disclosure are generally recombinant.

[0053] “Wild type” refers to the typical form of an organism, strain, gene, or characteristic as it occurs in nature, as distinguished from mutant or variant forms.

[0054] “Endogenous” refers to a nucleic acid or protein that is present or expressed in the wild-type or parental microorganism from which the microorganism of the disclosure is derived. For example, an endogenous gene is a gene that is natively present in the wild-type or parental microorganism from which the microorganism of the disclosure is derived. In one embodiment, the expression of an endogenous gene may be controlled by an exogenous regulatory element, such as an exogenous promoter.

[0055] “Exogenous” refers to a nucleic acid or protein that originates outside the microorganism of the disclosure. For example, an exogenous gene or enzyme may be artificially or recombinantly created and introduced to or expressed in the microorganism of the disclosure. An exogenous gene or enzyme may also be isolated from a heterologous microorganism and introduced to or expressed in the microorganism of the disclosure. Exogenous nucleic acids may be adapted to integrate into the genome of the microorganism of the disclosure or to remain in an extra-chromosomal state in the microorganism of the disclosure, for example, in a plasmid.

[0056] “Heterologous” refers to a nucleic acid or protein that is not present in the wild-type or parental microorganism from which the microorganism of the disclosure is derived. For example, a heterologous gene or enzyme may be derived from a different strain or species and introduced to or expressed in the microorganism of the disclosure. The heterologous gene or enzyme may be introduced to or expressed in the microorganism of the disclosure in the form in which it occurs in the different strain or species. Alternatively, the heterologous gene or enzyme may be modified in some way, e.g., by codon-optimizing it for expression in the microorganism of the disclosure or by engineering it to alter function, such as to reverse the direction of enzyme activity or to alter substrate specificity.

[0057] In particular, a heterologous nucleic acid or protein expressed in the microorganism described herein may be derived from *Bacillus*, *Clostridium*, *Cupriavidus*, *Escherichia*, *Gluconobacter*, *Hyphomicrobium*, *Lysinibacillus*, *Paenibacillus*, *Pseudomonas*, *Sedimenticola*, *Sporosarcina*, *Streptomyces*, *Thermithiobacillus*, *Thermotoga*, *Zea*, *Klebsiella*, *Mycobacterium*, *Salmonella*, *Mycobacteroides*, *Staphylococcus*, *Burkholderia*, *Listeria*, *Acinetobacter*, *Shigella*, *Neisseria*, *Bordetella*, *Streptococcus*, *Enterobacter*, *Vibrio*, *Legionella*, *Xanthomonas*, *Serratia*, *Cronobacter*, *Cupriavidus*, *Helicobacter*, *Yersinia*, *Cutibacterium*, *Francisella*, *Pectobacterium*, *Arcobacter*, *Lactobacillus*, *Shewanella*, *Erwinia*, *Sulfurospirillum*, *Peptococcaceae*, *Thermococcus*, *Saccharomyces*, *Pyrococcus*,

- Glycine, Homo, Ralstonia, Brevibacterium, Methylobacterium, Geobacillus, bos, gallus, Anaerococcus, Xenopus, Amblyrhynchus, rattus, mus, sus, Rhodococcus, Rhizobium, Megasphaera, Mesorhizobium, Peptococcus, Agrobacterium, Campylobacter, Acetobacterium, Alkalibaculum, Blautia, Butyrivacterium, Eubacterium, Moorella, Oxobacter, Sporomusa, Thermoanaerobacter, Schizosaccharomyces, Paenibacillus, Fictibacillus, Lysinibacillus, Ornithinibacillus, Halobacillus, Kurthia, Lentibacillus, Anoxybacillus, Solibacillus, Virgibacillus, Alicyclobacillus, Sporosarcina, Salimicrobium, Sporosarcina, Planococcus, Corynebacterium, Thermaerobacter, Sulfobacillus, or Symbiobacterium.*
- 10 **[0058]** 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
- 15 non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, 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
- 20 polynucleotide may comprise one or more modified nucleotides, such as methylated nucleotides or 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.
- 25 **[0059]** 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 products.”
- 30 **[0060]** 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, by 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.

[0061] “Enzyme activity,” or simply “activity,” refers broadly to enzymatic activity, including, but not limited, to the activity of an enzyme, the amount of an enzyme, or the availability of an enzyme to catalyze a reaction. Accordingly, “increasing” enzyme activity includes increasing the activity of an enzyme, increasing the amount of an enzyme, or increasing the availability of an enzyme to catalyze a reaction. Similarly, “decreasing” enzyme activity includes decreasing the activity of an enzyme, decreasing the amount of an enzyme, or decreasing the availability of an enzyme to catalyze a reaction.

[0062] “Mutated” refers to a nucleic acid or protein that has been modified in the microorganism of the disclosure compared to the wild-type or parental microorganism from which the microorganism of the disclosure is derived. In one embodiment, the mutation may be a deletion, insertion, or substitution in a gene encoding an enzyme. In another embodiment, the mutation may be a deletion, insertion, or substitution of one or more amino acids in an enzyme.

[0063] “Disrupted gene” refers to a gene that has been modified in some way to reduce or eliminate expression of the gene, regulatory activity of the gene, or activity of an encoded protein or enzyme. The disruption may partially inactivate, fully inactivate, or delete the gene or enzyme. The disruption may be a knockout (KO) mutation that fully eliminates the expression or activity of a gene, protein, or enzyme. The disruption may also be a knock-down that reduces, but does not entirely eliminate, the expression or activity of a gene, protein, or enzyme. The disruption may be anything that reduces, prevents, or blocks the biosynthesis of a product produced by an enzyme. The disruption may include, for example, a mutation in a gene encoding a protein or enzyme, a mutation in a genetic regulatory element involved in the expression of a gene encoding an enzyme, the introduction of a nucleic acid which produces a protein that reduces or inhibits the activity of an enzyme, or the introduction of a nucleic acid (e.g., antisense RNA, RNAi, TALEN, siRNA, CRISPR, or CRISPRi) or protein which inhibits the expression of a protein or enzyme. The disruption may be introduced using any method known in the art. For the purposes of the present disclosure, disruptions are laboratory-generated, not naturally occurring.

[0064] A “parental microorganism” is a microorganism used to generate a microorganism of the disclosure. The parental microorganism may be a naturally-occurring microorganism (i.e., a wild-type microorganism) or a microorganism that has been previously modified (i.e., a

mutant or recombinant microorganism). The microorganism of the disclosure may be modified to express or overexpress one or more enzymes that were not expressed or overexpressed in the parental microorganism. Similarly, the microorganism of the disclosure may be modified to contain one or more genes that were not contained by the parental microorganism. The microorganism of the disclosure may also be modified to not express or to express lower amounts of one or more enzymes that were expressed in the parental microorganism.

[0065] The microorganism of the disclosure may be derived from essentially any parental microorganism. In one embodiment, the microorganism of the disclosure may be derived from a parental microorganism selected from the group consisting of *Clostridium acetobutylicum*, *Clostridium beijerinckii*, *Escherichia coli*, and *Saccharomyces cerevisiae*. In other embodiments, the microorganism is derived from a parental microorganism selected from the group consisting of *Acetobacterium woodii*, *Alkalibaculum bacchii*, *Blautia product*, *Butyribacterium methylotrophicum*, *Clostridium aceticum*, *Clostridium autoethanogenum*, *Clostridium carboxidivorans*, *Clostridium coskatii*, *Clostridium drakei*, *Clostridium formicoaceticum*, *Clostridium ljungdahlii*, *Clostridium magnum*, *Clostridium ragsdalei*, *Clostridium scatologenes*, *Eubacterium limosum*, *Moorella thermautotrophica*, *Moorella thermoacetica*, *Oxobacter pfennigii*, *Sporomusa ovata*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, and *Thermoanaerobacter kivui*. In a preferred embodiment, the parental microorganism is *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, or *Clostridium ragsdalei*. In an especially preferred embodiment, the parental microorganism is *Clostridium autoethanogenum* LZ1561, which was deposited on June 7, 2010 with Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) located at Inhoffenstraße 7B, D-38124 Braunschweig, Germany on June 7, 2010 under the terms of the Budapest Treaty and accorded accession number DSM23693. This strain is described in International Patent Application No. PCT/NZ2011/000144, which published as WO 2012/015317.

[0066] The term “derived from” indicates that a nucleic acid, protein, or microorganism is modified or adapted from a different (e.g., a parental or wild-type) nucleic acid, protein, or microorganism, so as to produce a new nucleic acid, protein, or microorganism. Such modifications or adaptations typically include insertion, deletion, mutation, or substitution of nucleic acids or genes. Generally, the microorganism of the disclosure is derived from a parental microorganism. In one embodiment, the microorganism of the disclosure is derived from *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, or *Clostridium ragsdalei*. In a

preferred embodiment, the microorganism of the disclosure is derived from *Clostridium autoethanogenum* LZ1561, which is deposited under DSMZ accession number DSM23693.

- [0067] The microorganism of the disclosure may be further classified based on functional characteristics. For example, the microorganism of the disclosure may be or may be derived from a C1-fixing microorganism, an anaerobe, an acetogen, an ethanologen, a carboxydrotroph, and/or a methanotroph.

[0068] Table 1 provides a representative list of microorganisms and identifies their functional characteristics.

Table 1

	Wood-Ljungdahl	C1-fixing	Anaerobe	Acetogen	Ethanologen	Autotroph	Carboxydrotroph
<i>Acetobacterium woodii</i>	+	+	+	+	+/- ¹	+	-
<i>Alkalibaculum bacchii</i>	+	+	+	+	+	+	+
<i>Blautia producta</i>	+	+	+	+	-	+	+
<i>Butyribacterium methylotrophicum</i>	+	+	+	+	+	+	+
<i>Clostridium aceticum</i>	+	+	+	+	-	+	+
<i>Clostridium autoethanogenum</i>	+	+	+	+	+	+	+
<i>Clostridium carboxidivorans</i>	+	+	+	+	+	+	+
<i>Clostridium coskatii</i>	+	+	+	+	+	+	+
<i>Clostridium drakei</i>	+	+	+	+	-	+	+
<i>Clostridium formicoaceticum</i>	+	+	+	+	-	+	+
<i>Clostridium ljungdahlii</i>	+	+	+	+	+	+	+
<i>Clostridium magnum</i>	+	+	+	+	-	+	+/- ²
<i>Clostridium ragsdalei</i>	+	+	+	+	+	+	+
<i>Clostridium scatologenes</i>	+	+	+	+	-	+	+
<i>Eubacterium limosum</i>	+	+	+	+	-	+	+
<i>Moorella thermautotrophica</i>	+	+	+	+	+	+	+
<i>Moorella thermoacetica</i> (formerly <i>Clostridium thermoaceticum</i>)	+	+	+	+	- ³	+	+
<i>Oxobacter pfennigii</i>	+	+	+	+	-	+	+

<i>Sporomusa ovata</i>	+	+	+	+	-	+	+/- ⁴
<i>Sporomusa silvacetica</i>	+	+	+	+	-	+	+/- ⁵
<i>Sporomusa sphaeroides</i>	+	+	+	+	-	+	+/- ⁶
<i>Thermoanaerobacter kivui</i>	+	+	+	+	-	+	-

¹ *Acetobacterium woodii* can produce ethanol from fructose, but not from gas.

² It has not been investigated whether *Clostridium magnum* can grow on CO.

³ One strain of *Moorella thermoacetica*, *Moorella* sp. HUC22-1, has been reported to produce ethanol from gas.

⁴ It has not been investigated whether *Sporomusa ovata* can grow on CO.

⁵ It has not been investigated whether *Sporomusa silvacetica* can grow on CO.

⁶ It has not been investigated whether *Sporomusa sphaeroides* can grow on CO.

[0069] “Wood-Ljungdahl” refers to the Wood-Ljungdahl pathway of carbon fixation as described, e.g., by Ragsdale, *Biochim Biophys Acta*, 1784: 1873-1898, 2008. “Wood-Ljungdahl microorganisms” refers, predictably, to microorganisms containing the Wood-Ljungdahl pathway. Often, the microorganism of the disclosure contains a native Wood-Ljungdahl pathway. Herein, a Wood-Ljungdahl pathway may be a native, unmodified Wood-Ljungdahl pathway or it may be a Wood-Ljungdahl pathway with some degree of genetic modification (e.g., overexpression, heterologous expression, knockout, etc.) so long as it still functions to convert CO, CO₂, and/or H₂ to acetyl-CoA.

[0070] “C1” refers to a one-carbon molecule, for example, CO, CO₂, CH₄, or CH₃OH. “C1-oxygenate” refers to a one-carbon molecule that also comprises at least one oxygen atom, for example, CO, CO₂, or CH₃OH. “C1-carbon source” refers a one carbon-molecule that serves as a partial or sole carbon source for the microorganism of the disclosure. For example, a C1-carbon source may comprise one or more of CO, CO₂, CH₄, CH₃OH, or CH₂O₂. Preferably, the C1-carbon source comprises one or both of CO and CO₂. A “C1-fixing microorganism” is a microorganism that has the ability to produce one or more products from a C1-carbon source. Often, the microorganism of the disclosure is a C1-fixing bacterium. In a preferred embodiment, the microorganism of the disclosure is derived from a C1-fixing microorganism identified in Table 1.

[0071] An “anaerobe” is a microorganism that does not require oxygen for growth. An anaerobe may react negatively or even die if oxygen is present above a certain threshold.

However, some anaerobes are capable of tolerating low levels of oxygen (e.g., 0.000001-5% oxygen), sometimes referred to as “microoxic conditions.” Often, the microorganism of the disclosure is an anaerobe. In a preferred embodiment, the microorganism of the disclosure is derived from an anaerobe identified in Table 1.

- 5 [0072] “Acetogens” are obligately anaerobic bacteria that use the Wood-Ljungdahl pathway as their main mechanism for energy conservation and for synthesis of acetyl-CoA and acetyl-CoA-derived products, such as acetate (Ragsdale, *Biochim Biophys Acta*, 1784: 1873-1898, 2008). In particular, acetogens use the Wood-Ljungdahl pathway as a (1) mechanism for the reductive synthesis of acetyl-CoA from CO₂, (2) terminal electron-accepting, energy
10 conserving process, (3) mechanism for the fixation (assimilation) of CO₂ in the synthesis of cell carbon (Drake, *Acetogenic Prokaryotes*, In: *The Prokaryotes*, 3rd edition, p. 354, New York, NY, 2006). All naturally occurring acetogens are C1-fixing, anaerobic, autotrophic, and non-methanotrophic. Often, the microorganism of the disclosure is an acetogen. In a preferred embodiment, the microorganism of the disclosure is derived from an acetogen
15 identified in Table 1.

[0073] An “ethanologen” is a microorganism that produces or is capable of producing ethanol. Often, the microorganism of the disclosure is an ethanologen. In a preferred embodiment, the microorganism of the disclosure is derived from an ethanologen identified in Table 1.

- 20 [0074] An “autotroph” is a microorganism capable of growing in the absence of organic carbon. Instead, autotrophs use inorganic carbon sources, such as CO and/or CO₂. Often, the microorganism of the disclosure is an autotroph. In a preferred embodiment, the microorganism of the disclosure is derived from an autotroph identified in Table 1.

- [0075] A “carboxydrotroph” is a microorganism capable of utilizing CO as a sole source of
25 carbon and energy. Often, the microorganism of the disclosure is a carboxydrotroph. In a preferred embodiment, the microorganism of the disclosure is derived from a carboxydrotroph identified in Table 1.

- [0076] A “methanotroph” is a microorganism capable of utilizing methane as a sole source of carbon and energy. In certain embodiments, the microorganism of the disclosure is a
30 methanotroph or is derived from a methanotroph. In other embodiments, the microorganism of the disclosure is not a methanotroph or is not derived from a methanotroph.

