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(54) **MIXOTROPHIC FERMENTATION METHOD FOR MAKING ACETONE, ISOPROPANOL, AND OTHER BIOPRODUCTS, AND MIXTURES THEREOF**

MIXOTROPHE FERMENTATION ZUR HERSTELLUNG VON ACETON, ISOPROPANOL, UND ANDEREN BIOPRODUKTEN SOWIE MISCHUNGEN DAVON

PROCÉDÉ DE FERMENTATION MIXOTROPHE POUR PRODUIRE DE L'ACÉTONE, DE L'ISOPROPANOL ET D'AUTRE BIOPRODUITS, ET MÉLANGES DE CEUX-CI

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- **ALAN G FAST ET AL: "Stoichiometric and energetic analyses of non-photosynthetic CO2-fixation pathways to support synthetic biology strategies for production of fuels and chemicals", CURRENT OPINION IN CHEMICAL ENGINEERING, vol. 1, no. 4, November 2012 (2012-11), pages 380-395, XP055506464, Netherlands ISSN: 2211-3398, DOI: 10.1016/j.coche.2012.07.005**

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- **ALAN G FAST ET AL: "Acetogenic mixotrophy: novel options for yield improvement in biofuels and biochemicals production", CURRENT OPINION IN BIOTECHNOLOGY., vol. 33, June 2015 (2015-06), pages 60-72, XP055370103, GB ISSN: 0958-1669, DOI: 10.1016/j.copbio.2014.11.014**
- **ANITA S. GÖSSNER ET AL: "Carbon metabolism of the moderately acid-tolerant acetogen Clostridium drakei isolated from peat", FEMS MICROBIOLOGY LETTERS, vol. 287, no. 2, October 2008 (2008-10), pages 236-242, XP055505852, GB ISSN: 0378-1097, DOI: 10.1111/j.1574-6968.2008.01313.x**
- **FAST ET AL.: 'Acetogenic mixotrophy: novel options for yield improvement in biofuels and biochemicals production' CURRENT OPINION IN BIOTECHNOLOGY vol. 33, 01 June 2015, pages 60 - 72, XP055370103 DOI: 10.1016/J.COPBIO.2014.11.014**

Description

Sequence Listing

[0001] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format. . Said ASCII copy, created on February 24, 2016, is named P48210_SL.txt and is 104,007 bytes in size.

Field of Invention

[0002] The invention relates to the field of production of bioproducts such as alcohols, organic acids of less than 7 carbons, acetone, and mixtures thereof with microorganisms.

Background

[0003] The production costs for biofuels and certain other bioproducts via microbial fermentation is currently high, particularly compared to oil-derived fuels. Feedstock and feedstock pre-treatment costs for use in such methods can form 50-60% or more of total operating costs. Generally, these costs relate to the carbohydrates used as the carbon source in the production of the biofuels. Because these costs are so high, they are one of the primary factors affecting the economic viability of cellulosic and other next generation biofuel manufacturing processes. There is therefore a strong need for lowering these costs and for producing desired products at high yield and high titers. One way to mitigate high feedstock costs is by maximizing feedstock conversion to the product of interest.

[0004] However, conventional methods for maximizing feedstock conversion are fraught with difficulties. For example, attempts to ferment gaseous substrates with autotrophic organisms have been hindered by difficulties in reaching suitable concentrations of the substrate and by low titers, which increase isolation-related operating costs. Autotrophic fermentation has also been limited in the range of economically attainable products.

[0005] From a metabolic perspective, acetyl-CoA is a central building block and a link between glycolysis and fermentative alcohol production. Consequently acetyl-CoA serves as a focal point for biofuel production in microbial organisms. However, the ability to achieve metabolically efficient production of acetyl-CoA (and high mass yields) has historically been impeded by CO_2 loss during decarboxylation reactions involved in classical Embden-Meyerhof-Parnas (EMP) glycolysis. For example, one molecule of glucose (where glucose is the carbon source) under heterotrophic growth conditions may be used to generate two molecules of acetyl-CoA and excess ATP, but this occurs at the "expense" of two CO_2 molecules, which are lost in the conversion of pyruvate to acetyl-CoA. In contrast, two molecules of CO_2 (where gaseous CO_2 is the carbon source) under autotrophic growth conditions may generate one molecule of acetyl-CoA, but this scheme results in a net ATP formation of less than 1, and acetate production (from acetyl-CoA) is required to generate more ATP.

[0006] Accordingly, there is a need for fermentation methods and engineering metabolic pathways that minimize -- or ideally eliminate -- CO_2 losses and result in complete conversion of a carbohydrate source into acetyl-CoA without having to sacrifice the acetyl-CoA produced for further generation of ATP.

Prior Art

[0007] A. Fast et al.: "Stoichiometric and energetic analyses of non-photosynthetic C02-fixation pathways to support synthetic biology strategies for production of fuels and chemicals", CURRENT OPINION IN CHEMICAL ENGINEERING, vol. 1, no. 4, November 2012, pages 380-395, XP055506464 teaches and discusses the production of various bioproducts via mixotrophic fermentation with acetogenic microorganisms (Clostridium species), the need for genetic engineering of suitable strains, suitable carbon sources and reaction conditions. In particular mixotrophic butanol production using glucose and hydrogen is taught and the improvement of butanol yield compared to autotrophic fermentation.

[0008] A. Fast et al.: "Acetogenic mixotrophy: novel options for yield improvement in biofuels and biochemicals production", CURRENT OPINION IN BIOTECHNOLOGY., vol. 33, June 2015, pages 60-72, XP055370103, online publication: 10.12.2014 teaches methods for mixotrophic fermentation using sugars, H2, CO and CO2 as substrates with acetogenic microorganisms.

[0009] US-A1- 2011/059499 discloses the mixotrophic fermentation with Clostridium autoethanogenum in the presence of fructose or xylose together with CO/CO2. Acetate and ethanol were produced.

[0010] A. Gössner et al.: "Carbon metabolism of the moderately acid-tolerant acetogen Clostridium drakei isolated from peat", FEMS MICROBIOLOGY LETTERS, vol. 287, no. 2, October 2008, pages 236-242, XP055505852 teaches cultivation of an acetogenic Clostridium strain which is capable of mixotrophic fermentation. Suitable carbon sources are e.g. glucose, fructose, xylose, H2, and CO. The strain does not
metabolize e.g. maltose, sucrose and other carbon sources). Acetate was the main reduced end product formed.

[0011] US-A1- 2011/129904 discloses the production of 1,3-butanediol from glucose and methanol as carbon sources. The fermentation is a mixotrophic fermentation in the broad sense of the present application. The microorganism is a modified E.coli.

Summary of the Invention

[0012] Herein is provided a mixotrophic fermentation method according to claim 1.

[0013] The method yields a greater amount of the at least one bioproduct than the combined amounts of the at least on bioproduct produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions.

[0014] In an embodiment, the method may comprise production of at least one bioproduct and acetic acid as a second bioproduct, wherein the amount of acetic acid produced per biomass unit weight is less than about 50% of that produced in autotrophic fermentation with the same organism under the same conditions.

[0015] The carbon yield, based on the total amount of carbon in produced bioproducts divided by the total amount of carbon metabolized from said first feedstock, is at least 50%.

[0016] In an embodiment, the $^{13}C/^{12}C$ isotope ratio of the carbon present in the bioproduct may be less than that of atmospheric CO_2.

[0017] The carbon source is selected from sugars.

[0018] The organisms are either from *Clostridium ljungdahlii, Clostridium autoethanogenum* or from *Clostridium ragsdalei.*

[0019] In an embodiment, said organism may be genetically modified.

[0020] In an embodiment, said first feedstock and said second feedstock may be present in the fermentation medium at the same time.

[0021] In an embodiment, said fermentation medium may comprise a carbohydrate and at least one of CO, CO_2, and hydrogen.

[0022] In an embodiment, said fermentation medium comprises a steel mill produced CO composition.

[0023] In an embodiment, the culturing may be performed in whole or in part at a super-atmospheric pressure.

[0024] In an embodiment, said bioproduct may be selected from the group consisting of acetone, isopropanol, and combinations thereof.

[0025] In an embodiment, said bioproduct may be non-naturally occurring.

[0026] In an embodiment, the second feedstock may comprise CO, CO_2, carbonate, bicarbonate, methanol, or a combination thereof; and the $^{13}C/^{12}C$ isotope ratio of the carbon present in said second feedstock may be less than that of atmospheric CO_2.

[0027] In an embodiment, the method may comprise providing said fermentation medium with a mixture of CO_2 and hydrogen at a molar ratio in the range from 1:0.1 to 1:5.

[0028] In an embodiment, the method may further comprise steam reforming of a hydrocarbon to form said mixture of CO_2 and hydrogen.

[0029] In an embodiment, the first feedstock may comprise a sugar selected from glucose and sucrose, and the organism may metabolize CO_2 produced on metabolizing the sugar.

[0030] In an embodiment, the first feedstock may comprise a sugar selected from glucose and sucrose, the second feedstock may comprise at least one of H_2 and methanol, and the organism may metabolize CO_2 produced on metabolizing the sugar.

[0031] In an embodiment, said at least one bioproduct is acetone. In such an embodiment, the first feedstock may comprise a sugar selected from glucose and sucrose, and the organism may metabolize CO_2 produced on metabolizing the sugar.

[0032] In an embodiment, said at least one bioproduct is isopropanol. In such an embodiment, the first feedstock may comprise a sugar selected from glucose and sucrose, and the organism may metabolize CO_2 produced on metabolizing the sugar.

[0033] In an embodiment, the metabolizing of the first feedstock does not inhibit the metabolizing of the second feedstock.

[0034] In an embodiment, the first feedstock may comprise a non-preferred sugar and the second feedstock may comprise CO, CO_2, carbonate, bicarbonate, H_2, glycerol, methanol, formate, urea or a combination thereof.

[0035] Herein is also provided a mixotrophic fermentation method according to any one of claims 19-22.

Brief Description of the Drawings

[0036]

Fig. 1 shows ^{13}C labeling of acetate in C. *ljungdahlii* grown under autotrophic (A-Acetate) and mixotrophic (M-

Acetate) cultures.

Fig. 2 shows the percentage of ^{13}C labeling of acetate in C. *ljungdahlii* over time.

Fig. 3 shows the percentage of ^{13}C labeling of acetate in C. *autoethanogenum* over time.

Fig. 4 shows carbon yield with increasing amounts of H_2 in the headspace in a C. *ljungdahlii* strain with a deleted secondary alcohol dehydrogenase gene *(Clj* ΔSADH) and transformed with a plasmid expressing the genes for thiolase, acetoacetate transferase subunit A (COAT A), acetoacetate transferase subunit B (COAT B), and acetoacetate decarboxylase (acetone strain).

Fig. 5 shows product distributions of the acetone strain grown with increasing amounts of H_2 in the headspace. Carbon fraction is the amount of carbon in each bioproduct with the total being 1.0.

Detailed Description of the Invention

[0037] The particulars shown herein are by way of example and for purposes of illustrative discussion of the various embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0038] The present invention will now be described by reference to more detailed embodiments. This invention may, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

[0039] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for describing particular embodiments only and is not intended to be limiting of the invention. As used in the description of the invention and the appended claims, the singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0040] The numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

[0041] Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

[0042] One way to mitigate high feedstock costs is by maximizing feedstock conversion to the product of interest.

[0043] The inventors provide herein a mixotrophic fermentation method as described in claims 1 and 19 to 22.

[0044] The method may further comprise separating at least one bioproduct from said broth to form a separated bioproduct.

[0045] The method achieves greater production of the at least one bioproduct than the combined amounts produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions.

[0046] In an embodiment, also provided is a method as above, comprising production of at least one bioproduct and acetic acid.

[0047] In an embodiment, also provided is a method as above, wherein the amount of acetic acid produced per biomass unit weight is less than about 50% of that produced in autotrophic fermentation with the same organism under the same conditions.

[0048] In an embodiment, also provided is a method as above, wherein the carbon yield, based on the total amount of carbon in produced metabolites divided by the total amount of carbon metabolized from said first feedstock, is at least 50%.

[0049] In an embodiment, also provided is a method as above, wherein said bioproduct is characterized by a $^{13}C/^{12}C$ isotope ratio of less than that of atmospheric CO_2.

[0050] In an embodiment, also provided is a method as above, wherein said first feedstock and said second feedstock are present in the fermentation medium at the same time.

[0051] In an embodiment, also provided is a method as above, wherein said fermentation medium comprises a carbohydrate and at least one of CO, CO_2, and hydrogen.

[0052] In an embodiment, also provided is a method as above, wherein said fermentation medium comprises a steel mill produced CO composition.

[0053] In an embodiment, also provided is a method as above, wherein the first feedstock comprises juice extracted from at least one of sugarcane and sugarbeet.

[0054] In an embodiment, also provided is a method as above, wherein the culturing is performed in whole or in part at a super-atmospheric pressure.

[0055] In an embodiment, also provided is a method as above, wherein said bioproduct is selected from the group consisting of ethanol, crotyl alcohol, acetone, isopropanol, 2,3-butanediol, 1,3-propanediol and combinations thereof.

[0056] In an embodiment, also provided is a method as above, wherein said bioproduct is non-naturally occurring.

[0057] In an embodiment, also provided is a method as above, wherein said bioproduct comprises a composition having multiple compounds and wherein one of said compounds is acetone.

[0058] In an embodiment, also provided is a method as above, wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate methanol, and mixtures thereof; and wherein the $^{13}C/^{12}C$ isotope ratio of the carbon present in said second feedstock is less than that of atmospheric CO_2.

[0059] In an embodiment, also provided is a method as above, wherein said second feedstock comprises at least one of ammonium carbonate and ammonium bicarbonate. Also provided is such a method, further comprising adding pressurized CO_2 to said fermentation medium.

[0060] In an embodiment, also provided is a method as above, wherein providing a fermentation medium includes providing said fermentation medium with a mixture of CO_2 and hydrogen at a molar ratio in the range between 1:0.1 and 1:5. Also provided is such a method further comprising steam reforming of a hydrocarbon to form said mixture of CO_2 and hydrogen. Also provided is such a method, wherein said hydrocarbon comprises methane.

[0061] In an embodiment, also provided is a method as above, wherein the bioproduct comprises at least one of acetone or isopropanol.

[0062] Also provided is such a method, wherein the first feedstock comprises a sugar selected from glucose and sucrose, the second feedstock comprises at least one of H_2 and methanol, and the organism assimilates or metabolizes CO_2 produced on metabolizing the sugar. In an embodiment, the organism may metabolize CO_2 produced during glycolysis. In an embodiment, the organism may metabolize CO_2 produced via other metabolic pathways, for example, via the acetoacetate pathway and/or the 2-keto acid pathway and/or the α-acetolactate pathway.

[0063] Also described is a method, wherein the first feedstock comprises methanol and the second feedstock comprises a bicarbonate supplemented with CO_2. Also described is a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises glycerol. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2 and H_2. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2 and methanol. Also described is a method, wherein the first feedstock comprises methanol or glycerol and the second feedstock comprises CO_2 and H_2.

[0064] In an embodiment, also provided is a method as above, wherein said at least one bioproduct is acetone.

[0065] The microorganism is acetogenic.

[0066] The microorganism is acetogenic Clostridia.

[0067] Also provided is a method as above, wherein said organism expresses genes of the Wood-Ljungdahl pathway.

[0068] Also, in the method provided said organism is selected from the group consisting of *Clostridium ljungdahlii, Clostridium autoethanogenum,* and *Clostridium ragsdalei.*

[0069] Also provided is such a method, wherein said organism is genetically modified to have a primary alcohol dehydrogenase gene or a secondary alcohol dehydrogenase gene deleted from its genome.

[0070] Also provided is such a method, wherein said organism is genetically modified to have a butanediol dehydrogenase gene deleted from its genome.

[0071] Also provided is such a method, wherein the first feedstock comprises a sugar selected from glucose and sucrose, and the second feedstock comprises at least one of H_2, and methanol, and the organism assimilates CO_2 produced during glycolysis.

[0072] Also described is a method, wherein the first feedstock comprises methanol and the second feedstock comprises a bicarbonate supplemented with CO_2.

[0073] Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises glycerol.

[0074] Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2 and H_2.

[0075] Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2 and methanol.

[0076] Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2.

[0077] Also provided is such a method, wherein the first feedstock comprises glucose and the second feedstock comprises CO. A method as described above is provided, wherein the feedstock glucose and feedstock CO are present in a glucose/CO weight/weight ratio of from about 0.3 to about 0.8.

[0078] Also described is a method, wherein the first feedstock comprises methanol or glycerol and the second feedstock comprises CO_2 and H_2.

[0079] Also described is a method, wherein the first feedstock comprises a carbohydrate, wherein said fermentation medium comprises a non-fermentable impurity, and wherein the impurity and the carbohydrate are present in the fermentation medium in a weight/weight ratio of greater than 0.05. Said impurity may be a fermentation inhibitor.

[0080] Also provided is such a method, wherein said broth comprises a second bioproduct, wherein said second bioproduct is isopropanol and the molar ratio between acetone and said second bioproduct is greater than 5.

[0081] Also provided is such a method, further comprising separating acetone from said broth to form separated acetone. Said separating may comprise evaporation. Said method may further comprise catalytically converting said separated acetone into at least one acetone derivative. For example, comprising catalytically converting said separated acetone into one or more of mesitylene (1-3-5-trimethylbenzene), isophthalic acid, uvitic acid, and meta-xylene.

[0082] The method achieves greater production of acetone than the combined amounts of acetone produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions.

[0083] Also provided is such a method, wherein said acetone has a $^{13}C/^{12}C$ isotope ratio of less than that of atmospheric CO_2.

[0084] Also provided is such a method, wherein said first feedstock and said second feedstock are present in the fermentation medium at the same time.

[0085] Also provided is such a method, comprising production of acetone and acetic acid. For example, such a method is provided, wherein the amount of acetic acid formed per biomass unit weight is less than about 50% of that formed in autotrophic fermentation with the same organism under the same conditions.

[0086] Also provided is such a method, wherein said fermentation medium comprises at least one of CO, CO_2, and hydrogen.

[0087] Also provided is such a method, wherein said fermentation medium comprises a steel mill produced composition.

[0088] Also provided is such a method, wherein the first feedstock comprises glucose, and the second feedstock comprises methanol at a glucose/methanol molar ratio of about 1:6.

[0089] Also provided is such a method, wherein said separating comprises contacting said broth with an organic solvent comprising a C6-C12 alkanol.

[0090] Also provided is such a method, wherein said separating comprises contacting said broth with an organic solvent comprising an ester of butyric acid and a C4-C12 alkanol.

[0091] Also provided is such a method, wherein said fermentation medium has a pH of greater than 5.5 and comprises calcium carbonate for pH control.

[0092] Also provided is such a method, wherein said calcium carbonate is present in second feedstock and also serves as a carbon source.

[0093] Also provided is such a method, wherein said fermentation medium has a pH greater than 5.5, and comprises a calcium base for pH control and wherein said separating comprises acidulating with sulfuric acid.

[0094] Also provided is such a method, wherein said at least one bioproduct is isopropanol.

[0095] Also provided is such a method, wherein said broth comprises a second bioproduct, said second bioproduct is acetone, and the molar ratio of isopropanol to said second bioproduct in said broth is greater than 5.

[0096] Also provided is such a method, further comprising separating isopropanol from said broth to form separated isopropanol.

[0097] Also provided is such a method, wherein said separating comprises evaporation.

[0098] Also provided is such a method, further comprising catalytically converting said separated isopropanol into at least one isopropanol derivative.

[0099] Also provided is such a method, wherein said broth comprises more than one bioproduct and at least one bioproduct is isopropanol and another is acetone.

[0100] Also provided is such a method further comprising separating isopropanol and acetone from said broth to form a separated composition comprising isopropanol and acetone.

[0101] Also provided is such a method, wherein said separating comprises evaporation.

[0102] Also provided is such a method further comprising catalytically converting the isopropanol and/or acetone present in said separated composition into at least one derivative of isopropanol or acetone.

[0103] Said organism is acetogenic and said first feedstock may comprise at least one non-preferred sugar. Said first feedstock further may comprise at least one preferred sugar.

[0104] Said non-preferred sugar may be metabolized by a genetically modified acetogenic organism at a rate of at least 0.02 g/hr/g cell mass.

[0105] According to an embodiment, CO_2 is generated from metabolizing said non-preferred sugar and said generated CO_2 comprises at least a fraction of said second feedstock.

[0106] Said non-preferred sugar may be selected from the group consisting of glucose, mannose, galactose, arabinose, ribose, maltose, sucrose, lactose, cellobiose and mixtures thereof. Said non-preferred sugar may comprise glucose.

[0107] According to an embodiment, said organism selected from *Clostridium ljungdahlii, Clostridium autoethanogenum,* and *Clostridium ragdalei* is genetically modified to express at least one component of a phosphotransferase system (PTS). Said at least one component may be selected from the group consisting of enzymes EIIA, EIIB, EIIC, and combinations thereof.

[0108] The organism may be genetically modified to express a gene related to a sugar transport system other than genes associated with the phosphotransferase system. The gene may be selected from the group consisting of a symporter system utilizing a sodium ion (Na^+), a symporter system utilizing protons (H^+), a permease system and a combination thereof.

[0109] The rate of metabolizing said non-preferred sugar by said genetically modified organism may be greater than that of metabolizing said non-preferred sugar by the native form of the organism by a factor of at least 1.5.

[0110] According to an embodiment, said bioproduct is selected from the group consisting of acetone, isopropanol, and combinations thereof.

[0111] Described is a mixotrophic fermentation method comprising (i) providing an isolated organism capable of metabolizing CO_2 into acetyl-CoA; (ii) providing a first feedstock and a second feedstock for use in a fermentation medium, wherein said first feedstock comprises carbohydrates, glycerol, methanol or combinations thereof; and wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate, H_2, glycerol, methanol, formate, urea or mixtures thereof; (iii) culturing said organism in said fermentation medium, whereby both feedstocks are metabolized and a fermentation broth is formed, which broth comprises at least one bioproduct; and (iv) optionally separating said bioproduct from said broth.

[0112] The method provides a mixotrophic fermentation method that results in greater production of a target bioproduct or a combination of target bioproducts than the combined amounts produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions. Said method is exemplified by comparing three cases of fermenting with a given organism capable of and/or configured for use in the method. In the first case (referred to herein as heterotrophic fermentation), a microorganism is cultured in a fermentation medium comprising a first feedstock to form a heterotrophic fermentation broth. In the second case (referred to herein as autotrophic fermentation), the microorganism is cultured in a fermentation medium comprising a second feedstock to form an autotrophic fermentation broth. In the third case (referred to herein as mixotrophic fermentation), a microorganism is cultured in a fermentation medium comprising a mixture of the first feedstock and the second feedstock to form a mixotrophic fermentation broth. At the end of culturing time, the autotrophic fermentation broth is mixed with the heterotrophic fermentation broth to form a mixed fermentation broth. The mixotrophic fermentation method achieves greater production of a target bioproduct or a combination of target bioproducts than the combined amounts produced by heterotrophic and autotrophic fermentation with the same microorganism under the same conditions. The nature of bioproducts in said mixotrophic fermentation and/or the molar ratio between the bioproducts (in case of forming multiple bioproducts), may differ from those of the mixed fermentation broth.

[0113] The method may be characterized in that the amount of acetic acid formed per biomass unit weight is less than about 50% of that formed in autotrophic fermentation using the same organism, less than 40%, less than 30%, less than 20%, or less than 10%. Biomass refers to the total weight of solid biological material generated during fermentation. Biomass may be easily separated from the fermentation medium by, for example, centrifugation. Biomass does not include any solid biological material introduced into the fermentation medium by one or more feedstocks.

[0114] The method is characterized in carbon yield of at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 110%, at least 120%, at least 130%, at least 140%, at least 150%, or at least 160%. As used herein carbon yield may be calculated by dividing the total amount of carbon in bioproducts produced during fermentation by the total amount of carbon metabolized from the first feedstock during fermentation.

[0115] The method comprises providing an isolated, naturally occurring or non-naturally occurring organism capable of metabolizing CO_2. The organism may be autotrophic. The organism may be capable of assimilating CO, CO_2, methanol, etc., for growth. The organism may also be capable of utilizing glycolysis for growth. Any organism capable of metabolizing CO_2 is suitable. Said organism is acetogenic. In an embodiment, the organism is naturally acetogenic. An organism is "naturally acetogenic" if the wild-type (or native) organism is capable of metabolizing CO_2 into acetate using the Wood-Ljungdahl pathway (or reductive acetyl-CoA pathway). A naturally acetogenic organism may be a wild-type organism or genetically modified.

[0116] Said organism is acetogenic Clostridia. The organism is selected from the group consisting of *Clostridium ljungdahlii, Clostridium autoethanogenum,* and *Clostridium ragsdalei.*

[0117] The organism may be genetically modified. For example, the organism may be genetically modified to reduce or eliminate expression of a primary alcohol dehydrogenase or a secondary alcohol dehydrogenase. The organism may be genetically modified to have a primary alcohol dehydrogenase gene or a secondary alcohol dehydrogenase gene deleted from its genome. While a genomic deletion is preferred, any genomic mutation resulting in inactivation of the enzyme would be sufficient, including but not limited to partial gene deletion, nonsense mutation, transcriptional promoter deletion, etc. The transcriptional expression of this gene may be reduced by using antisense RNA.

[0118] Similarly, the organism may be genetically modified to reduce or eliminate nucleic acid and/or protein expression of butanediol dehydrogenase. The organism may be genetically modified to have a butanediol dehydrogenase gene deleted from its genome. The organism may be genetically modified to have a secondary alcohol dehydrogenase gene and a butanediol dehydrogenase gene deleted from its genome.

[0119] As used herein, a "secondary alcohol dehydrogenase" is an enzyme that catalyzes the reduction of a ketone to a secondary alcohol, for example, the reduction of acetone into 2-propanol (a.k.a. isopropanol). An exemplary amino acid sequence of the secondary alcohol dehydrogenase gene is the following amino acid sequence from C. *ljungdahlii* DSM 13528:

MKGFAMLGINKLGWIEKKNPVPGPYDAIVHPLAVSPCTSDIHTVFEGALGNRENMILGHEAVGEIAEVGSEV
KDFKVGDRVIVPCTTPDWRSLEVQAGFQQHSNGMLAGWKFSNFKDGVFADYFHVNDADMNLAILPDEIPLES
AVMMTDMMTTGFHGAELADIKMGSSVVVIGIGAVGLMGIAGSKLRGAGRIIGVGSRPVCVETAKFYGATDIV
NYKNGDIVEQIMDLTHGKGVDRVIMAGGGAETLAQAVTMVKPGGVISNINYHGSGDTLPIPRVQWGCGMAHK
TIRGGLCPGGRLRMEMLRDLVLYKRVDLSKLVTHVFDGAENIEKALLLMKNKPKDLIKSVVTF (SEQ ID NO:

[0120] An exemplary secondary alcohol dehydrogenase amino acid sequence may be an amino acid sequence which has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the above sequence, and which is capable of catalyzing the reduction of a ketone to a secondary alcohol.

[0121] As used herein, a "butanediol dehydrogenase" may be an oxidoreductase enzyme, with EC number 1.1.1.4, that catalyzes the reduction of a ketone group to an alcohol group, specifically converting acetoin into butanediol. An exemplary amino acid sequence encoded by the butanediol dehydrogenase gene is the following amino sequence from C. *ljungdahlii* DSM 13528:

MKAVLWYDKKDVRVEEIEEPKVKENAVKIKVKWCGICGSDLHEYLGGPIFIPVGTPHPLSKSTAPVVLGHEF
SGEVVEIGSKVTKFKAGDRVIVEPIVACGKCPACLEGKYNLCEALGFHGLCGSGGGFAEYTVFPEDFVHKIP
DTMDYEQAALVEPMAVALHSLRVGNFTTGNTALVLGAGPIGLATIQCLKASGARIVIVFQRKSVRQEYAKKF
GADVVLDPNEVDVIEEIKKLTGGVGVDTSFETTGANVGINTAIQALKYEGTAVITSVWEKNAEINPNDLVFT
EKKVVGTLAYRHEFPSTIALMNDGRIKTDGYITKRIALEDIVKEGFETLTGPEKKKHVKIIVTPDKSLL
(SEQ ID NO: 4)

[0122] An exemplary butanediol dehydrogenase amino acid sequence may be an amino acid sequence which has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the above sequence, and which is capable of catalyzing the reduction of a ketone to an alcohol, specifically acetoin to butanediol. An exemplary nucleic acid sequence that encodes a butanediol dehydrogenase, which is from C. *ljungdahlii* DSM 13528, is as follows:

ATGAAAGCTGTATTGTGGTATGATAAAAAAGATGTAAGAGTAGAGGAAATTGAGGAACCTAAGGTAAAAGAA
AATGCTGTAAAAATTAAAGTGAAATGGTGTGGTATATGTGGTTCTGACTTGCATGAGTATTTAGGAGGACCT
ATATTTATTCCAGTAGGTACGCCACATCCTTTAAGCAAGAGTACTGCACCAGTAGTTTTAGGACATGAGTTT
TCAGGAGAAGTAGTAGAAATAGGAAGCAAGGTTACAAAATTTAAAGCAGGAGATAGAGTTATTGTAGAACCT
ATAGTTGCCTGTGGAAAGTGTCCTGCTTGTCTTGAAGGAAAATATAATTTATGTGAAGCTTTGGGATTTCAT
GGACTTTGTGGAAGCGGCGGCGGATTTGCTGAATACACAGTATTTCCTGAAGATTTTGTCCATAAGATACCA
GATACTATGGACTATGAGCAGGCTGCACTTGTTGAGCCTATGGCAGTTGCCCTTCATTCTCTAAGAGTTGGA
AACTTTACTACAGGAAATACTGCTTTGGTTTTAGGTGCAGGACCTATAGGACTTGCAACTATTCAGTGTTTA
AAGGCATCAGGGGCAAGAATTGTAATTGTATTTCAGAGAAAATCTGTAAGACAGGAATATGCTAAGAAATTT
GGAGCAGATGTAGTTTTAGATCCAAATGAGGTAGATGTAATTGAAGAAATTAAAAAACTTACAGGCGGCGTA

GGCGTGGATACATCTTTTGAAACAACAGGTGCAAATGTAGGGATTAATACGGCAATTCAAGCTTTAAAATAT GAAGGTACTGCGGTAATAACCAGCGTATGGGAGAAAAATGCAGAAATCAATCCAAATGATCTTGTATTTACA GAAAAGAAGGTAGTTGGTACTCTTGCCTACAGACATGAATTTCCTTCTACAATAGCACTTATGAATGATGGA AGAATAAAGACAGACGGATATATTACAAAGAGAATAGCACTTGAGGACATTGTAAAAGAAGGATTTGAAACA CTTACAGGACCTGAAAAGAAAAAACATGTAAAAATAATTGTAACTCCTGACAAATCCTTATTGTAA (SEQ ID NO: 3).

[0123] An exemplary butanediol dehydrogenase nucleic acid sequence or an exemplary secondary alcohol dehydrogenase nucleic acid may be a nucleic acid sequence which has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to a corresponding butanediol dehydrogenase or secondary alcohol dehydrogenase nucleic acid sequence as disclosed herein.

[0124] The organism may express and/or comprise one or more biomolecules of the Wood-Ljungdahl pathway (or reductive acetyl-CoA pathway). Biomolecules of the Wood-Ljungdahl pathway include enzymes such as CO dehydrogenase and acetyl-CoA synthase, as well as genes encoding such enzymes. The Wood-Ljungdahl pathway metabolizes CO_2, which can be produced during glycolysis or fed exogenously, into acetyl-CoA. The acetyl-CoA is then fed into downstream pathways for production of other or additional bioproducts.

[0125] Rates and/or efficiencies relating to bioproduct formation and metabolite consumption achieved by the organism during the mixotrophic fermentation method of the invention may be higher than those achieved by the organism exposed to feedstocks comprising the same nutrients in the wild.

[0126] The method further comprises providing a fermentation medium comprising a first feedstock and a second feedstock. Providing a fermentation medium may comprise preparing an aqueous solution comprising said first feedstock and said second feedstock. Providing may comprise supplementing at least one of said first feedstock and said second feedstock during culturing. The fermentation medium may initially comprise only the first feedstock and then the second feedstock is supplemented. Supplementing said second feedstock may be done before the first feedstock is fully utilized, e.g., at the time the first feedstock is only 10%, 20%, 30% or 40% utilized.

[0127] According to an embodiment, the fermentation medium may further comprise a steel mill gas composition. For example, the fermentation medium may comprise a steel mill gas composition comprising 40-80% CO, 10-25% CO_2, 2-5% H_2, and 15-35% N_2. The fermentation medium may comprise a steel mill gas composition comprising 60-70% CO, 15-20% CO_2, 3-4% H_2, and 20-30% N_2. In an embodiment, the fermentation medium may comprise a steel mill gas composition comprising 43-55% CO, 17-20% CO_2, 2-3% H_2, and 25-34% N_2.

[0128] Said fermentation medium may comprise concurrently both said first feedstock and said second feedstock during at least a fraction of the culturing time, e.g., during at least 30% of the time, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the time.

[0129] The first feedstock of the provided method comprises a sugar. The second feedstock may comprise CO, CO_2, carbonate, bicarbonate, H_2, glycerol, methanol, formate, urea or mixtures thereof. When the first feedstock comprises glycerol or methanol, the second feedstock may or may not also comprise glycerol or methanol. If methanol is present in the first feedstock, it need not be present in the second feedstock.

[0130] According to an embodiment, said carbohydrate comprises monosaccharides, such as glucose, fructose and xylose, disaccharides, such as sucrose, oligosaccharides, such as dextrins, polysaccharides, such as starch, xylan, cellulose and hemicellulose and combinations thereof. According to an embodiment, said carbohydrate comprises hexoses, such as glucose and fructose, pentoses, such as xylose and arabinose and combinations thereof.

[0131] According to an embodiment, said second feedstock comprises a gaseous compound and said gaseous compound is supplemented to the fermentation medium, e.g., via bubbling the gaseous compound through the medium. The methods for supplementing the fermentation medium and/or the feedstock with a carbon source are not limited, and include, for example, exogenously feeding a gaseous compound, such as CO or CO_2 or adding a carbon source and/or feedstock and/or additional components to an initially provided fermentation medium or feedstock later in time during fermentation.

[0132] According to an embodiment, said fermentation medium is kept during at least a fraction of the culturing time at a super-atmospheric pressure, e.g., during at least 30% of the time, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the time. Said super-atmospheric pressure may be in the range between about 1.1 bar and about 10 bar.

[0133] Said second feedstock may comprise CO_2 and said CO_2 resulting from another fermentation process. Said another fermentation process may be a process for producing ethanol. According to another embodiment, said first feedstock comprises a carbohydrate, and metabolizing said carbohydrate by said autotrophic organism results in generating at least one of CO_2 and hydrogen, which then provides at least a fraction of said second feedstock, e.g., at least 50%, at least 60%, at least 70%, at least 80% or at least 90%.

[0134] According to an embodiment, said second feedstock comprises CO, CO_2, carbonate, bicarbonate, methanol and mixtures thereof and the $^{13}C/^{12}C$ isotope ratio of said second feedstock is less than that of atmospheric CO_2.

[0135] Said second feedstock may comprise at least one of ammonium carbonate and ammonium bicarbonate. The method may further comprise supplementing pressurized CO_2 to said fermentation medium. Providing said fermentation medium may comprise dissolving ammonium bicarbonate and/or ammonium carbonate, and optionally other components, in water and adjusting the pH to a selected level by introducing CO_2. A fraction of the CO_2 and/or carbonate may be metabolized during said culturing and the method may further comprise supplementing CO_2 in order to maintain the selected pH. The pH of the fermentation medium may be greater than 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, or 9.5. The pH of the fermentation medium may be in the range from 4 - 9.5, 5 - 8.5, or 5.5 - 7.5. Calcium carbonate may also be used as an agent for controlling pH. Calcium carbonate may serve as both a buffering agent and a source of carbon in a feedstock, including, for example, in the second feedstock.

[0136] According to an embodiment, said fermentation medium comprises a carbohydrate and carbon monoxide. According to an embodiment, said fermentation medium comprises a carbohydrate and carbon dioxide. According to an embodiment, said fermentation medium comprises a carbohydrate and hydrogen. According to an embodiment, said fermentation medium comprises a carbohydrate and at least one of carbon monoxide, carbon dioxide and hydrogen.

[0137] According to an embodiment, said first feedstock comprises a monosaccharide, said second feedstock comprises at least one of carbon monoxide and carbon dioxide and the weight ratio between said monosaccharide and said at least one of carbon monoxide and carbon dioxide is in the range from 0.1 to 10.

[0138] According to an embodiment, said providing a fermentation medium comprises providing said fermentation medium with a mixture of CO_2 and hydrogen at molar ratio in the range from about 1:0.1 to about 1:5. Said providing said mixture may further comprise steam reforming a hydrocarbon to form said mixture of CO_2 and hydrogen. Said hydrocarbon may comprise methane.

[0139] The provided method comprises culturing said organism in said fermentation medium, whereby both feedstocks are metabolized and a fermentation broth is formed, which broth comprises at least one bioproduct.

[0140] The consumption rate of said first feedstock may be in the range from 0.01 to 10 mM/hr/OD_{600}, where OD_{600} is the absorbance value of the culture read at a wavelength of 600 nm. The consumption rate of said second feedstock may be in the range from 0.01 to 100 mM/hr/OD_{600}, where OD_{600} is the absorbance value of the culture read at a wavelength of 600 nm.

[0141] According to an embodiment, the produced bioproduct is a metabolic derivative of acetyl-CoA.

[0142] Said bioproduct is selected from the group consisting of acetone and isopropanol, and combinations thereof.

[0143] According to an embodiment, said bioproduct is non-naturally occurring. As used herein a non-naturally occurring bioproduct is a product which is unattainable by said organism when cultured in autotrophic conditions or is produced from a metabolic pathway not native to said organism.

[0144] Said bioproduct may be a C_4 compound.

[0145] Said bioproduct may comprise multiple compounds and one of said compounds is acetone.

[0146] A $^{13}C/^{12}C$ isotope ratio may be used as an indicator of nutrient cycling. For example, according to an embodiment, said bioproduct is characterized by a $^{13}C/^{12}C$ isotope ratio of less than that of atmospheric CO_2. In such a case, the $^{13}C/^{12}C$ isotope ratio would be indicative of production of the bioproduct from a nonatmospheric CO_2 source, for example, CO, CO_2, carbonate, bicarbonate, methanol or mixtures thereof present in the second feedstock.

[0147] The mixtotrophic fermentation method may include methods that advantageously utilize CO_2 and/or H_2 produced via sugar consumption (glycolysis) by the organism. Bioproducts produced by such methods may be any molecule that has a NAD(P)H to acetyl-CoA ratio of less than 2. These products include, for example, acetone and isopropanol.

[0148] As the sugar is metabolized, CO_2 and H_2 evolved by the organism may be exhausted into the fermentation broth at the molecular level. This molecular-scale gas dispersion may provide an excellent source of CO_2 or H_2 for re-assimilation. No input energy is required for dissolving these molecules into the fermentation broth. No gas dispersion technology known to the inventors can achieve molecular-scale gas dispersion with zero energy input. The components of the first and second feedstock and the ratio of the components of the first and second feedstock may depend on the nature of the bioproduct targeted for production.

[0149] The organism may consume sugar and methanol in a particular molar ratio. Sugar consumption during fermentation is generally too electron deficient to achieve complete re-assimilation of glycolysis-derived CO_2. Thus, concurrent use of methanol and sugar in a given ratio may achieve complete CO_2 assimilation without the need for external gas delivery to the fermentation medium. The sugar to methanol molar ratio may range depending on the bioproduct targeted for production. For example, the sugar to methanol molar ratio may range from 1/1 to 1/3 to 1/6 to 1/12.

[0150] A bioproduct may be generated by mixotrophic fermentation in a fermentation medium comprising a first feedstock comprising a sugar such as glucose and a second feedstock comprising glycerol.

[0151] A bioproduct may be generated by mixotrophic fermentation in a fermentation medium comprising a first feedstock comprising glucose and a second feedstock comprising supplemented CO_2 and H_2.

[0152] A bioproduct may be generated by mixotrophic fermentation in a fermentation medium comprising a first feedstock comprising glucose and a second feedstock comprising methanol and supplemented CO_2.

[0153] A bioproduct may be generated by mixotrophic fermentation in a fermentation medium comprising a first feedstock comprising methanol or glycerol and a second feedstock comprising supplemented CO_2 and H_2.

[0154] Optionally said method further comprises separating said bioproduct from said broth. Any separation method is suitable. Separating may comprise distillation, solvent extraction, crystallization, ion-exchange, membrane separation and combinations thereof. The bioproduct may be separated by evaporation, wherein evaporation means any transfer into the vapor phase, e.g., distillation, stripping, etc. The bioproduct may be, for example, acetone, and the method may include catalytically converting said separated acetone into at least one acetone. Such a method may comprise catalytically converting said separated acetone into one or more of mesitylene (1-3-5-trimethylbenzene), isophthalic acid, uvitic acid, and meta-xylene.

Production of Acetone

[0155] Provided herein is a mixotrophic fermentation method for the production of acetone comprising (i) providing an isolated organism capable of metabolizing CO_2 into acetyl-CoA; (ii) providing a fermentation medium comprising a first feedstock and a second feedstock wherein said first feedstock comprises a sugar and wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate, H_2, glycerol, methanol, formate, urea or mixtures thereof; and (iii) culturing said organism in said fermentation medium, whereby both feedstocks are metabolized and a fermentation broth is formed, which broth comprises at least one bioproduct that includes acetone.

[0156] Said organism is acetogenic.

[0157] Said organism is selected from the group consisting of *Clostridium ljungdahlii, Clostridium autoethanogenum,* and *Clostridium ragsdalei.*

[0158] Also provided is such a method, wherein said organism is genetically modified to have a primary alcohol dehydrogenase gene or a secondary alcohol dehydrogenase gene deleted from its genome.

[0159] Also provided is such a method, wherein said organism is genetically modified to have butanediol dehydrogenase deleted from its genome.

[0160] Also provided is such a method, wherein the first feedstock comprises a sugar selected from glucose and sucrose, and the second feedstock comprises at least one of H_2, and methanol, and the organism assimilates CO_2 produced during glycolysis. Also provided is such a method, wherein the first feedstock comprises methanol and the second feedstock comprises a bicarbonate supplemented with CO_2. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises glycerol. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2 and H_2. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2 and methanol. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2. Also provided is such a method, wherein the first feedstock comprises glucose and the second feedstock comprises CO. A method as described above is provided, wherein the feedstock glucose and feedstock CO are present in a glucose/CO weight/weight ratio of from about 0.3 to about 0.8, e.g., from about 0.25 to about 0.85, from about 0.4 to about 0.7, from about 0.5 to about 0.6. Also described is such a method, wherein the first feedstock comprises methanol or glycerol and the second feedstock comprises CO_2 and H_2. Also provided is such a method, wherein the first feedstock comprises a carbohydrate, wherein said fermentation medium comprises a non-fermentable impurity and wherein the impurity and the carbohydrate are present in the fermentation medium in a weight/weight ratio of greater than 0.05. Said impurity may be a fermentation inhibitor.

[0161] Also provided is such a method, wherein said broth comprises a second bioproduct, wherein said second bioproduct is isopropanol and the molar ratio between acetone and said second bioproduct is greater than 5.

[0162] Also provided is such a method, further comprising separating acetone from said broth to form separated acetone. Said separation may comprise evaporation. Said method may further comprise catalytically converting said separated acetone into at least one acetone derivative. For example, such a method may comprise catalytically converting said separated acetone into one or more of mesitylene (1-3-5-trimethylbenzene), isophthalic acid, uvitic acid, and meta-xylene.

[0163] The method achieves greater production of acetone than the combined amounts of acetone produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions.

[0164] Also provided is such a method, wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate, methanol, and mixtures thereof; and wherein the $^{13}C/^{12}C$ isotope ratio of the carbon present in said second feedstock is less than that of atmospheric CO_2.

[0165] Also provided is such a method, wherein said acetone has a $^{13}C/^{12}C$ isotope ratio of less than that of atmospheric CO_2.

[0166] Also provided is such a method, wherein said first feedstock and said second feedstock are present in the fermentation medium at the same time.

[0167] Also provided is such a method, wherein the culturing is performed in whole or in part at a superatmospheric pressure.

[0168] Also provided is such a method, wherein providing a fermentation medium comprises providing said fermentation medium with a mixture of CO_2 and hydrogen at a molar ratio in the range between 1:0.1 and

[0169] Also provided is such a method, comprising production of acetone and acetic acid. For example, such a method is provided, wherein the amount of acetic acid formed per biomass unit weight is less than about 50% of that formed in autotrophic fermentation with the same organism under the same conditions.

[0170] Also provided is such a method, wherein said fermentation medium comprises at least one of CO, CO_2, and hydrogen.

[0171] Also provided is such a method, wherein said fermentation medium comprises a steel mill produced composition.

Production of Butyric Acid - described for illustrative purposes, not forming part of the present invention

[0172] A mixotrophic fermentation method for the production of butyric acid comprising (i) providing an isolated organism capable of metabolizing CO_2 into acetyl-CoA; (ii) providing a fermentation medium comprising a first feedstock and a second feedstock wherein said first feedstock comprises carbohydrates, glycerol, methanol, or combinations thereof; and wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate, H_2, glycerol, methanol, formate, urea or mixtures thereof; and (iii) culturing said organism in said fermentation medium, whereby both feedstocks are metabolized and a fermentation broth is formed, which broth comprises at least one bioproduct that includes butyric acid.

[0173] Also provided is such a method wherein said organism is selected from the group consisting of *Clostridium ljungdahlii, Clostridium autoethanogenum, Clostridium ragsdalei, Eubacterium limosum, Butyribacterium methylotrophicum, Moorella thermoacetica, Clostridium aceticum, Acetobacterium woodii, Clostridium carboxidivorans, Alkalibaculum bacchi, Clostridium drakei, Clostridium formicoaceticum, Clostridium scatologenes, Moorella thermoautotrophica, Acetonema longum, Blautia producta, Clostridium glycolicum, Clostridium magnum, Clostridium mayombei, Clostridium methoxybenzovorans, Oxobacter pfennigii,* and *Thermoanaerobacter kivui.*

[0174] Also in such a method the first feedstock comprises a sugar selected from glucose and sucrose, and the second feedstock comprises at least one of H_2 and methanol and the organism assimilates CO_2 produced during glycolysis.

[0175] Also in such a method the first feedstock comprises glucose, and the second feedstock comprises methanol at a glucose/methanol molar ratio of about 1:6, e.g., in a range from about 1:3 to about 1:4, from about 1:5 to about 1:6, from about 1:7 to about 1:9.

[0176] Also in such a method the first feedstock comprises methanol and the second feedstock comprises a bicarbonate supplemented with CO_2.

[0177] Also in such a method the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises glycerol.

[0178] Also in such a method the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2 and H_2.

[0179] Also in such a method the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2 and methanol.

[0180] Also in such a method the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2

[0181] Also in such a method the first feedstock comprises glucose and the second feedstock comprises CO. In an embodiment, such a method is provided wherein the feedstock glucose and feedstock CO are present in a glucose/CO weight/weight ratio of from about 0.3 to about 0.8, e.g., from about 0.25 to about 0.85, from about 0.4 to about 0.7, from about 0.5 to about 0.6.

[0182] Also in such a method the first feedstock comprises methanol or glycerol and the second feedstock comprises CO_2 and H_2.

[0183] Also in such a method the first feedstock comprises a carbohydrate, wherein said fermentation medium comprises a non-fermentable impurity and the impurity and the carbohydrate are present in the fermentation medium in a weight/weight ratio of greater than 0.05. The impurity may be a fermentation inhibitor.

[0184] Also such a method further comprises separating butyric acid from said broth to form separated butyric acid. The butyric acid may be separated by utilizing an organic solvent comprising a C6-C12 alkanol, a C4-C12 alkanol, and/or an ester of butyric acid.

[0185] Also in such a method said fermentation medium has a pH of greater than 5.5 and comprises calcium carbonate for pH control. Calcium carbonate may be present in the said second feedstock and also serves as a carbon source. Said fermentation medium may comprise a calcium base for pH control.

[0186] Also such a method further comprises separating butyric acid from said broth wherein said separating comprises acidulating with sulfuric acid.

[0187] Also such a method further comprises catalytically converting said separated butyric acid into at least one butyric acid derivative. Said catalytically converting may comprise hydrogenation and said at least one butyric acid derivative may comprise butanol.

Production of Isopropanol

[0188] Provided herein is a mixotrophic fermentation method for the production of isopropanol (also known a 2-propanol) comprising (i) providing an isolated organism capable of metabolizing CO_2 into acetyl-CoA; (ii) providing a fermentation medium comprising a first feedstock and a second feedstock wherein said first feedstock comprises a sugar; and wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate, H_2, glycerol, methanol, formate, urea or mixtures thereof; and (iii) culturing said organism in said fermentation medium, whereby both feedstocks are metabolized and a fermentation broth is formed, which broth comprises at least one bioproduct that includes isopropanol.

[0189] Said organism is acetogenic.

[0190] Said organism is selected from the group consisting of *Clostridium ljungdahlii, Clostridium autoethanogenum,* and *Clostridium ragsdalei.*

[0191] Also provided is such a method, wherein the first feedstock comprises a sugar selected from glucose and sucrose, and the second feedstock comprises at least one of H_2, and methanol, and the organism assimilates CO_2 produced during glycolysis. Also described is such a method, wherein the first feedstock comprises methanol and the second feedstock comprises a bicarbonate supplemented with CO_2. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises glycerol. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2 and H_2. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2 and methanol. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2. Also provided is such a method, wherein the first feedstock comprises glucose and the second feedstock comprises CO. A method as described above is provided, wherein the feedstock glucose and feedstock CO are present in a glucose/CO weight/weight ratio of from about 0.3 to about 0.8, e.g., from about 0.25 to about 0.85, from about 0.4 to about 0.7, from about 0.5 to about 0.6. Also described is such a method, wherein the first feedstock comprises methanol or glycerol and the second feedstock comprises CO_2 and H_2. Also provided is such a method, wherein the first feedstock comprises a carbohydrate, wherein said fermentation medium comprises a non-fermentable impurity and wherein the impurity and the carbohydrate are present in the fermentation medium in a weight/weight ratio of greater than 0.05. Said impurity may be a fermentation inhibitor.

[0192] Also provided is such a method, wherein said broth comprises a second bioproduct, wherein said second bioproduct is acetone and the molar ratio between isopropanol and said second bioproduct is greater than 5.

[0193] Also provided is such a method, further comprising separating isopropanol from said broth to form separated isopropanol. Said may comprise evaporation. Said method may further comprise catalytically converting said separated isopropanol into at least one isopropanol derivative. For example, herein is provided such a method comprising catalytically converting said separated isopropanol into acetone.

[0194] The method achieves greater production of isopropanol than the combined amounts of isopropanol produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions.

[0195] Also provided is such a method, wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate, methanol, and mixtures thereof; and wherein the $^{13}C/^{12}C$ isotope ratio of the carbon present in said second feedstock is less than that of atmospheric CO_2.

[0196] Also provided is such a method, wherein said isopropanol has a $^{13}C/^{12}C$ isotope ratio of less than that of atmospheric CO_2.

[0197] Also provided is such a method, wherein said first feedstock and said second feedstock are present in the fermentation medium at the same time.

[0198] Also provided is such a method, wherein the culturing is performed in whole or in part at a superatmospheric pressure.

[0199] Also provided is such a method, wherein providing a fermentation medium comprises providing said fermentation medium with a mixture of CO_2 and hydrogen at a molar ratio in the range between 1:0.1 and

[0200] Also provided is such a method, comprising production of isopropanol and acetic acid. For example, in an embodiment, such a method is provided, wherein the amount of acetic acid formed per biomass unit weight is less than about 50% of that formed in autotrophic fermentation with the same organism under the same conditions.

[0201] Also provided is such a method, wherein said fermentation medium comprises at least one of CO, CO_2, and hydrogen.

[0202] Also provided is such a method, wherein said fermentation medium comprises a steel mill produced composition.

Production of Isopropanol and Acetone

[0203] Provided herein is a mixotrophic fermentation method for the production of acetone and isopropanol comprising (i) providing an isolated organism capable of metabolizing CO_2 into acetyl-CoA; (ii) providing a fermentation medium comprising a first feedstock and a second feedstock wherein said first feedstock comprises a sugar; and wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate, H_2, glycerol, methanol, formate, urea or mixtures thereof; and (iii) culturing said organism in said fermentation medium, whereby both feedstocks are metabolized and a fermentation broth is formed, which broth comprises more than one bioproduct and at least one bioproduct is isopropanol and another is acetone.

[0204] Said organism is acetogenic.

[0205] Said organism is selected from the group consisting of *Clostridium ljungdahlii, Clostridium autoethanogenum,* and *Clostridium ragsdalei.*

[0206] Also provided is such a method, wherein the first feedstock comprises a sugar selected from glucose and sucrose, and the second feedstock comprises at least one of H_2, and methanol, and the organism assimilates CO_2 produced during glycolysis. Also described is such a method, wherein the first feedstock comprises methanol and the second feedstock comprises a bicarbonate supplemented with CO_2. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises glycerol. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2 and H_2. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2 and methanol. Also provided is such a method, wherein the first feedstock comprises at least one of glucose and sucrose and the second feedstock comprises CO_2. Also provided is such a method, wherein the first feedstock comprises glucose and the second feedstock comprises CO. The feedstock glucose and feedstock CO may be present in a glucose/CO weight/weight ratio of from about 0.3 to about 0.8, e.g., from about 0.25 to about 0.85, from about 0.4 to about 0.7, from about 0.5 to about 0.6. Also described is such a method, wherein the first feedstock comprises methanol or glycerol and the second feedstock comprises CO_2 and H_2. Also provided is such a method, wherein the first feedstock comprises a carbohydrate, wherein said fermentation medium comprises a non-fermentable impurity and wherein the impurity and the carbohydrate are present in the fermentation medium in a weight/weight ratio of greater than 0.05. Said impurity may be a fermentation inhibitor.

[0207] Also provided is such a method, further comprising separating acetone and isopropanol from said broth to form separated acetone and isopropanol. In an embodiment, said separating comprises evaporation. Said method may further comprise catalytically converting said separated acetone and separated isopropanol into at least one acetone or isopropanol derivative.

[0208] The method achieves greater production of acetone and isopropanol than the combined amounts of acetone and isopropanol produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions.

[0209] Also provided is such a method, wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate, methanol, and mixtures thereof; and wherein the $^{13}C/^{12}C$ isotope ratio of the carbon present in said second feedstock is less than that of atmospheric CO_2.

[0210] Also provided is such a method, wherein said isopropanol has a $^{13}C/^{12}C$ isotope ratio of less than that of atmospheric CO_2.

[0211] Also provided is such a method, wherein said first feedstock and said second feedstock are present in the fermentation medium at the same time.

[0212] Also provided is such a method, wherein the culturing is performed in whole or in part at a superatmospheric pressure.

[0213] Also provided is such a method, wherein providing a fermentation medium comprises providing said fermentation medium with a mixture of CO_2 and hydrogen at a molar ratio in the range between 1:0.1 and

[0214] Also provided is such a method, comprising production of acetone, isopropanol and acetic acid. For example, such a method is provided, wherein the amount of acetic acid formed per biomass unit weight is less than about 50% of that formed in autotrophic fermentation with the same organism under the same conditions.

[0215] Also provided is such a method, wherein said fermentation medium comprises at least one of CO, CO_2, and hydrogen.

[0216] Also provided is such a method, wherein said fermentation medium comprises a steel mill produced composition.

Production of Crotyl Alcohol - described for illustrative purposes, not forming part of the present invention

[0217] Described herein is a method of producing crotyl alcohol, comprising culturing a microbial organism on a growth substrate under conditions to form a broth comprising crotyl alcohol, wherein the microbial organism is capable of converting acetyl-CoA into crotyl alcohol and comprises at least one exogenous nucleic acid encoding one or more of the following crotyl alcohol pathway enzymes:

A. Acetyl-CoA acetyltransferase (also known as thiolase) (THL)
B. 3-hydroxybutyryl-CoA dehydrogenase (HBD)
C. 3-hydroxybutyryl-CoA dehydratase (also known as crotonase) (CRT)
D. Acetaldehyde/alcohol dehydrogenase (ADHE)
E. Butanol dehydrogenase (BDH)
F. CoA-transferase subunit A (COAT-A)
G. CoA-transferase subunit B (COAT-B)
H. Aldehyde:ferredoxin oxidoreductase (AOR),

and wherein said microbial organism produces more crotyl alcohol compared with a naturally occurring microbial organism of the same genus and species lacking said exogenous nucleic acid.

[0218] Also provided herein is an acetogenic microbial organism or a microbial organism naturally capable of converting acetyl-CoA into crotonyl-CoA, the microbial organism comprising at least one exogenous nucleic acid encoding one or more of the following crotyl alcohol pathway enzymes:

A. Acetyl-CoA acetyltransferase (also known as thiolase) (THL)
B. 3-hydroxybutyryl-CoA dehydrogenase (HBD)
C. 3-hydroxybutyryl-CoA dehydratase (also known as crotonase) (CRT)
D. Acetaldehyde/alcohol dehydrogenase (ADHE)
E. Butanol dehydrogenase (BDH)
F. CoA-transferase subunit A (COAT-A)
G. CoA-transferase subunit B (COAT-B)
H. Aldehyde:ferredoxin oxidoreductase (AOR),

wherein said microbial organism produces more crotyl alcohol compared with a naturally occurring microbial organism of the same genus and species lacking said exogenous nucleic acid.

[0219] Such a microbial organism, which is capable of converting acetyl-CoA into isopropanol, may further comprise at least a second exogenous nucleic acid, the second exogenous nucleic acid encoding one or more isopropanol pathway enzymes. The one or more isopropanol pathway enzymes comprises: A. THL, F. COAT-A, G. COAT-B, I. ADC, and/or J. secondary alcohol dehydrogenase (SADH). Said microbial organism may comprise exogenous nucleic acids encoding each of the enzymes A, B, C, D, F, G, I, J. Said microbial organism may comprise exogenous nucleic acids encoding each of the enzymes A, B, C, D, E, F, G, I, J. Said microbial organism may comprise exogenous nucleic acids encoding each of the enzymes A, B, C, E, F, G, H, I, J.

[0220] A microbial organism as described may comprise two, three, four, five, six, seven, eight, nine, or ten exogenous nucleic acids.

[0221] Also provided herein is such a microbial organism, wherein the exogenous nucleic acid is a heterologous nucleic acid.

[0222] Also provided is such a method for further producing acetone, comprising culturing said microbial organism comprising at least one exogenous nucleic acid on a growth substrate to form a broth comprising crotyl alcohol and acetone, wherein said microbial organism is capable of converting acetyl-CoA into acetone, the microbial organism further comprising at least a second exogenous nucleic acid, the second exogenous nucleic acid encoding one or more acetone pathway enzymes. Such a method may be performed, wherein a crotyl alcohol to acetone molar ratio in said broth is in the range from 5 to 10.

[0223] Also provided is such a method for further producing isopropanol, comprising culturing said microbial organism comprising at least one exogenous nucleic acid on a growth substrate to form a broth comprising crotyl alcohol and isopropanol, wherein said microbial organism is capable of converting acetyl-CoA into isopropanol, the microbial organism further comprising at least a second exogenous nucleic acid, the second exogenous nucleic acid encoding one or more isopropanol pathway enzymes. Such a method may be performed, wherein a crotyl alcohol to isopropanol molar ratio in said broth is in the range from 5 to 10.

[0224] Also provided is such a method, wherein said growth substrate comprises a carbohydrate.

[0225] Also provided is such a method, wherein said growth substrate comprises a one-carbon molecule. In an embodiment, such a method may be performed, wherein said one-carbon molecule is exogenously added. In an embodiment, said one-carbon molecule is selected from a group consisting of CO, CO_2, CH_3OH, carbonate, bicarbonate and combinations thereof.

[0226] Also provided is such a method, wherein said growth substrate comprises at least one gaseous compound. Said gaseous compound may be exogenously added. Said at least one gaseous compound may be selected from a group consisting of CO, CO_2, H_2 and combinations thereof.

[0227] Also provided herein is such a method, wherein said growth substrate comprises a carbohydrate in combination with at least one of a one-carbon molecule and a gaseous compound.

[0228] Also provided herein is such a method, wherein said growth substrate comprises a carbohydrate, exogenously added CO_2 and exogenously added H_2, and wherein at least 2 moles of H_2 are added per mole of CO_2.

[0229] Also provided herein is such a method, comprising steam reforming of a hydrocarbon, whereby CO_2 and H_2 are formed and used in said growth substrate.

[0230] Also provided herein is such a method, wherein carbon yield is at least 42 wt%.

[0231] Also provided herein is such a method, comprising providing pressurized CO_2, pressurized CO, pressurized H_2, or a combination thereof to said growth substrate.

[0232] Also provided herein is such a method, wherein said culturing is conducted at a pressure in the range between 1 atm and 5 atm.

[0233] Also provided herein is such a method, comprising providing at least one of ammonium carbonate and ammonium bicarbonate to said growth substrate.

[0234] Also provided herein is such a method, comprising at least partially separating crotyl alcohol from said broth.

[0235] Also provided herein is such a method, comprising at least partially separating acetone from said broth.

[0236] Also provided herein is such a method, comprising at least partially separating isopropanol from said broth.

[0237] Also provided herein is such a method, wherein said separating comprises liquid-liquid extraction. The method may further comprise dehydrating said separated crotyl alcohol to form butadiene.

[0238] Also provided is such a method, which comprises culturing the microbial organism on a growth substrate for at least 1 hour under conditions to form a broth comprising at least 1 g/L crotyl alcohol.

[0239] Also provided is such a method, which comprises culturing the microbial organism on a growth substrate for at least 1 hour under conditions to form a broth comprising at least 1 g/L crotyl alcohol and at least 0.1 g/L acetone.

[0240] Also provided is such a method, which comprises culturing the microbial organism on a growth substrate for at least 1 hour under conditions to form a broth comprising at least 1 g/L crotyl alcohol and at least 0.1 g/L isopropanol.

[0241] Provided herein is also a non-naturally occurring microbial organism capable of converting acetyl-CoA into crotyl alcohol, wherein butyryl-CoA dehydrogenase (BCD) nucleic acid expression and/or BCD protein translation in the microbial organism is disrupted or silenced. Said expression silencing may comprise at least one of gene disruption, gene deletion and gene mutation. Said protein translation silencing may comprise RNA interference. Such a microbial organism may comprise at least one exogenous nucleic acid encoding one or more of the following crotyl alcohol pathway enzymes:

A. Acetyl-CoA acetyltransferase (also known as thiolase) (THL)
B. 3-hydroxybutyryl-CoA dehydrogenase (HBD)
C. 3-hydroxybutyryl-CoA dehydratase (also known as crotonase) (CRT)
D. Acetaldehyde/alcohol dehydrogenase (ADHE)
E. Butanol dehydrogenase (BDH)
F. CoA-transferase subunit A (COAT-A)
G. CoA-transferase subunit B (COAT-B)
H. Aldehyde:ferredoxin oxidoreductase (AOR),

wherein said microbial organism produces more crotyl alcohol compared with a naturally occurring microbial organism of the same genus and species lacking said exogenous nucleic acid.

[0242] Also provided herein is such a microbial organism, which is capable of converting acetyl-CoA into acetone, the microbial organism further comprising at least one exogenous nucleic acid encoding one or more acetone pathway enzymes. Said one or more acetone pathway enzymes may comprise A. THL, F. COAT-A, G. COAT-B, and/or I. acetoacetate decarboxylase (ADC).

[0243] Also provided herein is such a microbial organism, which is capable of converting acetyl-CoA into isopropanol, the microbial organism further comprising at least one exogenous nucleic acid encoding one or more isopropanol pathway enzymes. Said one or more isopropanol pathway enzymes may comprise : A. THL, F. COAT-A, G. COAT-B, I. ADC, and/or J. secondary alcohol dehydrogenase (SADH).

[0244] Said microbial organism may comprise two, three, four, five, six, seven, eight, nine or ten exogenous nucleic acids.

[0245] Said microbial organism may comprise exogenous nucleic acids encoding each of the enzymes A, B, C, D, F, G, I. Said microbial organism may comprise exogenous nucleic acids encoding each of the enzymes A, B, C, D, E, F, G, I. Said microbial organism may comprise exogenous nucleic acids encoding each of the enzymes A, B, C, E, F, G, H, I. Said microbial organism may comprise exogenous nucleic acids encoding each of the enzymes A, B, C, D, F, G, I, J.

[0246] Also provided herein is such a microbial organism, wherein at least one exogenous nucleic acid is a heterologous nucleic acid.

[0247] Also provided herein is such a microbial organism, wherein said organism is an acetogenic bacterium.

[0248] Herein is further provided a method of producing crotyl alcohol, comprising culturing a non-naturally occurring microbial organism on a growth substrate under conditions to form a broth comprising crotyl alcohol, wherein the microbial organism is capable of converting acetyl-CoA into crotyl alcohol and wherein butyryl-CoA dehydrogenase (BCD) nucleic acid expression and/or BCD protein translation in the microbial organism is disrupted or silenced.

Utilization of Non-preferred Carbon Source

[0249] Said organism is acetogenic and said first feedstock may comprise at least one non-preferred carbon source, for example, a non-preferred sugar. As used herein, the term non-preferred carbon source refers to a carbon source that is metabolized by the native form of the organism at a rate of less than 0.01 g/hr/g cell mass. Such a carbon source may be a carbohydrate, a sugar (e.g., glucose) or glycerol. Such a non-preferred carbon source may also be methanol. The non-preferred carbon source may also be an oxygen-containing organic compound. Said non-preferred carbon source may comprise at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of said first feedstock. The concentration of said non-preferred carbon source in said provided fermentation medium may be in a range between 2 g/L and 50 g/L.

[0250] Said non-preferred sugar may be selected from the group consisting of glucose, mannose, galactose, arabinose, ribose, maltose, sucrose, lactose, cellobiose, and mixtures thereof. A non-preferred sugar is a sugar that is metabolized by the native form of the organism at a rate of less than 0.01 g/hr/g cell mass. Said non-preferred sugar may comprise glucose. Said non-preferred sugar may form at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of said first feedstock. The concentration of said non-preferred sugar in said provided fermentation medium may be in a range between 2 g/L and 50 g/L.

[0251] Said first feedstock may further comprise at least one preferred sugar. As used herein, the term preferred sugar refers to a sugar that is metabolized by the native form of the organism at a rate greater than 0.01 g/hr/g cell mass.

[0252] Said preferred sugar may be selected from the group consisting of fructose, xylose, and mixtures thereof. Said provided fermentation medium may comprise said preferred sugar and said non-preferred sugar concurrently. Said provided fermentation medium may comprise first said preferred sugar and then said non-preferred sugar.

[0253] Said non-preferred sugar may be metabolized at a rate greater than 0.01 g/hr/g cell mass. Metabolism rates of a non-preferred sugar of greater than 0.01 g/hr/g cell mass may be achieved by an organism that has been genetically modified for increased non-preferred sugar metabolism. Said non-preferred sugar may be metabolized by a genetically modified organism at a rate greater than 0.02 g/hr/g, greater than 0.04 g/hr/g cell mass, greater than 0.06 g/hr/g, greater than 0.08 g/hr/g cell mass, greater than 0.1 g/hr/g, greater than 0.12 g/hr/g cell mass, greater than 0.14 g/hr/g, greater than 0.16 g/hr/g cell mass, greater than 0.18 g/hr/g, greater than 0.2 g/hr/g cell mass, or greater than 0.26 g/hr/g.

[0254] CO_2 may be generated from metabolism of said non-preferred sugar and said generated CO_2 comprises at least a fraction of said fermentation medium second feedstock. Said generated CO_2 may comprise at least 20% of said fermentation medium second feedstock, at least 40%, at least 60%, at least 80% or at least 90%.

[0255] Said acetogenic organism metabolizing said non-preferred sugar is acetogenic Clostridia. Said organism metabolizing said non-preferred sugar is selected from the group consisting of *Clostridium ljungdahlii, Clostridium autoethanogenum,* and *Clostridium ragsdalei.*

[0256] According to an embodiment, said organism metabolizing said non-preferred sugar is genetically modified to express at least one component of a phosphotransferase system (PTS), also known as PEP group translocation. Said at least one component may be selected from the group consisting of enzymes EIIA, EIIB, EIIC, and combinations thereof.

[0257] Said organism may be genetically modified to express a gene related to a sugar transport system other than genes associated with the phosphotransferase system. Said organism may be genetically modified to express a gene selected from the group consisting of a symporter system utilizing a sodium ion (Na^+), a symporter system utilizing protons (H^+), a permease system, and a combination thereof. For example, the organism may be transformed with a Gnt-II system transporter (*gntP* gene), a glycoside-pentoside-hexuronide (GPH):cation symporter family gene (GPH gene) or a fucose-galactose-glucose (FGH):H^+ symporter family gene (FGH gene).

[0258] The sugar transport system is not particularly limited. For example, said *gntP* gene may be obtained from one or more various organisms including *Clostridium acetobutylicum* ATCC 824 and *Escherichia coli* K-12. An exemplary nucleic acid sequence that encodes a *gntP* gene, from C. *acetobutylicum,* is as follows:

ATGCCATTATTAATTGTTGTTATTGGCGTCGCATTACTATTACTACTTATGATTAAATTCAAAGTAAACGGA

TTCATATCCCTAATTCTTGTAGCTTTGGTTGTTGGTATCGCCGAAGGTATGAATCCTGCAAAAGCTGTTTCT
TCAATTCAAAACGGTGTTGGAAGCACCTTAAGCAGTTTGGCACTAATTTTAGGTTTTGGTGCTATGTTTGGA
AAATTAATAGCTGATTCTGGTGCTGCTCAAAGGATTTCTAGAAGTTTAATTAATAAATTTGGTGTAAAAAAA
ATTCAATGGGCTGTTGTATTAACGGGTTTCATAGTTGGCATTGCTATGTTCTATGAGGTAGGTTTTGTTCTA
CTTATACCTCTTGTTTTTACTATTGCTGAATTCACAGAACTTCCTCTTTTATACATAGGCGTTCCTATGGCT
GCAGCTTTATCTGTCACTCACGGATTTTTACCTCCTCACCCTGGACCTGTTGCAATAGCTACAATATATGGT
GCAAGCATTAGCATGACTCTTGTATATGGAATTGTAATAGCTATACCTACAGTAATAGTTGCAGGACCTGTT
TTGACTAAGTTTTTAAAACGTTTTGATCATAAATCTTCAAAAAACCTTTTTAAAACTAAGGTCTTTGATGAA
GATGAAATGCCAAGTTTCTCATTAAGCGTATTAACTGCTATTGTTCCTCCTATTCTTATGGCCTTTTCAGCT
GTTTGTGAAATCACACTACCAAAAACATCTCCTATAAGACATTTTGCAGAATTCGTTGGAAGTCCTATGATG
GCAATGTTTATATCAATCATTGTAGCTATCTTTACTCTTGGTATAATGCGCGGAAAGAAAATGGAAGAAATA
ATGAGAACTTTAGCTGAAGCCGCAAGTTCCATTGCAATGATCCTTTTAATAGTAGCTGGAGGTGGTGCCTTC
AAGCAAGTACTAATAGACAGTGGTGTTGGAAAATATATCGCTTCTATTATGGTTGGAAGTAATATATCTCCT
CTAATCTTGGCTTGGGCGATTGCAGCAATTTTAAGATTATCTCTTGGTTCTGCCACTGTTTCTGCTATGACT
ACTGCCGGTATAGTACTTCCTCTTATTCCTTCAACCCATGCAAACCCAGCATTAATGGTTTTAGCAACTGGC
GCAGGTAGTCTTATTTTCTCTCATGTAAACGATCCAGGTTTCTGGATGTTCAAAGAATATTTTGGACTTAGC
ATAGGAGAAACAATGGCTTCATGGTCTACTTTAGAAACTATAATTTCAATTATGGGGTTAATTGGTGTTTTA
GCTTTAAATATGGTTGGATAG (SEQ ID NO: 5).