[0077] In a preferred embodiment, the microorganism of the disclosure is derived from the cluster of *Clostridia* comprising the species *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, and *Clostridium ragsdalei*. These species were first reported and characterized by

Abrini, *Arch Microbiol*, 161: 345-351, 1994 (*Clostridium autoethanogenum*), Tanner, *Int J System Bacteriol*, 43: 232-236, 1993 (*Clostridium ljungdahlii*), and Huhnke, WO 2008/028055 (*Clostridium ragsdalei*).

[0078] These three species have many similarities. In particular, these species are all C1-fixing, anaerobic, acetogenic, ethanologenic, and carboxydutrophic members of the genus *Clostridium*. These species have similar genotypes and phenotypes and modes of energy conservation and fermentative metabolism. Moreover, these species are clustered in clostridial rRNA homology group I with 16S rRNA DNA that is more than 99% identical, have a DNA G + C content of about 22-30 mol%, are gram-positive, have similar morphology and size (logarithmic growing cells between 0.5-0.7 x 3-5 µm), are mesophilic (grow optimally at 30-37 °C), have similar pH ranges of about 4-7.5 (with an optimal pH of about 5.5-6), lack cytochromes, and conserve energy via an Rnf complex. Also, reduction of carboxylic acids into their corresponding alcohols has been shown in these species (Perez, *Biotechnol Bioeng*, 110:1066-1077, 2012). Importantly, these species also all show strong autotrophic growth on CO-containing gases, produce ethanol and acetate (or acetic acid) as main fermentation products, and produce small amounts of 2,3-butanediol and lactic acid under certain conditions.

[0079] However, these three species also have a number of differences. These species were isolated from different sources: *Clostridium autoethanogenum* from rabbit gut, *Clostridium ljungdahlii* from chicken yard waste, and *Clostridium ragsdalei* from freshwater sediment. These species differ in utilization of various sugars (e.g., rhamnose, arabinose), acids (e.g., gluconate, citrate), amino acids (e.g., arginine, histidine), and other substrates (e.g., betaine, butanol). Moreover, these species differ in auxotrophy to certain vitamins (e.g., thiamine, biotin). These species have differences in nucleic and amino acid sequences of Wood-Ljungdahl pathway genes and proteins, although the general organization and number of these genes and proteins has been found to be the same in all species (Köpke, *Curr Opin Biotechnol*, 22: 320-325, 2011).

[0080] Thus, in summary, many of the characteristics of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, or *Clostridium ragsdalei* are not specific to that species, but are rather general characteristics for this cluster of C1-fixing, anaerobic, acetogenic, ethanologenic, and carboxydutrophic members of the genus *Clostridium*. However, since these species are, in fact, distinct, the genetic modification or manipulation of one of these species may not have an identical effect in another of these species. For instance, differences in growth, performance, or product production may be observed.

[0081] The microorganism of the disclosure may also be derived from an isolate or mutant of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, or *Clostridium ragsdalei*. Isolates and mutants of *Clostridium autoethanogenum* include JA1-1 (DSM10061) (Abrini, *Arch Microbiol*, 161: 345-351, 1994), LBS1560 (DSM19630) (WO 2009/064200), and LZ1561
5 (DSM23693) (WO 2012/015317). Isolates and mutants of *Clostridium ljungdahlii* include ATCC 49587 (Tanner, *Int J Syst Bacteriol*, 43: 232-236, 1993), PETCT (DSM13528, ATCC 55383), ERI-2 (ATCC 55380) (US 5,593,886), C-01 (ATCC 55988) (US 6,368,819), O-52 (ATCC 55989) (US 6,368,819), and OTA-1 (Tirado-Acevedo, Production of bioethanol from synthesis gas using *Clostridium ljungdahlii*, PhD thesis, North Carolina State University,
10 2010). Isolates and mutants of *Clostridium ragsdalei* include PI 1 (ATCC BAA-622, ATCC PTA-7826) (WO 2008/028055).

[0082] As described above, however, the microorganism of the disclosure may also be derived from essentially any parental microorganism, such as a parental microorganism selected from the group consisting of *Clostridium acetobutylicum*, *Clostridium beijerinckii*,
15 *Escherichia coli*, and *Saccharomyces cerevisiae*.

[0083] The disclosure provides microorganisms capable of producing ethylene glycol, glyoxylate, and glycolate as well as methods of producing ethylene glycol, glyoxylate, and glycolate comprising culturing the microorganism of the disclosure in the presence of a substrate, whereby the microorganism produces ethylene glycol.

[0084] A microorganism of the disclosure may comprise an enzyme that converts acetyl-CoA, such as acetyl-CoA produced by the Wood-Ljungdahl pathway, to pyruvate (reaction 1 of Figure 1). This enzyme may be a pyruvate synthase (PFOR) [1.2.7.1] or an ATP:pyruvate, orthophosphate phosphotransferase [1.2.7.1]. In some embodiments, the enzyme that converts acetyl-CoA to pyruvate is an endogenous enzyme.

[0085] A microorganism of the disclosure may comprise an enzyme that converts pyruvate to oxaloacetate (reaction 2 of Figure 1). This enzyme may be a pyruvate:carbon-dioxide ligase [ADP-forming] [6.4.1.1]. In some embodiments, the enzyme that converts pyruvate to oxaloacetate is an endogenous enzyme. In some embodiments, the enzyme that converts pyruvate to oxaloacetate is overexpressed.

[0086] A microorganism of the disclosure may comprise an enzyme that converts oxaloacetate to citryl-CoA (reaction 3 of Figure 1). This enzyme may be a citryl-CoA lyase [4.1.3.34]. In some embodiments, the enzyme that converts oxaloacetate to citryl-CoA is an endogenous enzyme.

[0087] A microorganism of the disclosure may comprise an enzyme that converts citryl-CoA to citrate (reaction 4 of Figure 1). This enzyme may be a citrate-CoA transferase [2.8.3.10]. In some embodiments, the enzyme that converts citryl-CoA to citrate is an endogenous enzyme.

- 5 [0088] A microorganism of the disclosure may comprise an enzyme that converts oxaloacetate to citrate (reaction 5 of Figure 1). This enzyme may be a citrate [Si]-synthase [2.3.3.1], an ATP citrate synthase [2.3.3.8], or a citrate (Re)-synthase [2.3.3.3]. In some embodiments, the enzyme that converts oxaloacetate to citrate is an endogenous enzyme. In other embodiments, the enzyme that converts oxaloacetate to citrate is a heterologous
- 10 enzyme. For example, in some embodiments, a microorganism of the disclosure comprises citrate synthase 1 [EC 2.3.3.16] from *B. subtilis*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 1, which encodes the amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, a microorganism of the disclosure comprises citrate (Re)-synthase from *C. kluyveri*, such that the microorganism comprises a nucleotide
- 15 sequence set forth in SEQ ID NO: 3, which encodes the amino acid sequence set forth in SEQ ID NO: 4. In some embodiments, a microorganism of the disclosure comprises citrate (Si)-synthase from *Clostridium sp.*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 5, which encodes the amino acid sequence set forth in SEQ ID NO: 6. In some embodiments, a microorganism of the disclosure comprises citrate synthase 2
- 20 from *B. subtilis*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 7, which encodes the amino acid sequence set forth in SEQ ID NO: 8. In some embodiments, the enzyme that converts oxaloacetate to citrate is overexpressed.

- [0089] A microorganism of the disclosure may comprise an enzyme that converts citrate to aconitate and aconitate to iso-citrate (reactions 6 of Figure 1). This enzyme may be an
- 25 aconitate hydratase [4.2.1.3]. In some embodiments, the enzyme that converts citrate to aconitate and aconitate to iso-citrate is an endogenous enzyme. In some embodiments, the enzyme that converts citrate to aconitate and aconitate to iso-citrate is overexpressed.

- [0090] A microorganism of the disclosure may comprise an enzyme that converts isocitrate to glyoxylate (reaction 7 of Figure 1). This enzyme may be an isocitrate lyase [4.1.3.1]. In
- 30 some embodiments, a microorganism of the disclosure comprises isocitrate lyase from *Z. mays*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 9, which encodes the amino acid sequence set forth in SEQ ID NO: 10. In some embodiments, a microorganism of the disclosure comprises isocitrate lyase from *E. coli*, such

that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 11, which encodes the amino acid sequence set forth in SEQ ID NO: 12. In some embodiments

[0091] A microorganism of the disclosure may comprise an enzyme that converts glyoxylate to glycolate (reaction 8 of Figure 1). This enzyme may be a glycerate dehydrogenase

5 [1.1.1.29], a glyoxylate reductase [1.1.1.26/79], or a glycolate dehydrogenase [1.1.99.14]. In some embodiments, the enzyme that converts glyoxylate to glycolate is an endogenous enzyme. In some embodiments, the enzyme that converts glyoxylate to glycolate is overexpressed.

[0092] A microorganism of the disclosure may comprise an enzyme that converts glycolate
10 to glycolaldehyde (reaction 9 of Figure 1). This enzyme may be a glycolaldehyde dehydrogenase [1.2.1.21], a lactaldehyde dehydrogenase [1.2.1.22], a succinate-semialdehyde dehydrogenase [1.2.1.24], a 2,5-dioxovalerate dehydrogenase [1.2.1.26], an aldehyde dehydrogenase [1.2.1.3/4/5], a betaine-aldehyde dehydrogenase [1.2.1.8], or an aldehyde ferredoxin oxidoreductase [1.2.7.5]. In some embodiments, the enzyme that converts
15 glycolate to glycolaldehyde is an endogenous enzyme. In other embodiments, the enzyme that converts glycolate to glycolaldehyde is a heterologous enzyme. For example, in some embodiments, a microorganism of the disclosure comprises a gamma-aminobutyraldehyde dehydrogenase from *E. coli*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 49, which encodes the amino acid sequence set forth in SEQ ID NO: 50.
20 In some embodiments, a microorganism of the disclosure comprises an aldehyde dehydrogenase from *E. coli*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 51, which encodes the amino acid sequence set forth in SEQ ID NO: 52. In some embodiments, a microorganism of the disclosure comprises an NADP-dependent succinate-semialdehyde dehydrogenase I from *E. coli*, such that the microorganism comprises
25 a nucleotide sequence set forth in SEQ ID NO: 53, which encodes the amino acid sequence set forth in SEQ ID NO: 54. In some embodiments, a microorganism of the disclosure comprises a lactaldehyde dehydrogenase/glycolaldehyde dehydrogenase from *G. oxydans*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 55, which encodes the amino acid sequence set forth in SEQ ID NO: 56. In some embodiments, a
30 microorganism of the disclosure comprises an aldehyde dehydrogenase A from *P. fluorescens*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 57 or SEQ ID NO: 59, which encodes the amino acid sequence set forth in SEQ ID NO: 58 or SEQ ID NO: 60, respectively. Additional non-limiting examples of enzymes that convert glycolate to glycolaldehyde can be found in GenBank Accession Nos.

WP_003202098, WP_003182567, ACT39044, ACT39074, WP_041112005, and ACT40170.

In some embodiments, the enzyme that converts glycolate to glycolaldehyde is overexpressed.

[0093] A microorganism of the disclosure may comprise an enzyme that converts glycolaldehyde to ethylene glycol (reaction 10 of Figure 1). This enzyme may be a lactaldehyde reductase [1.1.1.77], an alcohol dehydrogenase [1.1.1.1], an alcohol dehydrogenase (NADP+) [1.1.1.2], a glycerol dehydrogenase [1.1.1.72], a glycerol-3-phosphate dehydrogenase [1.1.1.8], or an aldehyde reductase [1.1.1.21]. In some embodiments, the enzyme that converts glycolaldehyde to ethylene glycol is an endogenous enzyme. In some embodiments, the endogenous enzyme that converts glycolaldehyde to ethylene glycol is overexpressed. In other embodiments, the enzyme that converts glycolaldehyde to ethylene glycol is a heterologous enzyme. In some embodiments, a microorganism of the disclosure comprises a lactaldehyde reductase from *C. saccharoperbutylacetonicum*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 61, which encodes the amino acid sequence set forth in SEQ ID NO: 62. In some embodiments, a microorganism of the disclosure comprises a lactaldehyde reductase from *C. ljungdahlii*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 63, which encodes the amino acid sequence set forth in SEQ ID NO: 64. In some embodiments, a microorganism of the disclosure comprises a lactaldehyde reductase from *E. coli*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 65, which encodes the amino acid sequence set forth in SEQ ID NO: 66. In some embodiments, a microorganism of the disclosure comprises a lactaldehyde reductase from *C. beijerinckii*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 67, which encodes the amino acid sequence set forth in SEQ ID NO: 68. In some embodiments, the heterologous enzyme that converts glycolaldehyde to ethylene glycol is overexpressed.

[0094] A microorganism of the disclosure may comprise an enzyme that converts pyruvate to malate (reaction 11 of Figure 1). This enzyme may be a malate dehydrogenase [1.1.1.37], a malate dehydrogenase (oxaloacetate-decarboxylating) [1.1.1.38], a malate dehydrogenase (decarboxylating) [1.1.1.39], a malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) [1.1.1.40], a malate dehydrogenase (NADP+) [1.1.1.82], a D-malate dehydrogenase (decarboxylating) [1.1.1.83], a dimethylmalate dehydrogenase [1.1.1.84], a 3-isopropylmalate dehydrogenase [1.1.1.85], a malate dehydrogenase [NAD(P)+] [1.1.1.299], or a malate dehydrogenase (quinone) [1.1.5.4]. In some embodiments, the enzyme that

converts pyruvate to malate is an endogenous enzyme. In other embodiments, the enzyme that converts pyruvate to malate is a heterologous enzyme. For example, in some embodiments, a microorganism of the disclosure comprises a malate dehydrogenase from *C. autoethanogenum*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 23, which encodes the amino acid sequence set forth in SEQ ID NO: 24. In some embodiments, a microorganism of the disclosure comprises an NAD-dependent malic enzyme from *C. autoethanogenum*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 25, which encodes the amino acid sequence set forth in SEQ ID NO: 26.

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10 **[0095]** A microorganism of the disclosure may comprise an enzyme that converts malate to glyoxylate (reaction 12 of Figure 1). This enzyme may be a malate synthase [2.3.3.9] or an isocitrate lyase [4.1.3.1]. In some embodiments, the enzyme that converts malate to glyoxylate is a heterologous enzyme. For example, in some embodiments, a microorganism of the disclosure comprises a malate synthase G from *Sporosarcina sp.*, such that the

15 microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 27 or SEQ ID NO: 33, which encodes the amino acid sequence set forth in SEQ ID NO: 28 or SEQ ID NO: 34, respectively. In some embodiments, a microorganism of the disclosure comprises a malate synthase G from *Bacillus sp.*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 29 or SEQ ID NO: 35, which encodes the amino acid sequence set

20 forth in SEQ ID NO: 30 or SEQ ID NO: 36, respectively. In some embodiments, a microorganism of the disclosure comprises a malate synthase from *S. coelicolor*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 31, which encodes the amino acid sequence set forth in SEQ ID NO: 32. In some embodiments, a microorganism of the disclosure comprises a malate synthase G from *B. infantis*, such that the

25 microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 37, which encodes the amino acid sequence set forth in SEQ ID NO: 38. In some embodiments, a microorganism of the disclosure comprises a malate synthase from *C. cochlearium*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 39, which encodes the amino acid sequence set forth in SEQ ID NO: 40. In some embodiments, a

30 microorganism of the disclosure comprises a malate synthase G from *B. megaterium*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 41, which encodes the amino acid sequence set forth in SEQ ID NO: 42. In some embodiments, a microorganism of the disclosure comprises a malate synthase from *Paenibacillus sp.*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 43, which

encodes the amino acid sequence set forth in SEQ ID NO: 44. In some embodiments, a microorganism of the disclosure comprises a malate synthase from *Lysinibacillus sp.*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 45, which encodes the amino acid sequence set forth in SEQ ID NO: 46. In some embodiments, a microorganism of the disclosure comprises a malate synthase from *B. cereus*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 47, which encodes the amino acid sequence set forth in SEQ ID NO: 48.