[0259] The encoded *gntP* amino acid sequence is as follows:

MPLLIVVIGVALLLLLMIKFKVNGFISLILVALVVGIAEGMNPAKAVSSIQNGVGSTLSSLALILGFGAMFG

KLIADSGAAORISRSLINKFGVKKIOWAVVLTGFIVGIAMFYEVGFVLLIPLVFTIAEFTELPLLYIGVPMA
AALSVTHGFLPPHPGPVAIATTYGASISMTLVYGIVIAIPTVIVAGPVLTKFLKRFDHKSSKNLFKTKVFDE
DEMPSFSLSVLTAIVPPILMAFSAVCEITLPKTSPIRHFAEFVGSPMMAMFISIIVAIFTLGIMRGKKMEEI

MRTLAEAASSIAMILLIVAGGGAFKQVLIDSGVGKYIASIMVGSNISPLILAWAIAAILRLSLGSATVSAMT

TAGIVLPLIPSTHANPALMVLATGAGSLIFSHVNDPGFWMFKEYFGLSIGETMASWSTLETIISIMGLIGVL
ALNMVG (SEQ ID NO: 6).

[0260] An exemplary *gntP* amino acid sequence may be an amino acid sequence which has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the above sequence, and which is capable of transporting gluconate or glucose. The corresponding *gntP* polynucleotide sequence may be a polynucleotide sequence encoding an amino acid sequence which has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the above sequence. The corresponding GPH polynucleotide sequence may also be a sequence which is 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the above GPH polynucleotide sequence.

[0261] An exemplary nucleic acid sequence that encodes a GPH gene, from C. *acetobutylicum,* is as follows:

ATGAAAAAGTTAAGCTTAAAGGAAAAAATCTCTTATGGACTTGGCGATTTTGGAAATGGTTTCATGTTTGAT

TTGGGTCAATCATATCTGTTAAAATTCTATACAGACGTCGTAGGTATAGCTGCAGGAGCGGCGGGAGGAATA
TTCTTCTTCACTAAAATATTTGATGCTTTCATGGATCCTATAGCTGGAACAATAATAGATTCAAGGAAACCA
GGTAAAAACGGTAAATTCAAACCTATTATGTTCTTTGCAAGTATAGTACTTGCTATATTGACAGTAATAACG
TTTACTAACCCTGGAAAAACTGCTACATCAAAACTATTATTTGCATATGCAACATATATGATATGGGGACTT
GGATACTCATTTACAAATGTTCCGTATGGATCTCTTGGATCAGTTATAACTCAAGATGTTCAAGAAAGAACT
TCGTTGGCGACTTTTAGACAGATAGGTTCTTCAGGAGCTCTTCTTATAACAAGTGTTATATTTATGCCTCTT
GTTTTAGTATTTCATAACCCAGCAATAGGTTATCCAGTAGTTGCGGGTATAATGGGGTTAATAGGAATATTA
TCATTCTACATGACATACAAAAATACTAGAGAAGTTGTTGCGCCAGCTGAAAACGTTAAGAAGGAAAAAATA
ACACCAAAGTCAATTGCGGTTACAATATTTACAAATAGAGCATTATTAACATTAATATTAATGACTATATTC
TCTATTTCGGCTTACAATATTAGAAGTTCATTAATTGTTTATTACTGCCAATATAATCTTGGAAACGTTACT
TTATTACCATATATAAATTTCTTCACTATAGGATGTGCTGTTTTAGGTGTTTCTTTCATGCCAAAGCTAGTT
GGTAGATTTGGTAAAAAAAGAACTGCTATCATAGGATTTTTGATAAGTGTTATTGCAGATAGTATAAACTTT
CTTCTTCCAGGAAATATATATACTTTCACAATATTATTAGCAATTGGATTTATAGGTATAAGCATTCCTAAT
GGAATAACTTGGGCTTTTGTATCAGACAGTATCGATTATGGTGAGTGGAGAACAGGAACTAGAAGAGAAGGA
ATAACTTACTCTGTATTTAATTTCGCAAGAAAACTTGCTCAGTCAATAGCTGGATTATTATCAGGATGGGGA
CTTGGATTTGTTGGTTATGTAGCTAACAAGAAACAAAGTGCACATGCATTATTTGGAATAAAAGCATTATTG
ATGGCTTATCCAGCGGTAGCGCTTTTAGTAGCAGCATTAATAATTGGTTTATTGTACAACCTTTCAGATAAG
AAATTTACTGAAATAATAGAAGAATTAGACGCTAGAAAAGGTAAAACAGTTTAA (SEQ ID NO: 7).

[0262] The encoded GPH amino acid sequence is as follows:

MKKLSLKEKISYGLGDFGNGFMFDLGQSYLLKFYTDVVGIAAGAAGGIFFFTKIFDAFMDPIAGTIIDSRKP

GKNGKFKPIMFFASIVLAILTVITFTNPGKTATSKLLFAYATYMIWGLGYSFTNVPYGSLGSVITQDVQERT
SLATFRQIGSSGALLITSVIFMPLVLVFHNPAIGYPVVAGIMGLIGILSFYMTYKNTREVVAPAENVKKEKI
TPKSIAVTIFTNRALLTLILMTIFSISAYNIRSSLIVYYCQYNLGNVTLLPYINFFTIGCAVLGVSFMPKLV
GRFGKKRTAIIGFLISVIADSINFLLPGNIYTFTILLAIGFIGISIPNGITWAFVSDSIDYGEWRTGTRREG
ITYSVFNFARKLAQSIAGLLSGWGLGFVGYVANKKQSAHALFGIKALLMAYPAVALLVAALIIGLLYNLSDK
KFTEIIEELDARKGKTV (SEQ ID NO: 8).

[0263] An exemplary GPH amino acid sequence may be an amino acid sequence which has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the above sequence, and which is capable of transporting glucose. The corresponding GPH polynucleotide sequence may be a polynucleotide sequence encoding an amino acid sequence which has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the above sequence. The corresponding GPH polynucleotide sequence may also be a sequence which is 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the above GPH polynucleotide sequence.

[0264] An exemplary nucleic acid sequence that encodes an FGH gene, from *E. coli*, is as follows:

ATGGGAAACACATCAATACAAACGCAGAGTTACCGTGCGGTAGATAAAGATGCAGGGCAAAGCAGAAGTTAC
ATTATTCCATTCGCGCTGCTGTGCTCACTGTTTTTCTTTGGGCGGTAGCCAATAACCTTAACGACATTTTA
TTACCTCAATTCCAGCAGGCTTTTACGCTGACAAATTTCCAGGCTGGCCTGATCCAATCGGCCTTTTACTTT
GGTTATTTCATTATCCCAATCCCTGCTGGGATATTGATGAAAAAACTCAGTTATAAAGCAGGGATTATTACC
GGGTTATTTTTATATGCCTTGGGTGCTGCATTATTCTGGCCCGCCGCAGAAATAATGAACTACACCTTGTTT
TTAGTTGGCCTATTTATTATTGCAGCCGGATTAGGTTGTCTGGAAACTGCCGCAAACCCTTTTGTTACGGTA
TTAGGGCCGGAAAGTAGTGGTCACTTCCGCTTAAATCTTGCGCAAACATTTAACTCGTTTGGCGCAATTATC
GCGGTTGTCTTTGGGCAAAGTCTTATTTTGTCTAACGTGCCACATCAATCGCAAGACGTTCTCGATAAAATG
TCTCCAGAGCAATTGAGTGCGTATAAACACAGCCTGGTATTATCGGTACAGACACCTTATATGATCATCGTG
GCTATCGTGTTACTGGTCGCCCTGCTGATCATGCTGACGAAATTCCCGGCATTGCAGAGTGATAATCACAGT
GACGCCAAACAAGGATCGTTCTCCGCATCGCTTTCTCGCCTGGCGCGTATTCGCCACTGGCGCTGGGCGGTA
TTAGCGCAATTCTGCTATGTCGGCGCACAAACGGCCTGCTGGAGCTATTTGATTCGCTACGCTGTAGAAGAA
ATTCCAGGTATGACTGCAGGCTTTGCCGCTAACTATTTAACCGGAACCATGGTGTGCTTCTTTATTGGTCGT
TTCACCGGTACCTGGCTCATCAGTCGCTTCGCACCACACAAAGTCCTGGCCGCCTACGCATTAATCGCTATG
GCACTGTGCCTGATCTCAGCCTTCGCTGGCGGTCATGTGGGCTTAATAGCCCTGACTTTATGCAGCGCCTTT
ATGTCGATTCAGTACCCAACAATCTTCTCGCTGGGCATTAAGAATCTCGGCCAGGACACCAAATATGGTTCG
TCCTTCATCGTTATGACCATTATTGGCGGCGGTATTGTCACTCCGGTCATGGGTTTTGTCAGTGACGCGGCG
GGCAACATCCCCACTGCTGAACTGATCCCCGCACTCTGCTTCGCGGTCATCTTTATCTTTGCCCGTTTCCGT
TCTCAAACGGCAACTAACTGA (SEQ ID NO: 9).

[0265] The encoded FGH amino acid sequence is as follows:

MGNTSIQTQSYRAVDKDAGQSRSYIIPFALLCSLFFLWAVANNLNDILLPQFQQAFTLTNFQAGLIQSAFYF
GYFIIPIPAGILMKKLSYKAGIITGLFLYALGAALFWPAAEIMNYTLFLVGLFIIAAGLGCLETAANPFVTV
LGPESSGHFRLNLAQTFNSFGAIIAVVFGQSLILSNVPHQSQDVLDKMSPEQLSAYKHSLVLSVQTPYMIIV
AIVLLVALLIMLTKFPALQSDNHSDAKQGSFSASLSRLARIRHWRWAVLAQFCYVGAQTACWSYLIRYAVEE
IPGMTAGFAANYLTGTMVCFFIGRFTGTWLISRFAPHKVLAAYALIAMALCLISAFAGGHVGLIALTLCSAF
MSIQYPTIFSLGIKNLGQDTKYGSSFIVMTIIGGGIVTPVMGFVSDAAGNIPTAELIPALCFAVIFIFARFR
SQTATN (SEQ ID NO: 10).

[0266] An exemplary FGH amino acid sequence may be an amino acid sequence which has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the above sequence, and which is capable of transporting glucose. The corresponding FGH polynucleotide sequence may be a polynucleotide sequence encoding an amino acid sequence which has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the above sequence. The corresponding FGH polynucleotide sequence may also be a sequence which is 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the above FGH polynucleotide sequence.

[0267] Said organism metabolizing said non-preferred sugar may express genes of the Wood-Ljungdahl pathway.

[0268] Said organism metabolizing said non-preferred sugar may be genetically modified to have a primary alcohol dehydrogenase gene or a secondary alcohol dehydrogenase gene deleted from its genome.

[0269] Said organism metabolizing said non-preferred sugar may be genetically modified to have butanediol dehydrogenase deleted from its genome.

[0270] The rate of metabolizing said non-preferred sugar by said genetically modified organism may be greater than that of metabolizing said non-preferred sugar by the native form of the organism by a factor of at least 1.5, at least 2, at least 5, at least 8, at least 10, at least 12, at least 15, or at least 20.

[0271] Culturing said organism in said non-preferred sugar comprising fermentation medium, forms a fermentation broth comprising a bioproduct selected from the group consisting of acetone, isopropanol, and combinations thereof.

[0272] Culturing said organism in said non-preferred sugar comprising fermentation medium, may form a fermentation broth comprising a non-naturally occurring bioproduct.

[0273] Culturing said organism in said non-preferred sugar comprising fermentation medium achieves greater production of the at least one bioproduct than the combined amounts produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions.

[0274] Culturing said organism in said non-preferred sugar comprising fermentation medium, the carbon yield, based on the total amount of carbon in produced bioproducts divided by the total amount of carbon metabolized in said first feedstock, is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 110%, at least

120%, at least 130%, at least 140%, at least 150%, or at least 160%.

[0275] According to an embodiment, providing a non-preferred sugar comprising fermentation medium includes providing said fermentation medium with a mixture of CO_2 and hydrogen at a molar ratio in the range from 1:0.1 to 1:5.

[0276] Provided herein is a mixotrophic fermentation method comprising (i) providing an isolated naturally acetogenic organism, (ii) providing a first feedstock and a second feedstock wherein said first feedstock comprises a carbon source that is metabolized by the native form of the organism at a rate of less than 0.01 g/hr/g cell mass; and wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate, H_2, glycerol, methanol, formate, urea or a combination thereof; and (iii) culturing said organism in a fermentation medium, whereby both feedstocks are metabolized and a fermentation broth is formed, which broth comprises at least one bioproduct.

[0277] The carbon yield, based on the total amount of carbon in produced bioproducts divided by the total amount of carbon metabolized from said first feedstock, is at least 50%.

[0278] The $^{13}C/^{12}C$ isotope ratio of the carbon present in the bioproduct may be less than that of atmospheric CO_2.

[0279] Said organism is a *Clostridia* which may be genetically modified.

[0280] In an embodiment, said first feedstock and said second feedstock may be present in the fermentation medium at the same time.

[0281] In an embodiment, said fermentation medium may comprise a carbohydrate and at least one of CO, CO_2, and hydrogen.

[0282] In an embodiment, said fermentation medium may comprise a steel mill produced CO composition.

[0283] In an embodiment, the culturing may be performed in whole or in part at a super-atmospheric pressure.

[0284] In an embodiment, said bioproduct may be selected from the group consisting of acetone, isopropanol, and combinations thereof.

[0285] In an embodiment, said bioproduct may be non-naturally occurring.

[0286] In an embodiment, the second feedstock may comprise CO, CO_2, carbonate, bicarbonate, methanol, or a combination thereof; and the $^{13}C/^{12}C$ isotope ratio of the carbon present in said second feedstock may be less than that of atmospheric CO_2.

[0287] In an embodiment, the method may comprise providing said fermentation medium with a mixture of CO_2 and hydrogen at a molar ratio in the range from 1:0.1 to 1:5.

[0288] In an embodiment, the method may further comprise steam reforming of a hydrocarbon to form said mixture of CO_2 and hydrogen.

[0289] In an embodiment, the first feedstock may comprise a sugar selected from glucose and sucrose, and the organism may metabolize CO_2 produced during glycolysis.

[0290] In an embodiment, the first feedstock may comprise a sugar selected from glucose and sucrose, the second feedstock may comprise at least one of H_2 and methanol, and the organism may metabolize CO_2 produced during glycolysis.

[0291] In an embodiment, said at least one bioproduct is acetone. In such an embodiment, the first feedstock may comprise a sugar selected from glucose and sucrose, and the organism may metabolize CO_2 produced during glycolysis.

[0292] In an embodiment, said at least one bioproduct is isopropanol. In such an embodiment, the first feedstock may comprise a sugar selected from glucose and sucrose, and methanol, and the organism may metabolize CO_2 produced during glycolysis.

[0293] In an embodiment, the metabolizing of the first feedstock does not inhibit the metabolizing of the second feedstock. In such a case, inhibition is defined as a decrease in the metabolizing rate of the second feedstock in the presence of the first feedstock compared to the metabolizing rate of the second feedstock in the absence of the first feedstock. The first feedstock may inhibit the metabolizing of the second feedstock by less than 10%. The first feedstock may inhibit the metabolizing of the second feedstock by less than 1%, less than 5%, less than 15%, less than 20%, or less than 30%.

[0294] In an embodiment, the first feedstock may comprise a non-preferred sugar and the second feedstock may comprise CO, CO_2, carbonate, bicarbonate, H_2, glycerol, methanol, formate, urea or a combination thereof.

Additional Exemplary Polynucleotide and Amino Acids Sequences

[0295] Exemplary amino acid and nucleic acid sequences for performing methods are disclosed herein.

[0296] An exemplary acetyl-CoA acetyltransferase (also known as thiolase) (THL) for use in the present invention catalyzes the condensation of two (2) acetyl-CoA molecules into acetoacetyl-CoA and the release of one (1) coenzyme-A (CoA) molecule. Exemplary THL nucleic acid and amino acid sequences (from C. *acetobutylicum* ATCC 824) are set forth below:

EC number: 2.3.1.9

[0297] Example nucleic acid sequence:

ATGAAAGAAGTTGTAATAGCTAGTGCAGTAAGAACAGCGATTGGATCTTATGGAAAGTCTCTTAAGGATGTA
CCAGCAGTAGATTTAGGAGCTACAGCTATAAAGGAAGCAGTTAAAAAAGCAGGAATAAAACCAGAGGATGTT
AATGAAGTCATTTTAGGAAATGTTCTTCAAGCAGGTTTAGGACAGAATCCAGCAAGACAGGCATCTTTTAAA
GCAGGATTACCAGTTGAAATTCCAGCTATGACTATTAATAAGGTTTGTGGTTCAGGACTTAGAACAGTTAGC
TTAGCAGCACAAATTATAAAAGCAGGAGATGCTGACGTAATAATAGCAGGTGGTATGGAAAATATGTCTAGA
GCTCCTTACTTAGCGAATAACGCTAGATGGGGATATAGAATGGGAAACGCTAAATTTGTTGATGAAATGATC
ACTGACGGATTGTGGGATGCATTTAATGATTACCACATGGGAATAACAGCAGAAAACATAGCTGAGAGATGG
AACATTTCAAGAGAAGAACAAGATGAGTTTGCTCTTGCATCACAAAAAAAAGCTGAAGAAGCTATAAAATCA
GGTCAATTTAAAGATGAAATAGTTCCTGTAGTAATTAAAGGCAGAAAGGGAGAAACTGTAGTTGATACAGAT
GAGCACCCTAGATTTGGATCAACTATAGAAGGACTTGCAAAATTAAAACCTGCCTTCAAAAAAGATGGAACA
GTTACAGCTGGTAATGCATCAGGATTAAATGACTGTGCAGCAGTACTTGTAATCATGAGTGCAGAAAAAGCT
AAAGAGCTTGGAGTAAAACCACTTGCTAAGATAGTTTCTTATGGTTCAGCAGGAGTTGACCCAGCAATAATG
GGATATGGACCTTTCTATGCAACAAAAGCAGCTATTGAAAAAGCAGGTTGGACAGTTGATGAATTAGATTTA
ATAGAATCAAATGAAGCTTTTGCAGCTCAAAGTTTAGCAGTAGCAAAAGATTTAAAATTTGATATGAATAAA
GTAAATGTAAATGGAGGAGCTATTGCCCTTGGTCATCCAATTGGAGCATCAGGTGCAAGAATACTCGTTACT
CTTGTACACGCAATGCAAAAAAGAGATGCAAAAAAAGGCTTAGCAACTTTATGTATAGGTGGCGGACAAGGA
ACAGCAATATTGCTAGAAAAGTGCTAG (SEQ ID NO: 11)

[0298] Example amino acid sequence:

MKEVVIASAVRTAIGSYGKSLKDVPAVDLGATAIKEAVKKAGIKPEDVNEVILGNVLQAGLGQNPARQASFK
AGLPVEIPAMTINKVCGSGLRTVSLAAQIIKAGDADVIIAGGMENMSRAPYLANNARWGYRMGNAKFVDEMI
TDGLWDAFNDYHMGITAENIAERWNISREEQDEFALASQKKAEEAIKSGQFKDEIVPVVIKGRKGETVVDTD
EHPRFGSTIEGLAKLKPAFKKDGTVTAGNASGLNDCAAVLVIMSAEKAKELGVKPLAKIVSYGSAGVDPAIM
GYGPFYATKAAIEKAGWTVDELDLIESNEAFAAQSLAVAKDLKFDMNKVNVNGGAIALGHPIGASGARILVT
LVHAMQKRDAKKGLATLCIGGGQGTAILLEKC (SEQ ID NO: 12)

[0299] An exemplary 3-hydroxybutyryl-CoA dehydrogenase (HBD) for use in the present invention catalyzes the conversion of acetoacetyl-CoA into 3-hydroxybutyryl-CoA. 3-Hydroxybutyryl-CoA can be either the (S) or the (R) enantiomer. This reaction typically requires a coenzyme, such as NADH or NADPH. Exemplary HBD nucleic acid and amino acid sequences (from *C. acetobutylicum* ATCC 824) are set forth below:
EC number: 1.1.1.36 or 1.1.1.35 or 1.1.1.157

[0300] Example nucleic acid sequence:

ATGAAAAAGGTATGTGTTATAGGTGCAGGTACTATGGGTTCAGGAATTGCTCAGGCATTTGCAGCTAAAGGA
TTTGAAGTAGTATTAAGAGATATTAAAGATGAATTTGTTGATAGAGGATTAGATTTTATCAATAAAAATCTT
TCTAAATTAGTTAAAAAAGGAAAGATAGAAGAAGCTACTAAAGTTGAAATCTTAACTAGAATTTCCGGAACA
GTTGACCTTAATATGGCAGCTGATTGCGATTTAGTTATAGAAGCAGCTGTTGAAAGAATGGATATTAAAAAG
CAGATTTTTGCTGACTTAGACAATATATGCAAGCCAGAAACAATTCTTGCATCAAATACATCATCACTTTCA
ATAACAGAAGTGGCATCAGCAACTAAAAGACCTGATAAGGTTATAGGTATGCATTTCTTTAATCCAGCTCCT
GTTATGAAGCTTGTAGAGGTAATAAGAGGAATAGCTACATCACAAGAAACTTTTGATGCAGTTAAAGAGACA
TCTATAGCAATAGGAAAAGATCCTGTAGAAGTAGCAGAAGCACCAGGATTTGTTGTAAATAGAATATTAATA
CCAATGATTAATGAAGCAGTTGGTATATTAGCAGAAGGAATAGCTTCAGTAGAAGACATAGATAAAGCTATG
AAACTTGGAGCTAATCACCCAATGGGACCATTAGAATTAGGTGATTTTATAGGTCTTGATATATGTCTTGCT
ATAATGGATGTTTTATACTCAGAAACTGGAGATTCTAAGTATAGACCACATACATTACTTAAGAAGTATGTA
AGAGCAGGATGGCTTGGAAGAAAATCAGGAAAAGGTTTCTACGATTATTCAAAATAA (SEQ ID NO: 13)

[0301] Example amino acid sequence:

MKKVCVIGAGTMGSGIAQAFAAKGFEVVLRDIKDEFVDRGLDFINKNLSKLVKKGKIEEATKVEILTRISGT
VDLNMAADCDLVIEAAVERMDIKKQIFADLDNICKPETILASNTSSLSITEVASATKRPDKVIGMHFFNPAP
VMKLVEVIRGIATSQETFDAVKETSIAIGKDPVEVAEAPGFVVNRILIPMINEAVGILAEGIASVEDIDKAM
KLGANHPMGPLELGDFIGLDICLAIMDVLYSETGDSKYRPHTLLKKYVRAGWLGRKSGKGFYDYSK (SEQ ID NO: 14)

[0302] An exemplary 3-hydroxybutyryl-CoA dehydratase (also known as crotonase) (CRT) for use in the present invention catalyzes the dehydration of 3-hydroxybutyryl-CoA into crotonyl-CoA and a water molecule. It can act upon either the (S) or the (R) enantiomer of 3-hydroxybutyryl-CoA. Exemplary CRT nucleic acid and amino acid sequences (from *C. acetobutylicum* ATCC 824) are set forth below:
EC number: 4.2.1.17 or 4.2.1.55

[0303] Example nucleic acid sequence:

ATGGAACTAAACAATGTCATCCTTGAAAAGGAAGGTAAAGTTGCTGTAGTTACCATTAACAGACCTAAAGCA
TTAAATGCGTTAAATAGTGATACACTAAAAGAAATGGATTATGTTATAGGTGAAATTGAAAATGATAGCGAA
GTACTTGCAGTAATTTTAACTGGAGCAGGAGAAAAATCATTTGTAGCAGGAGCAGATATTTCTGAGATGAAG
GAAATGAATACCATTGAAGGTAGAAAATTCGGGATACTTGGAAATAAAGTGTTTAGAAGATTAGAACTTCTT
GAAAAGCCTGTAATAGCAGCTGTTAATGGTTTTGCTTTAGGAGGCGGATGCGAAATAGCTATGTCTTGTGAT
ATAAGAATAGCTTCAAGCAACGCAAGATTTGGTCAACCAGAAGTAGGTCTCGGAATAACACCTGGTTTTGGT
GGTACACAAAGACTTTCAAGATTAGTTGGAATGGGCATGGCAAAGCAGCTTATATTTACTGCACAAAATATA
AAGGCAGATGAAGCATTAAGAATCGGACTTGTAAATAAGGTAGTAGAACCTAGTGAATTAATGAATACAGCA
AAAGAAATTGCAAACAAAATTGTGAGCAATGCTCCAGTAGCTGTTAAGTTAAGCAAACAGGCTATTAATAGA
GGAATGCAGTGTGATATTGATACTGCTTTAGCATTTGAATCAGAAGCATTTGGAGAATGCTTTTCAACAGAG
GATCAAAAGGATGCAATGACAGCTTTCATAGAGAAAAGAAAAATTGAAGGCTTCAAAAATAGATAG (SEQ ID NO: 15)

[0304] Example amino acid sequence:

MELNNVILEKEGKVAVVTINRPKALNALNSDTLKEMDYVIGEIENDSEVLAVILTGAGEKSFVAGADISEMK
EMNTIEGRKFGILGNKVFRRLELLEKPVIAAVNGFALGGGCEIAMSCDIRIASSNARFGQPEVGLGITPGFG
GTQRLSRLVGMGMAKQLIFTAQNIKADEALRIGLVNKVVEPSELMNTAKEIANKIVSNAPVAVKLSKQAINR
GMQCDIDTALAFESEAFGECFSTEDQKDAMTAFIEKRKIEGFKNR (SEQ ID NO: 16)

[0305] An exemplary acetaldehyde/alcohol dehydrogenase (ADHE) for use in the present invention is a bifunctional enzyme that catalyzes two reactions sequentially. The first reaction is a CoA-acylating reaction in which crotonyl-CoA is converted into crotonaldehyde. The second reaction is a dehydrogenase reaction in which crotonaldehyde is converted into crotyl alcohol. Any similar substrates can also be used, such as acetyl-CoA, butyryl-CoA, and others. This reaction typically requires a coenzyme, such as NADH or NADPH. Exemplary ADHE nucleic acid and amino acid sequences (from *C. acetobutylicum* ATCC 824) are set forth below:
EC number: For the first reaction (1.2.1.10 or 1.2.1.57); for the second reaction (1.1.1.1)

[0306] Example nucleic acid sequence:

ATGAAAGTCACAACAGTAAAGGAATTAGATGAAAAACTCAAGGTAATTAAAGAAGCTCAAAAAAAATTCTCT
TGTTACTCGCAAGAAATGGTTGATGAAATCTTTAGAAATGCAGCAATGGCAGCAATCGACGCAAGGATAGAG
CTAGCAAAAGCAGCTGTTTTGGAAACCGGTATGGGCTTAGTTGAAGACAAGGTTATAAAAAATCATTTTGCA
GGCGAATACATCTATAACAAATATAAGGATGAAAAAACCTGCGGTATAATTGAACGAAATGAACCCTACGGA
ATTACAAAAATAGCAGAACCTATAGGAGTTGTAGCTGCTATAATCCCTGTAACAAACCCCACATCAACAACA
ATATTTAAATCCTTAATATCCCTTAAAACTAGAAATGGAATTTTCTTTTCGCCTCACCCAAGGGCAAAAAAA
TCCACAATACTAGCAGCTAAAACAATACTTGATGCAGCCGTTAAGAGTGGTGCCCCGGAAAATATAATAGGT
TGGATAGATGAACCTTCAATTGAACTAACTCAATATTTAATGCAAAAAGCAGATATAACCCTTGCAACTGGT
GGTCCCTCACTAGTTAAATCTGCTTATTCTTCCGGAAAACCAGCAATAGGTGTTGGTCCGGGTAACACCCCA
GTAATAATTGATGAATCTGCTCATATAAAAATGGCAGTAAGTTCAATTATATTATCCAAAACCTATGATAAT

GGTGTTATATGTGCTTCTGAACAATCTGTAATAGTCTTAAAATCCATATATAACAAGGTAAAAGATGAGTTC
CAAGAAAGAGGAGCTTATATAATAAAGAAAAACGAATTGGATAAAGTCCGTGAAGTGATTTTTAAAGATGGA
TCCGTAAACCCTAAAATAGTCGGACAGTCAGCTTATACTATAGCAGCTATGGCTGGCATAAAAGTACCTAAA
ACCACAAGAATATTAATAGGAGAAGTTACCTCCTTAGGTGAAGAAGAACCTTTTGCCCACGAAAAACTATCT
CCTGTTTTGGCTATGTATGAGGCTGACAATTTTGATGATGCTTTAAAAAAAGCAGTAACTCTAATAAACTTA
GGAGGCCTCGGCCATACCTCAGGAATATATGCAGATGAAATAAAAGCACGAGATAAAATAGATAGATTTAGT
AGTGCCATGAAAACCGTAAGAACCTTTGTAAATATCCCAACCTCACAAGGTGCAAGTGGAGATCTATATAAT
TTTAGAATACCACCTTCTTTCACGCTTGGCTGCGGATTTTGGGGAGGAAATTCTGTTTCCGAGAATGTTGGT
CCAAAACATCTTTTGAATATTAAAACCGTAGCTGAAAGGAGAGAAAACATGCTTTGGTTTAGAGTTCCACAT
AAAGTATATTTTAAGTTCGGTTGTCTTCAATTTGCTTTAAAAGATTTAAAAGATCTAAAGAAAAAAAGAGCC
TTTATAGTTACTGATAGTGACCCCTATAATTTAAACTATGTTGATTCAATAATAAAAATACTTGAGCACCTA
GATATTGATTTTAAAGTATTTAATAAGGTTGGAAGAGAAGCTGATCTTAAAACCATAAAAAAAGCAACTGAA
GAAATGTCCTCCTTTATGCCAGACACTATAATAGCTTTAGGTGGTACCCCTGAAATGAGCTCTGCAAAGCTA
ATGTGGGTACTATATGAACATCCAGAAGTAAAATTTGAAGATCTTGCAATAAAATTTATGGACATAAGAAAG
AGAATATATACTTTCCCAAAACTCGGTAAAAAGGCTATGTTAGTTGCAATTACAACTTCTGCTGGTTCCGGT
TCTGAGGTTACTCCTTTTGCTTTAGTAACTGACAATAACACTGGAAATAAGTACATGTTAGCAGATTATGAA
ATGACACCAAATATGGCAATTGTAGATGCAGAACTTATGATGAAAATGCCAAAGGGATTAACCGCTTATTCA
GGTATAGATGCACTAGTAAATAGTATAGAAGCATACACATCCGTATATGCTTCAGAATACACAAACGGACTA
GCACTAGAGGCAATACGATTAATATTTAAATATTTGCCTGAGGCTTACAAAAACGGAAGAACCAATGAAAAA
GCAAGAGAGAAAATGGCTCACGCTTCAACTATGGCAGGTATGGCATCCGCTAATGCATTTCTAGGTCTATGT
CATTCCATGGCAATAAAATTAAGTTCAGAACACAATATTCCTAGTGGCATTGCCAATGCATTACTAATAGAA
GAAGTAATAAAATTTAACGCAGTTGATAATCCTGTAAAACAAGCCCCTTGCCCACAATATAAGTATCCAAAC
ACCATATTTAGATATGCTCGAATTGCAGATTATATAAAGCTTGGAGGAAATACTGATGAGGAAAAGGTAGAT
CTCTTAATTAACAAAATACATGAACTAAAAAAAGCTTTAAATATACCAACTTCAATAAAGGATGCAGGTGTT
TTGGAGGAAAACTTCTATTCCTCCCTTGATAGAATATCTGAACTTGCACTAGATGATCAATGCACAGGCGCT
AATCCTAGATTTCCTCTTACAAGTGAGATAAAAGAAATGTATATAAATTGTTTTAAAAAACAACCTTAA
(SEQ ID NO: 17)

[0307] Example amino acid sequence:

MKVTTVKELDEKLKVIKEAQKKFSCYSQEMVDEIFRNAAMAAIDARIELAKAAVLETGMGLVEDKVIKNHFA
GEYIYNKYKDEKTCGIIERNEPYGITKIAEPIGVVAAIIPVTNPTSTTIFKSLISLKTRNGIFFSPHPRAKK
STILAAKTILDAAVKSGAPENIIGWIDEPSIELTQYLMQKADITLATGGPSLVKSAYSSGKPAIGVGPGNTP
VIIDESAHIKMAVSSIILSKTYDNGVICASEQSVIVLKSIYNKVKDEFQERGAYIIKKNELDKVREVIFKDG
SVNPKIVGQSAYTIAAMAGIKVPKTTRILIGEVTSLGEEEPFAHEKLSPVLAMYEADNFDDALKKAVTLINL
GGLGHTSGIYADEIKARDKIDRFSSAMKTVRTFVNIPTSQGASGDLYNFRIPPSFTLGCGFWGGNSVSENVG
PKHLLNIKTVAERRENMLWFRVPHKVYFKFGCLQFALKDLKDLKKKRAFIVTDSDPYNLNYVDSIIKILEHL
DIDFKVFNKVGREADLKTIKKATEEMSSFMPDTIIALGGTPEMSSAKLMWVLYEHPEVKFEDLAIKFMDIRK
RIYTFPKLGKKAMLVAITTSAGSGSEVTPFALVTDNNTGNKYMLADYEMTPNMAIVDAELMMKMPKGLTAYS
GIDALVNSIEAYTSVYASEYTNGLALEAIRLIFKYLPEAYKNGRTNEKAREKMAHASTMAGMASANAFLGLC
HSMAIKLSSEHNIPSGIANALLIEEVIKFNAVDNPVKQAPCPQYKYPNTIFRYARIADYIKLGGNTDEEKVD
LLINKIHELKKALNIPTSIKDAGVLEENFYSSLDRISELALDDQCTGANPRFPLTSEIKEMYINCFKKQP
(SEQ ID NO: 18)

[0308] An exemplary butanol dehydrogenase (BDH) for use in the present invention catalyzes the dehydrogenation of an aldehyde into an alcohol, particularly crotonaldehyde into crotyl alcohol, though any aldehyde can be a substrate. This reaction typically requires a coenzyme, such as NADH or NADPH. Exemplary butanol dehydrogenase nucleic acid and amino acid sequences (from C. *acetobutylicum* ATCC 824) are set forth below:
EC number: 1.1.1.1

[0309] Example nucleic acid sequence:

GTGGTTGATTTCGAATATTCAATACCAACTAGAATTTTTTTCGGTAAAGATAAGATAAATGTACTTGGAAGA
GAGCTTAAAAAATATGGTTCTAAAGTGCTTATAGTTTATGGTGGAGGAAGTATAAAGAGAAATGGAATATAT
GATAAAGCTGTAAGTATACTTGAAAAAAACAGTATTAAATTTTATGAACTTGCAGGAGTAGAGCCAAATCCA
AGAGTAACTACAGTTGAAAAAGGAGTTAAAATATGTAGAGAAAATGGAGTTGAAGTAGTACTAGCTATAGGT
GGAGGAAGTGCAATAGATTGCGCAAAGGTTATAGCAGCAGCATGTGAATATGATGGAAATCCATGGGATATT
GTGTTAGATGGCTCAAAAATAAAAAGGGTGCTTCCTATAGCTAGTATATTAACCATTGCTGCAACAGGATCA
GAAATGGATACGTGGGCAGTAATAAATAATATGGATACAAACGAAAAACTAATTGCGGCACATCCAGATATG
GCTCCTAAGTTTTCTATATTAGATCCAACGTATACGTATACCGTACCTACCAATCAAACAGCAGCAGGAACA
GCTGATATTATGAGTCATATATTTGAGGTGTATTTTAGTAATACAAAAACAGCATATTTGCAGGATAGAATG
GCAGAAGCGTTATTAAGAACTTGTATTAAATATGGAGGAATAGCTCTTGAGAAGCCGGATGATTATGAGGCA
AGAGCCAATCTAATGTGGGCTTCAAGTCTTGCGATAAATGGACTTTTAACATATGGTAAAGACACTAATTGG
AGTGTACACTTAATGGAACATGAATTAAGTGCTTATTACGACATAACACACGGCGTAGGGCTTGCAATTTTA
ACACCTAATTGGATGGAGTATATTTTAAATAATGATACAGTGTACAAGTTTGTTGAATATGGTGTAAATGTT
TGGGGAATAGACAAAGAAAAAAATCACTATGACATAGCACATCAAGCAATACAAAAAACAAGAGATTACTTT
GTAAATGTACTAGGTTTACCATCTAGACTGAGAGATGTTGGAATTGAAGAAGAAAAATTGGACATAATGGCA
AAGGAATCAGTAAAGCTTACAGGAGGAACCATAGGAAACCTAAGACCAGTAAACGCCTCCGAAGTCCTACAA
ATATTCAAAAAATCTGTGTAA (SEQ ID NO: 19)