[0096] A microorganism of the disclosure may comprise an enzyme that converts pyruvate to phosphoenolpyruvate (reaction 13 of Figure 1). This enzyme may be a pyruvate kinase [2.7.1.40], a pyruvate, phosphate dikinase [2.7.9.1], or a pyruvate, water dikinase [2.7.9.2]. In some embodiments, the enzyme that converts pyruvate to phosphoenolpyruvate is an endogenous enzyme.

[0097] A microorganism of the disclosure may comprise an enzyme that converts phosphoenolpyruvate to 2-phospho-D-glycerate (reaction 14 of Figure 1). This enzyme may be a phosphopyruvate hydratase [4.2.1.11]. In some embodiments, the enzyme that converts phosphoenolpyruvate to 2-phospho-D-glycerate is an endogenous enzyme.

[0098] A microorganism of the disclosure may comprise an enzyme that converts 2-phospho-D-glycerate to 3-phospho-D-glycerate (reaction 15 of Figure 1). This enzyme may be a phosphoglycerate mutase [5.4.2.11/12]. In some embodiments, the enzyme that converts 2-phospho-D-glycerate to 3-phospho-D-glycerate is an endogenous enzyme.

[0099] A microorganism of the disclosure may comprise an enzyme that converts 3-phospho-D-glycerate to 3-phosphonooxypyruvate (reaction 16 of Figure 1). This enzyme may be a phosphoglycerate dehydrogenase [1.1.1.95]. In some embodiments, the enzyme that converts 3-phospho-D-glycerate to 3-phosphonooxypyruvate is an endogenous enzyme.

[0100] A microorganism of the disclosure may comprise an enzyme that converts 3-phosphonooxypyruvate to 3-phospho-L-serine (reaction 17 of Figure 1). This enzyme may be a phosphoserine transaminase [2.6.1.52]. In some embodiments, the enzyme that converts 3-phosphonooxypyruvate to 3-phospho-L-serine is an endogenous enzyme.

[0101] A microorganism of the disclosure may comprise an enzyme that converts 3-phospho-L-serine to serine (reaction 18 of Figure 1). This enzyme may be a phosphoserine phosphatase [3.1.3.3]. In some embodiments, the enzyme that converts 3-phospho-L-serine to serine is an endogenous enzyme.

[0102] A microorganism of the disclosure may comprise an enzyme that converts serine to glycine (reaction 19 of Figure 1). This enzyme may be a glycine hydroxymethyltransferase

[2.1.2.1]. In some embodiments, the enzyme that converts serine to glycine is an endogenous enzyme. In some embodiments, the enzyme that converts serine to glycine is overexpressed.

[0103] A microorganism of the disclosure may comprise an enzyme that converts glycine to glyoxylate (reaction 20 of Figure 1). This enzyme may be an alanine-glyoxylate

5 aminotransferase/transaminase [2.6.1.44], a serine-glyoxylate aminotransferase/transaminase [2.6.1.45], a serine-pyruvate aminotransferase/transaminase [2.6.1.51], a glycine-oxaloacetate aminotransferase/transaminase [2.6.1.35], a glycine transaminase [2.6.1.4], a glycine dehydrogenase [1.4.1.10], an alanine dehydrogenase [1.4.1.1], or a glycine dehydrogenase [1.4.2.1]. In some embodiments, the enzyme that converts glycine to glyoxylate is an
10 endogenous enzyme. In other embodiments, the enzyme that converts glycine to glyoxylate is a heterologous enzyme. For example, in some embodiments, a microorganism of the disclosure comprises serine-glyoxylate aminotransferase from *H. methylovorum*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 13, which encodes the amino acid sequence set forth in SEQ ID NO: 14. In some embodiments, a
15 microorganism of the disclosure comprises alanine-glyoxylate aminotransferase from *S. thiotaaurini*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 15, which encodes the amino acid sequence set forth in SEQ ID NO: 16. In some embodiments, a microorganism of the disclosure comprises alanine-glyoxylate aminotransferase from *T. tepidarius*, such that the microorganism comprises a nucleotide
20 sequence set forth in SEQ ID NO: 17, which encodes the amino acid sequence set forth in SEQ ID NO: 18. In some embodiments, a microorganism of the disclosure comprises a Class V aminotransferase from *C. acidurici*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 19, which encodes the amino acid sequence set forth in SEQ ID NO: 20. In some embodiments, a microorganism of the disclosure comprises a
25 serine-pyruvate aminotransferase from *T. maritima*, such that the microorganism comprises a nucleotide sequence set forth in SEQ ID NO: 21, which encodes the amino acid sequence set forth in SEQ ID NO: 22. In some embodiments, the enzyme that converts glycine to glyoxylate is overexpressed.

[0104] A microorganism of the disclosure may comprise an enzyme that converts serine to
30 hydroxypyruvate (reaction 21 of Figure 1). This enzyme may be a serine-pyruvate transaminase [2.6.1.51], a serine-glyoxylate transaminase [2.6.1.45], an alanine dehydrogenase [1.4.1.1], an L-amino-acid dehydrogenase [1.4.1.5], a serine 2-dehydrogenase [1.4.1.7], an alanine transaminase [2.6.1.2], a glutamine-pyruvate transaminase [2.6.1.15], a D-amino-acid transaminase [2.6.1.21], an alanine-glyoxylate transaminase [2.6.1.44], or a

serine-pyruvate transaminase [2.6.1.51]. In some embodiments, the enzyme that converts serine to hydroxypyruvate is an endogenous enzyme. In other embodiments, the enzyme that converts serine to hydroxypyruvate is a heterologous enzyme. Non-limiting examples of enzymes capable of converting serine to hydroxypyruvate can be found in GenBank

5 Accession Nos. WP_009989311 and NP_511062.1. In some embodiments, the enzyme that converts serine to hydroxypyruvate is overexpressed.

[0105] A microorganism of the disclosure may comprise an enzyme that converts hydroxypyruvate to glycolaldehyde (reaction 22 of Figure 1). This enzyme may be a hydroxypyruvate decarboxylase [4.1.1.40] or a pyruvate decarboxylase [4.1.1.1]. This
10 enzyme may also be any other decarboxylase [4.1.1.-]. In some embodiments, the enzyme that converts hydroxypyruvate to glycolaldehyde is a heterologous enzyme. Non-limiting examples of enzymes capable of converting hydroxypyruvate to glycolaldehyde can be found in GenBank Accession Nos. CCG28866, SVF98953, PA0096, CAA54522, KRU13460, and KLA26356.

[0106] A microorganism of the disclosure may comprise an enzyme that converts D-glycerate to hydroxypyruvate (reaction 23 of Figure 1). This enzyme may be a glyoxylate reductase [EC 1.1.1.26], a glycerate dehydrogenase [EC 1.1.1.29], or a hydroxypyruvate reductase [EC 1.1.1.81]. In some embodiments, the enzyme that converts D-glycerate to hydroxypyruvate is a heterologous enzyme. Non-limiting examples of enzymes capable of
20 converting D-glycerate to hydroxypyruvate can be found in GenBank Accession Nos. SUK16841, RPK22618, KPA02240, AGW90762, CAC11987, Q9CA90, and Q9UBQ7.

[0107] A microorganism of the disclosure may comprise a complex of enzymes that converts 5,10-methylenetetrahydrofolate to glycine (reaction 24 of Figure 1). 5,10-methylenetetrahydrofolate is a cofactor in the reductive branch of the Wood-Ljungdahl
25 pathway and acts as a scaffold in the production of acetyl-CoA. This complex may be a glycine cleavage system comprising a glycine dehydrogenase [1.4.4.2], a dihydrolipoyl dehydrogenase [1.8.1.4], and an aminomethyltransferase (glycine synthase) [2.1.2.10]. In some embodiments, the enzymes of the complex that converts 5,10-methylenetetrahydrofolate to glycine are endogenous enzymes. In some embodiments, the
30 enzymes of the glycine cleavage system are overexpressed.

[0108] A microorganism of the disclosure may comprise an enzyme that converts phosphoenolpyruvate to oxaloacetate (reaction 25 of Figure 1). This enzyme may be a phosphoenolpyruvate carboxykinase (ATP) [4.1.1.49] or (GTP) [4.1.1.32]. In some embodiments, the enzyme that converts phosphoenolpyruvate to oxaloacetate is an

endogenous enzyme. In other embodiments, the enzyme that converts phosphoenolpyruvate to oxaloacetate is a heterologous enzyme. In some embodiments, the enzyme that converts phosphoenolpyruvate to oxaloacetate is overexpressed.

[0109] In some embodiments, a microorganism comprising an enzyme that converts acetyl-CoA to pyruvate (reaction 1 of Figure 1), an enzyme that converts pyruvate to oxaloacetate (reaction 2 of Figure 1), an enzyme that converts oxaloacetate to citrate (reaction 5 of Figure 1), an enzyme that converts citrate to aconitate and aconitate to iso-citrate (reactions 6 of Figure 1), an enzyme that converts isocitrate to glyoxylate (reaction 7 of Figure 1), an enzyme that converts glyoxylate to glycolate (reaction 8 of Figure 1), an enzyme that converts glycolate to glycolaldehyde (reaction 9 of Figure 1), and an enzyme that converts glycolaldehyde to ethylene glycol (reaction 10 of Figure 1) produces ethylene glycol. In a non-limiting example, the enzyme that converts oxaloacetate to citrate may be a citrate synthase from *B. subtilis* (SEQ ID NOs: 1-2). In a non-limiting example, the enzyme that converts iso-citrate to glyoxylate may be an isocitrate lyase from *E. coli* (SEQ ID NOs: 11-12). In a non-limiting example, the enzyme that converts glycolate to glycolaldehyde may be a glycolaldehyde dehydrogenase from *G. oxydans* (SEQ ID NOs: 55-56) or an aldehyde dehydrogenase from *P. fluorescens* (SEQ ID NOs: 57-58). One or more of the enzymes catalyzing reactions 2, 5, 6, 8, 9, and 10, as shown in Figure 1, may be overexpressed. See, e.g., Example 1 and Figure 3B.

[0110] In some embodiments, a microorganism comprising an enzyme that converts acetyl-CoA to pyruvate (reaction 1 of Figure 1), an enzyme that converts pyruvate to phosphoenolpyruvate (reaction 13 of Figure 1), an enzyme that converts phosphoenolpyruvate to 2-phospho-D-glycerate (reaction 14 of Figure 1), an enzyme that converts 2-phospho-D-glycerate to 3-phospho-D-glycerate (reaction 15 of Figure 1), an enzyme that converts 3-phospho-D-glycerate to 3-phosphonooxypyruvate (reaction 16 of Figure 1), an enzyme that converts 3-phosphonooxypyruvate to 3-phospho-L-serine (reaction 17 of Figure 1), an enzyme that converts 3-phospho-L-serine to serine (reaction 18 of Figure 1), an enzyme that converts serine to glycine (reaction 19 of Figure 1), an enzyme that converts glycine to glyoxylate (reaction 20 of Figure 1), an enzyme that converts glyoxylate to glycolate (reaction 8 of Figure 1), an enzyme that converts glycolate to glycolaldehyde (reaction 9 of Figure 1), and an enzyme that converts glycolaldehyde to ethylene glycol (reaction 10 of Figure 1) produces ethylene glycol. In a non-limiting example, the enzyme that converts glycine to glyoxylate may be an alanine-glyoxylate aminotransferase from *S. thiotaaurini* (SEQ ID NOs: 15-16) or a class V aminotransferase from *C. acidurici* (SEQ ID

NOs: 19-20). In a non-limiting example, the enzyme that converts glycolate to glycolaldehyde may be a glycolaldehyde dehydrogenase from *G. oxydans* (SEQ ID NOs: 55-56) or an aldehyde dehydrogenase from *P. fluorescens* (SEQ ID NOs: 57-58). One of more of the enzymes catalyzing the reactions of steps 19, 20, 8, 9, and 10, as shown in Figure 1, may be overexpressed. See, e.g., Examples 2-4 and Figures 4B, 5B, and 6B.

[0111] In some embodiments, a microorganism comprising an enzyme that converts acetyl-CoA to pyruvate (reaction 1 of Figure 1), an enzyme that converts pyruvate to oxaloacetate (reaction 2 of Figure 1), an enzyme that converts oxaloacetate to citryl-CoA (reaction 3 of Figure 1), an enzyme that converts citryl-CoA to citrate (reaction 4 of Figure 1), an enzyme that converts citrate to aconitate and aconitate to iso-citrate (reactions 6 of Figure 1), an enzyme that converts isocitrate to glyoxylate (reaction 7 of Figure 1), an enzyme that converts glyoxylate to glycolate (reaction 8 of Figure 1), an enzyme that converts glycolate to glycolaldehyde (reaction 9 of Figure 1), and an enzyme that converts glycolaldehyde to ethylene glycol (reaction 10 of Figure 1) produces ethylene glycol. In a non-limiting example, the enzyme that converts iso-citrate to glyoxylate may be an isocitrate lyase from *E. coli* (SEQ ID NOs: 11-12). In a non-limiting example, the enzyme that converts iso-citrate to glyoxylate may be an isocitrate lyase from *E. coli* (SEQ ID NOs: 11-12). In a non-limiting example, the enzyme that converts glycolate to glycolaldehyde may be a glycolaldehyde dehydrogenase from *G. oxydans* (SEQ ID NOs: 55-56) or an aldehyde dehydrogenase from *P. fluorescens* (SEQ ID NOs: 57-58). One or more of the enzymes catalyzing reactions 2, 6, 8, 9, and 10, as shown in Figure 1, may be overexpressed.

[0112] In some embodiments, a microorganism comprising an enzyme that converts acetyl-CoA to pyruvate (reaction 1 of Figure 1), an enzyme that converts pyruvate to malate (reaction 11 of Figure 1), an enzyme that converts malate to glyoxylate (reaction 12 of Figure 1), an enzyme that converts glyoxylate to glycolate (reaction 8 of Figure 1), an enzyme that converts glycolate to glycolaldehyde (reaction 9 of Figure 1), and an enzyme that converts glycolaldehyde to ethylene glycol (reaction 10 of Figure 1) produces ethylene glycol. In a non-limiting example, the enzyme that converts glycolate to glycolaldehyde may be a glycolaldehyde dehydrogenase from *G. oxydans* (SEQ ID NOs: 55-56) or an aldehyde dehydrogenase from *P. fluorescens* (SEQ ID NOs: 57-58). One of more of the enzymes catalyzing the reactions of steps 8, 9, and 10, as shown in Figure 1, may be overexpressed.

[0113] In some embodiments, a microorganism comprising a complex of enzymes that converts 5,10-methylenetetrahydrofolate to glycine (reaction 24 of Figure 1), an enzyme that converts glycine to glyoxylate (reaction 20 of Figure 1), an enzyme that converts glyoxylate

to glycolate (reaction 8 of Figure 1), an enzyme that converts glycolate to glycolaldehyde (reaction 9 of Figure 1), and an enzyme that converts glycolaldehyde to ethylene glycol (reaction 10 of Figure 1) produces ethylene glycol. In a non-limiting example, the enzyme that converts glycine to glyoxylate may be an alanine-glyoxylate aminotransferase from *S. thiotaurini* (SEQ ID NOs: 15-16) or a class V aminotransferase from *C. acidurici* (SEQ ID NOs: 19-20). In a non-limiting example, the enzyme that converts glycolate to glycolaldehyde may be a glycolaldehyde dehydrogenase from *G. oxydans* (SEQ ID NOs: 55-56) or an aldehyde dehydrogenase from *P. fluorescens* (SEQ ID NOs: 57-58). One or more of the enzymes catalyzing the reactions of steps 8, 9, 10, 20, and 24 may be overexpressed.

10 **[0114]** In some embodiments, a microorganism comprising an enzyme that converts acetyl-CoA to pyruvate (reaction 1 of Figure 1), an enzyme that converts pyruvate to phosphoenolpyruvate (reaction 13 of Figure 1), an enzyme that converts phosphoenolpyruvate to oxaloacetate (reaction 25 of Figure 1), an enzyme that converts oxaloacetate to citryl-CoA (reaction 3 of Figure 1), an enzyme that converts citryl-CoA to citrate (reaction 4 of Figure 1), an enzyme that converts citrate to aconitate and aconitate to iso-citrate (reactions 6 of Figure 1), an enzyme that converts isocitrate to glyoxylate (reaction 7 of Figure 1), an enzyme that converts glyoxylate to glycolate (reaction 8 of Figure 1), an enzyme that converts glycolate to glycolaldehyde (reaction 9 of Figure 1), and an enzyme that converts glycolaldehyde to ethylene glycol (reaction 10 of Figure 1) produces ethylene glycol. In a non-limiting example, the enzyme that converts iso-citrate to glyoxylate may be an isocitrate lyase from *E. coli* (SEQ ID NOs: 11-12). In a non-limiting example, the enzyme that converts glycolate to glycolaldehyde may be a glycolaldehyde dehydrogenase from *G. oxydans* (SEQ ID NOs: 55-56) or an aldehyde dehydrogenase from *P. fluorescens* (SEQ ID NOs: 57-58). One or more of the enzymes catalyzing reactions 2, 6, 8, 9, 10, and 25, as

25 shown in Figure 1, may be overexpressed.

[0115] In some embodiments, a microorganism comprising an enzyme that converts acetyl-CoA to pyruvate (reaction 1 of Figure 1), an enzyme that converts pyruvate to phosphoenolpyruvate (reaction 13 of Figure 1), an enzyme that converts phosphoenolpyruvate to oxaloacetate (reaction 25 of Figure 1), an enzyme that converts oxaloacetate to citrate (reaction 5 of Figure 1), an enzyme that converts citrate to aconitate and aconitate to iso-citrate (reactions 6 of Figure 1), an enzyme that converts isocitrate to glyoxylate (reaction 7 of Figure 1), an enzyme that converts glyoxylate to glycolate (reaction 8 of Figure 1), an enzyme that converts glycolate to glycolaldehyde (reaction 9 of Figure 1), and an enzyme that converts glycolaldehyde to ethylene glycol (reaction 10 of Figure 1)

produces ethylene glycol. In a non-limiting example, the enzyme that converts oxaloacetate to citrate may be a citrate synthase from *B. subtilis* (SEQ ID NOs: 1-2). In a non-limiting example, the enzyme that converts iso-citrate to glyoxylate may be an isocitrate lyase from *E. coli* (SEQ ID NOs: 11-12). In a non-limiting example, the enzyme that converts glycolate to glycolaldehyde may be a glycolaldehyde dehydrogenase from *G. oxydans* (SEQ ID NOs: 55-56) or an aldehyde dehydrogenase from *P. fluorescens* (SEQ ID NOs: 57-58). One or more of the enzymes catalyzing reactions 5, 6, 8, 9, 10, and 25, as shown in Figure 1, may be overexpressed.

[0116] In some embodiments, a microorganism comprising an enzyme that converts acetyl-CoA to pyruvate (reaction 1 of Figure 1), an enzyme that converts pyruvate to phosphoenolpyruvate (reaction 13 of Figure 1), an enzyme that converts phosphoenolpyruvate to 2-phospho-D-glycerate (reaction 14 of Figure 1), an enzyme that converts 2-phospho-D-glycerate to 3-phospho-D-glycerate (reaction 15 of Figure 1), an enzyme that converts 3-phospho-D-glycerate to 3-phosphonooxypyruvate (reaction 16 of Figure 1), an enzyme that converts 3-phosphonooxypyruvate to 3-phospho-L-serine (reaction 17 of Figure 1), an enzyme that converts 3-phospho-L-serine to serine (reaction 18 of Figure 1), comprise an enzyme that converts serine to hydroxypyruvate (reaction 21 of Figure 1), an enzyme that converts hydroxypyruvate to glycolaldehyde (reaction 22 of Figure 1), and an enzyme that converts glycolaldehyde to ethylene glycol (reaction 10 of Figure 1) produces ethylene glycol. The enzyme catalyzing the conversion of glycolaldehyde to ethylene glycol may be overexpressed.

[0117] In some embodiments, a microorganism comprising an enzyme that converts D-glycerate to hydroxypyruvate (reaction 23 of Figure 1), an enzyme that converts hydroxypyruvate to glycolaldehyde (reaction 22 of Figure 1), and an enzyme that converts glycolaldehyde to ethylene glycol (reaction 10 of Figure 1) produces ethylene glycol. The enzyme catalyzing the conversion of glycolaldehyde to ethylene glycol may be overexpressed.

[0118] The enzymes of the disclosure may be codon optimized for expression in the microorganism of the disclosure. "Codon optimization" refers to the mutation of a nucleic acid, such as a gene, for optimized or improved translation of the nucleic acid in a particular strain or species. Codon optimization may result in faster translation rates or higher translation accuracy. In a preferred embodiment, the genes of the disclosure are codon optimized for expression in the microorganism of the disclosure. Although codon optimization refers to the underlying genetic sequence, codon optimization often results in

improved translation and, thus, improved enzyme expression. Accordingly, the enzymes of the disclosure may also be described as being codon optimized.

[0119] One or more of the enzymes of the disclosure may be overexpressed.

“Overexpressed” refers to an increase in expression of a nucleic acid or protein in the microorganism of the disclosure compared to the wild-type or parental microorganism from which the microorganism of the disclosure is derived. Overexpression may be achieved by any means known in the art, including modifying gene copy number, gene transcription rate, gene translation rate, or enzyme degradation rate. As described above, one or more of the enzymes catalyzing reactions 2, 5, 6, 8, 9, 10, 19, 20, 24, or 25 of Figure 1 may be overexpressed.

[0120] The enzymes of the disclosure may comprise a disruptive mutation. A “disruptive mutation” refers to a mutation that reduces or eliminates (i.e., “disrupts”) the expression or activity of a gene or enzyme. The disruptive mutation may partially inactivate, fully inactivate, or delete the gene or enzyme. The disruptive mutation may be a knockout (KO) mutation. The disruptive mutation may be any mutation that reduces, prevents, or blocks the biosynthesis of a product produced by an enzyme. The disruptive mutation may include, for example, a mutation in a gene encoding an enzyme, a mutation in a genetic regulatory element involved in the expression of a gene encoding an enzyme, the introduction of a nucleic acid which produces a protein that reduces or inhibits the activity of an enzyme, or the introduction of a nucleic acid (e.g., antisense RNA, siRNA, CRISPR) or protein which inhibits the expression of an enzyme. The disruptive mutation may be introduced using any method known in the art.

[0121] In one embodiment, the microorganism of the disclosure comprises a disruptive mutation in a gene encoding diol dehydratase.

25 **[0122]** In some embodiments, the microorganism of the disclosure comprises a disruptive mutation in isocitrate dehydrogenase [1.1.1.41]. Isocitrate dehydrogenase converts iso-citrate to 2-oxoglutarate. Disruption of isocitrate dehydrogenase, such as by deleting isocitrate dehydrogenase, results in increased levels of iso-citrate.

[0123] In some embodiments, the microorganism of the disclosure comprises a disruptive mutation in glycerate dehydrogenase [1.1.1.29]. Glycerate dehydrogenase converts glyoxylate to glycolate. Disruption of glycerate dehydrogenase, such as by deleting isocitrate dehydrogenase, results in increased levels of glyoxylate.

[0124] In some embodiments, the microorganism of the disclosure comprises a disruptive mutation in glycolate dehydrogenase [1.1.99.14]. Glycolate dehydrogenase converts

glyoxylate to glycolate. Disruption of glycolate dehydrogenase, such as by deleting glycolate dehydrogenase, results in increased levels of glyoxylate.

[0125] In some embodiments, the microorganism of the disclosure comprises a disruptive mutation in aldehyde ferredoxin oxidoreductase [1.2.7.5]. Aldehyde ferredoxin

5 oxidoreductase converts glycolate to glycolaldehyde. Disruption of aldehyde ferredoxin oxidoreductase, such as by deleting aldehyde ferredoxin oxidoreductase, results in increased levels of glycolate.

[0126] In some embodiments, the microorganism of the disclosure comprises a disruptive mutation in aldehyde dehydrogenase [1.2.1.3/1.2.3.4/1.2.3.5]. Aldehyde dehydrogenase

10 converts glycolate to glycolaldehyde. Disruption of aldehyde dehydrogenase, such as by deleting aldehyde dehydrogenase, results in increased levels of glycolate.

[0127] Introduction of a disruptive mutation results in a microorganism of the disclosure that produces no target product or substantially no target product or a reduced amount of target product compared to the parental microorganism from which the microorganism of the

15 disclosure is derived. For example, the microorganism of the disclosure may produce no target product or at least about 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% less target product than the parental microorganism. For example, the microorganism of the disclosure may produce less than about 0.001, 0.01, 0.10, 0.30, 0.50, or 1.0 g/L target product.

20 **[0128]** Although exemplary sequences and sources for enzymes are provided herein, the disclosure is by no means limited to these sequences and sources – it also encompasses variants. The term “variants” includes nucleic acids and proteins whose sequence varies from the sequence of a reference nucleic acid and protein, such as a sequence of a reference nucleic acid and protein disclosed in the prior art or exemplified herein. The disclosure may

25 be practiced using variant nucleic acids or proteins that perform substantially the same function as the reference nucleic acid or protein. For example, a variant protein may perform substantially the same function or catalyze substantially the same reaction as a reference protein. A variant gene may encode the same or substantially the same protein as a reference gene. A variant promoter may have substantially the same ability to promote the expression
30 of one or more genes as a reference promoter.

[0129] Such nucleic acids or proteins may be referred to herein as “functionally equivalent variants.” By way of example, functionally equivalent variants of a nucleic acid may include allelic variants, fragments of a gene, mutated genes, polymorphisms, and the like.

Homologous genes from other microorganisms are also examples of functionally equivalent

variants. These include homologous genes in species such as *Clostridium acetobutylicum*, *Clostridium beijerinckii*, or *Clostridium ljungdahlii*, the details of which are publicly available on websites such as Genbank or NCBI. Functionally equivalent variants also include nucleic acids whose sequence varies as a result of codon optimization for a particular microorganism. A functionally equivalent variant of a nucleic acid will preferably have at least approximately 70%, approximately 80%, approximately 85%, approximately 90%, approximately 95%, approximately 98%, or greater nucleic acid sequence identity (percent homology) with the referenced nucleic acid. A functionally equivalent variant of a protein will preferably have at least approximately 70%, approximately 80%, approximately 85%, approximately 90%, approximately 95%, approximately 98%, or greater amino acid identity (percent homology) with the referenced protein. The functional equivalence of a variant nucleic acid or protein may be evaluated using any method known in the art.

[0130] “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). “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.

[0131] “Hybridization” refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson Crick base pairing, Hoogsteen binding, or in any other sequence specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of PCR, or the cleavage of a polynucleotide by an enzyme. A sequence capable of hybridizing with a given sequence is referred to as the “complement” of the given sequence.

[0132] Nucleic acids may be delivered to a microorganism of the disclosure using any method known in the art. For example, nucleic acids may be delivered as naked nucleic acids or may be formulated with one or more agents, such as liposomes. The nucleic acids may be DNA, RNA, cDNA, or combinations thereof, as is appropriate. Restriction inhibitors may be used in certain embodiments. Additional vectors may include plasmids, viruses, bacteriophages, cosmids, and artificial chromosomes. In a preferred embodiment, nucleic acids are delivered to the microorganism of the disclosure using a plasmid. By way of example, transformation (including transduction or transfection) may be achieved by electroporation, ultrasonication, polyethylene glycol-mediated transformation, chemical or natural competence, protoplast transformation, prophage induction, or conjugation. In certain embodiments having active restriction enzyme systems, it may be necessary to methylate a nucleic acid before introduction of the nucleic acid into a microorganism.

[0133] Furthermore, nucleic acids may be designed to comprise a regulatory element, such as a promoter, to increase or otherwise control expression of a particular nucleic acid. The promoter may be a constitutive promoter or an inducible promoter. Ideally, the promoter is a Wood-Ljungdahl pathway promoter, a ferredoxin promoter, a pyruvate ferredoxin oxidoreductase promoter, an Rnf complex operon promoter, an ATP synthase operon promoter, or a phosphotransacetylase/acetate kinase operon promoter.

[0134] It should be appreciated that the disclosure may be practiced using nucleic acids whose sequence varies from the sequences specifically exemplified herein provided they perform substantially the same function. For nucleic acid sequences that encode a protein or peptide this means that the encoded protein or peptide has substantially the same function. For nucleic acid sequences that represent promoter sequences, the variant sequence will have the ability to promote expression of one or more genes. Such nucleic acids may be referred to herein as “functionally equivalent variants.” By way of example, functionally equivalent variants of a nucleic acid include allelic variants, fragments of a gene, genes which include mutations (deletion, insertion, nucleotide substitutions and the like) and/or polymorphisms and the like. Homologous genes from other microorganisms may also be considered as examples of functionally equivalent variants of the sequences specifically exemplified herein.

[0135] These include homologous genes in species such as *Clostridium ljungdahlii*, *Chloroflexus aurantiacus*, *Metallosphaera* or *Sulfolobus* spp, details of which are publicly available on websites such as Genbank or NCBI. The phrase “functionally equivalent variants” should also be taken to include nucleic acids whose sequence varies as a result of codon optimisation for a particular organism. “Functionally equivalent variants” of a nucleic

acid herein will preferably have at least approximately 70%, preferably approximately 80%, more preferably approximately 85%, preferably approximately 90%, preferably approximately 95% or greater nucleic acid sequence identity with the nucleic acid identified.

[0136] It should also be appreciated that the disclosure may be practiced using polypeptides whose sequence varies from the amino acid sequences specifically exemplified herein. These variants may be referred to herein as “functionally equivalent variants.” A functionally equivalent variant of a protein or a peptide includes those proteins or peptides that share at least 40%, preferably 50%, preferably 60%, preferably 70%, preferably 75%, preferably 80%, preferably 85%, preferably 90%, preferably 95% or greater amino acid identity with the protein or peptide identified and has substantially the same function as the peptide or protein of interest. Such variants include within their scope fragments of a protein or peptide wherein the fragment comprises a truncated form of the polypeptide wherein deletions may be from 1 to 5, to 10, to 15, to 20, to 25 amino acids, and may extend from residue 1 through 25 at either terminus of the polypeptide, and wherein deletions may be of any length within the region; or may be at an internal location. Functionally equivalent variants of the specific polypeptides herein should also be taken to include polypeptides expressed by homologous genes in other species of bacteria, for example as exemplified in the previous paragraph.

[0137] The microorganisms of the disclosure may be prepared from a parental microorganism and one or more exogenous nucleic acids using any number of techniques known in the art for producing recombinant microorganisms. By way of example only, transformation (including transduction or transfection) may be achieved by electroporation, ultrasonication, polyethylene glycol-mediated transformation, chemical or natural competence, or conjugation. Suitable transformation techniques are described for example in, Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A laboratory Manual, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, 1989.