[0310] Example amino acid sequence:

MVDFEYSIPTRIFFGKDKINVLGRELKKYGSKVLIVYGGGSIKRNGIYDKAVSILEKNSIKFYELAGVEPNP
RVTTVEKGVKICRENGVEVVLAIGGGSAIDCAKVIAAACEYDGNPWDIVLDGSKIKRVLPIASILTIAATGS
EMDTWAVINNMDTNEKLIAAHPDMAPKFSILDPTYTYTVPTNQTAAGTADIMSHIFEVYFSNTKTAYLQDRM
AEALLRTCIKYGGIALEKPDDYEARANLMWASSLAINGLLTYGKDTNWSVHLMEHELSAYYDITHGVGLAIL
TPNWMEYILNNDTVYKFVEYGVNVWGIDKEKNHYDIAHQAIQKTRDYFVNVLGLPSRLRDVGIEEEKLDIMA
KESVKLTGGTIGNLRPVNASEVLQIFKKSV (SEQ ID NO: 20)

[0311] An exemplary CoA-transferase subunit A (COAT-A) for use in the present invention catalyzes the transfer of coenzyme-A (CoA) between two molecules. For example, from acetoacetyl-CoA to acetate to form acetoacetate and acetyl-CoA or from acetoacetyl-CoA to butyrate to form acetoacetate and butyryl-CoA. Exemplary COAT-A , i.e., subunit A nucleic acid and amino acid sequences (from *C. acetobutylicum* ATCC 824) are set forth below:
EC number: 2.8.3.8 or 2.8.3.9 or other related enzymes

[0312] Example nucleic acid sequence:

ATGAACTCTAAAATAATTAGATTTGAAAATTTAAGGTCATTCTTTAAAGATGGGATGACAATTATGATTGGA
GGTTTTTTAAACTGTGGCACTCCAACCAAATTAATTGATTTTTTAGTTAATTTAAATATAAAGAATTTAACG
ATTATAAGTAATGATACATGTTATCCTAATACAGGTATTGGTAAGTTAATATCAAATAATCAAGTAAAAAAG
CTTATTGCTTCATATATAGGCAGCAACCCAGATACTGGCAAAAAACTTTTTAATAATGAACTTGAAGTAGAG

CTCTCTCCCCAAGGAACTCTAGTGGAAAGAATACGTGCAGGCGGATCTGGCTTAGGTGGTGTACTAACTAAA
ACAGGTTTAGGAACTTTGATTGAAAAAGGAAAGAAAAAAATATCTATAAATGGAACGGAATATTTGTTAGAG
CTACCTCTTACAGCCGATGTAGCATTAATTAAAGGTAGTATTGTAGATGAGGCCGGAAACACCTTCTATAAA
GGTACTACTAAAAACTTTAATCCCTATATGGCAATGGCAGCTAAAACCGTAATAGTTGAAGCTGAAAATTTA
GTTAGCTGTGAAAAACTAGAAAAGGAAAAAGCAATGACCCCCGGAGTTCTTATAAATTATATAGTAAAGGAG
CCTGCATAA (SEQ ID NO: 21)

[0313] Example amino acid sequence:

MNSKIIRFENLRSFFKDGMTIMIGGFLNCGTPTKLIDFLVNLNIKNLTIISNDTCYPNTGIGKLISNNQVKK
LIASYIGSNPDTGKKLFNNELEVELSPQGTLVERIRAGGSGLGGVLTKTGLGTLIEKGKKKISINGTEYLLE
LPLTADVALIKGSIVDEAGNTFYKGTTKNFNPYMAMAAKTVIVEAENLVSCEKLEKEKAMTPGVLINYIVKE
PA (SEQ ID NO: 22)

[0314] An exemplary CoA-transferase subunit B (COAT-B) for use in the present invention catalyzes the transfer of coenzyme-A (CoA) between two molecules. For example, from acetoacetyl-CoA to acetate to form acetoacetate and acetyl-CoA or from acetoacetyl-CoA to butyrate to form acetoacetate and butyryl-CoA. Exemplary COAT-B , i.e., subunit B nucleic acid and amino acid sequences (from *C. acetobutylicum* ATCC 824) are set forth below:
EC number: 2.8.3.8 or 2.8.3.9 or other related enzymes

[0315] Example nucleic acid sequence:

ATGATTAATGATAAAAACCTAGCGAAAGAAATAATAGCCAAAAGAGTTGCAAGAGAATTAAAAAATGGTCAA
CTTGTAAACTTAGGTGTAGGTCTTCCTACCATGGTTGCAGATTATATACCAAAAAATTTCAAAATTACTTTC
CAATCAGAAAACGGAATAGTTGGAATGGGCGCTAGTCCTAAAATAAATGAGGCAGATAAAGATGTAGTAAAT
GCAGGAGGAGACTATACAACAGTACTTCCTGACGGCACATTTTTCGATAGCTCAGTTTCGTTTTCACTAATC
CGTGGTGGTCACGTAGATGTTACTGTTTTAGGGGCTCTCCAGGTAGATGAAAAGGGTAATATAGCCAATTGG
ATTGTTCCTGGAAAAATGCTCTCTGGTATGGGTGGAGCTATGGATTTAGTAAATGGAGCTAAGAAAGTAATA
ATTGCAATGAGACATACAAATAAAGGTCAACCTAAAATTTTAAAAAAATGTACACTTCCCCTCACGGCAAAG
TCTCAAGCAAATCTAATTGTAACAGAACTTGGAGTAATTGAGGTTATTAATGATGGTTTACTTCTCACTGAA
ATTAATAAAAACACAACCATTGATGAAATAAGGTCTTTAACTGCTGCAGATTTACTCATATCCAATGAACTT
AGACCCATGGCTGTTTAG (SEQ ID NO: 23)

[0316] Example amino acid sequence:

MINDKNLAKEIIAKRVARELKNGQLVNLGVGLPTMVADYIPKNFKITFQSENGIVGMGASPKINEADKDVVN
AGGDYTTVLPDGTFFDSSVSFSLIRGGHVDVTVLGALQVDEKGNIANWIVPGKMLSGMGGAMDLVNGAKKVI
IAMRHTNKGQPKILKKCTLPLTAKSQANLIVTELGVIEVINDGLLLTEINKNTTIDEIRSLTAADLLISNEL
RPMAV (SEQ ID NO: 24)

[0317] An exemplary aldehyde:ferredoxin oxidoreductase (AOR) for use in the present invention catalyzes the reduction of a carboxylic acid into its corresponding aldehyde. For example, crotonic acid into crotonaldehyde. This reaction typically requires a coenzyme, such as ferredoxin. Exemplary AOR nucleic acid and amino acid sequences (from *C. ljungdahlii* DSM 13528) are set forth below:
EC number: 1.2.7.5

[0318] Example nucleic acid sequence:

ATGTACGGATATAAGGGTAAGGTATTAAGAATTAATCTAAGTAGTAAAACTTATATAGTGGAAGAATTGAAA
ATTGACAAAGCTAAAAAATTTATAGGTGCAAGAGGGTTAGGCGTAAAAACCTTATTTGACGAAGTAGATCCA
AAGGTAGATCCATTATCACCTGATAACAAATTTATTATAGCAGCGGGACCACTTACAGGTGCACCTGTTCCA
ACAAGCGGAAGATTCATGGTAGTTACTAAATCACCTTTAACAGGAACTATTGCTATTGCAAATTCAGGTGGA
AAATGGGGAGCAGAATTCAAAGCAGCTGGATACGATATGATAATCGTTGAAGGTAAATCTGATAAAGAAGTT
TATGTAAATATAGTAGATGATAAAGTAGAATTTAGGGATGCTTCTCATGTTTGGGGAAAACTAACAGAAGAA
ACTACAAAAATGCTTCAACAGGAAACAGATTCGAGAGCTAAGGTTTTATGCATAGGACCAGCTGGGGAAAAG
TTATCACTTATGGCAGCAGTTATGAATGATGTTGATAGAACAGCAGGACGTGGTGGTGTTGGAGCTGTTATG
GGTTCAAAGAACTTAAAAGCTATTGTAGTTAAAGGAAGCGGAAAAGTAAAATTATTTGATGAACAAAAAGTG
AAGGAAGTAGCACTTGAGAAAACAAATATTTTAAGAAAAGATCCAGTAGCTGGTGGAGGACTTCCAACATAC
GGAACAGCTGTACTTGTTAATATTATAAATGAAAATGGTGTACATCCAGTAAAGAATTTTCAAAAATCTTAT
ACAGATCAAGCAGATAAGATCAGTGGAGAAACTTTAACTAAAGATTGCTTAGTTAGAAAAAATCCTTGCTAT
AGGTGTCCAATTGCCTGTGGAAGATGGGTAAAACTTGATGATGGAACTGAATGTGGAGGACCAGAATATGAA
ACATTATGGTCATTTGGATCTGATTGTGATGTATACGATATAAATGCTGTAAATACAGCAAATATGTTGTGT
AATGAATATGGATTAGATACCATTACAGCAGGATGTACTATTGCAGCAGCTATGGAACTTTATCAAAGAGGT
TATATTAAGGATGAAGAAATAGCAGCAGATGGATTGTCACTTAATTGGGGAGATGCTAAGTCCATGGTTGAA
TGGGTAAAGAAAATGGGACTTAGAGAAGGATTTGGAGACAAGATGGCAGATGGTTCATACAGACTTTGTGAC
TCATACGGTGTACCTGAGTATTCAATGACTGTAAAAAAACAGGAACTTCCAGCATATGACCCAAGAGGAATA
CAGGGACATGGTATTACTTATGCTGTTAACAATAGGGGAGGATGTCACATTAAGGGATATATGGTAAGTCCT
GAAATACTTGGCTATCCAGAAAAACTTGATAGACTTGCAGTGGAAGGAAAAGCAGGATATGCTAGAGTATTC
CATGATTTAACAGCTGTTATAGATTCACTTGGATTATGTATTTTTACAACATTTGGTCTTGGTGCACAGGAT
TATGTTGATATGTATAATGCAGTAGTTGGTGGAGAATTACATGATGTAAATTCTTTAATGTTAGCTGGAGAT
AGAATATGGACTTTAGAAAAAATATTTAACTTAAAGGCAGGCATAGATAGTTCACAGGATACTCTTCCAAAG
AGATTGCTTGAAGAACAAATTCCAGAAGGACCATCAAAAGGAGAAGTTCATAAGTTAGATGTACTACTACCT
GAATATTATTCAGTACGTGGATGGGATAAAAATGGTATTCCTACAGAGGAAACGTTAAAGAAATTAGGATTA
GATGAATACGTAGGTAAGCTTTAG (SEQ ID NO: 25)

[0319] Example amino acid sequence:

MYGYKGKVLRINLSSKTYIVEELKIDKAKKFIGARGLGVKTLFDEVDPKVDPLSPDNKFIIAAGPLTGAPVP
TSGRFMVVTKSPLTGTIAIANSGGKWGAEFKAAGYDMIIVEGKSDKEVYVNIVDDKVEFRDASHVWGKLTEE
TTKMLQQETDSRAKVLCIGPAGEKLSLMAAVMNDVDRTAGRGGVGAVMGSKNLKAIVVKGSGKVKLFDEQKV
KEVALEKTNILRKDPVAGGGLPTYGTAVLVNIINENGVHPVKNFQKSYTDQADKISGETLTKDCLVRKNPCY
RCPIACGRWVKLDDGTECGGPEYETLWSFGSDCDVYDINAVNTANMLCNEYGLDTITAGCTIAAAMELYQRG
YIKDEEIAADGLSLNWGDAKSMVEWVKKMGLREGFGDKMADGSYRLCDSYGVPEYSMTVKKQELPAYDPRGI
QGHGITYAVNNRGGCHIKGYMVSPEILGYPEKLDRLAVEGKAGYARVFHDLTAVIDSLGLCIFTTFGLGAQD
YVDMYNAVVGGELHDVNSLMLAGDRIWTLEKIFNLKAGIDSSQDTLPKRLLEEQIPEGPSKGEVHKLDVLLP
EYYSVRGWDKNGIPTEETLKKLGLDEYVGKL (SEQ ID NO: 26)

[0320] An exemplary acetoacetate decarboxylase (ADC) for use in the present invention catalyzes the decarboxylation of acetoacetate into acetone and CO_2. Exemplary ADC nucleic acid and amino acid sequences (from *C. acetobutylicum* ATCC 824) are set forth below:

EC number: 4.1.1.4

[0321] Example nucleic acid sequence:

ATGTTAAAGGATGAAGTAATTAAACAAATTAGCACGCCATTAACTTCGCCTGCATTTCCTAGAGGACCCTAT
AAATTTCATAATCGTGAGTATTTTAACATTGTATATCGTACAGATATGGATGCACTTCGTAAAGTTGTGCCA
GAGCCTTTAGAAATTGATGAGCCCTTAGTCAGGTTTGAAATTATGGCAATGCATGATACGAGTGGACTTGGT
TGTTATACAGAAAGCGGACAGGCTATTCCCGTAAGCTTTAATGGAGTTAAGGGAGATTATCTTCATATGATG
TATTTAGATAATGAGCCTGCAATTGCAGTAGGAAGGGAATTAAGTGCATATCCTAAAAAGCTCGGGTATCCA
AAGCTTTTTGTGGATTCAGATACTTTAGTAGGAACTTTAGACTATGGAAAACTTAGAGTTGCGACAGCTACA
ATGGGGTACAAACATAAAGCCTTAGATGCTAATGAAGCAAAGGATCAAATTTGTCGCCCTAATTATATGTTG
AAAATAATACCCAATTATGATGGAAGCCCTAGAATATGTGAGCTTATAAATGCGAAAATCACAGATGTTACC
GTACATGAAGCTTGGACAGGACCAACTCGACTGCAGTTATTTGATCACGCTATGGCGCCACTTAATGATTTG
CCAGTAAAAGAGATTGTTTCTAGCTCTCACATTCTTGCAGATATAATATTGCCTAGAGCTGAAGTTATATAT
GATTATCTTAAGTAA (SEQ ID NO: 27)

[0322] Example amino acid sequence:

MLKDEVIKQISTPLTSPAFPRGPYKFHNREYFNIVYRTDMDALRKVVPEPLEIDEPLVRFEIMAMHDTSGLG
CYTESGQAIPVSFNGVKGDYLHMMYLDNEPAIAVGRELSAYPKKLGYPKLFVDSDTLVGTLDYGKLRVATAT
MGYKHKALDANEAKDQICRPNYMLKIIPNYDGSPRICELINAKITDVTVHEAWTGPTRLQLFDHAMAPLNDL
PVKEIVSSSHILADIILPRAEVIYDYLK (SEQ ID NO: 28)

[0323] An exemplary secondary alcohol dehydrogenase (SADH) for use in the present invention catalyzes the reduction of a ketone into a secondary alcohol. For example, acetone into 2-propanol (a.k.a. isopropanol). The exemplary SADH may have EC number 1.1.1.1. Exemplary SADH nucleic acid and amino acid sequences (from *C. ljungdahlii* DSM 13528) are set forth in SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

[0324] An exemplary butyryl-CoA dehydrogenase (BCD) for use in the present invention catalyzes the reduction of crotonyl-CoA into butyryl-CoA by reducing the carbon-carbon double bond in crotonyl-CoA. This enzyme requires an electron-transfer flavoprotein. Exemplary BCD nucleic acid and amino acid sequences (from *C. acetobutylicum* ATCC 824) are set forth below:

EC number: 1.3.8.1

[0325] Example nucleic acid sequence:

ATGGATTTTAATTTAACAAGAGAACAAGAATTAGTAAGACAGATGGTTAGAGAATTTGCTGAAAATGAAGTT
AAACCTATAGCAGCAGAAATTGATGAAACAGAAAGATTTCCAATGGAAAATGTAAAGAAAATGGGTCAGTAT
GGTATGATGGGAATTCCATTTTCAAAAGAGTATGGTGGCGCAGGTGGAGATGTATTATCTTATATAATCGCC
GTTGAGGAATTATCAAAGGTTTGCGGTACTACAGGAGTTATTCTTTCAGCACATACATCACTTTGTGCTTCA
TTAATAAATGAACATGGTACAGAAGAACAAAAACAAAAATATTTAGTACCTTTAGCTAAAGGTGAAAAAATA

GGTGCTTATGGATTGACTGAGCCAAATGCAGGAACAGATTCTGGAGCACAACAAACAGTAGCTGTACTTGAA
GGAGATCATTATGTAATTAATGGTTCAAAAATATTCATAACTAATGGAGGAGTTGCAGATACTTTTGTTATA
TTTGCAATGACTGACAGAACTAAAGGAACAAAAGGTATATCAGCATTTATAATAGAAAAAGGCTTCAAAGGT
TTCTCTATTGGTAAAGTTGAACAAAAGCTTGGAATAAGAGCTTCATCAACAACTGAACTTGTATTTGAAGAT
ATGATAGTACCAGTAGAAAACATGATTGGTAAAGAAGGAAAAGGCTTCCCTATAGCAATGAAAACTCTTGAT
GGAGGAAGAATTGGTATAGCAGCTCAAGCTTTAGGTATAGCTGAAGGTGCTTTCAACGAAGCAAGAGCTTAC
ATGAAGGAGAGAAAACAATTTGGAAGAAGCCTTGACAAATTCCAAGGTCTTGCATGGATGATGGCAGATATG
GATGTAGCTATAGAATCAGCTAGATATTTAGTATATAAAGCAGCATATCTTAAACAAGCAGGACTTCCATAC
ACAGTTGATGCTGCAAGAGCTAAGCTTCATGCTGCAAATGTAGCAATGGATGTAACAACTAAGGCAGTACAA
TTATTTGGTGGATACGGATATACAAAAGATTATCCAGTTGAAAGAATGATGAGAGATGCTAAGATAACTGAA
ATATATGAAGGAACTTCAGAAGTTCAGAAATTAGTTATTTCAGGAAAAATTTTTAGATAA (SEQ ID NO: 29)

[0326] Example amino acid sequence:

MDFNLTREQELVRQMVREFAENEVKPIAAEIDETERFPMENVKKMGQYGMMGIPFSKEYGGAGGDVLSYIIA
VEELSKVCGTTGVILSAHTSLCASLINEHGTEEQKQKYLVPLAKGEKIGAYGLTEPNAGTDSGAQQTVAVLE
GDHYVINGSKIFITNGGVADTFVIFAMTDRTKGTKGISAFIIEKGFKGFSIGKVEQKLGIRASSTTELVFED
MIVPVENMIGKEGKGFPIAMKTLDGGRIGIAAQALGIAEGAFNEARAYMKERKQFGRSLDKFQGLAWMMADM
DVAIESARYLVYKAAYLKQAGLPYTVDAARAKLHAANVAMDVTTKAVQLFGGYGYTKDYPVERMMRDAKITE
IYEGTSEVQKLVISGKIFR (SEQ ID NO: 30)

[0327] An exemplary trans-2-enoyl-CoA reductase (TER) for use in the present invention catalyzes the reduction of crotonyl-CoA into butyryl-CoA by reducing the carbon-carbon double bond in crotonyl-CoA. Exemplary TER nucleic acid and amino acid sequences from *Euglena gracilis* are set forth below:
EC number: 1.3.1.44

[0328] Example nucleic acid sequence:

ATGATAGTAAAAGCAAAGTTTGTAAAAGGATTTATCAGAGATGTACATCCTTATGGTTGCAGAAGGGAAGTA
CTAAATCAAATAGATTATTGTAAGAAGGCTATTGGGTTTAGGGGACCAAAGAAGGTTTTAATTGTTGGAGCC
TCATCTGGGTTTGGTCTTGCTACTAGAATTTCAGTTGCATTTGGAGGTCCAGAAGCTCACACAATTGGAGTA
TCCTATGAAACAGGAGCTACAGATAGAAGAATAGGAACAGCGGGATGGTATAATAACATATTTTTTAAAGAA
TTTGCTAAAAAAAAAGGATTAGTTGCAAAAAACTTCATTGAGGATGCCTTTTCTAATGAAACCAAAGATAAA
GTTATTAAGTATATAAAGGATGAATTTGGTAAAATAGATTTATTTGTTTATAGTTTAGCTGCGCCTAGGAGA
AAGGACTATAAAACTGGAAATGTTTATACTTCAAGAATAAAAACAATTTTAGGAGATTTTGAGGGACCGACT
ATTGATGTTGAAAGAGACGAGATTACTTTAAAAAAGGTTAGTAGTGCTAGCATTGAAGAAATTGAAGAAACT
AGAAAGGTAATGGGTGGAGAGGATTGGCAAGAGTGGTGTGAAGAGCTGCTTTATGAAGATTGTTTTTCGGAT
AAAGCAACTACCATAGCATACTCGTATATAGGATCCCCAAGAACCTACAAGATATATAGAGAAGGTACTATA
GGAATAGCTAAAAAGGATCTTGAAGATAAGGCTAAGCTTATAAATGAAAAACTTAACAGAGTTATAGGTGGT
AGAGCCTTTGTGTCTGTGAATAAAGCATTAGTTACAAAAGCAAGTGCATATATTCCAACTTTTCCTCTTTAT
GCAGCTATTTTATATAAGGTCATGAAAGAAAAAAATATTCATGAAAATTGTATTATGCAAATTGAGAGAATG
TTTTCTGAAAAAATATATTCAAATGAAAAAATACAATTTGATGACAAGGGAAGATTAAGGATGGACGATTTA
GAGCTTAGAAAAGACGTTCAAGACGAAGTTGATAGAATATGGAGTAATATTACTCCTGAAAATTTTAAGGAA
TTATCTGATTATAAGGGATACAAAAAAGAATTCATGAACTTAAACGGTTTTGATCTAGATGGGGTTGATTAT
AGTAAAGACCTGGATATAGAATTATTAAGAAAATTAGAACCTTAA (SEQ ID NO: 31)

[0329] Example amino acid sequence:

MIVKAKFVKGFIRDVHPYGCRREVLNQIDYCKKAIGFRGPKKVLIVGASSGFGLATRISVAFGGPEAHTIGV
SYETGATDRRIGTAGWYNNIFFKEFAKKKGLVAKNFIEDAFSNETKDKVIKYIKDEFGKIDLFVYSLAAPRR
KDYKTGNVYTSRIKTILGDFEGPTIDVERDEITLKKVSSASIEEIEETRKVMGGEDWQEWCEELLYEDCFSD
KATTIAYSYIGSPRTYKIYREGTIGIAKKDLEDKAKLINEKLNRVIGGRAFVSVNKALVTKASAYIPTFPLY
AAILYKVMKEKNIHENCIMQIERMFSEKIYSNEKIQFDDKGRLRMDDLELRKDVQDEVDRIWSNITPENFKE
LSDYKGYKKEFMNLNGFDLDGVDYSKDLDIELLRKLEP (SEQ ID NO: 32)

[0330] Exemplary secondary alcohol dehydrogenase (SADH) nucleic acid and amino acid sequences from *Clostridium beijerinckii* DSM 6423 are set forth below:

Example nucleic acid sequence:

ATGAAAGGTTTTGCAATGCTAGGTATTAATAAGTTAGGATGGATCGAAAAAGAAAGGCCAGTTGCGGGTTCA
TATGATGCTATTGTACGCCCATTAGCAGTATCTCCGTGTACATCAGATATACATACTGTTTTTGAGGGAGCT
CTTGGAGATAGGAAGAATATGATTTTAGGGCATGAAGCTGTAGGTGAAGTTGTTGAAGTAGGAAGTGAAGTG
AAGGATTTTAAACCTGGTGACAGAGTTATAGTTCCTTGTACAACTCCAGATTGGAGATCTTTGGAAGTTCAA
GCTGGTTTTCAACAGCACTCAAACGGTATGCTCGCAGGATGGAAATTTTCAAATTTCAAGGATGGAGTTTTT
GGTGAATATTTTCATGTAAATGATGCGGATATGAATCTTGCGATTCTACCTAAAGACATGCCATTAGAAAAT
GCTGTTATGATAACAGATATGATGACTACTGGATTTCATGGAGCAGAACTTGCAGATATTCAAATGGGTTCA
AGTGTTGTGGTAATTGGCATTGGAGCTGTTGGCTTAATGGGAATAGCAGGTGCTAAATTACGTGGAGCAGGT
AGAATAATTGGAGTGGGGAGCAGGCCGATTTGTGTTGAGGCTGCAAAATTTTATGGAGCAACAGATATTCTA
AATTATAAAAATGGTCATATAGTTGATCAAGTTATGAAATTAACGAATGGAAAAGGCGTTGACCGCGTAATT
ATGGCAGGCGGTGGTTCTGAAACATTATCCCAAGCAGTATCTATGGTTAAACCAGGAGGAATAATTTCTAAT
ATAAATTATCATGGAAGTGGAGATGCTTTACTAATACCACGTGTAGAATGGGGATGTGGAATGGCTCACAAG
ACTATAAAAGGAGGTCTTTGTCCTGGGGGACGTTTGAGAGCAGAAATGTTAAGAGATATGGTAGTATATAAT
CGTGTTGATCTAAGTAAATTAGTTACACATGTATATCATGGATTTGATCACATAGAAGAAGCACTGTTATTA
ATGAAAGACAAGCCAAAAGACTTAATTAAAGCAGTAGTTATATTATAA (SEQ ID NO: 33)

Example amino acid sequence:

MKGFAMLGINKLGWIEKERPVAGSYDAIVRPLAVSPCTSDIHTVFEGALGDRKNMILGHEAVGEVVEVGSEV
KDFKPGDRVIVPCTTPDWRSLEVQAGFQQHSNGMLAGWKFSNFKDGVFGEYFHVNDADMNLAILPKDMPLEN
AVMITDMMTTGFHGAELADIQMGSSVVVIGIGAVGLMGIAGAKLRGAGRIIGVGSRPICVEAAKFYGATDIL
NYKNGHIVDQVMKLTNGKGVDRVIMAGGGSETLSQAVSMVKPGGIISNINYHGSGDALLIPRVEWGCGMAHK
TIKGGLCPGGRLRAEMLRDMVVYNRVDLSKLVTHVYHGFDHIEEALLLMKDKPKDLIKAVVIL (SEQ ID NO: 34)

[0331] Exemplary PTS nucleic acid and amino acid sequences (from *C. acetobutylicum* ATCC 824) are set forth below:

Example nucleic acid sequence:

ATGGGTAATAAGATATTTGCCGTACTTCAAAAAATCGGTAAATCTTTAATGCTTCCAGTATCTGTTCTACCG
GCGGCTGGAATTCTATTGAGACTTGGACAGCCAGATTTGCTTAATATGCCTTATGTTCAAGCAGCAGGAAAT
GCTATTTTTAATAATTTACCTTTAATTTTTGCGGTTGGAGTTGCTATAGGTTTTTCAGGCGGAGAAGGTGTT
GCAGCACTTGCAGCTGTAATTGGAGAACTAATACTTGAGGCGGTTGAAAAAACAGCAGGTGATACGGCAGCA
GCAGCTTTAGCAAAAACAGCGGCAGCTTCACATCATATGACGCTTGCAGCATTTCAAAAAACTCAAGAATAC

AGTAACATTGTAACTAAAACAACTATTAGTATGGGTGTTTTTGGCGGTATAATAATCGGTCTTACAGCAGCT
ATTTTATATAATAAGTTCCATGATATAAAGATGCCTCAAGTTTTGGGTTTCTTTGGTGGAAAACGTTTCGTT
CCAATTATAACTTCAATTTCAGCTCTTATTATTGCAACTATAGGAGTAAATATTTGGTTGCCAATACAAGCT
GGAATAAATTCACTAGCAGCATTTGCAACTACATCACCAATTGGACCTGCAATGTATGCTGGAGGAAAAAGA
CTTTTAATTCCACTAGGACTTCATCACATTTATTATCCATTGTTCTTATATCAATTTGGTCACTTTGTTTCA
AATGGAGTTACTTATGTAGGAGATACAGCAAGATACTTCCATGGGGATCCTACTGCCGGAAACTTCATGGCA
GCAGAGTATCCAATACTAATGTTTGGTCTTCCAGGAGCTGCTCTAGCTATGATTGCAGCTGCTAAAAAAGAA
AAGAGAAAAGAAATGGCTGGAATGATGATTTCAGCAGCATTTGTAGCATTTGTAACAGGTATTACAGAGCCA
ATTGAATTCTCATTCATATTTGTTGCTCCAGTATTATTTGTGTTCCACGTACTTGCTGCATTCGCATCTGGT
CTTATTACAAGTTATTTACACATAAGATTAGGGTATACTTTCTCAGCATCCTTCATAGATTATGTTTTAGGA
TTCAAATATGCAGGTCATCCATTACTTATATGGCTTGTAGGTATAGGGTTCTTTGTATTGTACTTTGTTGTA
TTCTACTTCACAATTAAAGCAATGAACATTAAGACACCAGGTAGAGAAGATGATGATGCAGAAGGTGTTAAG
AAGATAAATGTAAAAGGAAAAGCTAAGGCAGCTAAGGTGCTTGAAGCTATAGGCGGAAAAGATAATATAAAA
GTACTTGATGCATGTATAACAAGATTAAGACTTAACTTAAATGATCCTTCTTTGGTTGATAAAGCTACACTT
AAAGCTCTTGGAGCAGCTGGAGTAATGACAGCAGAAGATAGTGTTCAAGTAGTTTTTGGAACTGAAGCTGAA
AGAATAAAAGATGACATAAAAGCAATTATACAAAATGGTGGATATGTTGAAGATGATTCAGATAAGGAAGAA
GAAGTTCAAGAGGATAAGCAAATTTCTAAAGGTGCACACGAACTTTTAAGTCCAGCTGATGGAGAAGTAGTT
GGTATTGAGAGTGTTCCGGATAGTACATTTGCTGAAAAAATGCTTGGAGACGGTTTTGCAGTAATACCTTCA
GGAAATGAAGTACACTCACCAGCTGATGGAGAAGTATCAGTTTTATTCCCAACTAAGCATGCATTTGCAATA
ACAACAGAAGGCGGACTTGAACTTTTAATACATGTTGGAATTGATACTGTAGCATTAAATGGTGAAGGTTTC
ACAGCACATGTAAAACAAGGAGATAAAGTTAAAAAAGGAGATTTGATTTTAACTTTAGATACTGAGTTTATA
AAGAGCAAAGGTAAAAACCTTATAACTCCAGTGATTGTAACTAACATGGATGTTGTAGGAAATATAGATGTT
AAATTAGGAAACGTTAAAAATTCCGAGAAAGCTGCGGATGTTACCGTAAAATAA (SEQ ID NO: 35)

Example amino acid sequence:

MGNKIFAVLQKIGKSLMLPVSVLPAAGILLRLGQPDLLNMPYVQAAGNAIFNNLPLIFAVGVAIGFSGGEGV
AALAAVIGELILEAVEKTAGDTAAAALAKTAAASHHMTLAAFQKTQEYSNIVTKTTISMGVFGGIIIGLTAA
ILYNKFHDIKMPQVLGFFGGKRFVPIITSISALIIATIGVNIWLPIQAGINSLAAFATTSPIGPAMYAGGKR
LLIPLGLHHIYYPLFLYQFGHFVSNGVTYVGDTARYFHGDPTAGNFMAAEYPILMFGLPGAALAMIAAAKKE
KRKEMAGMMISAAFVAFVTGITEPIEFSFIFVAPVLFVFHVLAAFASGLITSYLHIRLGYTFSASFIDYVLG
FKYAGHPLLIWLVGIGFFVLYFVVFYFTIKAMNIKTPGREDDDAEGVKKINVKGKAKAAKVLEAIGGKDNIK
VLDACITRLRLNLNDPSLVDKATLKALGAAGVMTAEDSVQVVFGTEAERIKDDIKAIIQNGGYVEDDSDKEE
EVQEDKQISKGAHELLSPADGEVVGIESVPDSTFAEKMLGDGFAVIPSGNEVHSPADGEVSVLFPTKHAFAI
TTEGGLELLIHVGIDTVALNGEGFTAHVKQGDKVKKGDLILTLDTEFIKSKGKNLITPVIVTNMDVVGNIDV
KLGNVKNSEKAADVTVK (SEQ ID NO: 36)

[0332] Exemplary PTS nucleic acid and amino acid sequences (from *C. saccharobutylicum* DSM 13864) are set forth below:

Example nucleic acid sequence:

ATGTTAGTTGATGCTTTTTTGCGCACAAAGCATCTAAAACATCCCTATATACAAAAAATATTTAAAAAAGGG
GAAAATGTTATGAAAGACAAAATTTTTGGTATTTTACAGCGTGTAGGAAGATCTTTTATGCTTCCAATAGCT
ATTTTACCAGTAGCTGGATTATTTCTTGGTTTAGGTGGATCATTTACCAATGAAACAATGATTCAAGCTTAT
GGACTTACTGGGTTAATCGGACCTGGTACATTTATTTATTCAATCCTTTCTGTAATGAATGCAGCAGGAAAT
GTAGTGTTTGGCAATTTGCCTTTATTATTTGCAATGGGTGTTGCAATTGGTATGGCTAAAAAGGAAAAAGAT
GTTGCAGCTTTATCAGCAGCAATTGCATTCCTTATAATGCATGCATCAATAAGTGCTATGATTAATATTAAT
GGTGGAACTGATGCTCTTCTTAGTGGTGCATCAACTTCAGTACTTGGTATTACTTCATTACAAATGGGTGTT

TTTGGTGGTATTATTGTAGGGCTTGGAGTAGCAGCGTTACATAATAAATTCTATAAAATTGAATTACCACAG
GTATTATCATTCTTTGGTGGAACTAGATTTGTTCCAATTGTAAGTGCAATAACATATTTAATTGTTGGAATT
TTAATGTTTTATATTTGGCCTCCAATTCAAGGTGGTATTTATAAAGTTGGAGATCTTGTATTAAGATCAGGA
TATGCAGGAACATGGCTTTATGGTTTAATGGAACGTTTATTAATACCTTTTGGTCTTCATCATGTATTTTAC
TTACCATTCTGGCAAACAGCAGTTGGTGGTACAGCTACAGTAGGTGGGAAAGTTATTGAAGGTGCTCAAAAT
ATTTTCTTTGCTGAACTTGGAACTCCAGGAATAACACACTTTAGTGTTTCAGCAACAAGATTTATGTCAGGT
AAATTCCCACTTATGATATTTGGTTTACCTGGAGCAGCGCTTGCGATGTACAGATGTGCAAAACCAGAAAAG
AGAAAAGTAGTAGGTGGATTATTATTATCAGCAGCATTAACTTCGATGTTAACTGGTATTACAGAACCAATT
GAATTTACATTCTTATTTGTTGCACCATTATTATATGGAATTCACTGCGTATTTGCTGGACTAGCTTATATG
TTTATGCATATGTTAAATGTTGGAGTTGGTATGACTTTCTCTGGTGGATTTATAGATTTATTCTTATTTGGT
ATTTTACAAGGAAATGCTAAGACAAGTTGGATTTGGGTTGCAGTTGTAGGTATTGCATATTTTGTAGTATAC
TATGTATTGTTCTCTTTCTTAATTAAGAAGCTTGACTTAAAGACTCCAGGTCGTGATGATTCTGAAGAAGTT
AAACTTTATCGTAGAAGCGATTTAGATGCAAAGAAAAAAGGTAATAATGCAGATAATGGAGAAAGTGAAAGT
ATAGATGAATTATCAGCTATGATCTGTCAAGGTCTTGGTGGTAAGAAGAATATTTCAGATGTTGACTGTTGC
GCAACTAGATTACGTTGTACAGTTGTTAAATCAGAATTAGTAAATGAAGCTTTATTAAAACAAACTGGAGCA
TCAGGAGTAGTTCATAAAGGCGTAGGTGTTCAAATTATATATGGACCAAGAGTAACAGTTATAAAATCTAAT
TTAGAAGATTATTTAATTGCAGCACCTGATGAAGAAGTTGCTATAGATGCAGTAGAAGAAAAAGCACCTGAA
ATGCCAACTGAAAAGGAAGCGGAAGGAAAAGTTGTTAATACAATAGTTTTAAGCAGTCCATTAACAGGAGAT
GCTAAAGATTTATCAGAAGCTCCAGATGAAGCATTTGCAAGCAAAATGATGGGAGACGGAGCGGTAGTAATT
CCAAGTAATGGAGATGTTGTAGCACCAGCAGATGGTGAGGTGAGTTTTGTATTCCCATCAAAACATGCAGTA
GGATTAACAACAACTGATGGTCTTGAATTACTTATTCATATAGGAATAGATACAGTAAAGCTTGATGGAAAA
GGCTTTGAAACTTTCGTAAAAGAAGGAGACAAAGTTAAAAAAGGTGATAAATTATTAAGCTTTGACTTAGAA
TTTATAAAAGAAAATGCACCATCTATTGCATCACCATGCATTTGTACAGCATTAAGCAGCAAACAAAAAGTA
CGTTTGTTAAAAACAGGTGATATAAAGGCAGGAGAAGACTTAATAGCAATTGATGTGCTTGAATAA (SEQ ID NO: 37)

Example amino acid sequence:

MLVDAFLRTKHLKHPYIQKIFKKGENVMKDKIFGILQRVGRSFMLPIAILPVAGLFLGLGGSFTNETMIQAY
GLTGLIGPGTFIYSILSVMNAAGNVVFGNLPLLFAMGVAIGMAKKEKDVAALSAAIAFLIMHASISAMININ
GGTDALLSGASTSVLGITSLQMGVFGGIIVGLGVAALHNKFYKIELPQVLSFFGGTRFVPIVSAITYLIVGI
LMFYIWPPIQGGIYKVGDLVLRSGYAGTWLYGLMERLLIPFGLHHVFYLPFWQTAVGGTATVGGKVIEGAQN
IFFAELGTPGITHFSVSATRFMSGKFPLMIFGLPGAALAMYRCAKPEKRKVVGGLLLSAALTSMLTGITEPI
EFTFLFVAPLLYGIHCVFAGLAYMFMHMLNVGVGMTFSGGFIDLFLFGILQGNAKTSWIWVAVVGIAYFVVY
YVLFSFLIKKLDLKTPGRDDSEEVKLYRRSDLDAKKKGNNADNGESESIDELSAMICQGLGGKKNISDVDCC
ATRLRCTVVKSELVNEALLKQTGASGVVHKGVGVQIIYGPRVTVIKSNLEDYLIAAPDEEVAIDAVEEKAPE
MPTEKEAEGKVVNTIVLSSPLTGDAKDLSEAPDEAFASKMMGDGAVVIPSNGDVVAPADGEVSFVFPSKHAV
GLTTTDGLELLIHIGIDTVKLDGKGFETFVKEGDKVKKGDKLLSFDLEFIKENAPSIASPCICTALSSKQKV
RLLKTGDIKAGEDLIAIDVLE (SEQ ID NO: 38)

[0333] Amino acid sequences as described herein may include amino acid sequences having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity the disclosed wild type sequences. A corresponding polynucleotide sequence may be a polynucleotide sequence encoding a wild type amino acid sequence as described herein, and may further include a polynucleotide sequence which encodes a protein having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to a wild type amino acid sequence as described herein. A polynucleotide sequence as described herein may include a sequence which has 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the described wild type polynucleotide sequence.

Examples

Example 1

[0334] Acetogenic clostridia strain *C. ljungdahlii* was cultured under three conditions: 5 g/l of fructose (first feedstock) with a N_2 headspace at 20 psig (referred to as heterotrophic fermentation), no fructose with a CO (second feedstock) headspace at 20 psig (autotrophic fermentation), and 5 g/l of fructose with a CO headspace at 20 psig (mixotrophic fermentation). Three biological replicates were prepared, grown at 37°C and shaken at 225 rpm. Table 1 shows the metabolite profiles and carbon yields achieved. Carbon yield for this experiment is calculated by dividing the total amount of carbon in produced bioproducts by the total amount of carbon metabolized from the first feedstock during fermentation.

Table 1.

5 g/l fructose with N_2 headspace Concentration (mM)						
Hour	Fructose	Acetate	Ethanol	2,3-butanediol	Lactate	Carbon yield
0	27.52	3.74	0.29	0.11	0.00	-
41	25.36	8.28	1.98	0.13	0.00	67%
49	19.06	16.58	5.77	0.19	0.00	65%
56	10.19	29.27	8.29	0.36	0.00	62%
65	0.62	44.19	12.63	0.63	0.00	64%
73	0.05	45.07	13.60	0.66	0.00	65%
80	0.03	45.11	14.81	0.72	0.00	67%
5 g/l fructose with CO headspace Concentration (mM)						
Hour	Fructose	Acetate	Ethanol	2,3-butanediol	Lactate	Carbon yield
0	27.23	3.74	0.00	0.11	0.00	-
49	25.86	4.16	0.48	0.19	0.16	32%
56	25.12	4.30	0.75	0.19	0.12	26%
65	25.07	5.14	1.49	0.35	0.14	55%
73	22.39	5.85	2.15	0.39	0.17	35%
80	22.32	7.96	4.19	0.55	0.23	65%
85	20.36	9.76	5.62	1.77	0.21	74%
89	19.69	12.14	5.24	2.46	0.49	84%
97	16.56	17.62	9.26	4.49	1.00	104%
104	9.68	19.22	14.75	8.70	1.38	94%
113	3.07	27.38	23.10	16.53	2.59	115%
121	0.00	29.65	22.65	17.85	2.63	108%
CO headspace (no fructose) Concentration (mM)						
Hour	Fructose	Acetate	Ethanol	2.3-butanediol	Lactate	
0	0.00	3.72	0.00	0.12	0.00	
41	0.00	4.02	0.47	0.14	0.00	
49	0.00	4.17	0.78	0.11	0.00	
56	0.00	4.32	0.46	0.10	0.25	
65	0.00	4.65	0.64	0.00	0.22	
73	0.00	4.71	0.52	0.08	0.20	
80	0.00	4.94	0.69	0,14	0.19	
89	0.00	5.63	0.74	0.14	0.18	
97	0.00	7.12	1.03	0.20	0.00	
104	0.00	8.26	1.07	0.24	0.19	
113	0.00	13.50	1.81	0.51	0.75	
121	0.00	17.62	2.51	0.93	0.32	

[0335] These results exemplify the non-additive, i.e., synergistic nature of the mixotrophic fermentation. Combining the heterotrophic fermentation broth with the autotrophic fermentation broth, the molar ratios of acetate, ethanol, 2,3-butanediol and lactate are: 0.77, 0.20, 0.02, and 0.004, respectively. In comparison, those for the mixotrophic fermentation are 0.40, 0.31, 0.24, and 0.04, respectively. The proportion of the more reduced products, ethanol, lactic acid and 2.3-butanediol is increased, while that of acetate decreases. Thus, the fraction of 2,3-butanediol in the mixotrophic fermentation is more than 10 times greater than that in the combination and that of acetate is about one half. In comparison, in the autotrophic fermentation, the molecular fraction of acetate is 0.82.

[0336] Additionally, the results show that, by the time the carbohydrate is metabolized, the mixotrophic fermentation has a much greater carbon yield compared to the heterotrophic fermentation.

[0337] This example demonstrates the ability to increase carbon efficiencies and increase the yield of reduced product with mixotrophic fermentation.

Example 2

[0338] Acetogenic clostridia strain *C. ljungdahlii* was cultured under two conditions: 10 g/l of fructose (first feedstock) with a headspace of the gas mixture of CO, CO_2, H_2, and N_2 (55%, 10%, 20%, 15%, respectively) (second feedstock) at 30 psig (referred to as mixotrophic fermentation) and no fructose with a headspace of the gas mixture of CO, CO_2, H_2, and N_2 (55%, 10%, 20%, 15%, respectively) (second feedstock) at 30 psig (referred to as autotrophic fermentation). Two biological replicates were prepared, grown at 37°C and shaken at 225 rpm. The CO and CO_2 were labeled with ^{13}C, allowing the ability to track the uptake and incorporation of the carbon substrates.

[0339] Fig. 1 shows the percentage of ^{13}C labeling of the metabolite acetate over time in both cultures. Average ^{13}C labeling of acetate for autotrophic (A) and mixotrophic (M) cultures between two biological replicates is shown. For the mixotrophic cultures, fructose was never depleted over the time sampled. The final concentration of fructose at timepoint 168 hr was 7.9 g/1.

[0340] The only way acetate could be labeled with ^{13}C is if the labeled gas, either ^{13}CO or $^{13}CO_2$, was utilized by the Wood-Ljungdahl pathway and used to form acetyl-CoA. For the autotrophic cultures, >90% of the acetate was labeled with ^{13}C, indicating that less than 10% of the carbon came from the inoculum culture and yeast extract in the medium. For the mixotrophic cultures, ~80% of the acetate was labeled, even in the presence of excess fructose. This indicates that *C. ljungdahlii* is able to utilize and consume gas in the presence of excess sugar.

Example 3 (comparative)

[0341] The test of Example 1 was repeated using the acetogenic clostridia strain *C. autoethanogenum*. Table 2 shows the metabolite profiles and carbon yields.

[0342] Similar to the first example, the cultures with both fructose and a CO headspace had greater carbon efficiencies, indicating gas consumption. In addition, the mixotrophic cultures produced greater amounts of 2,3-butanediol. Compared to the pure gas culture, the mixotrophic culture produced bioproducts at a much faster rate and produced less acetate, relative to other bioproducts.

Table 2.

5 g/l fructose with N_2 headspace Concentration (mM)					
Hour	Fructose	Acetate	Ethanol	2,3-butanediol	Carbon yield
0	27.55	6.71	3.46	0.00	-
84	26.89	9.15	4.74	0.00	120%
94	23.43	12.69	7.14	0.38	77%
120	3.80	42.39	23.84	0.79	80%
170	0.00	46.77	25.92	0.96	77%
5 g/l fructose with CO headspace Concentration (mM)					
Hour	Fructose	Acetate	Ethanol	2,3-butanediol	Carbon yield
0	24.62	7.08	3.81	0.19	-
53	23.75	9.11	6.26	0.19	170%
58	22.35	10.62	10.09	0.36	149%
63	20.73	12.69	14.25	0.64	145%
68	17.37	15.67	25.45	1.51	151%
77	9.78	43.77	27.77	4.68	156%
84	3.98	69.20	20.78	5.18	144%
94	0.00	79.86	21.54	5.55	137%
CO headspace (no fructose) Concentration (mM)					
Hour	Fructose	Acetate	Ethanol	2,3-butanediol	
0	0.00	6.82	3.68	0.00	
84	0.00	7.22	3.95	0.00	

(continued)

CO headspace (no fructose) Concentration (mM)				
Hour	Fructose	Acetate	Ethanol	2,3-butanediol
94	0.00	8.07	4.35	0.00
120	0.00	9.43	8.58	0.00
170	0.00	60.24	1.12	1.82

[0343] As in Example 1, the proportion of 2,3-butanediol is greater in the mixotrophic fermentation compared with that in either heterotrophic fermentation or autotrophic fermentation. The carbon yield at the time of carbohydrate exhausting in case of mixotrophic fermentation is nearly double that in autotrophic fermentation.

Example 4

[0344] A strain of *C. ljungdahlii* was constructed with a recombinant pathway expressing a thiolase (also known as acetyl-CoA acetyltransferase) gene, an acetoacetate transferase subunit A (also known as CoA-transferase subunit A, or COAT A) gene, an acetoacetate transferase subunit B (also known as CoA-transferase subunit B, or COAT B) gene, an acetoacetate decarboxylase (ADC) gene, and a secondary alcohol dehydrogenase (SADH) gene. All genes were derived from *C. acetobutylicum* ATCC 824 except for the secondary alcohol dehydrogenase gene, which came from *C. beijerinckii* DSM 6423. Three biological replicates of this strain were grown anaerobically in media containing initially 5 g/l of fructose and were fed additional fructose over time. The headspace consisted of N_2, CO_2, and H_2 (85%, 10%, 5%, respectively) at 1 atm.

[0345] These cultures produced three main metabolites: isopropanol, acetone, and acetate. Acetone is an intermediate metabolite of isopropanol, and so the titers and yields of these two metabolites are combined together. Table 3 shows the metabolite production of these cultures.

Table 3.

Biological Replicate	Acetone & Isopropanol titer (g/L)	Acetate titer (g/L)	Acetate in metabolite pool (wt%)	Sugar metabolized (g/L)	Acetone & isopropanol mass yield (wt%)	Acetone & isopropanol yield if all acetate were re-assimilated (wt%)
#1	3.90	0.75	19.3%	8.89	41.5%	45.4%
#2	3.76	0.85	22.5%	8.64	41.1%	45.6%
#3	3.77	0.96	25.6%	8.78	40.6%	45.6%

[0346] Mass yield is calculated by dividing total concentration of products produced by the total amount of sugar metabolized. As can be seen, a mass yield of ~41% was achieved for acetone and isopropanol.

[0347] This mass yield is greater than could theoretically be from fructose alone. Table 4 outlines different theoretical mass yields based on different substrates.

Table 4.

Mode of fermentation	Fructose only - no mixotrophy	Limited mixotrophy	H_2 supplemented mixotrophy	Full mixotrophy (CO only)	Full mixotrophy (CO_2 & H_2)
Acetone mass yield on sugar (wt%)	30.0%	47.1%	62.8%	90%	95.5%

[0348] If no gas was metabolized (fructose only - no mixotrophy) the maximum yield for acetone is only 30%, while the recombinant strain producing acetone/isopropanol had a mass yield of 41%. If all the acetate produced by the recombinant strain were reassimilated into acetone and isopropanol, the mass yields increase to 45%, close to the theoretical maximum of limited mixotrophy (47.1%). This limited mixotrophy is defined as sugar and gas consumption, where the only source of gas is the CO_2 evolved during metabolism of the sugar, and potentially hydrogen generated from pyruvate ferredoxin oxidoreductase activity. There is no exogenous CO, CO_2 or H_2 fed to the fermentation. These mass yields can be further increased with other modes of mixotrophy, such as:

H_2 supplemented mixotrophy - H_2 supplemented mixotrophy is defined as sugar and gas consumption, where the only source of CO_2 comes from glycolysis, and H_2 is minimally provided by pyruvate ferredoxin oxidoreductase activity and largely provided by exogenous H_2.

Full supplemented mixotrophy - Full supplemented mixotrophy is defined as sugar and gas consumption, where CO_2, CO and/or H_2 can be provided exogenously in addition to the gas evolved by glycolysis and/or pyruvate ferredoxin oxidoreductase activity.

Example 5

[0349] *C. ljungdahlii* DSM 13528 has a native secondary alcohol dehydrogenase gene (accession number CLJU_c24860) with a nucleic acid sequence of:

ATGAAAGGTTTTGCAATGTTAGGTATTAACAAATTAGGATGGATTGAAAAGAAAAACCCAGTGCCAGGTCCT
TATGATGCGATTGTACATCCTCTAGCTGTATCCCCATGTACATCAGATATACATACGGTTTTTGAAGGAGCA
CTTGGTAATAGGGAAAATATGATTTTAGGCCATGAAGCTGTAGGTGAAATAGCCGAAGTTGGCAGCGAAGTT
AAAGATTTTAAAGTTGGCGATAGAGTTATCGTACCATGCACAACACCTGACTGGAGATCTTTAGAAGTCCAA
GCTGGTTTTCAGCAGCATTCAAACGGTATGCTTGCAGGATGGAAGTTTTCCAATTTTAAAGATGGTGTATTT
GCAGATTACTTTCATGTAAACGATGCAGATATGAATCTTGCCATACTCCCAGATGAAATACCTTTAGAAAGT
GCAGTTATGATGACAGACATGATGACTACTGGTTTTCATGGAGCAGAACTTGCAGACATAAAAATGGGCTCC
AGCGTTGTAGTAATTGGTATAGGAGCTGTTGGATTAATGGGAATAGCCGGTTCCAAACTTCGAGGAGCAGGC
AGAATTATCGGTGTTGGAAGCAGACCTGTTTGTGTTGAAACAGCTAAATTTTATGGAGCAACTGATATTGTA
AATTATAAAAATGGTGATATAGTTGAACAAATCATGGACTTAACTCATGGTAAAGGTGTAGACCGTGTAATC
ATGGCAGGCGGTGGTGCTGAAACACTAGCACAAGCAGTAACTATGGTTAAACCTGGCGGCGTAATTTCTAAC
ATCAACTACCATGGAAGCGGTGATACTTTACCAATACCTCGTGTTCAATGGGGCTGCGGCATGGCTCACAAA
ACTATAAGAGGAGGATTATGCCCCGGCGGACGTCTTAGAATGGAAATGCTAAGAGATCTTGTTCTATATAAA
CGTGTTGATTTGAGTAAACTTGTTACTCATGTATTTGATGGTGCAGAAAATATTGAAAAGGCCCTTTTGCTT
ATGAAAAATAAGCCAAAAGATTTAATTAAATCAGTAGTTACATTCTAA (SEQ ID NO: 1).

[0350] This gene was deleted from the chromosome and replaced with a chloramphenicol acetyltransferase (CAT) gene, which confers resistance to the antibiotic chloramphenicol or thiamphenicol. This new strain is termed *Clj* ΔSADH.

[0351] A plasmid expressing the genes for thiolase (also known as acetyl-CoA acetyltransferase), acetoacetate transferase subunit A (COAT A), acetoacetate transferase subunit B (COAT B), and acetoacetate decarboxylase (ADC) was transformed into this deletion strain. All genes came from C. *acetobutylicum* ATCC 824.

[0352] The deletion strain with the plasmid was grown anaerobically in media containing 5 g/l of fructose. The headspace consisted of N_2, CO_2, and H_2 (85%, 10%, 5%, respectively) at 1 atm.

[0353] This strain produced primarily acetone and acetate. Table 5 shows the metabolite production of this strain.

Table 5.

Acetone titer (g/L)	Acetate titer (g/L)	Acetate in metabolite pool (wt%)	Sugar metabolized (g/L)	Acetone mass yield (wt%)	Acetone yield if all acetate were re-assimilated (wt%)
2.65	0.77	22.0%	6.79	39.1%	45.6%

[0354] Mass yield is calculated as in Example 4. The mass yield for this strain is 39.1% for acetone, which is greater than can be achieved on sugar alone (Table 4). This demonstrates the ability of this strain to produce enhanced mass yields over sugar alone.

Example 6

[0355] A plasmid was constructed to overexpress a glucose-specific EIIABC gene from *Clostridium acetobutylicum* ATCC 824 (CA C0570). This plasmid was transformed into *Clostridium ljungdahlii* DSM 13528 to make the strain *Clj* (pCAC-EIIABC). Biological replicates of this strain were grown in 10 ml of PETC medium with 5 g/l of fructose and thiamphenicol (5 μg/ml). Once the cultures reached an OD600 of 1.0, 1 ml (10 % inoculum) was used to inoculate new tubes of 10 ml of PETC medium with 5 g/l of glucose and thiamphenicol (5 μg/ml). The metabolite profile of these glucose-grown cultures is shown in Table 6.

Table 6.

Replicate	Day	Concentration (g/l) Glucose	Fructose	Acetate	Ethanol	Glucose metabolized (g/l)	Rate of consumption (g/hr/g cell mass)
#1	0	5.19	0.33	0.32	0.98	-	-
	3	4.62	0.00	1.71	0.67	0.57	-
	4	4.16	0.00	2.12	0.67	1.03	-
	6	3.09	0.00	3.11	0.70	2.10	0.07
#2	0	5.21	0.33	0.33	0.99	-	-
	3	4.97	0.00	1.35	0.69	0.24	-
	4	4.73	0.00	1.60	0.70	0.47	-
	6	3.79	0.00	2.53	0.69	1.42	0.07

[0356] Replicate #1 metabolized 2.1 g/l of glucose over 6 days, while replicate #2 metabolized 1.42 g/l of glucose over the same period. A plasmid control culture metabolized no glucose over this same time period. The strains also metabolized the residual fructose from the inoculum (0.33 g/l in each case) and some ethanol from the antibiotic (~0.3 g/1). However, the majority of the carbon metabolized was glucose. The maximum rate of consumption of glucose was 0.07 g/hr/g cell mass. This was calculated between Days 4 and 6, after the cultures reached their maximum cell density ($OD_{600} \approx 1.0$). Replicate #1 reached its maximum cell density by Day 3, while Replicate #2 reached this by Day 4. Thus Replicate #1 metabolized a greater amount of glucose than Replicate #2, though they both had the same maximum rate of consumption. In this particular example, the measured rate is about 65% less than the typical batch consumption rate of fructose.

<u>Example 7</u>

[0357] Two acetogenic clostridial strains were tested for mixotrophic growth: *Clostridium ljungdahlii* DSM 13528 and *Clostridium autoethanogenum* DSM 10061. Both strains were cultured under three conditions: 10 g/l of fructose (first feedstock) with a N_2 headspace at 20 psig (referred to as heterotrophic fermentation), no fructose with a syngas headspace ($CO:CO_2:H_2:N_2$, 55:10:20:15, second feedstock) headspace at 30 psig (autotrophic fermentation), and 10 g/l of fructose with a syngas headspace ($CO:CO_2:H_2:N_2$, 55:10:20:15, second feedstock) headspace at 30 psig (mixotrophic fermentation). In addition, a control culture of C. *acetobutylicum* ATCC 824, that cannot metabolize CO_2, was prepared using the heterotrophic conditions to compare against the two acetogens. Three biological replicates of each strain were prepared, grown at 37°C in standard PETC medium and shaken at 200 rpm. The pH was actively controlled with 6M NaOH to keep the pH between 5 and 6. Headspace volumes for the autotrophic and mixotrophic fermentations was replenished every 2-3 days.

[0358] Metabolite profiles and carbon yields are shown in the Tables below. Carbon yield is calculated in this example by dividing the total amount of carbon in produced bioproducts by the total amount of carbon metabolized from the sugar in the first feedstock. In the case for metabolites derived from acetyl-CoA, the theoretical maximum without CO_2 fixation is 67%.

Table 7. Heterotrophic fermentation of *C. ljungdahlii.*

Heterotrophic culture (10 g/l fructose with N_2 headspace)						
	Concentration (mM)					
Hour	Fructose	Acetate	Ethanol	2,3-Butanediol	Lactate	Carbon yield
0	55.78	0.00	0.00	0.00	0.14	-
29	50.08	10.69	3.87	0.00	0.12	86%
47	40.57	34.45	5.82	0.00	0.07	88%
70	27.47	68.20	4.78	0.00	0.12	86%
97	13.98	98.18	5.09	0.10	0.16	83%
121	4.80	120.33	6.21	0.17	0.23	83%
144	0.25	129.28	7.51	0.19	0.33	83%

Table 8. Mixotrophic fermentation of *C. ljungdahlii.*

Mixotrophic culture (10 g/l fructose with syngas headspace)						
	Concentration (mM)					
Hour	Fructose	Acetate	Ethanol	2,3-Butanediol	Lactate	Carbon yield
0	57.41	0.00	0.00	0.00	0.16	-
19	57.19	1.01	0.14	0.00	0.21	220%
42	49.60	20.52	1.87	0.03	0.20	97%
67	37.20	86.87	4.93	0.92	0.14	155%
93	34.56	112.37	5.56	3.55	0.72	184%
114	33.32	120.78	7.15	5.48	1.57	195%
138	31.38	127.57	7.90	6.64	1.89	194%
162	29.34	134.32	8.78	7.39	2.29	192%

Table 9. Autotrophic fermentation of *C. ljungdahlii.*

Autotrophic culture (syngas headspace)						
	Concentration (mM)					
Hour	Fructose	Acetate	Ethanol	2,3-Butanediol	Lactate	Carbon yield*
0	0.00	0.00	0.00	0.00	0.16	-
25	0.00	0.43	0.00	0.00	0.16	-
41	0.00	1.18	0.10	0.00	0.17	-
65	0.00	8.15	0.98	0.18	0.32	-
90	0.00	38.05	4.17	1.86	0.51	-
113	0.00	39.85	3.43	1.85	0.36	-
138	0.00	39.25	7.27	1.98	0.29	
164	0.00	31.32	23.67	3.18	0.35	-
185	0.00	58.88	20.79	3.93	0.17	-
209	0.00	68.27	18.09	4.03	0.11	-

(continued)

Autotrophic culture (syngas headspace)						
	Concentration (mM)					
Hour	Fructose	Acetate	Ethanol	2,3-Butanediol	Lactate	Carbon yield*
233	0.00	57.82	33.30	4.90	0.36	-
* Carbon yield cannot be determined for autotrophic fermentations because there is no sugar substrate.						

Table 10. Heterotrophic fermentation of *C. autoethanogenum.*

Heterotrophic culture (10 g/l fructose with N_2 headspace)						
	Concentration (mM)					
Hour	Fructose	Acetate	Ethanol	2,3-Butanediol	Lactate	Carbon yield
0	54.93	0.00	0.00	0.00	0.14	-
23	54.04	4.50	0.52	0.00	0.14	195%
50	44.38	15.91	8.52	0.03	0.14	78%
74	23.82	37.47	23.84	1.10	0.42	69%
97	0.50	71.35	34.07	2.57	1.18	69%
123	0.12	77.12	32.10	2.75	1.20	71%

Table 11. Mixotrophic fermentation of *C. autoethanogenum.*

Mixotrophic culture (10 g/l fructose with syngas headspace)						
	Concentration (mM)					
Hour	Fructose	Acetate	Ethanol	2,3-Butanediol	Lactate	Carbon yield
0	58.11	0.00	0.00	0.00	0.16	-
19	58.27	0.20	0.00	0.00	0.16	-
42	52.92	8.23	3.60	0.00	0.21	78%
67	35.95	69.41	17.77	0.63	0.25	134%
93	26.40	92.50	31.13	3.10	0.76	138%
114	14.39	121.79	44.71	8.18	2.44	142%
138	6.71	164.11	50.85	13.05	3.86	160%
162	1.16	168.14	67.42	17.04	4.98	162%

Table 12. Autotrophic fermentation of *C. autoethanogenum.*

Autotrophic culture (syngas headspace)						
	Concentration (mM)					
Hour	Fructose	Acetate	Ethanol	2,3-Butanediol	Lactate	Carbon yield*
0	0.00	0.00	0.00	0.00	0.16	-
25	0.00	1.84	0.00	0.00	0.13	-
41	0.00	8.24	1.48	0.00	0.13	-
65	0.00	41.96	6.72	0.21	0.04	-

(continued)

Autotrophic culture (syngas headspace)						
	Concentration (mM)					
Hour	Fructose	Acetate	Ethanol	2,3-Butanediol	Lactate	Carbon yield*
90	0.00	38.55	10.56	0.30	0.13	-
113	0.00	39.52	12.44	0.31	0.13	-
138	0.00	37.94	19.51	0.69	0.20	
164	0.00	25.02	39.80	3.27	0.55	-
185	0.00	69.78	26.19	5.00	0.13	-
209	0.00	72.45	25.86	5.12	0.12	-
233	0.00	67.61	32.90	5.29	0.19	-
* Carbon yield cannot be determined for autotrophic fermentations because there is no sugar substrate.						

Table 13. Heterotrophic fermentation of *C. acetobutylicum.*

Heterotrophic culture (10 g/l fructose with N_2 headspace)								
	Concentration (mM)							
Hour	Glucose/ Fructose	Acetate	Butyrate	Ethanol	Butanol	Lactate	Acetoin	Carbon yield
0	90.97	3.31	0.65	0.00	0.00	0.4	0.00	-
12	67.88	10.99	13.06	2.21	0.36	4.20	2.19	66%
24	47.06	16.84	24.02	2.55	1.73	10.10	1.95	64%
48	47.13	17.00	23.74	2.34	2.09	10.09	2.46	65%

[0359] The results for the two acetogens exemplify the non-additive, i.e., synergistic nature of the mixotrophic fermentation. Combining the heterotrophic fermentation broth with the autotrophic fermentation broth, the mixotrophic fermentation broth is not achieved. For example, adding the endpoints of heterotrophic and autotrophic for *C. ljungdahlii,* the molar ratios of acetate, ethanol, 2,3-butanediol, and lactate are: 0.80, 0.18, 0.02, and 0.003, respectively. In comparison, the ratios for mixotrophic fermentation are 0.88, 0.06, 0.05, and 0.01, respectively. The fraction of both 2,3-butanediol and lactate increase, while the fraction of ethanol decreases. The same is true for *C. autoethanogenum.*

[0360] Additionally, the carbon efficiencies under mixotrophic fermentation demonstrate that both sugar and gases are being metabolized, since the efficiencies are >100%. Even under heterotrophic conditions for the two acetogens, the carbon efficiencies are greater than the theoretically possible 67%, demonstrating that some of the evolved CO_2 from glycolysis is being metabolized into bioproducts. In comparison, the carbon efficiencies for *C. acetobutylicum* are only ~65%, which is the maximum without being able to metabolize CO_2.

Example 8

[0361] To further demonstrate concurrent gas and sugar utilization, the acetogens *Clostridium ljungdahlii* DSM 13528 and *Clostridium autoethanogenum* DSM 10061 were again cultured under mixotrophic conditions: 10 g/l of fructose (first feedstock) with a headspace of the gas mixture of CO, CO_2, H_2, and N_2 (55%, 10%, 20%, 15%, respectively) (second feedstock) at 30 psig. In this case though, both CO and CO_2 were labeled with ^{13}C, allowing the quantification of the amount of gas metabolized versus the amount of sugar, labeled with ^{12}C, is metabolized. As a control, an autotrophic culture was also prepared with the ^{13}C-labeled syngas at 30 psig. Two biological replicates for each strain were prepared, grown at 37°C and shaken at 200 rpm.

[0362] Fig. 2 and Fig. 3 show the percentage of ^{13}C labeling of the metabolite acetate over time under mixotrophic conditions in *C. ljungdahlii* and *C. autoethanogenum,* respectively.

[0363] For *C. ljungdahlii,* between 73% to 80% of acetate, the primary metabolite, exhibited ^{13}C-labeling over the course of the fermentation. Even at the earliest time point (t=24hr), when there is still 10.5 g/l fructose present in the media, over 70% of acetate was derived from the labeled syngas rather than fructose. The majority of growth occurred during the first 72 hours. After which, the low pH of the culture begins to inhibit growth. *C. autoethanogenum* displayed a similar ^{13}C- incorporation profile, with over 50% of acetate being labeled with ^{13}C even at 24hr. In addition to acetate, *C. autoethanogenum* also produces ethanol, so that the pH does not drop as quickly, which allowed the culture consume the majority of fructose. The method for quantification of ^{13}C-labeling prevents quantification of ^{13}C-labeled ethanol.

Example 9

[0364] The acetone strain created in Example 5 was grown on standard PETC medium with 5 g/l of fructose and with different amounts of H_2 in the headspace: 0%, 20% or 40% (in triplicate). As seen in Figure 4, increasing the amount of H_2 in the headspace led to an increase in total carbon metabolized and converted into bioproducts. Carbon yield is the amount of carbon in the produced bioproducts divided by the amount of carbon metabolized.

[0365] The product distributions of these cultures shows that almost all CO_2 is metabolized into bioproducts.

[0366] Figure 5 shows distributions of the bioproducts produced by the acetone strain grown with increasing amounts of H_2 in the headspace. Carbon fraction is the amount of carbon in each bioproduct with the total being 1.0.

[0367] In addition to metabolizing almost all the CO_2, the increased reductant in the headspace led to an increased production of reduced products, particularly ethanol.

Example 10

[0368] A plasmid was constructed to overexpress a glucose-specific EIIABC gene from *C. acetobutylicum* ATCC 824 (CA C0570). This plasmid was transformed into *C. ljungdahlii* DSM 13528 to make the strain Clj (pCAC-EIIABC). Four biological replicates of this strain were grown in 10 ml of PETC medium with 5 g/l of fructose and thiamphenicol (5 μg/ml). Once the cultures reached an OD_{600} of 1.0, the cells were harvested, resuspended in 10 ml of PETC medium without any carbon source, and 1 ml (10 % inoculum) was transferred to new tubes of 10 ml of PETC medium with 10 g/l of glucose and thiamphenicol (5 μg/ml). The average metabolite profile of these glucose-grown cultures is shown in Table 16.

Table 16.

Day	Concentration (g/l)				Glucose metabolized (g/l)	Rate of consumption (g/hr/g cell mass)
	Glucose	Fructose	Acetate	Ethanol		
0	9.19	0.00	0.05	0.70	-	-
3	7.59	0.00	2.95	0.17	1.60	0.07
7	6.05	0.00	4.42	0.19	3.15	0.05
11	5.41	0.00	5.28	0.19	3.78	0.02

[0369] After 11 days, the average amount of glucose consumed was 3.78 g/1. A plasmid control culture metabolized no glucose over this same time period. The strains also metabolized some ethanol from the antibiotic (~0.5 g/1), the majority of the carbon metabolized was glucose. The maximum rate of metabolism of glucose was 0.07 g/hr/g cell mass. This was calculated between Days 0 and 3, after the cultures reached their maximum cell density ($OD_{600} \approx 1.0$).

Example 11

[0370] A plasmid was constructed to overexpress a glucose-specific EIIABC gene from C. *saccharobutylicum* DSM 13864 (CLSA c10070). This plasmid was transformed into *C. ljungdahlii* DSM 13528 to make the strain Clj (pCSB-EIIABC). Four biological replicates of this strain were grown in 10 ml of PETC medium with 5 g/l of fructose and thiamphenicol (5 μg/ml). Once the cultures reached an OD_{600} of 1.0, the cells were harvested, resuspended in 10 ml of PETC medium without any carbon source, and 1 ml (10 % inoculum) was transferred to new tubes of 10 ml of PETC medium with 10 g/l of glucose and thiamphenicol (5 μg/ml). The average metabolite profile of these glucose-grown cultures is shown in Table 17.

Table 17.

Day	Concentration (g/l)				Glucose metabolized (g/l)	Rate of consumption (g/hr/g cell mass)
	Glucose	Fructose	Acetate	Ethanol		
0	9.15	0.00	0.04	0.68	-	-
3	8.69	0.00	1.79	0.11	0.47	0.02
7	6.82	0.00	3.82	0.15	2.33	0.06
11	5.68	0.00	5.16	0.14	3.47	0.04

[0371] After 11 days, the average amount of glucose metabolized was 3.47 g/l. A plasmid control culture metabolized no glucose over this same time period. The strains also metabolized some ethanol from the antibiotic (~0.5 g/1), but as in Example 11, the majority of the carbon metabolized was glucose. The maximum rate of metabolism of glucose was 0.06 g/hr/g cell mass. This was calculated between Days 3 and 7, after the cultures reached their maximum cell density ($OD_{600} \approx 1.0$).

Example 12: Crotyl Alcohol Production in *C. ljundgahlii* (comparative)

[0372] Wild-type *C. ljungdahlii* does not produce crotyl alcohol. Wild-type *C. ljungdahlii* was therefore engineered to produce crotyl alcohol. A plasmid, called pTHCA, over expressing the genes *thl* (CA_C2783), *hbd* (CA C2708), *crt* (CA C2712), and *adhE1* (CA P0162) was transformed into strain *C. ljungdahlii* DSM 13528.

[0373] *C. ljungdahlii* DSM 13528 [WT] and *C. ljungdahlii* (pTHCA) [Clj (pTHCA)] were then grown in standard PETC medium with 5 g/l of fructose anaerobically at 37°C for 6 days. Metabolite concentrations are presented in Table 18.

Table 18. End point metabolite concentrations of crotyl alcohol producing strains of *C. ljungdahlii.*

Strain	Concentration of crotyl alcohol (mg/l)
WT	0.0
Clj (pTHCA)	40.6

[0374] As can be seen from Table 18, *C. ljungdahlii* was genetically engineered to overexpress *thl, hbd, crt,* and *adhE1,* and thereby exhibited the ability to produce at least 40.6 mg/l of crotyl alcohol under the appropriate fermentation conditions.