[0138] In certain embodiments, due to the restriction systems which are active in the microorganism to be transformed, it is necessary to methylate the nucleic acid to be introduced into the microorganism. This can be done using a variety of techniques, including those described below, and further exemplified in the Examples section herein after.

[0139] By way of example, in one embodiment, a recombinant microorganism of the disclosure is produced by a method comprises the following steps: introduction into a shuttle microorganism of (i) of an expression construct/vector as described herein and (ii) a methylation construct/vector comprising a methyltransferase gene; expression of the methyltransferase gene; isolation of one or more constructs/vectors from the shuttle

microorganism; and, introduction of the one or more construct/vector into a destination microorganism.

[0140] In one embodiment, the methyltransferase gene of step B is expressed constitutively.

In another embodiment, expression of the methyltransferase gene of step B is induced.

- 5 [0141] The shuttle microorganism is a microorganism, preferably a restriction negative microorganism that facilitates the methylation of the nucleic acid sequences that make up the expression construct/vector. In a particular embodiment, the shuttle microorganism is a restriction negative *E. coli*, *Bacillus subtilis*, or *Lactococcus lactis*.

- 10 [0142] The methylation construct/vector comprises a nucleic acid sequence encoding a methyltransferase.

- [0143] Once the expression construct/vector and the methylation construct/vector are introduced into the shuttle microorganism, the methyltransferase gene present on the methylation construct/vector is induced. Induction may be by any suitable promoter system although in one particular embodiment of the disclosure, the methylation construct/vector
15 comprises an inducible lac promoter and is induced by addition of lactose or an analogue thereof, more preferably isopropyl- β -D-thiogalactoside (IPTG). Other suitable promoters include the ara, tet, or T7 system. In a further embodiment of the disclosure, the methylation construct/vector promoter is a constitutive promoter.

- [0144] In a particular embodiment, the methylation construct/vector has an origin of
20 replication specific to the identity of the shuttle microorganism so that any genes present on the methylation construct/vector are expressed in the shuttle microorganism. Preferably, the expression construct/vector has an origin of replication specific to the identity of the destination microorganism so that any genes present on the expression construct/vector are expressed in the destination microorganism.

- 25 [0145] Expression of the methyltransferase enzyme results in methylation of the genes present on the expression construct/vector. The expression construct/vector may then be isolated from the shuttle microorganism according to any one of a number of known methods. By way of example only, the methodology described in the Examples section described hereinafter may be used to isolate the expression construct/vector.

- 30 [0146] In one particular embodiment, both construct/vector are concurrently isolated.

[0147] The expression construct/vector may be introduced into the destination microorganism using any number of known methods. However, by way of example, the methodology described in the Examples section hereinafter may be used. Since the expression construct/vector is methylated, the nucleic acid sequences present on the

expression construct/vector are able to be incorporated into the destination microorganism and successfully expressed.

[0148] It is envisaged that a methyltransferase gene may be introduced into a shuttle microorganism and over-expressed. Thus, in one embodiment, the resulting

5 methyltransferase enzyme may be collected using known methods and used in vitro to methylate an expression plasmid. The expression construct/vector may then be introduced into the destination microorganism for expression. In another embodiment, the methyltransferase gene is introduced into the genome of the shuttle microorganism followed by introduction of the expression construct/vector into the shuttle microorganism, isolation of
10 one or more constructs/vectors from the shuttle microorganism and then introduction of the expression construct/vector into the destination microorganism.

[0149] It is envisaged that the expression construct/vector and the methylation construct/vector as defined above may be combined to provide a composition of matter.

Such a composition has particular utility in circumventing restriction barrier mechanisms to
15 produce the recombinant microorganisms of the disclosure.

[0150] In one particular embodiment, the expression construct/vector and/or the methylation construct/vector are plasmids.

[0151] Persons of ordinary skill in the art will appreciate a number of suitable methyltransferases of use in producing the microorganisms of the disclosure. However, by
20 way of example the Bacillus subtilis phage Φ T1 methyltransferase and the methyltransferase described in the Examples herein after may be used. Nucleic acids encoding suitable methyltransferases will be readily appreciated having regard to the sequence of the desired methyltransferase and the genetic code.

[0152] Any number of constructs/vectors adapted to allow expression of a methyltransferase
25 gene may be used to generate the methylation construct/vector.

[0153] In one embodiment, the substrate comprises CO. In one embodiment, the substrate comprises CO₂ and CO. In another embodiment, the substrate comprises CO₂ and H₂. In another embodiment, the substrate comprises CO₂ and CO and H₂.

[0154] "Substrate" refers to a carbon and/or energy source for the microorganism of the
30 disclosure. Often, the substrate is gaseous and comprises a C1-carbon source, for example, CO, CO₂, and/or CH₄. Preferably, the substrate comprises a C1-carbon source of CO or CO + CO₂. The substrate may further comprise other non-carbon components, such as H₂, N₂, or electrons. In other embodiments, however, the substrate may be a carbohydrate, such as sugar, starch, fiber, lignin, cellulose, or hemicellulose or a combination thereof. For example,

the carbohydrate may be fructose, galactose, glucose, lactose, maltose, sucrose, xylose, or some combination thereof. In some embodiments, the substrate does not comprise (D)-xylose (Alkim, *Microb Cell Fact*, 14: 127, 2015). In some embodiments, the substrate does not comprise a pentose such as xylose (Pereira, *Metab Eng*, 34: 80-87, 2016). In some
5 embodiments, the substrate may comprise both gaseous and carbohydrate substrates (mixotrophic fermentation). The substrate may further comprise other non-carbon components, such as H₂, N₂, or electrons.

[0155] The gaseous substrate generally comprises at least some amount of CO, such as about 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 mol% CO. The gaseous substrate may
10 comprise a range of CO, such as about 20-80, 30-70, or 40-60 mol% CO. Preferably, the gaseous substrate comprises about 40-70 mol% CO (e.g., steel mill or blast furnace gas), about 20-30 mol% CO (e.g., basic oxygen furnace gas), or about 15-45 mol% CO (e.g., syngas). In some embodiments, the gaseous substrate may comprise a relatively low amount of CO, such as about 1-10 or 1-20 mol% CO. The microorganism of the disclosure typically
15 converts at least a portion of the CO in the gaseous substrate to a product. In some embodiments, the gaseous substrate comprises no or substantially no (< 1 mol%) CO.

[0156] The gaseous substrate may comprise some amount of H₂. For example, the gaseous substrate may comprise about 1, 2, 5, 10, 15, 20, or 30 mol% H₂. In some embodiments, the gaseous substrate may comprise a relatively high amount of H₂, such as about 60, 70, 80, or
20 90 mol% H₂. In further embodiments, the gaseous substrate comprises no or substantially no (< 1 mol%) H₂.

[0157] The gaseous substrate may comprise some amount of CO₂. For example, the gaseous substrate may comprise about 1-80 or 1-30 mol% CO₂. In some embodiments, the gaseous substrate may comprise less than about 20, 15, 10, or 5 mol% CO₂. In another embodiment,
25 the gaseous substrate comprises no or substantially no (< 1 mol%) CO₂.

[0158] The gaseous substrate may also be provided in alternative forms. For example, the gaseous substrate may be dissolved in a liquid or adsorbed onto a solid support.

[0159] The gaseous substrate and/or C1-carbon source may be a waste gas or an off gas obtained as a byproduct of an industrial process or from some other source, such as from
30 automobile exhaust fumes or biomass gasification. In certain embodiments, the industrial process is selected from the group consisting of ferrous metal products manufacturing, such as a steel mill manufacturing, non-ferrous products manufacturing, petroleum refining, coal gasification, electric power production, carbon black production, ammonia production, methanol production, and coke manufacturing. In these embodiments, the gaseous substrate

and/or C1-carbon source may be captured from the industrial process before it is emitted into the atmosphere, using any convenient method.

[0160] The gaseous substrate and/or C1-carbon source may be syngas, such as syngas obtained by gasification of coal or refinery residues, gasification of biomass or

5 lignocellulosic material, or reforming of natural gas. In another embodiment, the syngas may be obtained from the gasification of municipal solid waste or industrial solid waste.

[0161] The substrate and/or C1-carbon source may be a waste gas obtained as a byproduct of an industrial process or from another source, such as automobile exhaust fumes, biogas, landfill gas, direct air capture, or from electrolysis. The substrate and/or C1-carbon source
10 may be syngas generated by pyrolysis, torrefaction, or gasification. In other words, carbon in waste material may be recycled by pyrolysis, torrefaction, or gasification to generate syngas which is used as the substrate and/or C1-carbon source. The substrate and/or C1-carbon source may be a gas comprising methane.

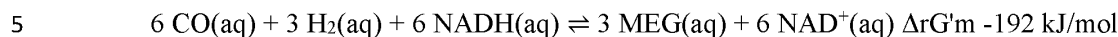
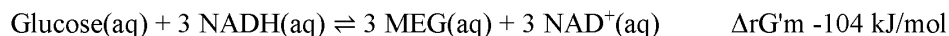
[0162] In certain embodiments, the industrial process is selected from ferrous metal products manufacturing, such as a steel manufacturing, non-ferrous products manufacturing, petroleum refining, electric power production, carbon black production, paper and pulp manufacturing, ammonia production, methanol production, coke manufacturing, petrochemical production, carbohydrate fermentation, cement making, aerobic digestion, anaerobic digestion, catalytic processes, natural gas extraction, cellulosic fermentation, oil extraction, geological reservoirs,
20 gas from fossil resources such as natural gas coal and oil, or any combination thereof.

Examples of specific processing steps within an industrial process include catalyst regeneration, fluid catalyst cracking, and catalyst regeneration. Air separation and direct air capture are other suitable industrial processes. Specific examples in steel and ferroalloy manufacturing include blast furnace gas, basic oxygen furnace gas, coke oven gas, direct
25 reduction of iron furnace top-gas, and residual gas from smelting iron. In these embodiments, the substrate and/or C1-carbon source may be captured from the industrial process before it is emitted into the atmosphere, using any known method.

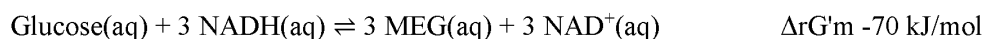
[0163] The substrate and/or C1-carbon source may be synthesis gas known as syngas, which may be obtained from reforming, partial oxidation, or gasification processes. Examples of
30 gasification processes include gasification of coal, gasification of refinery residues, gasification of petroleum coke, gasification of biomass, gasification of lignocellulosic material, gasification of waste wood, gasification of black liquor, gasification of municipal solid waste, gasification of municipal liquid waste, gasification of industrial solid waste, gasification of industrial liquid waste, gasification of refuse derived fuel, gasification of

- sewerage, gasification of sewerage sludge, gasification of sludge from wastewater treatment, gasification of biogas. Examples of reforming processes include, steam methane reforming, steam naphtha reforming, reforming of natural gas, reforming of biogas, reforming of landfill gas, naphtha reforming, and dry methane reforming. Examples of partial oxidation processes
- 5 include thermal and catalytic partial oxidation processes, catalytic partial oxidation of natural gas, partial oxidation of hydrocarbons. Examples of municipal solid waste include tires, plastics, fibers, such as in shoes, apparel, and textiles. Municipal solid waste may be simply landfill-type waste. The municipal solid waste may be sorted or unsorted. Examples of biomass may include lignocellulosic material and may also include microbial biomass.
- 10 Lignocellulosic material may include agriculture waste and forest waste.
- [0164]** The substrate and/or C1-carbon source may be a gas stream comprising methane. Such a methane containing gas may be obtained from fossil methane emission such as during fracking, wastewater treatment, livestock, agriculture, and municipal solid waste landfills. It is also envisioned that the methane may be burned to produce electricity or heat, and the C1
- 15 byproducts may be used as the substrate or carbon source.
- [0165]** The composition of the gaseous substrate may have a significant impact on the efficiency and/or cost of the reaction. For example, the presence of oxygen (O₂) may reduce the efficiency of an anaerobic fermentation process. Depending on the composition of the substrate, it may be desirable to treat, scrub, or filter the substrate to remove any undesired
- 20 impurities, such as toxins, undesired components, or dust particles, and/or increase the concentration of desirable components.
- [0166]** In certain embodiments, the fermentation is performed in the absence of carbohydrate substrates, such as sugar, starch, fiber, lignin, cellulose, or hemicellulose.
- [0167]** In some embodiments, the overall energetics of CO and H₂ to ethylene glycol (MEG)
- 25 are preferable to those from glucose to ethylene glycol, as shown below, wherein the more negative Gibbs free energy, $\Delta_r G'^m$, values for CO and H₂ indicate a larger driving force towards ethylene glycol. Calculations of overall reaction delta G for the comparison of glucose vs CO as a substrate were performed using equilibrator (<http://equilibrator.weizmann.ac.il/>), which is a standard method for evaluating the overall feasibility of a pathway or individual steps in pathways in
- 30 biological systems (Flamholz, E. Noor, A. Bar-Even, R. Milo (2012) eQuilibrator - the biochemical thermodynamics calculator *Nucleic Acids Res* 40:D770-5; Noor, A. Bar-Even, A. Flamholz, Y. Lubling, D. Davidi, R. Milo (2012) An integrated open framework for thermodynamics of reactions that combines accuracy and coverage *Bioinformatics* 28:2037-2044; Noor, H.S. Haraldsdóttir, R. Milo, R.M.T. Fleming (2013) Consistent Estimation of Gibbs Energy Using Component

Contributions PLoS Comput Biol 9(7): e1003098; Noor, A. Bar-Even, A. Flamholz, E. Reznik, W. Liebermeister, R. Milo (2014) Pathway Thermodynamics Highlights Kinetic Obstacles in Central Metabolism PLoS Comput Biol 10(2):e1003483). The calculations are as follows:



Physiological conditions:



[0168] In addition to ethylene glycol, glyoxylate, and/or glycolate, the microorganism of the disclosure may be cultured to produce one or more co-products products. For instance, the microorganism of the disclosure may produce or may be engineered to produce ethanol (WO 2007/117157), acetate (WO 2007/117157), 1-butanol (WO 2008/115080, WO 2012/053905, and WO 2017/066498), butyrate (WO 2008/115080), 2,3-butanediol (WO 2009/151342 and WO 2016/094334), lactate (WO 2011/112103), butene (WO 2012/024522), butadiene (WO 2012/024522), methyl ethyl ketone (2-butanone) (WO 2012/024522 and WO 2013/185123), ethylene (WO 2012/026833), acetone (WO 2012/115527), isopropanol (WO 2012/115527), lipids (WO 2013/036147), 3-hydroxypropionate (3-HP) (WO 2013/180581), terpenes, including isoprene (WO 2013/180584), fatty acids (WO 2013/191567), 2-butanol (WO 2013/185123), 1,2-propanediol (WO 2014/036152), 1-propanol (WO 2017/066498), 1-hexanol (WO 2017/066498), 1-octanol (WO 2017/066498), chorismate-derived products (WO 2016/191625), 3-hydroxybutyrate (WO 2017/066498), 1,3-butanediol (WO 2017/066498), 2-hydroxyisobutyrate or 2-hydroxyisobutyric acid (WO 2017/066498), isobutylene (WO 2017/066498), adipic acid (WO 2017/066498), 1,3-hexanediol (WO 2017/066498), 3-methyl-2-butanol (WO 2017/066498), 2-buten-1-ol (WO 2017/066498), isovalerate (WO 2017/066498), isoamyl alcohol (WO 2017/066498), and/or monoethylene glycol (WO 2019/126400) in addition to 2-phenylethanol. In some embodiments, in addition to ethylene glycol, the microorganism of the disclosure also produces ethanol, 2,3-butanediol, and/or succinate. In certain embodiments, microbial biomass itself may be considered a product. These products may be further converted to produce at least one component of diesel, jet fuel, and/or gasoline. In certain embodiments, 2-phenylethanol may be used as an ingredient in fragrances, essential oils, flavors, and soaps. Additionally, the microbial biomass may be further processed to produce a single cell protein (SCP) by any method or combination of methods known in the art. In addition to one or more target products, the microorganism of the disclosure may also produce ethanol, acetate, and/or 2,3-butanediol.