SEQUENCE LISTING

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        35                  40                  45

Leu Gly Asn Arg Glu Asn Met Ile Leu Gly His Glu Ala Val Gly Glu
    50                  55                  60

Ile Ala Glu Val Gly Ser Glu Val Lys Asp Phe Lys Val Gly Asp Arg
65                  70                  75                  80

Val Ile Val Pro Cys Thr Thr Pro Asp Trp Arg Ser Leu Glu Val Gln
                85                  90                  95

Ala Gly Phe Gln Gln His Ser Asn Gly Met Leu Ala Gly Trp Lys Phe
            100                 105                 110

Ser Asn Phe Lys Asp Gly Val Phe Ala Asp Tyr Phe His Val Asn Asp
        115                 120                 125

Ala Asp Met Asn Leu Ala Ile Leu Pro Asp Glu Ile Pro Leu Glu Ser
    130                 135                 140

Ala Val Met Met Thr Asp Met Met Thr Thr Gly Phe His Gly Ala Glu
145                 150                 155                 160

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Ala Val Gly Leu Met Gly Ile Ala Gly Ser Lys Leu Arg Gly Ala Gly
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Phe Tyr Gly Ala Thr Asp Ile Val Asn Tyr Lys Asn Gly Asp Ile Val
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Glu Gln Ile Met Asp Leu Thr His Gly Lys Gly Val Asp Arg Val Ile
225                 230                 235                 240

Met Ala Gly Gly Gly Ala Glu Thr Leu Ala Gln Ala Val Thr Met Val
                245                 250                 255

Lys Pro Gly Gly Val Ile Ser Asn Ile Asn Tyr His Gly Ser Gly Asp
            260                 265                 270

Thr Leu Pro Ile Pro Arg Val Gln Trp Gly Cys Gly Met Ala His Lys
        275                 280                 285

Thr Ile Arg Gly Gly Leu Cys Pro Gly Gly Arg Leu Arg Met Glu Met
    290                 295                 300

Leu Arg Asp Leu Val Leu Tyr Lys Arg Val Asp Leu Ser Lys Leu Val
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Thr His Val Phe Asp Gly Ala Glu Asn Ile Glu Lys Ala Leu Leu Leu
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    50                  55                  60

Pro Val Val Leu Gly His Glu Phe Ser Gly Glu Val Val Glu Ile Gly
65                  70                  75                  80

Ser Lys Val Thr Lys Phe Lys Ala Gly Asp Arg Val Ile Val Glu Pro
                85                  90                  95

Ile Val Ala Cys Gly Lys Cys Pro Ala Cys Leu Glu Gly Lys Tyr Asn
            100                 105                 110

Leu Cys Glu Ala Leu Gly Phe His Gly Leu Cys Gly Ser Gly Gly Gly
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Phe Ala Glu Tyr Thr Val Phe Pro Glu Asp Phe Val His Lys Ile Pro
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Leu Val Leu Gly Ala Gly Pro Ile Gly Leu Ala Thr Ile Gln Cys Leu
            180                 185                 190

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        195                 200                 205

Arg Gln Glu Tyr Ala Lys Lys Phe Gly Ala Asp Val Val Leu Asp Pro
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Asn Glu Val Asp Val Ile Glu Glu Ile Lys Lys Leu Thr Gly Gly Val
225                 230                 235                 240

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        275                 280                 285

Glu Lys Lys Val Val Gly Thr Leu Ala Tyr Arg His Glu Phe Pro Ser
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305                 310                 315                 320

Thr Lys Arg Ile Ala Leu Glu Asp Ile Val Lys Glu Gly Phe Glu Thr
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Ala Ala Leu Ser Val Thr His Gly Phe Leu Pro Pro His Pro Gly Pro
145   150   155   160

Val Ala Ile Ala Thr Ile Tyr Gly Ala Ser Ile Ser Met Thr Leu Val
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Tyr Gly Ile Val Ile Ala Ile Pro Thr Val Ile Val Ala Gly Pro Val
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        195   200   205

Phe Lys Thr Lys Val Phe Asp Glu Asp Glu Met Pro Ser Phe Ser Leu
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Ser Val Leu Thr Ala Ile Val Pro Pro Ile Leu Met Ala Phe Ser Ala
225   230   235   240

Val Cys Glu Ile Thr Leu Pro Lys Thr Ser Pro Ile Arg His Phe Ala
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Glu Phe Val Gly Ser Pro Met Met Ala Met Phe Ile Ser Ile Ile Val
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Ala Ile Phe Thr Leu Gly Ile Met Arg Gly Lys Lys Met Glu Glu Ile
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        35                  40                  45

Phe Phe Phe Thr Lys Ile Phe Asp Ala Phe Met Asp Pro Ile Ala Gly
    50                  55                  60

Thr Ile Ile Asp Ser Arg Lys Pro Gly Lys Asn Gly Lys Phe Lys Pro
65                  70                  75                  80

Ile Met Phe Phe Ala Ser Ile Val Leu Ala Ile Leu Thr Val Ile Thr
                85                  90                  95

Phe Thr Asn Pro Gly Lys Thr Ala Thr Ser Lys Leu Leu Phe Ala Tyr
            100                 105                 110

Ala Thr Tyr Met Ile Trp Gly Leu Gly Tyr Ser Phe Thr Asn Val Pro
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Ser Leu Ala Thr Phe Arg Gln Ile Gly Ser Ser Gly Ala Leu Leu Ile
145                 150                 155                 160

Thr Ser Val Ile Phe Met Pro Leu Val Leu Val Phe His Asn Pro Ala
                165                 170                 175

Ile Gly Tyr Pro Val Val Ala Gly Ile Met Gly Leu Ile Gly Ile Leu
            180                 185                 190

Ser Phe Tyr Met Thr Tyr Lys Asn Thr Arg Glu Val Val Ala Pro Ala
        195                 200                 205

Glu Asn Val Lys Lys Glu Lys Ile Thr Pro Lys Ser Ile Ala Val Thr
    210                 215                 220

Ile Phe Thr Asn Arg Ala Leu Leu Thr Leu Ile Leu Met Thr Ile Phe
225                 230                 235                 240

Ser Ile Ser Ala Tyr Asn Ile Arg Ser Ser Leu Ile Val Tyr Tyr Cys
                245                 250                 255

Gln Tyr Asn Leu Gly Asn Val Thr Leu Leu Pro Tyr Ile Asn Phe Phe
            260                 265                 270

Thr Ile Gly Cys Ala Val Leu Gly Val Ser Phe Met Pro Lys Leu Val
        275                 280                 285

Gly Arg Phe Gly Lys Lys Arg Thr Ala Ile Ile Gly Phe Leu Ile Ser
    290                 295                 300

Val Ile Ala Asp Ser Ile Asn Phe Leu Leu Pro Gly Asn Ile Tyr Thr
305                 310                 315                 320

Phe Thr Ile Leu Leu Ala Ile Gly Phe Ile Gly Ile Ser Ile Pro Asn
                325                 330                 335

Gly Ile Thr Trp Ala Phe Val Ser Asp Ser Ile Asp Tyr Gly Glu Trp
            340                 345                 350

Arg Thr Gly Thr Arg Arg Glu Gly Ile Thr Tyr Ser Val Phe Asn Phe
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        370                 375                 380

Leu Gly Phe Val Gly Tyr Val Ala Asn Lys Lys Gln Ser Ala His Ala
385                 390                 395                 400

Leu Phe Gly Ile Lys Ala Leu Leu Met Ala Tyr Pro Ala Val Ala Leu
                405                 410                 415

Leu Val Ala Ala Leu Ile Ile Gly Leu Leu Tyr Asn Leu Ser Asp Lys
            420                 425                 430

Lys Phe Thr Glu Ile Ile Glu Glu Leu Asp Ala Arg Lys Gly Lys Thr
        435                 440                 445

Val
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<210> 9
<211> 1317
<212> DNA
<213> Escherichia coli

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gccaataacc ttaacgacat tttattacct caattccagc aggcttttac gctgacaaat 180
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tttgttacgg tattagggcc ggaaagtagt ggtcacttcc gcttaaatct tgcgcaaaca 480
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gtgccacatc aatcgcaaga cgttctcgat aaaatgtctc cagagcaatt gagtgcgtat 600
aaacacagcc tggtattatc ggtacagaca ccttatatga tcatcgtggc tatcgtgtta 660
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<210> 10
<211> 438
<212> PRT
<213> Escherichia coli

<400> 10

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Met Gly Asn Thr Ser Ile Gln Thr Gln Ser Tyr Arg Ala Val Asp Lys
1               5                   10                  15

Asp Ala Gly Gln Ser Arg Ser Tyr Ile Ile Pro Phe Ala Leu Leu Cys
            20                  25                  30

Ser Leu Phe Phe Leu Trp Ala Val Ala Asn Asn Leu Asn Asp Ile Leu
        35                  40                  45

Leu Pro Gln Phe Gln Gln Ala Phe Thr Leu Thr Asn Phe Gln Ala Gly
    50                  55                  60

Leu Ile Gln Ser Ala Phe Tyr Phe Gly Tyr Phe Ile Ile Pro Ile Pro
65                  70                  75                  80

Ala Gly Ile Leu Met Lys Lys Leu Ser Tyr Lys Ala Gly Ile Ile Thr
                85                  90                  95

Gly Leu Phe Leu Tyr Ala Leu Gly Ala Ala Leu Phe Trp Pro Ala Ala
            100                 105                 110

Glu Ile Met Asn Tyr Thr Leu Phe Leu Val Gly Leu Phe Ile Ile Ala
        115                 120                 125

Ala Gly Leu Gly Cys Leu Glu Thr Ala Ala Asn Pro Phe Val Thr Val
    130                 135                 140

Leu Gly Pro Glu Ser Ser Gly His Phe Arg Leu Asn Leu Ala Gln Thr
145                 150                 155                 160

Phe Asn Ser Phe Gly Ala Ile Ile Ala Val Val Phe Gly Gln Ser Leu
                165                 170                 175

Ile Leu Ser Asn Val Pro His Gln Ser Gln Asp Val Leu Asp Lys Met
            180                 185                 190
```

Ser Pro Glu Gln Leu Ser Ala Tyr Lys His Ser Leu Val Leu Ser Val
195 200 205

Gln Thr Pro Tyr Met Ile Ile Val Ala Ile Val Leu Leu Val Ala Leu
210 215 220

Leu Ile Met Leu Thr Lys Phe Pro Ala Leu Gln Ser Asp Asn His Ser
225 230 235 240

Asp Ala Lys Gln Gly Ser Phe Ser Ala Ser Leu Ser Arg Leu Ala Arg
245 250 255

Ile Arg His Trp Arg Trp Ala Val Leu Ala Gln Phe Cys Tyr Val Gly
260 265 270

Ala Gln Thr Ala Cys Trp Ser Tyr Leu Ile Arg Tyr Ala Val Glu Glu
275 280 285

Ile Pro Gly Met Thr Ala Gly Phe Ala Ala Asn Tyr Leu Thr Gly Thr
290 295 300

Met Val Cys Phe Phe Ile Gly Arg Phe Thr Gly Thr Trp Leu Ile Ser
305 310 315 320

Arg Phe Ala Pro His Lys Val Leu Ala Ala Tyr Ala Leu Ile Ala Met
325 330 335

Ala Leu Cys Leu Ile Ser Ala Phe Ala Gly Gly His Val Gly Leu Ile
340 345 350

Ala Leu Thr Leu Cys Ser Ala Phe Met Ser Ile Gln Tyr Pro Thr Ile
355 360 365

Phe Ser Leu Gly Ile Lys Asn Leu Gly Gln Asp Thr Lys Tyr Gly Ser
370 375 380

Ser Phe Ile Val Met Thr Ile Ile Gly Gly Gly Ile Val Thr Pro Val
385 390 395 400

Met Gly Phe Val Ser Asp Ala Ala Gly Asn Ile Pro Thr Ala Glu Leu
405 410 415

Ile Pro Ala Leu Cys Phe Ala Val Ile Phe Ile Phe Ala Arg Phe Arg
420 425 430

Ser Gln Thr Ala Thr Asn

435

<210> 11
<211> 1179
<212> DNA
<213> Clostridium acetobutylicum

<400> 11

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gcaggaataa aaccagagga tgttaatgaa gtcattttag gaaatgttct tcaagcaggt    180
ttaggacaga atccagcaag acaggcatct tttaaagcag gattaccagt tgaaattcca    240
gctatgacta ttaataaggt ttgtggttca ggacttagaa cagttagctt agcagcacaa    300
attataaaag caggagatgc tgacgtaata atagcaggtg gtatggaaaa tatgtctaga    360
gctccttact tagcgaataa cgctagatgg ggatatagaa tgggaaacgc taaatttgtt    420
gatgaaatga tcactgacgg attgtgggat gcatttaatg attaccacat gggaataaca    480
gcagaaaaca tagctgagag atggaacatt tcaagagaag aacaagatga gtttgctctt    540
gcatcacaaa aaaaagctga agaagctata aaatcaggtc aatttaaaga tgaaatagtt    600
cctgtagtaa ttaaaggcag aaagggagaa actgtagttg atacagatga gcaccctaga    660
tttggatcaa ctatagaagg acttgcaaaa ttaaaacctg ccttcaaaaa agatggaaca    720
gttacagctg gtaatgcatc aggattaaat gactgtgcag cagtacttgt aatcatgagt    780
gcagaaaaag ctaaagagct tggagtaaaa ccacttgcta agatagtttc ttatggttca    840
gcaggagttg acccagcaat aatgggatat ggacctttct atgcaacaaa agcagctatt    900
gaaaaagcag gttggacagt tgatgaatta gatttaatag aatcaaatga agcttttgca    960
gctcaaagtt tagcagtagc aaaagattta aaatttgata tgaataaagt aaatgtaaat   1020
ggaggagcta ttgcccttgg tcatccaatt ggagcatcag gtgcaagaat actcgttact   1080
cttgtacacg caatgcaaaa aagagatgca aaaaaaggct tagcaacttt atgtataggt   1140
ggcggacaag gaacagcaat attgctagaa aagtgctag                          1179
```

<210> 12
<211> 392
<212> PRT
<213> Clostridium acetobutylicum

<400> 12

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Met Lys Glu Val Val Ile Ala Ser Ala Val Arg Thr Ala Ile Gly Ser
1               5                   10                  15

Tyr Gly Lys Ser Leu Lys Asp Val Pro Ala Val Asp Leu Gly Ala Thr
            20                  25                  30
```

```
Ala Ile Lys Glu Ala Val Lys Lys Ala Gly Ile Lys Pro Glu Asp Val
        35                  40                  45

Asn Glu Val Ile Leu Gly Asn Val Leu Gln Ala Gly Leu Gly Gln Asn
    50                  55                  60

Pro Ala Arg Gln Ala Ser Phe Lys Ala Gly Leu Pro Val Glu Ile Pro
65                  70                  75                  80

Ala Met Thr Ile Asn Lys Val Cys Gly Ser Gly Leu Arg Thr Val Ser
                85                  90                  95

Leu Ala Ala Gln Ile Ile Lys Ala Gly Asp Ala Asp Val Ile Ile Ala
            100                 105                 110

Gly Gly Met Glu Asn Met Ser Arg Ala Pro Tyr Leu Ala Asn Asn Ala
        115                 120                 125

Arg Trp Gly Tyr Arg Met Gly Asn Ala Lys Phe Val Asp Glu Met Ile
    130                 135                 140

Thr Asp Gly Leu Trp Asp Ala Phe Asn Asp Tyr His Met Gly Ile Thr
145                 150                 155                 160

Ala Glu Asn Ile Ala Glu Arg Trp Asn Ile Ser Arg Glu Glu Gln Asp
                165                 170                 175

Glu Phe Ala Leu Ala Ser Gln Lys Lys Ala Glu Glu Ala Ile Lys Ser
            180                 185                 190

Gly Gln Phe Lys Asp Glu Ile Val Pro Val Val Ile Lys Gly Arg Lys
        195                 200                 205

Gly Glu Thr Val Val Asp Thr Asp Glu His Pro Arg Phe Gly Ser Thr
    210                 215                 220

Ile Glu Gly Leu Ala Lys Leu Lys Pro Ala Phe Lys Lys Asp Gly Thr
225                 230                 235                 240

Val Thr Ala Gly Asn Ala Ser Gly Leu Asn Asp Cys Ala Ala Val Leu
                245                 250                 255

Val Ile Met Ser Ala Glu Lys Ala Lys Glu Leu Gly Val Lys Pro Leu
            260                 265                 270

Ala Lys Ile Val Ser Tyr Gly Ser Ala Gly Val Asp Pro Ala Ile Met
        275                 280                 285
```

Gly Tyr Gly Pro Phe Tyr Ala Thr Lys Ala Ala Ile Glu Lys Ala Gly
290 295 300

Trp Thr Val Asp Glu Leu Asp Leu Ile Glu Ser Asn Glu Ala Phe Ala
305 310 315 320

Ala Gln Ser Leu Ala Val Ala Lys Asp Leu Lys Phe Asp Met Asn Lys
325 330 335

Val Asn Val Asn Gly Gly Ala Ile Ala Leu Gly His Pro Ile Gly Ala
340 345 350

Ser Gly Ala Arg Ile Leu Val Thr Leu Val His Ala Met Gln Lys Arg
355 360 365

Asp Ala Lys Lys Gly Leu Ala Thr Leu Cys Ile Gly Gly Gly Gln Gly
370 375 380

Thr Ala Ile Leu Leu Glu Lys Cys
385 390

<210> 13
<211> 849
<212> DNA
<213> Clostridium acetobutylicum

<400> 13

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ttagatttta tcaataaaaa tctttctaaa ttagttaaaa aaggaaagat agaagaagct     180
actaaagttg aaatcttaac tagaatttcc ggaacagttg accttaatat ggcagctgat     240
tgcgatttag ttatagaagc agctgttgaa agaatggata ttaaaaagca gatttttgct     300
gacttagaca atatatgcaa gccagaaaca attcttgcat caaatacatc atcactttca     360
ataacagaag tggcatcagc aactaaaaga cctgataagg ttataggtat gcatttcttt     420
aatccagctc ctgttatgaa gcttgtagag gtaataagag gaatagctac atcacaagaa     480
acttttgatg cagttaaaga gacatctata gcaataggaa aagatcctgt agaagtagca     540
gaagcaccag gatttgttgt aaatagaata ttaataccaa tgattaatga agcagttggt     600
atattagcag aaggaatagc ttcagtagaa gacatagata aagctatgaa acttggagct     660
aatcacccaa tgggaccatt agaattaggt gattttatag gtcttgatat atgtcttgct     720
ataatggatg ttttatactc agaaactgga gattctaagt atagaccaca tacattactt     780
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tcaaaataa                                                             849
```

<210> 14
<211> 282
<212> PRT
<213> Clostridium acetobutylicum

<400> 14

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Met Lys Lys Val Cys Val Ile Gly Ala Gly Thr Met Gly Ser Gly Ile
1               5                   10                  15

Ala Gln Ala Phe Ala Ala Lys Gly Phe Glu Val Val Leu Arg Asp Ile
            20                  25                  30

Lys Asp Glu Phe Val Asp Arg Gly Leu Asp Phe Ile Asn Lys Asn Leu
        35                  40                  45

Ser Lys Leu Val Lys Lys Gly Lys Ile Glu Glu Ala Thr Lys Val Glu
    50                  55                  60

Ile Leu Thr Arg Ile Ser Gly Thr Val Asp Leu Asn Met Ala Ala Asp
65                  70                  75                  80

Cys Asp Leu Val Ile Glu Ala Ala Val Glu Arg Met Asp Ile Lys Lys
                85                  90                  95

Gln Ile Phe Ala Asp Leu Asp Asn Ile Cys Lys Pro Glu Thr Ile Leu
            100                 105                 110

Ala Ser Asn Thr Ser Ser Leu Ser Ile Thr Glu Val Ala Ser Ala Thr
        115                 120                 125

Lys Arg Pro Asp Lys Val Ile Gly Met His Phe Phe Asn Pro Ala Pro
    130                 135                 140

Val Met Lys Leu Val Glu Val Ile Arg Gly Ile Ala Thr Ser Gln Glu
145                 150                 155                 160

Thr Phe Asp Ala Val Lys Glu Thr Ser Ile Ala Ile Gly Lys Asp Pro
                165                 170                 175

Val Glu Val Ala Glu Ala Pro Gly Phe Val Val Asn Arg Ile Leu Ile
            180                 185                 190

Pro Met Ile Asn Glu Ala Val Gly Ile Leu Ala Glu Gly Ile Ala Ser
        195                 200                 205

Val Glu Asp Ile Asp Lys Ala Met Lys Leu Gly Ala Asn His Pro Met
```

```
        210                 215                 220

Gly Pro Leu Glu Leu Gly Asp Phe Ile Gly Leu Asp Ile Cys Leu Ala
225                 230                 235                 240

Ile Met Asp Val Leu Tyr Ser Glu Thr Gly Asp Ser Lys Tyr Arg Pro
                245                 250                 255

His Thr Leu Leu Lys Lys Tyr Val Arg Ala Gly Trp Leu Gly Arg Lys
            260                 265                 270

Ser Gly Lys Gly Phe Tyr Asp Tyr Ser Lys
        275                 280
```

<210> 15
<211> 786
<212> DNA
<213> Clostridium acetobutylicum

<400> 15

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ggtgaaattg aaaatgatag cgaagtactt gcagtaattt taactggagc aggagaaaaa      180
tcatttgtag caggagcaga tatttctgag atgaaggaaa tgaataccat tgaaggtaga      240
aaattcggga tacttggaaa taaagtgttt agaagattag aacttcttga aaagcctgta      300
atagcagctg ttaatggttt tgctttagga ggcggatgcg aaatagctat gtcttgtgat      360
ataagaatag cttcaagcaa cgcaagattt ggtcaaccag aagtaggtct cggaataaca      420
cctggttttg gtggtacaca aagactttca agattagttg gaatgggcat ggcaaagcag      480
cttatattta ctgcacaaaa tataaaggca gatgaagcat taagaatcgg acttgtaaat      540
aaggtagtag aacctagtga attaatgaat acagcaaaag aaattgcaaa caaaattgtg      600
agcaatgctc cagtagctgt taagttaagc aaacaggcta ttaatagagg aatgcagtgt      660
gatattgata ctgctttagc atttgaatca gaagcatttg gagaatgctt ttcaacagag      720
gatcaaaagg atgcaatgac agctttcata gagaaaagaa aaattgaagg cttcaaaaat      780
agatag                                                                 786
```

<210> 16
<211> 261
<212> PRT
<213> Clostridium acetobutylicum

<400> 16

```
Met Glu Leu Asn Asn Val Ile Leu Glu Lys Glu Gly Lys Val Ala Val
1               5                   10                  15
```

```
Val Thr Ile Asn Arg Pro Lys Ala Leu Asn Ala Leu Asn Ser Asp Thr
            20                  25                  30

Leu Lys Glu Met Asp Tyr Val Ile Gly Glu Ile Glu Asn Asp Ser Glu
        35                  40                  45

Val Leu Ala Val Ile Leu Thr Gly Ala Gly Glu Lys Ser Phe Val Ala
    50                  55                  60

Gly Ala Asp Ile Ser Glu Met Lys Glu Met Asn Thr Ile Glu Gly Arg
65                  70                  75                  80

Lys Phe Gly Ile Leu Gly Asn Lys Val Phe Arg Arg Leu Glu Leu Leu
                85                  90                  95

Glu Lys Pro Val Ile Ala Ala Val Asn Gly Phe Ala Leu Gly Gly Gly
            100                 105                 110

Cys Glu Ile Ala Met Ser Cys Asp Ile Arg Ile Ala Ser Ser Asn Ala
        115                 120                 125

Arg Phe Gly Gln Pro Glu Val Gly Leu Gly Ile Thr Pro Gly Phe Gly
    130                 135                 140

Gly Thr Gln Arg Leu Ser Arg Leu Val Gly Met Gly Met Ala Lys Gln
145                 150                 155                 160

Leu Ile Phe Thr Ala Gln Asn Ile Lys Ala Asp Glu Ala Leu Arg Ile
                165                 170                 175

Gly Leu Val Asn Lys Val Val Glu Pro Ser Glu Leu Met Asn Thr Ala
            180                 185                 190

Lys Glu Ile Ala Asn Lys Ile Val Ser Asn Ala Pro Val Ala Val Lys
        195                 200                 205

Leu Ser Lys Gln Ala Ile Asn Arg Gly Met Gln Cys Asp Ile Asp Thr
    210                 215                 220

Ala Leu Ala Phe Glu Ser Glu Ala Phe Gly Glu Cys Phe Ser Thr Glu
225                 230                 235                 240

Asp Gln Lys Asp Ala Met Thr Ala Phe Ile Glu Lys Arg Lys Ile Glu
                245                 250                 255

Gly Phe Lys Asn Arg
            260
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<210> 17
<211> 2589
<212> DNA
<213> Clostridium acetobutylicum

<400> 17

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gcagcaatcg acgcaaggat agagctagca aaagcagctg ttttggaaac cggtatgggc     180
ttagttgaag acaaggttat aaaaaatcat tttgcaggcg aatacatcta taacaaatat     240
aaggatgaaa aaacctgcgg tataattgaa cgaaatgaac cctacggaat tacaaaaata     300
gcagaaccta taggagttgt agctgctata atccctgtaa caaaccccac atcaacaaca     360
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agggcaaaaa aatccacaat actagcagct aaaacaatac ttgatgcagc cgttaagagt     480
ggtgccccgg aaaatataat aggttggata gatgaacctt caattgaact aactcaatat     540
ttaatgcaaa aagcagatat aacccttgca actggtggtc cctcactagt taaatctgct     600
tattcttccg gaaaaccagc aataggtgtt ggtccgggta acaccccagt aataattgat     660
gaatctgctc atataaaaat ggcagtaagt tcaattatat tatccaaaac ctatgataat     720
ggtgttatat gtgcttctga acaatctgta atagtcttaa aatccatata taacaaggta     780
aaagatgagt tccaagaaag aggagcttat ataataaaga aaaacgaatt ggataaagtc     840
cgtgaagtga tttttaaaga tggatccgta aaccctaaaa tagtcggaca gtcagcttat     900
actatagcag ctatggctgg cataaaagta cctaaaacca caagaatatt aataggagaa     960
gttacctcct taggtgaaga agaacctttt gcccacgaaa aactatctcc tgttttggct    1020
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ggtgcaagtg gagatctata taattttaga ataccacctt ctttcacgct tggctgcgga    1260
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cttgagcacc tagatattga ttttaaagta tttaataagg ttggaagaga agctgatctt    1560
aaaaccataa aaaaagcaac tgaagaaatg tcctccttta tgccagacac tataatagct    1620
ttaggtggta cccctgaaat gagctctgca aagctaatgt gggtactata tgaacatcca    1680
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gaagtaaaat ttgaagatct tgcaataaaa tttatggaca taagaaagag aatatatact     1740
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gcagattatg aaatgacacc aaatatggca attgtagatg cagaacttat gatgaaaatg     1920
ccaaagggat taaccgctta ttcaggtata gatgcactag taaatagtat agaagcatac     1980
acatccgtat atgcttcaga atacacaaac ggactagcac tagaggcaat acgattaata     2040
tttaaatatt tgcctgaggc ttacaaaaac ggaagaacca atgaaaaagc aagagagaaa     2100
atggctcacg cttcaactat ggcaggtatg gcatccgcta atgcatttct aggtctatgt     2160
cattccatgg caataaaatt aagttcagaa cacaatattc ctagtggcat tgccaatgca     2220
ttactaatag aagaagtaat aaaatttaac gcagttgata atcctgtaaa acaagcccct     2280
tgcccacaat ataagtatcc aaacaccata tttagatatg ctcgaattgc agattatata     2340
aagcttggag gaaatactga tgaggaaaag gtagatctct taattaacaa aatacatgaa     2400
ctaaaaaaag ctttaaatat accaacttca ataaaggatg caggtgtttt ggaggaaaac     2460
ttctattcct cccttgatag aatatctgaa cttgcactag atgatcaatg cacaggcgct     2520
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caaccttaa                                                             2589
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<210> 18
<211> 862
<212> PRT
<213> Clostridium acetobutylicum

<400> 18

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Met Lys Val Thr Thr Val Lys Glu Leu Asp Glu Lys Leu Lys Val Ile
1               5                   10                  15

Lys Glu Ala Gln Lys Lys Phe Ser Cys Tyr Ser Gln Glu Met Val Asp
            20                  25                  30

Glu Ile Phe Arg Asn Ala Ala Met Ala Ala Ile Asp Ala Arg Ile Glu
        35                  40                  45

Leu Ala Lys Ala Ala Val Leu Glu Thr Gly Met Gly Leu Val Glu Asp
    50                  55                  60

Lys Val Ile Lys Asn His Phe Ala Gly Glu Tyr Ile Tyr Asn Lys Tyr
65                  70                  75                  80

Lys Asp Glu Lys Thr Cys Gly Ile Ile Glu Arg Asn Glu Pro Tyr Gly
                85                  90                  95
```

Ile Thr Lys Ile Ala Glu Pro Ile Gly Val Val Ala Ala Ile Ile Pro
100 105 110

Val Thr Asn Pro Thr Ser Thr Thr Ile Phe Lys Ser Leu Ile Ser Leu
115 120 125

Lys Thr Arg Asn Gly Ile Phe Phe Ser Pro His Pro Arg Ala Lys Lys
130 135 140

Ser Thr Ile Leu Ala Ala Lys Thr Ile Leu Asp Ala Ala Val Lys Ser
145 150 155 160

Gly Ala Pro Glu Asn Ile Ile Gly Trp Ile Asp Glu Pro Ser Ile Glu
165 170 175

Leu Thr Gln Tyr Leu Met Gln Lys Ala Asp Ile Thr Leu Ala Thr Gly
180 185 190

Gly Pro Ser Leu Val Lys Ser Ala Tyr Ser Ser Gly Lys Pro Ala Ile
195 200 205

Gly Val Gly Pro Gly Asn Thr Pro Val Ile Ile Asp Glu Ser Ala His
210 215 220

Ile Lys Met Ala Val Ser Ser Ile Ile Leu Ser Lys Thr Tyr Asp Asn
225 230 235 240

Gly Val Ile Cys Ala Ser Glu Gln Ser Val Ile Val Leu Lys Ser Ile
245 250 255

Tyr Asn Lys Val Lys Asp Glu Phe Gln Glu Arg Gly Ala Tyr Ile Ile
260 265 270

Lys Lys Asn Glu Leu Asp Lys Val Arg Glu Val Ile Phe Lys Asp Gly
275 280 285

Ser Val Asn Pro Lys Ile Val Gly Gln Ser Ala Tyr Thr Ile Ala Ala
290 295 300

Met Ala Gly Ile Lys Val Pro Lys Thr Thr Arg Ile Leu Ile Gly Glu
305 310 315 320

Val Thr Ser Leu Gly Glu Glu Glu Pro Phe Ala His Glu Lys Leu Ser
325 330 335

Pro Val Leu Ala Met Tyr Glu Ala Asp Asn Phe Asp Asp Ala Leu Lys
340 345 350

Lys Ala Val Thr Leu Ile Asn Leu Gly Gly Leu Gly His Thr Ser Gly
355 360 365

Ile Tyr Ala Asp Glu Ile Lys Ala Arg Asp Lys Ile Asp Arg Phe Ser
370 375 380

Ser Ala Met Lys Thr Val Arg Thr Phe Val Asn Ile Pro Thr Ser Gln
385 390 395 400

Gly Ala Ser Gly Asp Leu Tyr Asn Phe Arg Ile Pro Pro Ser Phe Thr
405 410 415

Leu Gly Cys Gly Phe Trp Gly Gly Asn Ser Val Ser Glu Asn Val Gly
420 425 430

Pro Lys His Leu Leu Asn Ile Lys Thr Val Ala Glu Arg Arg Glu Asn
435 440 445

Met Leu Trp Phe Arg Val Pro His Lys Val Tyr Phe Lys Phe Gly Cys
450 455 460

Leu Gln Phe Ala Leu Lys Asp Leu Lys Asp Leu Lys Lys Lys Arg Ala
465 470 475 480

Phe Ile Val Thr Asp Ser Asp Pro Tyr Asn Leu Asn Tyr Val Asp Ser
485 490 495

Ile Ile Lys Ile Leu Glu His Leu Asp Ile Asp Phe Lys Val Phe Asn
500 505 510

Lys Val Gly Arg Glu Ala Asp Leu Lys Thr Ile Lys Lys Ala Thr Glu
515 520 525

Glu Met Ser Ser Phe Met Pro Asp Thr Ile Ile Ala Leu Gly Gly Thr
530 535 540

Pro Glu Met Ser Ser Ala Lys Leu Met Trp Val Leu Tyr Glu His Pro
545 550 555 560

Glu Val Lys Phe Glu Asp Leu Ala Ile Lys Phe Met Asp Ile Arg Lys
565 570 575

Arg Ile Tyr Thr Phe Pro Lys Leu Gly Lys Lys Ala Met Leu Val Ala
580 585 590

Ile Thr Thr Ser Ala Gly Ser Gly Ser Glu Val Thr Pro Phe Ala Leu

595 600 605

Val Thr Asp Asn Asn Thr Gly Asn Lys Tyr Met Leu Ala Asp Tyr Glu
610 615 620

Met Thr Pro Asn Met Ala Ile Val Asp Ala Glu Leu Met Met Lys Met
625 630 635 640

Pro Lys Gly Leu Thr Ala Tyr Ser Gly Ile Asp Ala Leu Val Asn Ser
645 650 655

Ile Glu Ala Tyr Thr Ser Val Tyr Ala Ser Glu Tyr Thr Asn Gly Leu
660 665 670

Ala Leu Glu Ala Ile Arg Leu Ile Phe Lys Tyr Leu Pro Glu Ala Tyr
675 680 685

Lys Asn Gly Arg Thr Asn Glu Lys Ala Arg Glu Lys Met Ala His Ala
690 695 700

Ser Thr Met Ala Gly Met Ala Ser Ala Asn Ala Phe Leu Gly Leu Cys
705 710 715 720

His Ser Met Ala Ile Lys Leu Ser Ser Glu His Asn Ile Pro Ser Gly
725 730 735

Ile Ala Asn Ala Leu Leu Ile Glu Glu Val Ile Lys Phe Asn Ala Val
740 745 750

Asp Asn Pro Val Lys Gln Ala Pro Cys Pro Gln Tyr Lys Tyr Pro Asn
755 760 765

Thr Ile Phe Arg Tyr Ala Arg Ile Ala Asp Tyr Ile Lys Leu Gly Gly
770 775 780

Asn Thr Asp Glu Glu Lys Val Asp Leu Leu Ile Asn Lys Ile His Glu
785 790 795 800

Leu Lys Lys Ala Leu Asn Ile Pro Thr Ser Ile Lys Asp Ala Gly Val
805 810 815

Leu Glu Glu Asn Phe Tyr Ser Ser Leu Asp Arg Ile Ser Glu Leu Ala
820 825 830

Leu Asp Asp Gln Cys Thr Gly Ala Asn Pro Arg Phe Pro Leu Thr Ser
835 840 845

Glu Ile Lys Glu Met Tyr Ile Asn Cys Phe Lys Lys Gln Pro
850 855 860

<210> 19
<211> 1173
<212> DNA
<213> Clostridium acetobutylicum

<400> 19

```
gtggttgatt tcgaatattc aataccaact agaatttttt tcggtaaaga taagataaat      60
gtacttggaa gagagcttaa aaaatatggt tctaaagtgc ttatagttta tggtggagga     120
agtataaaga gaaatggaat atatgataaa gctgtaagta tacttgaaaa aaacagtatt     180
aaattttatg aacttgcagg agtagagcca aatccaagag taactacagt tgaaaaagga     240
gttaaaatat gtagagaaaa tggagttgaa gtagtactag ctataggtgg aggaagtgca     300
atagattgcg caaaggttat agcagcagca tgtgaatatg atggaaatcc atgggatatt     360
gtgttagatg gctcaaaaat aaaaagggtg cttcctatag ctagtatatt aaccattgct     420
gcaacaggat cagaaatgga tacgtgggca gtaataaata atatggatac aaacgaaaaa     480
ctaattgcgg cacatccaga tatggctcct aagttttcta tattagatcc aacgtatacg     540
tataccgtac ctaccaatca aacagcagca ggaacagctg atattatgag tcatatattt     600
gaggtgtatt ttagtaatac aaaaacagca tatttgcagg atagaatggc agaagcgtta     660
ttaagaactt gtattaaata tggaggaata gctcttgaga agccggatga ttatgaggca     720
agagccaatc taatgtgggc ttcaagtctt gcgataaatg gacttttaac atatggtaaa     780
gacactaatt ggagtgtaca cttaatggaa catgaattaa gtgcttatta cgacataaca     840
cacggcgtag ggcttgcaat tttaacacct aattggatgg agtatatttt aaataatgat     900
acagtgtaca agtttgttga atatggtgta aatgtttggg gaatagacaa agaaaaaaat     960
cactatgaca tagcacatca agcaatacaa aaaacaagag attactttgt aaatgtacta    1020
ggtttaccat ctagactgag agatgttgga attgaagaag aaaaattgga cataatggca    1080
aaggaatcag taaagcttac aggaggaacc ataggaaacc taagaccagt aaacgcctcc    1140
gaagtcctac aaatattcaa aaaatctgtg taa                                  1173
```

<210> 20
<211> 390
<212> PRT
<213> Clostridium acetobutylicum

<400> 20

```
Met Val Asp Phe Glu Tyr Ser Ile Pro Thr Arg Ile Phe Phe Gly Lys
1               5                   10                  15

Asp Lys Ile Asn Val Leu Gly Arg Glu Leu Lys Lys Tyr Gly Ser Lys
            20                  25                  30
```

```
Val Leu Ile Val Tyr Gly Gly Gly Ser Ile Lys Arg Asn Gly Ile Tyr
        35                  40                  45

Asp Lys Ala Val Ser Ile Leu Glu Lys Asn Ser Ile Lys Phe Tyr Glu
    50                  55                  60

Leu Ala Gly Val Glu Pro Asn Pro Arg Val Thr Thr Val Glu Lys Gly
65                  70                  75                  80

Val Lys Ile Cys Arg Glu Asn Gly Val Glu Val Val Leu Ala Ile Gly
                85                  90                  95

Gly Gly Ser Ala Ile Asp Cys Ala Lys Val Ile Ala Ala Ala Cys Glu
            100                 105                 110

Tyr Asp Gly Asn Pro Trp Asp Ile Val Leu Asp Gly Ser Lys Ile Lys
        115                 120                 125

Arg Val Leu Pro Ile Ala Ser Ile Leu Thr Ile Ala Ala Thr Gly Ser
    130                 135                 140

Glu Met Asp Thr Trp Ala Val Ile Asn Asn Met Asp Thr Asn Glu Lys
145                 150                 155                 160

Leu Ile Ala Ala His Pro Asp Met Ala Pro Lys Phe Ser Ile Leu Asp
                165                 170                 175

Pro Thr Tyr Thr Tyr Thr Val Pro Thr Asn Gln Thr Ala Ala Gly Thr
            180                 185                 190

Ala Asp Ile Met Ser His Ile Phe Glu Val Tyr Phe Ser Asn Thr Lys
        195                 200                 205

Thr Ala Tyr Leu Gln Asp Arg Met Ala Glu Ala Leu Leu Arg Thr Cys
    210                 215                 220

Ile Lys Tyr Gly Gly Ile Ala Leu Glu Lys Pro Asp Asp Tyr Glu Ala
225                 230                 235                 240

Arg Ala Asn Leu Met Trp Ala Ser Ser Leu Ala Ile Asn Gly Leu Leu
                245                 250                 255

Thr Tyr Gly Lys Asp Thr Asn Trp Ser Val His Leu Met Glu His Glu
            260                 265                 270

Leu Ser Ala Tyr Tyr Asp Ile Thr His Gly Val Gly Leu Ala Ile Leu
```

```
            275                 280                 285

Thr Pro Asn Trp Met Glu Tyr Ile Leu Asn Asn Asp Thr Val Tyr Lys
    290                 295                 300

Phe Val Glu Tyr Gly Val Asn Val Trp Gly Ile Asp Lys Glu Lys Asn
305                 310                 315                 320

His Tyr Asp Ile Ala His Gln Ala Ile Gln Lys Thr Arg Asp Tyr Phe
                325                 330                 335

Val Asn Val Leu Gly Leu Pro Ser Arg Leu Arg Asp Val Gly Ile Glu
            340                 345                 350

Glu Glu Lys Leu Asp Ile Met Ala Lys Glu Ser Val Lys Leu Thr Gly
        355                 360                 365

Gly Thr Ile Gly Asn Leu Arg Pro Val Asn Ala Ser Glu Val Leu Gln
    370                 375                 380

Ile Phe Lys Lys Ser Val
385                 390
```

<210> 21
<211> 657
<212> DNA
<213> Clostridium acetobutylicum

<400> 21

```
atgaactcta aaataattag atttgaaaat ttaaggtcat tctttaaaga tgggatgaca      60

attatgattg gaggtttttt aaactgtggc actccaacca aattaattga tttttagtt      120

aatttaaata taaagaattt aacgattata agtaatgata catgttatcc taatacaggt      180

attggtaagt taatatcaaa taatcaagta aaaaagctta ttgcttcata tataggcagc      240

aacccagata ctggcaaaaa actttttaat aatgaacttg aagtagagct ctctccccaa      300

ggaactctag tggaaagaat acgtgcaggc ggatctggct taggtggtgt actaactaaa      360

acaggtttag gaactttgat tgaaaaagga aagaaaaaaa tatctataaa tggaacggaa      420

tatttgttag agctacctct tacagccgat gtagcattaa ttaaaggtag tattgtagat      480

gaggccggaa acaccttcta taaaggtact actaaaaact ttaatcccta tatggcaatg      540

gcagctaaaa ccgtaatagt tgaagctgaa aatttagtta gctgtgaaaa actagaaaag      600

gaaaaagcaa tgacccccgg agttcttata aattatatag taaaggagcc tgcataa       657
```

<210> 22

<211> 218
<212> PRT
<213> Clostridium acetobutylicum

<400> 22

```
Met Asn Ser Lys Ile Ile Arg Phe Glu Asn Leu Arg Ser Phe Phe Lys
1               5                   10                  15

Asp Gly Met Thr Ile Met Ile Gly Gly Phe Leu Asn Cys Gly Thr Pro
            20                  25                  30

Thr Lys Leu Ile Asp Phe Leu Val Asn Leu Asn Ile Lys Asn Leu Thr
        35                  40                  45

Ile Ile Ser Asn Asp Thr Cys Tyr Pro Asn Thr Gly Ile Gly Lys Leu
    50                  55                  60

Ile Ser Asn Asn Gln Val Lys Lys Leu Ile Ala Ser Tyr Ile Gly Ser
65                  70                  75                  80

Asn Pro Asp Thr Gly Lys Lys Leu Phe Asn Asn Glu Leu Glu Val Glu
                85                  90                  95

Leu Ser Pro Gln Gly Thr Leu Val Glu Arg Ile Arg Ala Gly Gly Ser
            100                 105                 110

Gly Leu Gly Gly Val Leu Thr Lys Thr Gly Leu Gly Thr Leu Ile Glu
        115                 120                 125

Lys Gly Lys Lys Lys Ile Ser Ile Asn Gly Thr Glu Tyr Leu Leu Glu
    130                 135                 140

Leu Pro Leu Thr Ala Asp Val Ala Leu Ile Lys Gly Ser Ile Val Asp
145                 150                 155                 160

Glu Ala Gly Asn Thr Phe Tyr Lys Gly Thr Thr Lys Asn Phe Asn Pro
                165                 170                 175

Tyr Met Ala Met Ala Ala Lys Thr Val Ile Val Glu Ala Glu Asn Leu
            180                 185                 190

Val Ser Cys Glu Lys Leu Glu Lys Glu Lys Ala Met Thr Pro Gly Val
        195                 200                 205

Leu Ile Asn Tyr Ile Val Lys Glu Pro Ala
    210                 215
```

<210> 23
<211> 666
<212> DNA
<213> Clostridium acetobutylicum

<400> 23

```
atgattaatg ataaaaacct agcgaaagaa ataatagcca aaagagttgc aagagaatta      60
aaaaatggtc aacttgtaaa cttaggtgta ggtcttccta ccatggttgc agattatata     120
ccaaaaaatt tcaaaattac tttccaatca gaaaacggaa tagttggaat gggcgctagt     180
cctaaaataa atgaggcaga taaagatgta gtaaatgcag gaggagacta tacaacagta     240
cttcctgacg gcacattttt cgatagctca gtttcgtttt cactaatccg tggtggtcac     300
gtagatgtta ctgttttagg ggctctccag gtagatgaaa agggtaatat agccaattgg     360
attgttcctg gaaaaatgct ctctggtatg ggtggagcta tggatttagt aaatggagct     420
aagaaagtaa taattgcaat gagacataca aataaaggtc aacctaaaat tttaaaaaaa     480
tgtacacttc ccctcacggc aaagtctcaa gcaaatctaa ttgtaacaga acttggagta     540
attgaggtta ttaatgatgg tttacttctc actgaaatta ataaaaacac aaccattgat     600
gaaataaggt ctttaactgc tgcagattta ctcatatcca atgaacttag acccatggct     660
gtttag                                                                666
```

<210> 24
<211> 221
<212> PRT
<213> Clostridium acetobutylicum

<400> 24

```
Met Ile Asn Asp Lys Asn Leu Ala Lys Glu Ile Ile Ala Lys Arg Val
1               5                   10                  15

Ala Arg Glu Leu Lys Asn Gly Gln Leu Val Asn Leu Gly Val Gly Leu
            20                  25                  30

Pro Thr Met Val Ala Asp Tyr Ile Pro Lys Asn Phe Lys Ile Thr Phe
        35                  40                  45

Gln Ser Glu Asn Gly Ile Val Gly Met Gly Ala Ser Pro Lys Ile Asn
    50                  55                  60

Glu Ala Asp Lys Asp Val Val Asn Ala Gly Gly Asp Tyr Thr Thr Val
65                  70                  75                  80

Leu Pro Asp Gly Thr Phe Phe Asp Ser Ser Val Ser Phe Ser Leu Ile
                85                  90                  95

Arg Gly Gly His Val Asp Val Thr Val Leu Gly Ala Leu Gln Val Asp
            100                 105                 110

Glu Lys Gly Asn Ile Ala Asn Trp Ile Val Pro Gly Lys Met Leu Ser
        115                 120                 125

Gly Met Gly Gly Ala Met Asp Leu Val Asn Gly Ala Lys Lys Val Ile
    130                 135                 140

Ile Ala Met Arg His Thr Asn Lys Gly Gln Pro Lys Ile Leu Lys Lys
145                 150                 155                 160

Cys Thr Leu Pro Leu Thr Ala Lys Ser Gln Ala Asn Leu Ile Val Thr
                165                 170                 175

Glu Leu Gly Val Ile Glu Val Ile Asn Asp Gly Leu Leu Leu Thr Glu
            180                 185                 190

Ile Asn Lys Asn Thr Thr Ile Asp Glu Ile Arg Ser Leu Thr Ala Ala
        195                 200                 205

Asp Leu Leu Ile Ser Asn Glu Leu Arg Pro Met Ala Val
    210                 215                 220
```

<210> 25
<211> 1824
<212> DNA
<213> Clostridium ljungdahlii

<400> 25

```
atgtacggat ataagggtaa ggtattaaga attaatctaa gtagtaaaac ttatatagtg      60
gaagaattga aaattgacaa agctaaaaaa tttataggtg caagagggtt aggcgtaaaa     120
accttatttg acgaagtaga tccaaaggta gatccattat cacctgataa caaatttatt     180
atagcagcgg gaccacttac aggtgcacct gttccaacaa gcggaagatt catggtagtt     240
actaaatcac ctttaacagg aactattgct attgcaaatt caggtggaaa atggggagca     300
gaattcaaag cagctggata cgatatgata atcgttgaag gtaaatctga taaagaagtt     360
tatgtaaata tagtagatga taaagtagaa tttagggatg cttctcatgt ttggggaaaa     420
ctaacagaag aaactacaaa aatgcttcaa caggaaacag attcgagagc taaggtttta     480
tgcataggac cagctgggga aaagttatca cttatggcag cagttatgaa tgatgttgat     540
agaacagcag gacgtggtgg tgttggagct gttatgggtt caaagaactt aaaagctatt     600
gtagttaaag gaagcggaaa agtaaaatta tttgatgaac aaaaagtgaa ggaagtagca     660
cttgagaaaa caaatatttt aagaaaagat ccagtagctg gtggaggact tccaacatac     720
ggaacagctg tacttgttaa tattataaat gaaaatggtg tacatccagt aaagaatttt     780
caaaaatctt atacagatca agcagataag atcagtggag aaactttaac taaagattgc     840
ttagttagaa aaaatccttg ctataggtgt ccaattgcct gtggaagatg ggtaaaactt     900
gatgatggaa ctgaatgtgg aggaccagaa tatgaaacat tatggtcatt tggatctgat     960
```

tgtgatgtat acgatataaa tgctgtaaat acagcaaata tgttgtgtaa tgaatatgga 1020

ttagatacca ttacagcagg atgtactatt gcagcagcta tggaacttta tcaaagaggt 1080

tatattaagg atgaagaaat agcagcagat ggattgtcac ttaattgggg agatgctaag 1140

tccatggttg aatgggtaaa gaaaatggga cttagagaag gatttggaga caagatggca 1200

gatggttcat acagactttg tgactcatac ggtgtacctg agtattcaat gactgtaaaa 1260

aaacaggaac ttccagcata tgacccaaga ggaatacagg gacatggtat tacttatgct 1320

gttaacaata ggggaggatg tcacattaag ggatatatgg taagtcctga aatacttggc 1380

tatccagaaa aacttgatag acttgcagtg gaaggaaaag caggatatgc tagagtattc 1440

catgatttaa cagctgttat agattcactt ggattatgta tttttacaac atttggtctt 1500

ggtgcacagg attatgttga tatgtataat gcagtagttg gtggagaatt acatgatgta 1560

aattctttaa tgttagctgg agatagaata tggactttag aaaaaatatt taacttaaag 1620

gcaggcatag atagttcaca ggatactctt ccaaagagat tgcttgaaga acaaattcca 1680

gaaggaccat caaaaggaga agttcataag ttagatgtac tactacctga atattattca 1740

gtacgtggat gggataaaaa tggtattcct acagaggaaa cgttaaagaa attaggatta 1800

gatgaatacg taggtaagct ttag 1824

<210> 26
<211> 607
<212> PRT
<213> Clostridium ljungdahlii

<400> 26

```
Met Tyr Gly Tyr Lys Gly Lys Val Leu Arg Ile Asn Leu Ser Ser Lys
1               5                   10                  15

Thr Tyr Ile Val Glu Glu Leu Lys Ile Asp Lys Ala Lys Lys Phe Ile
            20                  25                  30

Gly Ala Arg Gly Leu Gly Val Lys Thr Leu Phe Asp Glu Val Asp Pro
        35                  40                  45

Lys Val Asp Pro Leu Ser Pro Asp Asn Lys Phe Ile Ile Ala Ala Gly
    50                  55                  60

Pro Leu Thr Gly Ala Pro Val Pro Thr Ser Gly Arg Phe Met Val Val
65                  70                  75                  80

Thr Lys Ser Pro Leu Thr Gly Thr Ile Ala Ile Ala Asn Ser Gly Gly
                85                  90                  95

Lys Trp Gly Ala Glu Phe Lys Ala Ala Gly Tyr Asp Met Ile Ile Val
```

100 105 110

Glu Gly Lys Ser Asp Lys Glu Val Tyr Val Asn Ile Val Asp Asp Lys
115 120 125

Val Glu Phe Arg Asp Ala Ser His Val Trp Gly Lys Leu Thr Glu Glu
130 135 140

Thr Thr Lys Met Leu Gln Gln Glu Thr Asp Ser Arg Ala Lys Val Leu
145 150 155 160

Cys Ile Gly Pro Ala Gly Glu Lys Leu Ser Leu Met Ala Ala Val Met
165 170 175

Asn Asp Val Asp Arg Thr Ala Gly Arg Gly Gly Val Gly Ala Val Met
180 185 190

Gly Ser Lys Asn Leu Lys Ala Ile Val Val Lys Gly Ser Gly Lys Val
195 200 205

Lys Leu Phe Asp Glu Gln Lys Val Lys Glu Val Ala Leu Glu Lys Thr
210 215 220

Asn Ile Leu Arg Lys Asp Pro Val Ala Gly Gly Gly Leu Pro Thr Tyr
225 230 235 240

Gly Thr Ala Val Leu Val Asn Ile Ile Asn Glu Asn Gly Val His Pro
245 250 255

Val Lys Asn Phe Gln Lys Ser Tyr Thr Asp Gln Ala Asp Lys Ile Ser
260 265 270

Gly Glu Thr Leu Thr Lys Asp Cys Leu Val Arg Lys Asn Pro Cys Tyr
275 280 285

Arg Cys Pro Ile Ala Cys Gly Arg Trp Val Lys Leu Asp Asp Gly Thr
290 295 300

Glu Cys Gly Gly Pro Glu Tyr Glu Thr Leu Trp Ser Phe Gly Ser Asp
305 310 315 320

Cys Asp Val Tyr Asp Ile Asn Ala Val Asn Thr Ala Asn Met Leu Cys
325 330 335

Asn Glu Tyr Gly Leu Asp Thr Ile Thr Ala Gly Cys Thr Ile Ala Ala
340 345 350

```
Ala Met Glu Leu Tyr Gln Arg Gly Tyr Ile Lys Asp Glu Glu Ile Ala
        355                 360                 365

Ala Asp Gly Leu Ser Leu Asn Trp Gly Asp Ala Lys Ser Met Val Glu
    370                 375                 380

Trp Val Lys Lys Met Gly Leu Arg Glu Gly Phe Gly Asp Lys Met Ala
385                 390                 395                 400

Asp Gly Ser Tyr Arg Leu Cys Asp Ser Tyr Gly Val Pro Glu Tyr Ser
                405                 410                 415

Met Thr Val Lys Lys Gln Glu Leu Pro Ala Tyr Asp Pro Arg Gly Ile
            420                 425                 430

Gln Gly His Gly Ile Thr Tyr Ala Val Asn Asn Arg Gly Gly Cys His
        435                 440                 445

Ile Lys Gly Tyr Met Val Ser Pro Glu Ile Leu Gly Tyr Pro Glu Lys
    450                 455                 460

Leu Asp Arg Leu Ala Val Glu Gly Lys Ala Gly Tyr Ala Arg Val Phe
465                 470                 475                 480

His Asp Leu Thr Ala Val Ile Asp Ser Leu Gly Leu Cys Ile Phe Thr
                485                 490                 495

Thr Phe Gly Leu Gly Ala Gln Asp Tyr Val Asp Met Tyr Asn Ala Val
            500                 505                 510

Val Gly Gly Glu Leu His Asp Val Asn Ser Leu Met Leu Ala Gly Asp
        515                 520                 525

Arg Ile Trp Thr Leu Glu Lys Ile Phe Asn Leu Lys Ala Gly Ile Asp
    530                 535                 540

Ser Ser Gln Asp Thr Leu Pro Lys Arg Leu Leu Glu Glu Gln Ile Pro
545                 550                 555                 560

Glu Gly Pro Ser Lys Gly Glu Val His Lys Leu Asp Val Leu Leu Pro
                565                 570                 575

Glu Tyr Tyr Ser Val Arg Gly Trp Asp Lys Asn Gly Ile Pro Thr Glu
            580                 585                 590

Glu Thr Leu Lys Lys Leu Gly Leu Asp Glu Tyr Val Gly Lys Leu
        595                 600                 605
```

<210> 27
<211> 735
<212> DNA
<213> Clostridium acetobutylicum

<400> 27

```
atgttaaagg atgaagtaat taaacaaatt agcacgccat taacttcgcc tgcatttcct      60
agaggaccct ataaatttca taatcgtgag tattttaaca ttgtatatcg tacagatatg     120
gatgcacttc gtaaagttgt gccagagcct ttagaaattg atgagccctt agtcaggttt     180
gaaattatgg caatgcatga tacgagtgga cttggttgtt atacagaaag cggacaggct     240
attcccgtaa gctttaatgg agttaaggga gattatcttc atatgatgta tttagataat     300
gagcctgcaa ttgcagtagg aagggaatta agtgcatatc ctaaaaagct cgggtatcca     360
aagctttttg tggattcaga tactttagta ggaactttag actatggaaa acttagagtt     420
gcgacagcta caatggggta caaacataaa gccttagatg ctaatgaagc aaaggatcaa     480
atttgtcgcc ctaattatat gttgaaaata atacccaatt atgatggaag ccctagaata     540
tgtgagctta taaatgcgaa aatcacagat gttaccgtac atgaagcttg gacaggacca     600
actcgactgc agttatttga tcacgctatg gcgccactta atgatttgcc agtaaaagag     660
attgtttcta gctctcacat tcttgcagat ataatattgc ctagagctga agttatatat     720
gattatctta agtaa                                                      735
```

<210> 28
<211> 244
<212> PRT
<213> Clostridium acetobutylicum

<400> 28

```
Met Leu Lys Asp Glu Val Ile Lys Gln Ile Ser Thr Pro Leu Thr Ser
1               5                   10                  15

Pro Ala Phe Pro Arg Gly Pro Tyr Lys Phe His Asn Arg Glu Tyr Phe
            20                  25                  30

Asn Ile Val Tyr Arg Thr Asp Met Asp Ala Leu Arg Lys Val Val Pro
        35                  40                  45

Glu Pro Leu Glu Ile Asp Glu Pro Leu Val Arg Phe Glu Ile Met Ala
    50                  55                  60

Met His Asp Thr Ser Gly Leu Gly Cys Tyr Thr Glu Ser Gly Gln Ala
65                  70                  75                  80

Ile Pro Val Ser Phe Asn Gly Val Lys Gly Asp Tyr Leu His Met Met
                85                  90                  95

Tyr Leu Asp Asn Glu Pro Ala Ile Ala Val Gly Arg Glu Leu Ser Ala
            100                 105                 110

Tyr Pro Lys Lys Leu Gly Tyr Pro Lys Leu Phe Val Asp Ser Asp Thr
        115                 120                 125

Leu Val Gly Thr Leu Asp Tyr Gly Lys Leu Arg Val Ala Thr Ala Thr
    130                 135                 140

Met Gly Tyr Lys His Lys Ala Leu Asp Ala Asn Glu Ala Lys Asp Gln
145                 150                 155                 160

Ile Cys Arg Pro Asn Tyr Met Leu Lys Ile Ile Pro Asn Tyr Asp Gly
                165                 170                 175

Ser Pro Arg Ile Cys Glu Leu Ile Asn Ala Lys Ile Thr Asp Val Thr
            180                 185                 190

Val His Glu Ala Trp Thr Gly Pro Thr Arg Leu Gln Leu Phe Asp His
        195                 200                 205

Ala Met Ala Pro Leu Asn Asp Leu Pro Val Lys Glu Ile Val Ser Ser
    210                 215                 220

Ser His Ile Leu Ala Asp Ile Ile Leu Pro Arg Ala Glu Val Ile Tyr
225                 230                 235                 240

Asp Tyr Leu Lys
```

<210> 29
<211> 1140
<212> DNA
<213> Clostridium acetobutylicum

<400> 29

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atggatttta atttaacaag agaacaagaa ttagtaagac agatggttag agaatttgct      60
gaaaatgaag ttaaacctat agcagcagaa attgatgaaa cagaaagatt tccaatggaa     120
aatgtaaaga aaatgggtca gtatggtatg atgggaattc cattttcaaa agagtatggt     180
ggcgcaggtg gagatgtatt atcttatata atcgccgttg aggaattatc aaaggtttgc     240
ggtactacag gagttattct ttcagcacat acatcacttt gtgcttcatt aataaatgaa     300
catggtacag aagaacaaaa acaaaaatat ttagtacctt tagctaaagg tgaaaaaata     360
ggtgcttatg gattgactga gccaaatgca ggaacagatt ctggagcaca acaaacagta     420
gctgtacttg aaggagatca ttatgtaatt aatggttcaa aaatattcat aactaatgga     480
ggagttgcag atacttttgt tatatttgca atgactgaca gaactaaagg aacaaaaggt     540
atatcagcat ttataataga aaaaggcttc aaaggtttct ctattggtaa agttgaacaa     600
aagcttggaa taagagcttc atcaacaact gaacttgtat ttgaagatat gatagtacca     660
gtagaaaaca tgattggtaa agaaggaaaa ggcttcccta tagcaatgaa aactcttgat     720
ggaggaagaa ttggtatagc agctcaagct ttaggtatag ctgaaggtgc tttcaacgaa     780
gcaagagctt acatgaagga gagaaaacaa tttggaagaa gccttgacaa attccaaggt     840
cttgcatgga tgatggcaga tatggatgta gctatagaat cagctagata tttagtatat     900
aaagcagcat atcttaaaca agcaggactt ccatacacag ttgatgctgc aagagctaag     960
cttcatgctg caaatgtagc aatggatgta acaactaagg cagtacaatt atttggtgga    1020
tacggatata caaaagatta tccagttgaa agaatgatga gagatgctaa gataactgaa    1080
atatatgaag gaacttcaga agttcagaaa ttagttattt caggaaaaat ttttagataa    1140
```

<210> 30
<211> 379
<212> PRT
<213> Clostridium acetobutylicum

<400> 30

```
Met Asp Phe Asn Leu Thr Arg Glu Gln Glu Leu Val Arg Gln Met Val
1               5                   10                  15

Arg Glu Phe Ala Glu Asn Glu Val Lys Pro Ile Ala Ala Glu Ile Asp
            20                  25                  30

Glu Thr Glu Arg Phe Pro Met Glu Asn Val Lys Lys Met Gly Gln Tyr
        35                  40                  45

Gly Met Met Gly Ile Pro Phe Ser Lys Glu Tyr Gly Gly Ala Gly Gly
    50                  55                  60

Asp Val Leu Ser Tyr Ile Ile Ala Val Glu Glu Leu Ser Lys Val Cys
65                  70                  75                  80

Gly Thr Thr Gly Val Ile Leu Ser Ala His Thr Ser Leu Cys Ala Ser
                85                  90                  95

Leu Ile Asn Glu His Gly Thr Glu Glu Gln Lys Gln Lys Tyr Leu Val
            100                 105                 110

Pro Leu Ala Lys Gly Glu Lys Ile Gly Ala Tyr Gly Leu Thr Glu Pro
        115                 120                 125

Asn Ala Gly Thr Asp Ser Gly Ala Gln Gln Thr Val Ala Val Leu Glu
    130                 135                 140
```

```
Gly Asp His Tyr Val Ile Asn Gly Ser Lys Ile Phe Ile Thr Asn Gly
145                 150                 155                 160

Gly Val Ala Asp Thr Phe Val Ile Phe Ala Met Thr Asp Arg Thr Lys
                165                 170                 175

Gly Thr Lys Gly Ile Ser Ala Phe Ile Ile Glu Lys Gly Phe Lys Gly
            180                 185                 190

Phe Ser Ile Gly Lys Val Glu Gln Lys Leu Gly Ile Arg Ala Ser Ser
        195                 200                 205

Thr Thr Glu Leu Val Phe Glu Asp Met Ile Val Pro Val Glu Asn Met
    210                 215                 220

Ile Gly Lys Glu Gly Lys Gly Phe Pro Ile Ala Met Lys Thr Leu Asp
225                 230                 235                 240

Gly Gly Arg Ile Gly Ile Ala Ala Gln Ala Leu Gly Ile Ala Glu Gly
                245                 250                 255

Ala Phe Asn Glu Ala Arg Ala Tyr Met Lys Glu Arg Lys Gln Phe Gly
            260                 265                 270

Arg Ser Leu Asp Lys Phe Gln Gly Leu Ala Trp Met Met Ala Asp Met
        275                 280                 285

Asp Val Ala Ile Glu Ser Ala Arg Tyr Leu Val Tyr Lys Ala Ala Tyr
    290                 295                 300

Leu Lys Gln Ala Gly Leu Pro Tyr Thr Val Asp Ala Ala Arg Ala Lys
305                 310                 315                 320

Leu His Ala Ala Asn Val Ala Met Asp Val Thr Thr Lys Ala Val Gln
                325                 330                 335

Leu Phe Gly Gly Tyr Gly Tyr Thr Lys Asp Tyr Pro Val Glu Arg Met
            340                 345                 350

Met Arg Asp Ala Lys Ile Thr Glu Ile Tyr Glu Gly Thr Ser Glu Val
        355                 360                 365

Gln Lys Leu Val Ile Ser Gly Lys Ile Phe Arg
    370                 375
```

<210> 31
<211> 1197

<212> DNA
<213> Euglena gracilis

<400> 31

```
atgatagtaa aagcaaagtt tgtaaaagga tttatcagag atgtacatcc ttatggttgc      60
agaagggaag tactaaatca aatagattat tgtaagaagg ctattgggtt taggggacca     120
aagaaggttt taattgttgg agcctcatct gggtttggtc ttgctactag aatttcagtt     180
gcatttggag gtccagaagc tcacacaatt ggagtatcct atgaaacagg agctacagat     240
agaagaatag gaacagcggg atggtataat aacatatttt ttaaagaatt tgctaaaaaa     300
aaaggattag ttgcaaaaaa cttcattgag gatgcctttt ctaatgaaac caaagataaa     360
gttattaagt atataaagga tgaatttggt aaaatagatt tatttgttta tagtttagct     420
gcgcctagga gaaaggacta taaaactgga aatgtttata cttcaagaat aaaaacaatt     480
ttaggagatt ttgagggacc gactattgat gttgaaagag acgagattac tttaaaaaag     540
gttagtagtg ctagcattga agaaattgaa gaaactagaa aggtaatggg tggagaggat     600
tggcaagagt ggtgtgaaga gctgctttat gaagattgtt tttcggataa agcaactacc     660
atagcatact cgtatatagg atccccaaga acctacaaga tatatagaga aggtactata     720
ggaatagcta aaaaggatct tgaagataag gctaagctta taaatgaaaa acttaacaga     780
gttataggtg gtagagcctt tgtgtctgtg aataaagcat tagttacaaa agcaagtgca     840
tatattccaa cttttcctct ttatgcagct attttatata aggtcatgaa agaaaaaaat     900
attcatgaaa attgtattat gcaaattgag agaatgtttt ctgaaaaaat atattcaaat     960
gaaaaaatac aatttgatga caagggaaga ttaaggatgg acgatttaga gcttagaaaa    1020
gacgttcaag acgaagttga tagaatatgg agtaatatta ctcctgaaaa ttttaaggaa    1080
ttatctgatt ataagggata caaaaaagaa ttcatgaact taaacggttt tgatctagat    1140
ggggttgatt atagtaaaga cctggatata gaattattaa gaaaattaga accttaa       1197
```

<210> 32
<211> 398
<212> **PRT**
<213> Euglena gracilis

<400> 32

```
Met Ile Val Lys Ala Lys Phe Val Lys Gly Phe Ile Arg Asp Val His
1               5                   10                  15

Pro Tyr Gly Cys Arg Arg Glu Val Leu Asn Gln Ile Asp Tyr Cys Lys
            20                  25                  30

Lys Ala Ile Gly Phe Arg Gly Pro Lys Lys Val Leu Ile Val Gly Ala
        35                  40                  45
```

Ser Ser Gly Phe Gly Leu Ala Thr Arg Ile Ser Val Ala Phe Gly Gly
50 55 60

Pro Glu Ala His Thr Ile Gly Val Ser Tyr Glu Thr Gly Ala Thr Asp
65 70 75 80

Arg Arg Ile Gly Thr Ala Gly Trp Tyr Asn Asn Ile Phe Phe Lys Glu
85 90 95

Phe Ala Lys Lys Lys Gly Leu Val Ala Lys Asn Phe Ile Glu Asp Ala
100 105 110

Phe Ser Asn Glu Thr Lys Asp Lys Val Ile Lys Tyr Ile Lys Asp Glu
115 120 125

Phe Gly Lys Ile Asp Leu Phe Val Tyr Ser Leu Ala Ala Pro Arg Arg
130 135 140

Lys Asp Tyr Lys Thr Gly Asn Val Tyr Thr Ser Arg Ile Lys Thr Ile
145 150 155 160

Leu Gly Asp Phe Glu Gly Pro Thr Ile Asp Val Glu Arg Asp Glu Ile
165 170 175

Thr Leu Lys Lys Val Ser Ser Ala Ser Ile Glu Glu Ile Glu Glu Thr
180 185 190

Arg Lys Val Met Gly Gly Glu Asp Trp Gln Glu Trp Cys Glu Glu Leu
195 200 205

Leu Tyr Glu Asp Cys Phe Ser Asp Lys Ala Thr Thr Ile Ala Tyr Ser
210 215 220

Tyr Ile Gly Ser Pro Arg Thr Tyr Lys Ile Tyr Arg Glu Gly Thr Ile
225 230 235 240

Gly Ile Ala Lys Lys Asp Leu Glu Asp Lys Ala Lys Leu Ile Asn Glu
245 250 255

Lys Leu Asn Arg Val Ile Gly Gly Arg Ala Phe Val Ser Val Asn Lys
260 265 270

Ala Leu Val Thr Lys Ala Ser Ala Tyr Ile Pro Thr Phe Pro Leu Tyr
275 280 285

Ala Ala Ile Leu Tyr Lys Val Met Lys Glu Lys Asn Ile His Glu Asn
290 295 300

```
Cys Ile Met Gln Ile Glu Arg Met Phe Ser Glu Lys Ile Tyr Ser Asn
305                 310                 315                 320

Glu Lys Ile Gln Phe Asp Asp Lys Gly Arg Leu Arg Met Asp Asp Leu
                325                 330                 335

Glu Leu Arg Lys Asp Val Gln Asp Glu Val Asp Arg Ile Trp Ser Asn
            340                 345                 350

Ile Thr Pro Glu Asn Phe Lys Glu Leu Ser Asp Tyr Lys Gly Tyr Lys
        355                 360                 365

Lys Glu Phe Met Asn Leu Asn Gly Phe Asp Leu Asp Gly Val Asp Tyr
    370                 375                 380

Ser Lys Asp Leu Asp Ile Glu Leu Leu Arg Lys Leu Glu Pro
385                 390                 395
```

<210> 33
<211> 1056
<212> DNA
<213> Clostridium beijerinckii

<400> 33

```
atgaaaggtt ttgcaatgct aggtattaat aagttaggat ggatcgaaaa agaaaggcca      60
gttgcgggtt catatgatgc tattgtacgc ccattagcag tatctccgtg tacatcagat     120
atacatactg tttttgaggg agctcttgga gataggaaga atatgatttt agggcatgaa     180
gctgtaggtg aagttgttga agtaggaagt gaagtgaagg attttaaacc tggtgacaga     240
gttatagttc cttgtacaac tccagattgg agatctttgg aagttcaagc tggttttcaa     300
cagcactcaa acggtatgct cgcaggatgg aaattttcaa atttcaagga tggagttttt     360
ggtgaatatt ttcatgtaaa tgatgcggat atgaatcttg cgattctacc taaagacatg     420
ccattagaaa atgctgttat gataacagat atgatgacta ctggatttca tggagcagaa     480
cttgcagata ttcaaatggg ttcaagtgtt gtggtaattg gcattggagc tgttggctta     540
atgggaatag caggtgctaa attacgtgga gcaggtagaa taattggagt ggggagcagg     600
ccgatttgtg ttgaggctgc aaaattttat ggagcaacag atattctaaa ttataaaaat     660
ggtcatatag ttgatcaagt tatgaaatta acgaatggaa aaggcgttga ccgcgtaatt     720
atggcaggcg gtggttctga aacattatcc caagcagtat ctatggttaa accaggagga     780
ataatttcta atataaatta tcatggaagt ggagatgctt tactaatacc acgtgtagaa     840
tggggatgtg gaatggctca caagactata aaaggaggtc tttgtcctgg gggacgtttg     900
agagcagaaa tgttaagaga tatggtagta tataatcgtg ttgatctaag taaattagtt     960

acacatgtat atcatggatt tgatcacata gaagaagcac tgttattaat gaaagacaag    1020
ccaaaagact taattaaagc agtagttata ttataa                              1056
```

<210> 34
<211> 351
<212> PRT
<213> Clostridium beijerinckii

<400> 34

```
Met Lys Gly Phe Ala Met Leu Gly Ile Asn Lys Leu Gly Trp Ile Glu
1               5                   10                  15

Lys Glu Arg Pro Val Ala Gly Ser Tyr Asp Ala Ile Val Arg Pro Leu
            20                  25                  30