[0169] A “native product” is a product produced by a genetically unmodified microorganism. For example, ethanol, acetate, and 2,3-butanediol are native products of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, and *Clostridium ragsdalei*. A “non-native product” is a product that is produced by a genetically modified microorganism but is not produced by a genetically unmodified microorganism from which the genetically modified microorganism is derived. Ethylene glycol is not known to be produced by any naturally-occurring microorganism, such that it is a non-native product of all microorganisms.

[0170] “Selectivity” refers to the ratio of the production of a target product to the production of all fermentation products produced by a microorganism. The microorganism of the disclosure may be engineered to produce products at a certain selectivity or at a minimum selectivity. In one embodiment, a target product, such as ethylene glycol, accounts for at least about 5%, 10%, 15%, 20%, 30%, 50%, or 75% of all fermentation products produced by the microorganism of the disclosure. In one embodiment, ethylene glycol accounts for at least 10% of all fermentation products produced by the microorganism of the disclosure, such that the microorganism of the disclosure has a selectivity for ethylene glycol of at least 10%. In another embodiment, ethylene glycol accounts for at least 30% of all fermentation products produced by the microorganism of the disclosure, such that the microorganism of the disclosure has a selectivity for ethylene glycol of at least 30%.

[0171] At least one of the one or more fermentation products may be biomass produced by the culture. At least a portion of the microbial biomass may be converted to a single cell protein (SCP). At least a portion of the single cell protein may be utilized as a component of animal feed.

[0172] In one embodiment, the disclosure provides an animal feed comprising microbial biomass and at least one excipient, wherein the microbial biomass comprises a microorganism grown on a gaseous substrate comprising one or more of CO, CO₂, and H₂.

[0173] A “single cell protein” (SCP) refers to a microbial biomass that may be used in protein-rich human and/or animal feeds, often replacing conventional sources of protein supplementation such as soymeal or fishmeal. To produce a single cell protein, or other product, the process may comprise additional separation, processing, or treatments steps. For example, the method may comprise sterilizing the microbial biomass, centrifuging the microbial biomass, and/or drying the microbial biomass. In certain embodiments, the microbial biomass is dried using spray drying or paddle drying. The method may also comprise reducing the nucleic acid content of the microbial biomass using any method known in the art, since intake of a diet high in nucleic acid content may result in the

accumulation of nucleic acid degradation products and/or gastrointestinal distress. The single cell protein may be suitable for feeding to animals, such as livestock or pets. In particular, the animal feed may be suitable for feeding to one or more beef cattle, dairy cattle, pigs, sheep, goats, horses, mules, donkeys, deer, buffalo/bison, llamas, alpacas, reindeer, camels, bantengs, gayals, yaks, chickens, turkeys, ducks, geese, quail, guinea fowl, squabs/pigeons, fish, shrimp, crustaceans, cats, dogs, and rodents. The composition of the animal feed may be tailored to the nutritional requirements of different animals. Furthermore, the process may comprise blending or combining the microbial biomass with one or more excipients.

[0174] “Microbial biomass” refers biological material comprising microorganism cells. For example, microbial biomass may comprise or consist of a pure or substantially pure culture of a bacterium, archaea, virus, or fungus. When initially separated from a fermentation broth, microbial biomass generally contains a large amount of water. This water may be removed or reduced by drying or processing the microbial biomass.

[0175] An “excipient” may refer to any substance that may be added to the microbial biomass to enhance or alter the form, properties, or nutritional content of the animal feed. For example, the excipient may comprise one or more of a carbohydrate, fiber, fat, protein, vitamin, mineral, water, flavour, sweetener, antioxidant, enzyme, preservative, probiotic, or antibiotic. In some embodiments, the excipient may be hay, straw, silage, grains, oils or fats, or other plant material. The excipient may be any feed ingredient identified in Chiba, Section 18: Diet Formulation and Common Feed Ingredients, Animal Nutrition Handbook, 3rd revision, pages 575-633, 2014.

[0176] A “biopolymer” refers to natural polymers produced by the cells of living organisms. In certain embodiments, the biopolymer is PHA. In certain embodiments, the biopolymer is PHB.

[0177] A “bioplastic” refers to plastic materials produced from renewable biomass sources. A bioplastic may be produced from renewable sources, such as vegetable fats and oils, corn starch, straw, woodchips, sawdust, or recycled food waste.

[0178] Herein, reference to an acid (e.g., acetic acid or 2-hydroxyisobutyric acid) should be taken to also include the corresponding salt (e.g., acetate or 2-hydroxyisobutyrate).

[0179] Typically, the culture is performed in a bioreactor. The term “bioreactor” includes a culture/fermentation device consisting of one or more vessels, towers, or piping arrangements, such as a continuous stirred tank reactor (CSTR), immobilized cell reactor (ICR), trickle bed reactor (TBR), bubble column, gas lift fermenter, static mixer, or other vessel or other device suitable for gas-liquid contact. In some embodiments, the bioreactor

may comprise a first growth reactor and a second culture/fermentation reactor. The substrate may be provided to one or both of these reactors. As used herein, the terms “culture” and “fermentation” are used interchangeably. These terms encompass both the growth phase and product biosynthesis phase of the culture/fermentation process.

- 5 **[0180]** The culture is generally maintained in an aqueous culture medium that contains nutrients, vitamins, and/or minerals sufficient to permit growth of the microorganism. Preferably the aqueous culture medium is an anaerobic microbial growth medium, such as a minimal anaerobic microbial growth medium. Suitable media are well known in the art.
- 10 **[0181]** The culture/fermentation should desirably be carried out under appropriate conditions for production of ethylene glycol. If necessary, the culture/fermentation is performed under anaerobic conditions. Reaction conditions to consider include pressure (or partial pressure), temperature, gas flow rate, liquid flow rate, media pH, media redox potential, agitation rate (if using a continuous stirred tank reactor), inoculum level, maximum gas substrate concentrations to ensure that gas in the liquid phase does not become limiting, and maximum
- 15 product concentrations to avoid product inhibition. In particular, the rate of introduction of the substrate may be controlled to ensure that the concentration of gas in the liquid phase does not become limiting.
- 20 **[0182]** Operating a bioreactor at elevated pressures allows for an increased rate of gas mass transfer from the gas phase to the liquid phase. Accordingly, it is generally preferable to perform the culture/fermentation at pressures higher than atmospheric pressure. Also, since a given gas conversion rate is, in part, a function of the substrate retention time and retention time dictates the required volume of a bioreactor, the use of pressurized systems can greatly reduce the volume of the bioreactor required and, consequently, the capital cost of the culture/fermentation equipment. This, in turn, means that the retention time, defined as the
- 25 liquid volume in the bioreactor divided by the input gas flow rate, can be reduced when bioreactors are maintained at elevated pressure rather than atmospheric pressure. The optimum reaction conditions will depend partly on the particular microorganism used. However, in general, it is preferable to operate the fermentation at a pressure higher than atmospheric pressure. Also, since a given gas conversion rate is in part a function of substrate
- 30 retention time and achieving a desired retention time in turn dictates the required volume of a bioreactor, the use of pressurized systems can greatly reduce the volume of the bioreactor required, and consequently the capital cost of the fermentation equipment.
- [0183]** In certain embodiments, the fermentation is performed in the absence of light or in the presence of an amount of light insufficient to meet the energetic requirements of

photosynthetic microorganisms. In certain embodiments, the microorganism of the disclosure is a non-photosynthetic microorganism.

[0184] Target products may be separated or purified from a fermentation broth using any method or combination of methods known in the art, including, for example, fractional
5 distillation, evaporation, pervaporation, gas stripping, phase separation, and extractive fermentation, including for example, liquid-liquid extraction. In certain embodiments, target products are recovered from the fermentation broth by continuously removing a portion of the broth from the bioreactor, separating microbial cells from the broth (conveniently by filtration), and recovering one or more target products from the broth. Alcohols and/or
10 acetone may be recovered, for example, by distillation. Acids may be recovered, for example, by adsorption on activated charcoal. Separated microbial cells are preferably returned to the bioreactor. The cell-free permeate remaining after target products have been removed is also preferably returned to the bioreactor. Additional nutrients (such as B vitamins) may be added to the cell-free permeate to replenish the medium before it is returned to the bioreactor.
15 Purification techniques may include affinity tag purification (e.g. His, Twin-Strep, and FLAG), bead-based systems, a tip-based approach, and FPLC system for larger scale, automated purifications. Purification methods that do not rely on affinity tags (e.g. salting out, ion exchange, and size exclusion) are also disclosed.

[0185] The method of the disclosure may further comprise separating the ethylene glycol
20 from the fermentation broth. Ethylene glycol may be separated or purified from a fermentation broth using any method or combination of methods known in the art, including, for example, distillation, simulated moving bed processes, membrane treatment, evaporation, pervaporation, gas stripping, phase separation, ion exchange, or extractive fermentation, including for example, liquid-liquid extraction. In one embodiment, ethylene glycol may be
25 concentrated from the fermentation broth using reverse osmosis and/or pervaporation (US 5,552,023). Water may be removed by distillation and the bottoms (containing a high proportion of ethylene glycol) may then be recovered using distillation or vacuum distillation to produce a high purity ethylene glycol stream. Alternatively, with or without concentration by reverse osmosis and/or pervaporation, ethylene glycol may be further purified by reactive
30 distillation with an aldehyde (Atul, *Chem Eng Sci*, 59: 2881-2890, 2004) or azeotropic distillation using a hydrocarbon (US 2,218,234). In another approach, ethylene glycol may be trapped on an activated carbon or polymer absorbent from aqueous solution (with or without reverse osmosis and/or pervaporation) and recovered using a low boiling organic solvent (Chinn, Recovery of Glycols, Sugars, and Related Multiple -OH Compounds from Dilute-

Aqueous Solution by Regenerable Adsorption onto Activated Carbons, University of California Berkeley, 1999). Ethylene glycol can then be recovered from the organic solvent by distillation. In certain embodiments, ethylene glycol is recovered from the fermentation broth by continuously removing a portion of the broth from the bioreactor, separating
5 microbial cells from the broth (conveniently by filtration), and recovering ethylene glycol from the broth. Co-products, such as alcohols or acids may also be separated or purified from the broth. Alcohols may be recovered, for example, by distillation. Acids may be recovered, for example, by adsorption on activated charcoal. Separated microbial cells may be returned to the bioreactor in certain embodiments. The cell-free permeate remaining after target
10 products have been removed is also preferably returned to the bioreactor, in whole or in part. Additional nutrients (such as B vitamins) may be added to the cell-free permeate to replenish the medium before it is returned to the bioreactor.

[0186] Recovery of diols from aqueous media has been demonstrated a number of ways. Simulated moving bed (SMB) technology has been used to recover 2,3-butaendiol from an
15 aqueous mixture of ethanol and associated oxygenates (U.S. Patent 8,658,845). Reactive separation has also been demonstrated for effective diol recovery. In some embodiments, recovery of ethylene glycol is conducted by reaction of the diol-containing stream with aldehydes, fractionation and regeneration of the diol, final fractionation to recover a concentrated diol stream. *See, e.g.*, U.S. Patent 7,951,980.

[0187] The disclosure provides compositions comprising ethylene glycol produced by the microorganisms and according to the methods described herein. For example, the composition comprising ethylene glycol may be an antifreeze, preservative, dehydrating agent, or drilling fluid.

[0188] The disclosure also provides polymers comprising ethylene glycol produced by the microorganisms and according to the methods described herein. Such polymers may be, for
25 example, homopolymers such as polyethylene glycol or copolymers such as polyethylene terephthalate. Methods for the synthesis of these polymers are well-known in the art. *See, e.g.*, Herzberger *et al.*, *Chem Rev.*, 116(4): 2170-2243 (2016) and Xiao *et al.*, *Ind Eng Chem Res.* 54(22): 5862-5869 (2015).

[0189] The disclosure further provides compositions comprising polymers comprising ethylene glycol produced by the microorganisms and according to the methods described herein. For example, the composition may be a fiber, resin, film, or plastic.

[0190] One embodiment is directed to a genetically engineered microorganism capable of producing ethylene glycol or a precursor of ethylene glycol from a gaseous substrate comprising: a disruptive mutation in a gene encoding diol dehydratase.

[0191] The microorganism according to an embodiment, wherein the microorganism produces ethylene glycol or the precursor of ethylene glycol through one or more intermediates selected from the group consisting of 5,10-methylenetetrahydrofolate, oxaloacetate, citrate, malate, and glycine.

[0192] The microorganism according to an embodiment, wherein the microorganism comprises one or more of:

- 10 a. a nucleic acid encoding a heterologous enzyme capable of converting oxaloacetate to citrate;
- b. a nucleic acid encoding a heterologous enzyme capable of converting glycine to glyoxylate;
- c. a nucleic acid encoding a heterologous enzyme capable of converting iso-
- 15 citrate to glyoxylate; and
- d. a nucleic acid encoding a heterologous enzyme capable of converting glycolate to glycolaldehyde.

[0193] The microorganism according to an embodiment, wherein:

- 20 a. the heterologous enzyme capable of converting oxaloacetate to citrate is a citrate [Si]-synthase having the EC number 2.3.3.1, an ATP citrate synthase having the EC number 2.3.3.8; or a citrate (Re)-synthase having the EC number 2.3.3.3;
- 25 b. the heterologous enzyme capable of converting glycine to glyoxylate is an alanine-glyoxylate transaminase having the EC number 2.6.1.44, a serine-glyoxylate transaminase having the EC number 2.6.1.45, a serine-pyruvate transaminase having the EC number 2.6.1.51, a glycine-oxaloacetate transaminase having the EC number 2.6.1.35, a glycine transaminase having the EC number 2.6.1.4, a glycine dehydrogenase having the EC number 1.4.1.10, an alanine dehydrogenase having the EC number 1.4.1.1, or a glycine
- 30 dehydrogenase having the EC number 1.4.2.1;
- c. the heterologous enzyme capable of converting iso-citrate to glyoxylate is an isocitrate lyase having the EC number 4.1.3.1; and/or
- d. the heterologous enzyme capable of converting glycolate to glycolaldehyde is a glycolaldehyde dehydrogenase having the EC number 1.2.1.21, a

lactaldehyde dehydrogenase having the EC number 1.2.1.22, a succinate-semialdehyde dehydrogenase having the EC number 1.2.1.24, a 2,5-dioxovalerate dehydrogenase having the EC number 1.2.1.26, an aldehyde dehydrogenase having the EC number 1.2.1.3/4/5, a betaine-aldehyde dehydrogenase having the EC number 1.2.1.8, or an aldehyde ferredoxin oxidoreductase having the EC number 1.2.7.5.

[0194] The microorganism according to an embodiment, wherein one or more of the heterologous enzymes are derived from a genus selected from the group consisting of *Bacillus*, *Clostridium*, *Escherichia*, *Gluconobacter*, *Hyphomicrobium*, *Lysinibacillus*, *Paenibacillus*, *Pseudomonas*, *Sedimenticola*, *Sporosarcina*, *Streptomyces*, *Thermithiobacillus*, *Thermotoga*, *Cupriavidus*, and *Zea*.

[0195] The microorganism according to an embodiment, wherein one or more of the heterologous enzymes are codon-optimized for expression in the microorganism.

[0196] The microorganism according to an embodiment, wherein the microorganism further comprises one or more of an enzymes capable of converting acetyl-CoA to pyruvate; an enzyme capable of converting pyruvate to oxaloacetate; an enzyme capable of converting pyruvate to malate; an enzyme capable of converting pyruvate to phosphoenolpyruvate; an enzyme capable of converting oxaloacetate to citryl-CoA; an enzyme capable of converting citryl-CoA to citrate; an enzyme capable of converting citrate to aconitate and aconitate to iso-citrate; an enzyme capable of converting phosphoenolpyruvate to oxaloacetate; an enzyme capable of converting phosphoenolpyruvate to 2-phospho-D-glycerate; an enzyme capable of converting 2-phospho-D-glycerate to 3-phospho-D-glycerate; an enzyme capable of converting 3-phospho-D-glycerate to 3-phosphonooxypyruvate; an enzyme capable of converting 3-phosphonooxypyruvate to 3-phospho-L-serine; an enzyme capable of converting 3-phospho-L-serine to serine; an enzyme capable of converting serine to glycine; an enzyme capable of converting 5,10-methylenetetrahydrofolate to glycine; an enzyme capable of converting serine to hydroxypyruvate; an enzyme capable of converting D-glycerate to hydroxypyruvate; an enzyme capable of converting malate to glyoxylate; an enzyme capable of converting glyoxylate to glycolate; an enzyme capable of converting hydroxypyruvate to glycolaldehyde; and an enzyme capable of converting glycolaldehyde to ethylene glycol.

[0197] The microorganism according to an embodiment, wherein the microorganism overexpresses:

- a. the heterologous enzyme capable of converting oxaloacetate to citrate;

- b. the heterologous enzyme capable of converting glycine to glyoxylate; and/or
- c. the heterologous enzyme capable of converting glycolate to glycolaldehyde.

[0198] The microorganism according to an embodiment, wherein the microorganism overexpresses:

- 5 a. the enzyme capable of converting pyruvate to oxaloacetate;
- b. the enzyme capable of converting citrate to aconitate and aconitate to isocitrate;
- c. the enzyme capable of converting phosphoenolpyruvate to oxaloacetate;
- d. the enzyme capable of converting serine to glycine;
- 10 e. the enzyme capable of converting 5,10-methylenetetrahydrofolate to glycine;
- f. the enzyme capable of converting glyoxylate to glycolate; and/or
- g. the enzyme capable of converting glycolaldehyde to ethylene glycol.

[0199] The microorganism according to an embodiment, wherein the microorganism further comprises a disruptive mutation in one or more of isocitrate dehydrogenase, glycerate dehydrogenase, glycolate dehydrogenase, glycerate dehydrogenase, glycolate dehydrogenase, aldehyde ferredoxin oxidoreductase, and aldehyde dehydrogenase.

[0200] The microorganism according to an embodiment, wherein the microorganism is a member of a genus selected from the group consisting of *Acetobacterium*, *Alkalibaculum*, *Blautia*, *Butyribacterium*, *Clostridium*, *Cupriavidus*, *Eubacterium*, *Moorella*, *Oxobacter*, *Sporomusa*, and *Thermoanaerobacter*.

[0201] The microorganism according to an embodiment, wherein the microorganism is derived from a parental microorganism selected from the group consisting of *Acetobacterium woodii*, *Alkalibaculum bacchii*, *Blautia producta*, *Butyribacterium methylotrophicum*, *Clostridium aceticum*, *Clostridium autoethanogenum*, *Clostridium carboxidivorans*, *Clostridium coskatii*, *Clostridium drakei*, *Clostridium formicoaceticum*, *Clostridium ljungdahlii*, *Clostridium magnum*, *Clostridium ragsdalei*, *Clostridium scatologenes*, *Cupriavidus necator*, *Eubacterium limosum*, *Moorella thermautotrophica*, *Moorella thermoacetica*, *Oxobacter pfennigii*, *Sporomusa ovata*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, and *Thermoanaerobacter kiuvi*.

[0202] The microorganism according to an embodiment, wherein the microorganism is derived from a parental bacterium selected from the group consisting of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, and *Clostridium ragsdalei*.

[0203] The microorganism according to an embodiment, wherein the microorganism comprises a native or heterologous Wood-Ljungdahl pathway.

[0204] The microorganism according to an embodiment, wherein the precursor of ethylene glycol is glyoxylate or glycolate.

[0205] Another embodiment is directed to a method of producing ethylene glycol or a precursor of ethylene glycol comprising culturing the microorganism of claim 1 in a nutrient medium in the presence of a gaseous substrate, whereby the microorganism produces ethylene glycol or the precursor of ethylene glycol.

[0206] The method of one embodiment, wherein the gaseous substrate comprises one or more of CO, CO₂, and H₂.

[0207] The method of one embodiment, wherein the precursor of ethylene glycol is glyoxylate or glycolate.

[0208] The method of one embodiment, further comprising separating ethylene glycol or the precursor of ethylene glycol from the nutrient medium.

[0209] The method of one embodiment, wherein the microorganism further produces one or more of ethanol, 2,3-butanediol, and succinate.

[0210] Another embodiment is directed to a method of producing a polyethylene terephthalate (PET) product from a gaseous substrate comprising 1) forming at least one PET component, wherein the at least one PET component is selected from monoethylene glycol (MEG), a terephthalic acid (PTA), or any combinations thereof; 2) processing the at least one PET component into PET; 3) polymerizing the PET to form a PET resin; and 4) processing the PET resin into a PET product.

EXAMPLES

[0211] The following examples further illustrate the disclosure but, of course, should not be construed to limit its scope in any way.

Example 1: Construction of heterologous expression vector comprising B. subtilis citrate synthase, E. coli isocitrate lyase, and G. oxydans glycolaldehyde dehydrogenase for production of ethylene glycol from CO and/or CO₂ and H₂ in C. autoethanogenum.

[0212] Genes coding for citrate synthase from *B. subtilis* (*citZ*; SEQ ID NOs: 1-2), isocitrate lyase from *E. coli* (*icl*; SEQ ID NOs: 11-12), and glycolaldehyde dehydrogenase from *G. oxydans* (*aldA1*; SEQ ID NOs: 55-56) were codon-adapted and synthesized for expression in *C. autoethanogenum*. The adapted genes were cloned into an expression shuttle vector, pIPL12, using a standard BsaI golden gate cloning kit (New England Biolabs, Ipswich, MA). pIPL12 comprises an origin of replication for both *E. coli* and *C. autoethanogenum*, enabling it to replicate and be maintained in both species; pIPL12 also functions in most *Clostridia*.

pIPL12 further comprises 23S rRNA (adenine(2058)-N(6))-methyltransferase Erm(B) conferring erythromycin/clarithromycin resistance for positive selection, TraJ for conjugative transfer from *E. coli*, and a promoter for expression of heterologous genes. See Figure 2A. The expression vector created upon cloning of *citZ*, *icl*, and *aldA1* into pIPL12 is referred to as pMEG042 herein (Figure 2B).

Table 2: Oligos used to construct pMEG042 expression vector.

SEQ ID NO	Name	Sequence
69	pIPL12-bb-F	CACACCAGGTCTCAAACCATGGAGATCTCGAGG CCTG
70	pIPL12-bb-R	CACACCAGGTCTCACATATGATAAGAAGACTCT TGGC
71	citZ_Bs1-F	CACACCAGGTCTCACATATGACAGCAACAAGGG GCC
72	citZ_Bs1-R	CACACCAGGTCTCAATTGTAACACCTCCTTAATT AGTTATGCTCTTTCTTCTATAGGTACAAATTTT G
73	Icl_Ec-F	CACACCAGGTCTCACAATGAAAACAAGAACTCA ACAAATAG
74	Icl_Ec-R	CACACCAGGTCTCAGTGTTCTCCTATGTGTTCT TAAATTGAGATTCTTCAGTTGAACCTG
75	aldA1_Go-F	CACACCAGGTCTCAACACATATGACTGAAAAAA ATAATTTATTCATAAATGGATC
76	aldA1_Go-R	CACACCAGGTCTCAGGTTATGCATTTAGATATAT TGTTTTTGTCTGTACG

[0213] The pMEG042 construct was transformed into *C. autoethanogenum* via conjugation. The expression vector was first introduced into the conjugative donor strain, *E. coli* HB101+R702 (CA434) (Williams *et al.* 1990) (the donor), using standard heat shock transformation. Donor cells were recovered in SOC media at 37°C for 1 h before being plated onto LB media plates comprising 100 µg/mL spectinomycin and 500 µg/mL erythromycin

and incubated at 37°C overnight. The next day, 5 mL LB aliquots comprising 100 µg/mL spectinomycin and 500 µg/mL erythromycin were inoculated with several donor colonies and incubated at 37°C, shaking for approximately 4 h or until the culture was visibly dense but had not yet entered stationary phase. 1.5 mL of the donor culture was harvested by centrifugation at 4000 rpm and 20-25°C for 2 min, and the supernatant was discarded. The donor cells were gently resuspended in 500 µL sterile PBS buffer and centrifuged at 4000 rpm for 2 min, and the PBS supernatant was discarded.

[0214] The pellet was introduced into an anaerobic chamber and gently resuspended in 200 µL during late exponential phase of a *C. autoethanogenum* culture (the recipient). *C.*

autoethanogenum DSM10061 and DSM23693 (a derivative of DSM10061) were sourced from DSMZ (The German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7 B, 38124 Braunschweig, Germany). Strains were grown at 37°C in PETC medium (See U.S. Pat. No. 9,738,875) at pH 5.6 using standard anaerobic techniques (Hungate 1969; Wolfe 1971).

[0215] The conjugation mixture (the mix of donor and recipient cells) was spotted onto PETC-MES + fructose agar plates and left to dry. When the spots were no longer visibly wet, the plates were introduced into a pressure jar, pressurized with syngas (50% CO, 10% N₂, 30% CO₂, 10% H₂) to 25-30 psi, and incubated at 37°C for ~24 h. The conjugation mixture was then removed from the plates by gentle scraping using a 10 µL inoculation loop. The removed mixture was suspended in 200-300 µL PETC media. 100 µL aliquots of the conjugation mixture were plated onto PETC media agar plates supplemented 5 µg/mL clarithromycin to select for transformants bearing the plasmid.

[0216] Three distinct colonies of *C. autoethanogenum* bearing the pMEG042 plasmid were inoculated into 2 mL of PETC-MES media with 5 µg/mL clarithromycin and grown autotrophically at 37°C with 50% CO, 10% N₂, 30% CO₂, 10% H₂ and 100 rpm orbital shaking with for three days. Cultures were diluted to OD₆₀₀ of 0.05 in 10 mL PETC-MES medium with 5 µg/mL clarithromycin in serum bottles and grown autotrophically at 37°C with 50% CO, 10% N₂, 30% CO₂, 10% H₂ and 100 rpm orbital shaking for up to 20 days, sampling daily to measure biomass and metabolites (Figures 3A and 3B). Production of ethylene glycol was measured using gas chromatography mass spectrometry (GC-MS), and other metabolites were measured using high-performance liquid chromatography (HPLC), as described below.

[0217] Ethylene glycol concentrations were measured with a Thermo Scientific ISQ LT GCMS equipped an Agilent VF-WAXms column (15 m × 0.25 µm × 0.25 µm) and RSH

autosampler. Samples were prepared by diluting 200 μ L of broth with 200 μ L of methanol. The samples were vortexed then centrifuged for 3 min at 14,000 rpm; 200 μ L of the supernatant was transferred to a glass vial with insert. Samples were transferred to an autosampler for analysis using a 1.0 μ L injection, a split ratio of 5 to 1, and an inlet temperature of 240°C. Chromatography was performed with an oven program of 80°C with a 0.5 min hold to a ramp of 10°C/min to 150°C to a ramp of 25 °C/min to 220°C with a 3 min final hold. The column flow rate was 4.0 mL/min with a 0.5 min hold then dropping to 1.5 ml/min at a rate of 100 ml/min/min using helium as the carrier gas. The MS ion source was kept at 260°C with the transfer line set at 240°C. Quantitation was performed using a linear external standard calibration using 33.0 m/z as the quantitation peak and 31.0 + 62.0 m/z as the confirming peaks.

[0218] Ethanol, acetate, 2,3-butanediol, glyoxylate, and glycolate concentrations were measured by HPLC on an Agilent 1260 Infinity LC with Refractive Index (RI) detection at 35°C. Samples were prepared by heating for 5 min at 80°C, followed by a 3 min centrifugation at 14,000 rpm; the supernatant was transferred to a glass vial for analysis. Separation was carried out with a 10 μ L injection on to a Phenomenex Rezex™ ROA-Organic Acid H+ (8%) column (300 mm x 7.8 mm x 8 μ m) at 0.7 mL/min and 35°C under isocratic conditions, using 5 mM sulphuric acid mobile phase.

[0219] After approximately 3 days of autotrophic growth, the ethylene glycol precursor glycolate was observed, and after 10 days, production of ethylene glycol was observed (Figure 3B). Glycolate produced over time in *C. autoethanogenum* growing autotrophically and carrying expression vector pMEG042 (Figure 3C).

Example 2: Construction of heterologous expression vector comprising S. thiotaurini alanine-glyoxylate aminotransferase and P. fluorescens aldehyde dehydrogenase for production of ethylene glycol from CO and/or CO₂ and H₂ in C. autoethanogenum.

[0220] Genes coding for an alanine-glyoxylate aminotransferase from *S. thiotaurini* (*pucG*; SEQ ID NOs: 15-16) and aldehyde dehydrogenase from *P. fluorescens* Q8r1-96 (*aldA1*; SEQ ID NOs: 57-58) were codon-adapted and synthesized for expression in *C. autoethanogenum*. The codon-adapted genes were cloned into pIPL12 (Figure 2A), and the resulting expression vector, pMEG058, was introduced into *C. autoethanogenum*, as described in Example 1. See Figure 2C.

Table 3: Oligos used to construct pMEG058 expression vector.

SEQ ID NO	Name	Sequence
69	pIPL12-bb-F	CACACCAGGTCTCAAACCATGGAGATCTCGAGG CCTG
70	pIPL12-bb-R	CACACCAGGTCTCACATATGATAAGAAGACTCT TGGC
77	PucG_Sthi1-F	CACACCAGGTCTCACATATGCAATTTAGGCCTTT TAATCCACCA
78	PucG_Sthi1-R	CACACCAGGTCTCAGTGTTCTCCTATGTGTTCT TATGCTTGCGCAAGTGCCT
79	aldA1_Pfq8-F	CACACCAGGTCTCAACACATATGTCTTCAGTGCC TGTATTCCAG
80	aldA1_Pfq8-R	CACACCAGGTCTCAGGTAAAGACTGGAGATATA CTGCATGAG

- [0221] Two distinct colonies of *C. autoethanogenum* bearing the pMEG058 plasmid were inoculated into 2 mL of PETC-MES media with 5 µg/mL clarithromycin and grown autotrophically, as described in Example 1. *See* Figure 4A. After approximately 3 days of autotrophic growth, glycolate was observed, and after 8 days production of ethylene glycol was observed (Figure 4B).

- Example 3: Construction of heterologous expression vector comprising S. thiotaurini alanine-glyoxylate aminotransferase and G. oxydans glycolaldehyde dehydrogenase for production of ethylene glycol from CO and/or CO₂ and H₂ in C. autoethanogenum.*

- [0222] Genes coding for an alanine-glyoxylate aminotransferase from *S. thiotaurini* (*pucG*; SEQ ID NOs: 15-16) and glycolaldehyde dehydrogenase from *G. oxydans* (*aldA1*; SEQ ID NOs: 55-56) were codon-adapted and synthesized for expression in *C. autoethanogenum*. The codon-adapted genes were cloned into pIPL12 (Figure 2A), and the resulting expression vector, pMEG059, was introduced into *C. autoethanogenum*, as described in Example 1. *See* Figure 2D.

Table 4: Oligos used to construct pMEG059 expression vector.

SEQ ID NO	Name	Sequence
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69	pIPL12-bb-F	CACACCAGGTCTCAAACCATGGAGATCTCGAGG CCTG
70	pIPL12-bb-R	CACACCAGGTCTCACATATGATAAGAAGACTCT TGGC
77	PucG_Sthi1-F	CACACCAGGTCTCACATATGCAATTTAGGCCTTT TAATCCACCA
78	PucG_Sthi1-R	CACACCAGGTCTCAGTGTTCTCCTATGTGTTCT TATGCTTGCGCAAGTGCCT
75	aldA1_Go-F	CACACCAGGTCTCAACACATATGACTGAAAAAA ATAATTTATTCATAAATGGATC
76	aldA1_Go-R	CACACCAGGTCTCAGGTTATGCATTTAGATATAT TGTTTTTGTCTGTACG

[0223] Two distinct colonies of *C. autoethanogenum* bearing the pMEG059 plasmid were inoculated into 2 mL of PETC-MES medium with 5 µg/mL clarithromycin and grown autotrophically, as described in Example 1. *See* Figure 5A. After approximately 3 days of autotrophic growth, glycolate was observed, and after 10 days, production of ethylene glycol was observed (Figure 5B).

Example 4: Construction of heterologous expression vector comprising alanine-glyoxylate aminotransferase and aldehyde dehydrogenase for production of ethylene glycol from CO and/or CO₂ and H₂ in C. autoethanogenum.

[0224] Genes coding for class V aminotransferase from *C. acidurici* (*SgA*; SEQ ID NOs: 19, 20) and aldehyde dehydrogenase from *P. fluorescens* Q8r1-96 (*aldA1*; SEQ ID NOs: 57-58) were codon-adapted and synthesized for expression in *C. autoethanogenum*. The codon-adapted genes were cloned into pIPL12 (Figure 2A), and the resulting vector, pMEG061, was introduced into *C. autoethanogenum*, as described in Example 1. *See* Figure 2E.

Table 5: Oligos used to construct pMEG061 expression vector.

SEQ ID NO	Name	Sequence
69	pIPL12-bb-F	CACACCAGGTCTCAAACCATGGAGATCTCGAG GCCTG

70	pIPL12-bb-R	CACACCAGGTCTCACATATGATAAGAAGACTC TTGGC
81	SgaA_Caci1-F	CACACCAGGTCTCACATATGAGAAGTCCATT ATTATGAC
82	SgaA_Caci1-R	CACACCAGGTCTCAGTGTTCTCCTATGTGTTC CTAATCTACAAAGTGCTTG
79	aldA1_Pfq8-F	CACACCAGGTCTCAACACATATGTCTTCAGTG CCTGTATTCCAG
80	aldA1_Pfq8-R	CACACCAGGTCTCAGGTTAAGACTGGAGATAT ACTGCATGAG

[0225] Three distinct colonies of *C. autoethanogenum* bearing the pMEG061 plasmid were inoculated into 2 mL of PETC-MES medium with 5 µg/mL clarithromycin and grown autotrophically, as described in Example 1. *See* Figure 6A. After approximately 3 days of autotrophic growth, glycolate was observed, and after 16 days, production of ethylene glycol was observed (Figure 6B).

Example 5: Modeling of maximum yields of different routes to ethylene glycol

[0226] A genome-scale metabolic model of *Clostridium autoethanogenum* like the one described by Marcellin, *Green Chem*, 18: 3020-3028, 2016 was utilized to predict maximum yields of different routes to ethylene glycol. Heterologous metabolic reactions were added to the wild type *Clostridium autoethanogenum* model structure to represent the incorporation of the non-native compound production pathway. Although the model used for the experimental work described herein is based on *Clostridium autoethanogenum*, the results can reasonably be expected to apply to other Wood-Ljungdahl microorganisms as well, given similarities in metabolism.

[0227] Ethylene glycol production was simulated using constraint-based computational modeling techniques flux balance analysis (FBA) and linear minimization of metabolic adjustment (LMOMA) (Maia, *Proceedings of the Genetic and Evolutionary Computation Conference Companion on – GECCO '17*, New York, New York, ACM Press, 1661-1668, 2017) using cobrapy version 0.8.2 (Ebrahim., COBRApy: CONstraints-Based Reconstruction and Analysis for Python, *BMC Syst Biol*, 7: 74, 2013), with optlang version 1.2.3 (Jensen, Optlang: An Algebraic Modeling Language for Mathematical Optimization,” *The Journal of*

Open Source Software, 2, doi:10.21105/joss.00139, 2017) as the solver interface and Gurobi Optimizer version 7.0.2 as the optimization solver.

[0228] Modeling revealed a predicted yield of 0.37 mol ethylene glycol / mol CO by the pathways described herein in Examples 1-4. This is more than double the predicted yield by the hypothetical pathways described by Islam et al. *Metab Eng*, 41: 173-181, 2017, which require gluconeogenesis; the highest predicted yields were found to be ~0.44 g ethylene glycol/ g CO, which equals ~0.18 mol ethylene glycol / mol CO.

Example 6: Ethylene glycol production and diol dehydratase knockout

[0229] Biomass levels (g dry cell weight/L) of two different genotypes of *C. autoethanogenum*: one containing the identified native diol dehydratase (Base), and one with the diol dehydratase gene deleted (KO). Each strain has two variants, one carrying the pMEG042 expression vector, and one carrying no vector (negative control). Values shown are calculated from the average of 3 technical replicates. See Figure 7. Ethylene glycol (mg/L) produced over time in two different genotypes of *C. autoethanogenum*: one containing the identified native diol dehydratase (Base), and one with the diol dehydratase gene deleted (KO). Each strain has two variants, one carrying the pMEG042 expression vector, and one carrying no vector (negative control). Values shown are calculated from the average of 3 technical replicates. See Figure 8A. Ethylene glycol (mg/L/g dry cell weight) produced over time in different genotypes of *C. autoethanogenum*: one containing the identified native diol dehydratase (Base), and one with the diol dehydratase gene deleted (KO). Each strain has two variants, one carrying the pMEG042 expression vector, and one carrying no vector (negative control). Values shown are calculated from the average of 3 technical replicates. See Figure 8B.

[0230] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein. The reference to any prior art in this specification is not, and should not be taken as, an acknowledgement that that prior art forms part of the common general knowledge in the field of endeavour in any country.

[0231] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the disclosure (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and

“containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to”) unless otherwise noted. The term “consisting essentially of” limits the scope of a composition, process, or method to the specified materials or steps, or to those that do not materially affect the basic and novel characteristics of the composition, process, or method.

- 5 The use of the alternative (e.g., “or”) should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the term “about” means $\pm 20\%$ of the indicated range, value, or structure, unless otherwise indicated.

- [0232]** Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless
10 otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, any concentration range, percentage range, ratio range, integer range, size range, or thickness range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated.

- 15 **[0233]** All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the disclosure and does not pose a limitation on the scope of the disclosure unless otherwise claimed. No language in the specification should be construed as indicating
20 any non-claimed element as essential to the practice of the disclosure.

- [0234]** Preferred embodiments of this disclosure are described herein. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the disclosure to be practiced otherwise
25 than as specifically described herein. Accordingly, this disclosure includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the disclosure unless otherwise indicated herein or otherwise clearly contradicted by context.

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CLAIMS

1. A genetically engineered microorganism capable of producing ethylene glycol or a precursor of ethylene glycol from a gaseous substrate comprising: a disruptive mutation in a gene encoding diol dehydratase.
2. The microorganism of claim 1, wherein the microorganism produces ethylene glycol or the precursor of ethylene glycol through one or more intermediates selected from the group consisting of 5,10-methylenetetrahydrofolate, oxaloacetate, citrate, malate, and glycine.
3. The microorganism of claim 1 or 2, wherein the microorganism comprises one or more of:
 - a. a nucleic acid encoding a heterologous enzyme capable of converting oxaloacetate to citrate;
 - b. a nucleic acid encoding a heterologous enzyme capable of converting glycine to glyoxylate;
 - c. a nucleic acid encoding a heterologous enzyme capable of converting iso-citrate to glyoxylate; and
 - d. a nucleic acid encoding a heterologous enzyme capable of converting glycolate to glycolaldehyde.
4. The microorganism of claim 3, wherein:
 - a. the heterologous enzyme capable of converting oxaloacetate to citrate is a citrate [Si]-synthase having the EC number 2.3.3.1, an ATP citrate synthase having the EC number 2.3.3.8; or a citrate (Re)-synthase having the EC number 2.3.3.3;
 - b. the heterologous enzyme capable of converting glycine to glyoxylate is an alanine-glyoxylate transaminase having the EC number 2.6.1.44, a serine-glyoxylate transaminase having the EC number 2.6.1.45, a serine-pyruvate transaminase having the EC number 2.6.1.51, a glycine-oxaloacetate transaminase having the EC number 2.6.1.35, a glycine transaminase having the EC number 2.6.1.4, a glycine dehydrogenase having the EC number 1.4.1.10, an alanine dehydrogenase having the EC number 1.4.1.1, or a glycine dehydrogenase having the EC number 1.4.2.1;
 - c. the heterologous enzyme capable of converting iso-citrate to glyoxylate is an isocitrate lyase having the EC number 4.1.3.1; and/or

- d. the heterologous enzyme capable of converting glycolate to glycolaldehyde is a glycolaldehyde dehydrogenase having the EC number 1.2.1.21, a lactaldehyde dehydrogenase having the EC number 1.2.1.22, a succinate-semialdehyde dehydrogenase having the EC number 1.2.1.24, a 2,5-dioxovalerate dehydrogenase having the EC number 1.2.1.26, an aldehyde dehydrogenase having the EC number 1.2.1.3/4/5, a betaine-aldehyde dehydrogenase having the EC number 1.2.1.8, or an aldehyde ferredoxin oxidoreductase having the EC number 1.2.7.5.
5. The microorganism of claim 3 or 4, wherein one or more of the heterologous enzymes are derived from a genus selected from the group consisting of *Bacillus*, *Clostridium*, *Escherichia*, *Gluconobacter*, *Hyphomicrobium*, *Lysinibacillus*, *Paenibacillus*, *Pseudomonas*, *Sedimenticola*, *Sporosarcina*, *Streptomyces*, *Thermithiobacillus*, *Thermotoga*, *Cupriavidus*, and *Zea*.
6. The microorganism of any one of claims 3 to 5, wherein one or more of the heterologous enzymes are codon-optimized for expression in the microorganism.
7. The microorganism of any one of claims 1 to 6, wherein the microorganism further comprises one or more of an enzymes capable of converting acetyl-CoA to pyruvate; an enzyme capable of converting pyruvate to oxaloacetate; an enzyme capable of converting pyruvate to malate; an enzyme capable of converting pyruvate to phosphoenolpyruvate; an enzyme capable of converting oxaloacetate to citryl-CoA; an enzyme capable of converting citryl-CoA to citrate; an enzyme capable of converting citrate to aconitate and aconitate to iso-citrate; an enzyme capable of converting phosphoenolpyruvate to oxaloacetate; an enzyme capable of converting phosphoenolpyruvate to 2-phospho-D-glycerate; an enzyme capable of converting 2-phospho-D-glycerate to 3-phospho-D-glycerate; an enzyme capable of converting 3-phospho-D-glycerate to 3-phosphonooxypyruvate; an enzyme capable of converting 3-phosphonooxypyruvate to 3-phospho-L-serine; an enzyme capable of converting 3-phospho-L-serine to serine; an enzyme capable of converting serine to glycine; an enzyme capable of converting 5,10-methylenetetrahydrofolate to glycine; an enzyme capable of converting serine to hydroxypyruvate; an enzyme capable of converting D-glycerate to hydroxypyruvate; an enzyme capable of converting malate to glyoxylate; an enzyme capable of converting glyoxylate to glycolate; an enzyme capable of converting hydroxypyruvate to glycolaldehyde; and an enzyme capable of converting glycolaldehyde to ethylene glycol.

8. The microorganism of any one of claims 3 to 7, wherein the microorganism overexpresses:
- a. the heterologous enzyme capable of converting oxaloacetate to citrate;
 - b. the heterologous enzyme capable of converting glycine to glyoxylate; and/or
 - 5 c. the heterologous enzyme capable of converting glycolate to glycolaldehyde.
9. The microorganism of claim 7, wherein the microorganism overexpresses:
- a. the enzyme capable of converting pyruvate to oxaloacetate;
 - b. the enzyme capable of converting citrate to aconitate and aconitate to iso-citrate;
 - 10 c. the enzyme capable of converting phosphoenolpyruvate to oxaloacetate;
 - d. the enzyme capable of converting serine to glycine;
 - e. the enzyme capable of converting 5,10-methylenetetrahydrofolate to glycine;
 - f. the enzyme capable of converting glyoxylate to glycolate; and/or
 - g. the enzyme capable of converting glycolaldehyde to ethylene glycol.
- 15 10. The microorganism of any one of claims 1 to 9, wherein the microorganism further comprises a disruptive mutation in one or more of isocitrate dehydrogenase, glycerate dehydrogenase, glycolate dehydrogenase, glycerate dehydrogenase, glycolate dehydrogenase, aldehyde ferredoxin oxidoreductase, and aldehyde dehydrogenase.
11. The microorganism of any one of claims 1 to 10, wherein the microorganism is a
- 20 member of a genus selected from the group consisting of *Acetobacterium*, *Alkalibaculum*, *Blautia*, *Butyribacterium*, *Clostridium*, *Cupriavidus*, *Eubacterium*, *Moorella*, *Oxobacter*, *Sporomusa*, and *Thermoanaerobacter*.
12. The microorganism of any one of claims 1 to 11, wherein the microorganism is derived from a parental microorganism selected from the group consisting of *Acetobacterium*
- 25 *woodii*, *Alkalibaculum bacchii*, *Blautia producta*, *Butyribacterium methylotrophicum*, *Clostridium aceticum*, *Clostridium autoethanogenum*, *Clostridium carboxidivorans*, *Clostridium coskatii*, *Clostridium drakei*, *Clostridium formicoaceticum*, *Clostridium ljungdahlii*, *Clostridium magnum*, *Clostridium ragsdalei*, *Clostridium scatologenes*, *Cupriavidus necator*, *Eubacterium limosum*, *Moorella thermautotrophica*, *Moorella*
- 30 *thermoacetica*, *Oxobacter pfennigii*, *Sporomusa ovata*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, and *Thermoanaerobacter kiuvi*.
13. The microorganism of any one of claims 1 to 12, wherein the microorganism is derived from a parental bacterium selected from the group consisting of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, and *Clostridium ragsdalei*.

14. The microorganism of any one of claims 1 to 13, wherein the microorganism comprises a native or heterologous Wood-Ljungdahl pathway.
15. The microorganism of any one of claims 1 to 14, wherein the precursor of ethylene glycol is glyoxylate or glycolate.
- 5 16. A method of producing ethylene glycol or a precursor of ethylene glycol comprising culturing the microorganism of any one of claims 1 to 15 in a nutrient medium in the presence of a gaseous substrate, whereby the microorganism produces ethylene glycol or the precursor of ethylene glycol.
17. The method of claim 16, wherein the gaseous substrate comprises one or more of CO,
10 CO₂, and H₂.
18. The method of claim 16 or 17, wherein the precursor of ethylene glycol is glyoxylate or glycolate.
19. The method of any one of claims 16 to 18, further comprising separating ethylene glycol or the precursor of ethylene glycol from the nutrient medium.
- 15 20. The method of any one of claims 16 to 19, wherein the microorganism further produces one or more of ethanol, 2,3-butanediol, and succinate.