Ala Val Ser Pro Cys Thr Ser Asp Ile His Thr Val Phe Glu Gly Ala
        35                  40                  45

Leu Gly Asp Arg Lys Asn Met Ile Leu Gly His Glu Ala Val Gly Glu
    50                  55                  60

Val Val Glu Val Gly Ser Glu Val Lys Asp Phe Lys Pro Gly Asp Arg
65                  70                  75                  80

Val Ile Val Pro Cys Thr Thr Pro Asp Trp Arg Ser Leu Glu Val Gln
                85                  90                  95

Ala Gly Phe Gln Gln His Ser Asn Gly Met Leu Ala Gly Trp Lys Phe
            100                 105                 110

Ser Asn Phe Lys Asp Gly Val Phe Gly Glu Tyr Phe His Val Asn Asp
        115                 120                 125

Ala Asp Met Asn Leu Ala Ile Leu Pro Lys Asp Met Pro Leu Glu Asn
    130                 135                 140

Ala Val Met Ile Thr Asp Met Met Thr Thr Gly Phe His Gly Ala Glu
145                 150                 155                 160

Leu Ala Asp Ile Gln Met Gly Ser Ser Val Val Val Ile Gly Ile Gly
                165                 170                 175

Ala Val Gly Leu Met Gly Ile Ala Gly Ala Lys Leu Arg Gly Ala Gly
            180                 185                 190

Arg Ile Ile Gly Val Gly Ser Arg Pro Ile Cys Val Glu Ala Ala Lys
        195                 200                 205
```

```
Phe Tyr Gly Ala Thr Asp Ile Leu Asn Tyr Lys Asn Gly His Ile Val
    210                 215                 220

Asp Gln Val Met Lys Leu Thr Asn Gly Lys Gly Val Asp Arg Val Ile
225                 230                 235                 240

Met Ala Gly Gly Gly Ser Glu Thr Leu Ser Gln Ala Val Ser Met Val
                245                 250                 255

Lys Pro Gly Gly Ile Ile Ser Asn Ile Asn Tyr His Gly Ser Gly Asp
            260                 265                 270

Ala Leu Leu Ile Pro Arg Val Glu Trp Gly Cys Gly Met Ala His Lys
        275                 280                 285

Thr Ile Lys Gly Gly Leu Cys Pro Gly Gly Arg Leu Arg Ala Glu Met
    290                 295                 300

Leu Arg Asp Met Val Val Tyr Asn Arg Val Asp Leu Ser Lys Leu Val
305                 310                 315                 320

Thr His Val Tyr His Gly Phe Asp His Ile Glu Glu Ala Leu Leu Leu
                325                 330                 335

Met Lys Asp Lys Pro Lys Asp Leu Ile Lys Ala Val Val Ile Leu
            340                 345                 350
```

<210> 35
<211> 1998
<212> DNA
<213> Clostridium acetobutylicum

<400> 35

```
atgggtaata agatatttgc cgtacttcaa aaaatcggta aatctttaat gcttccagta     60
tctgttctac cggcggctgg aattctattg agacttggac agccagattt gcttaatatg    120
ccttatgttc aagcagcagg aaatgctatt tttaataatt tacctttaat ttttgcggtt    180
ggagttgcta taggtttttc aggcggagaa ggtgttgcag cacttgcagc tgtaattgga    240
gaactaatac ttgaggcggt tgaaaaaaca gcaggtgata cggcagcagc agctttagca    300
aaaacagcgg cagcttcaca tcatatgacg cttgcagcat ttcaaaaaac tcaagaatac    360
agtaacattg taactaaaac aactattagt atgggtgttt ttggcggtat aataatcggt    420
cttacagcag ctattttata taataagttc catgatataa agatgcctca agttttgggt    480
ttctttggtg gaaaacgttt cgttccaatt ataacttcaa tttcagctct tattattgca    540
actataggag taaatatttg gttgccaata caagctggaa taaattcact agcagcattt    600
```

```
gcaactacat caccaattgg acctgcaatg tatgctggag gaaaaagact tttaattcca      660
ctaggacttc atcacattta ttatccattg ttcttatatc aatttggtca ctttgtttca      720
aatggagtta cttatgtagg agatacagca agatacttcc atggggatcc tactgccgga      780
aacttcatgg cagcagagta tccaatacta atgtttggtc ttccaggagc tgctctagct      840
atgattgcag ctgctaaaaa agaaaagaga aaagaaatgg ctggaatgat gatttcagca      900
gcatttgtag catttgtaac aggtattaca gagccaattg aattctcatt catatttgtt      960
gctccagtat tatttgtgtt ccacgtactt gctgcattcg catctggtct tattacaagt     1020
tatttacaca taagattagg gtatactttc tcagcatcct tcatagatta tgttttagga     1080
ttcaaatatg caggtcatcc attacttata tggcttgtag gtatagggtt ctttgtattg     1140
tactttgttg tattctactt cacaattaaa gcaatgaaca ttaagacacc aggtagagaa     1200
gatgatgatg cagaaggtgt taagaagata aatgtaaaag gaaaagctaa ggcagctaag     1260
gtgcttgaag ctataggcgg aaaagataat ataaaagtac ttgatgcatg tataacaaga     1320
ttaagactta acttaaatga tccttctttg gttgataaag ctacacttaa agctcttgga     1380
gcagctggag taatgacagc agaagatagt gttcaagtag tttttggaac tgaagctgaa     1440
agaataaaag atgacataaa agcaattata caaaatggtg gatatgttga agatgattca     1500
gataaggaag aagaagttca agaggataag caaatttcta aaggtgcaca cgaactttta     1560
agtccagctg atggagaagt agttggtatt gagagtgttc cggatagtac atttgctgaa     1620
aaaatgcttg gagacggttt tgcagtaata ccttcaggaa atgaagtaca ctcaccagct     1680
gatggagaag tatcagtttt attcccaact aagcatgcat ttgcaataac aacagaaggc     1740
ggacttgaac ttttaataca tgttggaatt gatactgtag cattaaatgg tgaaggtttc     1800
acagcacatg taaaacaagg agataaagtt aaaaaaggag atttgatttt aactttagat     1860
actgagttta taaagagcaa aggtaaaaac cttataactc cagtgattgt aactaacatg     1920
gatgttgtag gaaatataga tgttaaatta ggaaacgtta aaaattccga gaaagctgcg     1980
gatgttaccg taaaataa                                                   1998
```

<210> 36
<211> 665
<212> PRT
<213> Clostridium acetobutylicum

<400> 36

```
Met Gly Asn Lys Ile Phe Ala Val Leu Gln Lys Ile Gly Lys Ser Leu
1               5                   10                  15

Met Leu Pro Val Ser Val Leu Pro Ala Ala Gly Ile Leu Leu Arg Leu
            20                  25                  30
```

```
Gly Gln Pro Asp Leu Leu Asn Met Pro Tyr Val Gln Ala Ala Gly Asn
        35                  40                  45

Ala Ile Phe Asn Asn Leu Pro Leu Ile Phe Ala Val Gly Val Ala Ile
    50                  55                  60

Gly Phe Ser Gly Gly Glu Gly Val Ala Ala Leu Ala Ala Val Ile Gly
65                  70                  75                  80

Glu Leu Ile Leu Glu Ala Val Glu Lys Thr Ala Gly Asp Thr Ala Ala
                85                  90                  95

Ala Ala Leu Ala Lys Thr Ala Ala Ala Ser His His Met Thr Leu Ala
            100                 105                 110

Ala Phe Gln Lys Thr Gln Glu Tyr Ser Asn Ile Val Thr Lys Thr Thr
        115                 120                 125

Ile Ser Met Gly Val Phe Gly Gly Ile Ile Ile Gly Leu Thr Ala Ala
    130                 135                 140

Ile Leu Tyr Asn Lys Phe His Asp Ile Lys Met Pro Gln Val Leu Gly
145                 150                 155                 160

Phe Phe Gly Gly Lys Arg Phe Val Pro Ile Ile Thr Ser Ile Ser Ala
                165                 170                 175

Leu Ile Ile Ala Thr Ile Gly Val Asn Ile Trp Leu Pro Ile Gln Ala
            180                 185                 190

Gly Ile Asn Ser Leu Ala Ala Phe Ala Thr Thr Ser Pro Ile Gly Pro
        195                 200                 205

Ala Met Tyr Ala Gly Gly Lys Arg Leu Leu Ile Pro Leu Gly Leu His
    210                 215                 220

His Ile Tyr Tyr Pro Leu Phe Leu Tyr Gln Phe Gly His Phe Val Ser
225                 230                 235                 240

Asn Gly Val Thr Tyr Val Gly Asp Thr Ala Arg Tyr Phe His Gly Asp
                245                 250                 255

Pro Thr Ala Gly Asn Phe Met Ala Ala Glu Tyr Pro Ile Leu Met Phe
            260                 265                 270

Gly Leu Pro Gly Ala Ala Leu Ala Met Ile Ala Ala Ala Lys Lys Glu
        275                 280                 285
```

```
Lys Arg Lys Glu Met Ala Gly Met Met Ile Ser Ala Ala Phe Val Ala
    290                 295                 300

Phe Val Thr Gly Ile Thr Glu Pro Ile Glu Phe Ser Phe Ile Phe Val
305                 310                 315                 320

Ala Pro Val Leu Phe Val Phe His Val Leu Ala Ala Phe Ala Ser Gly
                325                 330                 335

Leu Ile Thr Ser Tyr Leu His Ile Arg Leu Gly Tyr Thr Phe Ser Ala
            340                 345                 350

Ser Phe Ile Asp Tyr Val Leu Gly Phe Lys Tyr Ala Gly His Pro Leu
        355                 360                 365

Leu Ile Trp Leu Val Gly Ile Gly Phe Phe Val Leu Tyr Phe Val Val
    370                 375                 380

Phe Tyr Phe Thr Ile Lys Ala Met Asn Ile Lys Thr Pro Gly Arg Glu
385                 390                 395                 400

Asp Asp Asp Ala Glu Gly Val Lys Lys Ile Asn Val Lys Gly Lys Ala
                405                 410                 415

Lys Ala Ala Lys Val Leu Glu Ala Ile Gly Gly Lys Asp Asn Ile Lys
            420                 425                 430

Val Leu Asp Ala Cys Ile Thr Arg Leu Arg Leu Asn Leu Asn Asp Pro
        435                 440                 445

Ser Leu Val Asp Lys Ala Thr Leu Lys Ala Leu Gly Ala Ala Gly Val
    450                 455                 460

Met Thr Ala Glu Asp Ser Val Gln Val Val Phe Gly Thr Glu Ala Glu
465                 470                 475                 480

Arg Ile Lys Asp Asp Ile Lys Ala Ile Ile Gln Asn Gly Gly Tyr Val
                485                 490                 495

Glu Asp Asp Ser Asp Lys Glu Glu Glu Val Gln Glu Asp Lys Gln Ile
            500                 505                 510

Ser Lys Gly Ala His Glu Leu Leu Ser Pro Ala Asp Gly Glu Val Val
        515                 520                 525

Gly Ile Glu Ser Val Pro Asp Ser Thr Phe Ala Glu Lys Met Leu Gly
    530                 535                 540
```

```
Asp Gly Phe Ala Val Ile Pro Ser Gly Asn Glu Val His Ser Pro Ala
545                 550                 555                 560

Asp Gly Glu Val Ser Val Leu Phe Pro Thr Lys His Ala Phe Ala Ile
                565                 570                 575

Thr Thr Glu Gly Gly Leu Glu Leu Leu Ile His Val Gly Ile Asp Thr
            580                 585                 590

Val Ala Leu Asn Gly Glu Gly Phe Thr Ala His Val Lys Gln Gly Asp
        595                 600                 605

Lys Val Lys Lys Gly Asp Leu Ile Leu Thr Leu Asp Thr Glu Phe Ile
    610                 615                 620

Lys Ser Lys Gly Lys Asn Leu Ile Thr Pro Val Ile Val Thr Asn Met
625                 630                 635                 640

Asp Val Val Gly Asn Ile Asp Val Lys Leu Gly Asn Val Lys Asn Ser
                645                 650                 655

Glu Lys Ala Ala Asp Val Thr Val Lys
            660                 665
```

<210> 37
<211> 2226
<212> DNA
<213> Clostridium saccharobutylicum

<400> 37

atgttagttg atgctttttt gcgcacaaag catctaaaac atccctatat acaaaaaata 60

tttaaaaaag gggaaaatgt tatgaaagac aaaatttttg gtattttaca gcgtgtagga 120

agatctttta tgcttccaat agctatttta ccagtagctg gattatttct tggtttaggt 180

ggatcattta ccaatgaaac aatgattcaa gcttatggac ttactgggtt aatcggacct 240

ggtacattta tttattcaat cctttctgta atgaatgcag caggaaatgt agtgtttggc 300

aatttgcctt tattatttgc aatgggtgtt gcaattggta tggctaaaaa ggaaaaagat 360

gttgcagctt tatcagcagc aattgcattc cttataatgc atgcatcaat aagtgctatg 420

attaatatta atggtggaac tgatgctctt cttagtggtg catcaacttc agtacttggt 480

attacttcat tacaaatggg tgtttttggt ggtattattg tagggcttgg agtagcagcg 540

ttacataata aattctataa aattgaatta ccacaggtat tatcattctt tggtggaact 600

agatttgttc caattgtaag tgcaataaca tatttaattg ttggaatttt aatgttttat 660

atttggcctc caattcaagg tggtatttat aaagttggag atcttgtatt aagatcagga 720

```
tatgcaggaa catggcttta tggtttaatg gaacgtttat taataccttt tggtcttcat      780
catgtatttt acttaccatt ctggcaaaca gcagttggtg gtacagctac agtaggtggg      840
aaagttattg aaggtgctca aaatattttc tttgctgaac ttggaactcc aggaataaca      900
cactttagtg tttcagcaac aagatttatg tcaggtaaat tcccacttat gatatttggt      960
ttacctggag cagcgcttgc gatgtacaga tgtgcaaaac cagaaaagag aaaagtagta     1020
ggtggattat tattatcagc agcattaact tcgatgttaa ctggtattac agaaccaatt     1080
gaatttacat tcttatttgt tgcaccatta ttatatggaa ttcactgcgt atttgctgga     1140
ctagcttata tgtttatgca tatgttaaat gttggagttg gtatgacttt ctctggtgga     1200
tttatagatt tattcttatt tggtatttta caaggaaatg ctaagacaag ttggatttgg     1260
gttgcagttg taggtattgc atattttgta gtatactatg tattgttctc tttcttaatt     1320
aagaagcttg acttaaagac tccaggtcgt gatgattctg aagaagttaa actttatcgt     1380
agaagcgatt tagatgcaaa gaaaaaaggt aataatgcag ataatggaga aagtgaaagt     1440
atagatgaat tatcagctat gatctgtcaa ggtcttggtg gtaagaagaa tatttcagat     1500
gttgactgtt gcgcaactag attacgttgt acagttgtta aatcagaatt agtaaatgaa     1560
gctttattaa aacaaactgg agcatcagga gtagttcata aaggcgtagg tgttcaaatt     1620
atatatggac caagagtaac agttataaaa tctaatttag aagattattt aattgcagca     1680
cctgatgaag aagttgctat agatgcagta gaagaaaaag cacctgaaat gccaactgaa     1740
aaggaagcgg aaggaaaagt tgttaataca atagttttaa gcagtccatt aacaggagat     1800
gctaaagatt tatcagaagc tccagatgaa gcatttgcaa gcaaaatgat gggagacgga     1860
gcggtagtaa ttccaagtaa tggagatgtt gtagcaccag cagatggtga ggtgagtttt     1920
gtattcccat caaaacatgc agtaggatta acaacaactg atggtcttga attacttatt     1980
catataggaa tagatacagt aaagcttgat ggaaaaggct ttgaaacttt cgtaaaagaa     2040
ggagacaaag ttaaaaaagg tgataaatta ttaagctttg acttagaatt tataaaagaa     2100
aatgcaccat ctattgcatc accatgcatt tgtacagcat taagcagcaa acaaaaagta     2160
cgtttgttaa aaacaggtga tataaaggca ggagaagact taatagcaat tgatgtgctt     2220
gaataa                                                                2226
```

<210> 38
<211> 741
<212> PRT
<213> Clostridium saccharobutylicum

<400> 38

Met Leu Val Asp Ala Phe Leu Arg Thr Lys His Leu Lys His Pro Tyr
1   5   10   15

Ile Gln Lys Ile Phe Lys Lys Gly Glu Asn Val Met Lys Asp Lys Ile
20 25 30

Phe Gly Ile Leu Gln Arg Val Gly Arg Ser Phe Met Leu Pro Ile Ala
35 40 45

Ile Leu Pro Val Ala Gly Leu Phe Leu Gly Leu Gly Gly Ser Phe Thr
50 55 60

Asn Glu Thr Met Ile Gln Ala Tyr Gly Leu Thr Gly Leu Ile Gly Pro
65 70 75 80

Gly Thr Phe Ile Tyr Ser Ile Leu Ser Val Met Asn Ala Ala Gly Asn
85 90 95

Val Val Phe Gly Asn Leu Pro Leu Leu Phe Ala Met Gly Val Ala Ile
100 105 110

Gly Met Ala Lys Lys Glu Lys Asp Val Ala Ala Leu Ser Ala Ala Ile
115 120 125

Ala Phe Leu Ile Met His Ala Ser Ile Ser Ala Met Ile Asn Ile Asn
130 135 140

Gly Gly Thr Asp Ala Leu Leu Ser Gly Ala Ser Thr Ser Val Leu Gly
145 150 155 160

Ile Thr Ser Leu Gln Met Gly Val Phe Gly Gly Ile Ile Val Gly Leu
165 170 175

Gly Val Ala Ala Leu His Asn Lys Phe Tyr Lys Ile Glu Leu Pro Gln
180 185 190

Val Leu Ser Phe Phe Gly Gly Thr Arg Phe Val Pro Ile Val Ser Ala
195 200 205

Ile Thr Tyr Leu Ile Val Gly Ile Leu Met Phe Tyr Ile Trp Pro Pro
210 215 220

Ile Gln Gly Gly Ile Tyr Lys Val Gly Asp Leu Val Leu Arg Ser Gly
225 230 235 240

Tyr Ala Gly Thr Trp Leu Tyr Gly Leu Met Glu Arg Leu Leu Ile Pro
245 250 255

Phe Gly Leu His His Val Phe Tyr Leu Pro Phe Trp Gln Thr Ala Val
260 265 270

```
Gly Gly Thr Ala Thr Val Gly Gly Lys Val Ile Glu Gly Ala Gln Asn
        275                 280                 285

Ile Phe Phe Ala Glu Leu Gly Thr Pro Gly Ile Thr His Phe Ser Val
    290                 295                 300

Ser Ala Thr Arg Phe Met Ser Gly Lys Phe Pro Leu Met Ile Phe Gly
305                 310                 315                 320

Leu Pro Gly Ala Ala Leu Ala Met Tyr Arg Cys Ala Lys Pro Glu Lys
                325                 330                 335

Arg Lys Val Val Gly Gly Leu Leu Leu Ser Ala Ala Leu Thr Ser Met
            340                 345                 350

Leu Thr Gly Ile Thr Glu Pro Ile Glu Phe Thr Phe Leu Phe Val Ala
        355                 360                 365

Pro Leu Leu Tyr Gly Ile His Cys Val Phe Ala Gly Leu Ala Tyr Met
    370                 375                 380

Phe Met His Met Leu Asn Val Gly Val Gly Met Thr Phe Ser Gly Gly
385                 390                 395                 400

Phe Ile Asp Leu Phe Leu Phe Gly Ile Leu Gln Gly Asn Ala Lys Thr
                405                 410                 415

Ser Trp Ile Trp Val Ala Val Val Gly Ile Ala Tyr Phe Val Val Tyr
            420                 425                 430

Tyr Val Leu Phe Ser Phe Leu Ile Lys Lys Leu Asp Leu Lys Thr Pro
        435                 440                 445

Gly Arg Asp Asp Ser Glu Glu Val Lys Leu Tyr Arg Arg Ser Asp Leu
    450                 455                 460

Asp Ala Lys Lys Lys Gly Asn Asn Ala Asp Asn Gly Glu Ser Glu Ser
465                 470                 475                 480

Ile Asp Glu Leu Ser Ala Met Ile Cys Gln Gly Leu Gly Gly Lys Lys
                485                 490                 495

Asn Ile Ser Asp Val Asp Cys Cys Ala Thr Arg Leu Arg Cys Thr Val
            500                 505                 510

Val Lys Ser Glu Leu Val Asn Glu Ala Leu Leu Lys Gln Thr Gly Ala
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            515                 520                 525

Ser Gly Val Val His Lys Gly Val Gly Val Gln Ile Ile Tyr Gly Pro
        530                 535                 540

Arg Val Thr Val Ile Lys Ser Asn Leu Glu Asp Tyr Leu Ile Ala Ala
545                 550                 555                 560

Pro Asp Glu Glu Val Ala Ile Asp Ala Val Glu Glu Lys Ala Pro Glu
                565                 570                 575

Met Pro Thr Glu Lys Glu Ala Glu Gly Lys Val Val Asn Thr Ile Val
            580                 585                 590

Leu Ser Ser Pro Leu Thr Gly Asp Ala Lys Asp Leu Ser Glu Ala Pro
        595                 600                 605

Asp Glu Ala Phe Ala Ser Lys Met Met Gly Asp Gly Ala Val Val Ile
    610                 615                 620

Pro Ser Asn Gly Asp Val Val Ala Pro Ala Asp Gly Glu Val Ser Phe
625                 630                 635                 640

Val Phe Pro Ser Lys His Ala Val Gly Leu Thr Thr Thr Asp Gly Leu
                645                 650                 655

Glu Leu Leu Ile His Ile Gly Ile Asp Thr Val Lys Leu Asp Gly Lys
            660                 665                 670

Gly Phe Glu Thr Phe Val Lys Glu Gly Asp Lys Val Lys Lys Gly Asp
        675                 680                 685

Lys Leu Leu Ser Phe Asp Leu Glu Phe Ile Lys Glu Asn Ala Pro Ser
    690                 695                 700

Ile Ala Ser Pro Cys Ile Cys Thr Ala Leu Ser Ser Lys Gln Lys Val
705                 710                 715                 720

Arg Leu Leu Lys Thr Gly Asp Ile Lys Ala Gly Glu Asp Leu Ile Ala
                725                 730                 735

Ile Asp Val Leu Glu
            740
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Claims

1. A mixotrophic fermentation method comprising

   (i) providing

      (a) an isolated naturally acetogenic organism selected from wild type *Clostridium ljungdahlii, Clostridium autoethanogenum,* and *Clostridium ragsdalei,* the native form of the organism having a glucose metabolism rate of less than 0.01 g/hr/g cell mass,
      (b) an acetogenic organism selected from *Clostridium ljungdahlii, Clostridium autoethanogenum,* and *Clostridium ragsdalei* which has been genetically modified to express a thiolase, CoA-transferase subunit A, CoA-transferase subunit B, acetoacetate decarboxylase, and secondary alcohol dehydrogenase, or
      (c) an acetogenic organism selected from *Clostridium ljungdahlii, Clostridium autoethanogenum,* and *Clostridium ragsdalei* which has been genetically modified to reduce expression of a secondary alcohol dehydrogenase or to delete from the genome a secondary alcohol dehydrogenase, and which has been genetically modified to express a thiolase, CoA-transferase subunit A, CoA-transferase subunit B, and acetoacetate decarboxylase;

   (ii) providing a first feedstock and a second feedstock wherein said first feedstock comprises a sugar that is metabolized by the native form of the organism at a rate of less than 0.01 g/hr/g cell mass; and wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate, H_2, glycerol, methanol, formate, urea, or a combination thereof; and
   (iii) culturing said organism in a fermentation medium, whereby both feedstocks are metabolized and a fermentation broth is formed, which broth comprises at least one bioproduct selected from acetone, isopropanol, acetate, or a combination thereof;

   wherein the method yields a greater amount of the at least one bioproduct than the combined amounts of the at least one bioproduct produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions; and
   wherein the carbon yield, based on the total amount of carbon in produced bioproducts divided by the total amount of carbon metabolized from said first feedstock, is at least 50%.

2. A method according to claim 1, wherein the $^{13}C/^{12}C$ isotope ratio of the carbon present in the bioproduct is less than that of atmospheric CO_2.

3. A method according to claim 1, wherein said first feedstock and said second feedstock are present in the fermentation medium at the same time.

4. A method according to claim 1, wherein said fermentation medium comprises at least one of CO, CO_2, and hydrogen.

5. A method according to claim 1, wherein said fermentation medium comprises a steel mill produced CO composition.

6. A method according to claim 1, wherein the culturing is performed in whole or in part at a super-atmospheric pressure.

7. A method according to claim 1, wherein said at least one bioproduct is selected from acetone, isopropanol, or a combination thereof.

8. A method according to claim 1, wherein said at least one bioproduct is non-naturally occurring.

9. A method according to claim 1, wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate, methanol, or a combination thereof, and wherein the $^{13}C/^{12}C$ isotope ration of the carbon present in the second feedstock is less than that of atmospheric CO_2.

10. A method according to claim 1, comprising providing said fermentation medium with a mixture of CO_2 and hydrogen at a molar ratio in the range from 1:0.1 to 1:5.

11. A method according to claim 10, further comprising steam reforming of a hydrocarbon to form said mixture of CO_2 and hydrogen.

12. A method according to claim 1, wherein the first feedstock comprises a sugar selected from glucose and sucrose, the second feedstock comprises at least one of H_2 and methanol, and the organism metabolizes CO_2 produced on metabolizing the sugar.

13. A method according to claim 1, wherein at least one bioproduct is acetone.

14. A method according to claim 1, wherein said at least one bioproduct is acetate.

15. A method according to claim 1, wherein said at least one bioproduct is isopropanol.

16. A method according to claim 1, 13 or 15, wherein the first feedstock comprises a sugar selected from glucose and sucrose, and the organism metabolizes CO_2 produced on metabolizing the sugar.

17. A method according to claim 1, wherein the metabolizing of the first feedstock does not inhibit the metabolizing of the second feedstock.

18. A method according to claim 1, wherein the first feedstock comprises a non-preferred sugar and wherein said second feedstock comprises CO, CO_2, carbonate, bicarbonate, H_2, glycerol, methanol, formate, urea, or a combination thereof.

19. A mixotrophic fermentation method comprising

(i) providing an isolated naturally acetogenic organism of the species *Clostridium ljungdahlii,* wherein the organism has been genetically modified to express a thiolase, CoA-transferase subunit A, CoA-transferase subunit B, acetoacetate decarboxylase, and secondary alcohol dehydrogenase,
(ii) providing a fermentation medium comprising a first feedstock and a second feedstock wherein said first feedstock comprises a sugar and said second feedstock comprises CO, CO_2, or a combination thereof; and
(iii) culturing said organism in said fermentation medium, whereby both feedstocks are metabolized and a fermentation broth is formed, which fermentation broth comprises at least one bioproduct selected from isopropanol, acetone, and acetate;

wherein the method yields a greater amount of the at least one bioproduct than the combined amounts of the at least one bioproduct produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions; and
wherein the carbon yield, based on the total amount of carbon in produced bioproducts divided by the total amount of carbon metabolized from said first feedstock, is at least 50%.

20. A mixotrophic fermentation method comprising

(i) providing an isolated naturally acetogenic organism of the species *Clostridium ljungdahlii,* wherein the organism has been genetically modified to reduce expression of a secondary alcohol dehydrogenase or to delete from the genome a secondary alcohol dehydrogenase, and genetically modified to express a thiolase, CoA-transferase subunit A, CoA-transferase subunit B, and acetoacetate decarboxylase;
(ii) providing a fermentation medium comprising a first feedstock and a second feedstock wherein said first feedstock comprises a sugar that is metabolized by the native form of the organism at a rate of less than 0.01 g/hr/g cell mass; and wherein said second feedstock comprises CO, CO_2, H_2, or mixtures thereof; and
(iii) culturing said organism in said fermentation medium, whereby both feedstocks are metabolized and a fermentation broth is formed, which broth comprises at least one bioproduct comprising acetone;

wherein the method yields a greater amount of the at least one bioproduct than the combined amounts of the at least one bioproduct produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions; and
wherein the carbon yield, based on the total amount of carbon in produced bioproducts divided by the total amount of carbon metabolized from said first feedstock, is at least 50%.

21. A mixotrophic fermentation method comprising

(i) providing

(a) an isolated naturally acetogenic organism selected from *Clostridium ljungdahlii, Clostridium autoethanogenum,* the native form of the organism having a glucose metabolism rate of less than 0.01 g/hr/g cell mass, or
(b) an acetogenic organism selected from *Clostridium ljungdahlii, Clostridium autoethanogenum,* and *Clostridium ragsdalei* which has been genetically modified to express at least one component of a phosphotransferase system;

(ii) providing a first feedstock and a second feedstock wherein said first feedstock comprises a sugar; and wherein said second feedstock comprises CO, CO_2, H_2, or a combination thereof; and
(iii) culturing said organism in a fermentation medium, whereby both feedstocks are metabolized and a fermentation broth is formed, which broth comprises at least one bioproduct selected from acetone, ethanol, 2,3-butanediol, lactate, or a combination thereof;

wherein the method yields a greater amount of the at least one bioproduct than the combined amounts of the at least one bioproduct produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions; and
wherein the carbon yield, based on the total amount of carbon in produced bioproducts divided by the total amount of carbon metabolized from said first feedstock, is at least 50%.

22. A mixotrophic fermentation method comprising

(i) providing an isolated naturally acetogenic organism of the species *Clostridium ljungdahlii,* wherein the organism has been genetically modified to express a thiolase, 3-hydroxybutyryl-CoA dehydrogenase, 3-hydroxybutyryl-CoA dehydratase, and acetaldehyde/alcohol dehydrogenase;
(ii) providing a fermentation medium comprising a first feedstock and a second feedstock wherein said first feedstock comprises a sugar that is metabolized by the native form of the organism at a rate of less than 0.01 g/hr/g cell mass; and wherein said second feedstock comprises CO, CO_2, H_2, or mixtures thereof; and (iii) culturing said organism in said fermentation medium, whereby both feedstocks are metabolized and a fermentation broth is formed, which broth comprises at least one bioproduct comprising crotyl alcohol;

wherein the method yields a greater amount of the at least one bioproduct than the combined amounts of the at least one bioproduct produced by heterotrophic and autotrophic fermentation with the same organism under the same conditions; and
wherein the carbon yield, based on the total amount of carbon in produced bioproducts divided by the total amount of carbon metabolized from said first feedstock, is at least 50%.

Patentansprüche

1. Mixotrophes Fermentationsverfahren, umfassend

(i) Bereitstellen

(a) eines isolierten natürlich acetogenen Organismus, ausgewählt aus Wildtyp-Clostridium ljungdahlii, Clostridium autoethanogenum und Clostridium ragsdalei, wobei die native Form des Organismus eine Glukosemetabolismusrate von weniger als 0,01 g/h/g Zellmasse aufweist;
(b) eines acetogenen Organismus, ausgewählt aus Clostridium ljungdahlii, Clostridium autoethanogenum und Clostridium ragsdalei, der genetisch modifiziert wurde, um eine Thiolase, CoA-Transferase Untereinheit A, CoA-Transferase Untereinheit B, Acetoacetat-Decarboxylase und Sekundäralkoholdehydrogenase zu exprimieren, oder
(c) eines acetogenen Organismus, ausgewählt aus Clostridium ljungdahlii, Clostridium autoethanogenum und Clostridium ragsdalei, der genetisch modifiziert wurde, um die Expression einer Sekundäralkoholdehydrogenase zu reduzieren oder Sekundäralkoholdehydrogenase aus dem Genom zu deletieren, und der genetisch modifiziert wurde, um eine Thiolase, CoA-Transferase Untereinheit A, CoA-Transferase Untereinheit B, Acetoacetat-Decarboxylase und Sekundäralkoholdehydrogenase zu exprimieren;

(ii) Bereitstellen eines ersten Ausgangsmaterials und eines zweiten Ausgangsmaterials, wobei das erste Ausgangsmaterial einen Zucker umfasst, die von der nativen Form des Organismus mit einer Rate von weniger als

0,01 g/h/g Zellmasse metabolisiert wird; und wobei das zweite Ausgangsmaterial CO, CO_2, Carbonat, Bicarbonat, H_2, Glycerin, Methanol, Formiat, Harnstoff oder eine Kombination davon umfasst; und
(iii) Kultivieren des Organismus in einem Fermentationsmedium, wodurch beide Ausgangsmaterialien metabolisiert werden und eine Fermentationsbrühe gebildet wird, wobei die Brühe mindestens ein Bioprodukt ausgewählt aus Aceton, Isopropanol, Acetat oder einer Kombination davon umfasst;

wobei das Verfahren eine größere Menge des mindestens einen Bioprodukts ergibt als die kombinierten Mengen des mindestens einen Bioprodukts, das durch heterotrophe und autotrophe Fermentation mit dem gleichen Organismus unter den gleichen Bedingungen produziert wird; und
wobei die Kohlenstoffausbeute, basierend auf der Gesamtmenge an Kohlenstoff in produzierten Bioprodukten dividiert durch die Gesamtmenge an Kohlenstoff, die aus dem ersten Ausgangsmaterial metabolisiert wird, mindestens 50 % beträgt.

2. Verfahren nach Anspruch 1, wobei das $^{13}C/^{12}C$-Isotopenverhältnis des in dem Bioprodukt vorhandenen Kohlenstoffs geringer ist als das von atmosphärischem CO_2.

3. Verfahren nach Anspruch 1, wobei das erste Ausgangsmaterial und das zweite Ausgangsmaterial gleichzeitig in dem Fermentationsmedium vorhanden sind.

4. Verfahren nach Anspruch 1, wobei das Fermentationsmedium mindestens eines von CO, CO_2 und Wasserstoff umfasst.

5. Verfahren nach Anspruch 1, wobei das Fermentationsmedium eine im Stahlwerk hergestellte CO-Zusammensetzung umfasst.

6. Verfahren nach Anspruch 1, wobei das Kultivieren ganz oder teilweise bei überatmosphärischem Druck durchgeführt wird.

7. Verfahren nach Anspruch 1, wobei das mindestens eine Bioprodukt aus Aceton, Isopropanol oder einer Kombination davon ausgewählt ist.

8. Verfahren nach Anspruch 1, wobei das Bioprodukt nicht natürlich vorkommt.

9. Verfahren nach Anspruch 1, wobei das zweite Ausgangsmaterial CO, CO_2, Carbonat, Bicarbonat, Methanol oder eine Kombination davon umfasst; und wobei das $^{13}C/^{12}C$-Isotopenverhältnis des Kohlenstoffs, der in dem zweiten Ausgangsmaterial vorhanden ist, geringer ist als das von atmosphärischem CO_2.

10. Verfahren nach Anspruch 1, umfassend das Bereitstellen des Fermentationsmediums mit einer Mischung aus CO_2 und Wasserstoff in einem Molverhältnis im Bereich von 1:0,1 bis 1:5.

11. Verfahren nach Anspruch 10, ferner umfassend das Dampfreformieren eines Kohlenwasserstoffs, um das Gemisch aus CO_2 und Wasserstoff zu bilden.

12. Verfahren nach Anspruch 1, wobei das erste Ausgangsmaterial einen Zucker ausgewählt aus Glukose und Saccharose umfasst, das zweite Ausgangsmaterial mindestens eines von H_2 und Methanol umfasst und der Organismus das bei der Metabolisierung des Zuckers erzeugte CO_2 metabolisiert.

13. Verfahren nach Anspruch 1, wobei das mindestens eine Bioprodukt Aceton ist.

14. Verfahren nach Anspruch 1, wobei das mindestens eine Bioprodukt Acetat ist.

15. Verfahren nach Anspruch 1, wobei das mindestens eine Bioprodukt Isopropanol ist.

16. Verfahren nach Anspruch 1, 13 oder 15, wobei das erste Ausgangsmaterial einen Zucker ausgewählt aus Glukose und Saccharose umfasst und der Organismus das beim Metabolisieren des Zuckers erzeugte CO_2 metabolisiert.

17. Verfahren nach Anspruch 1, wobei die Metabolisierung des ersten Ausgangsmaterials die Metabolisierung des zweiten Ausgangsmaterials nicht hemmt.

18. Verfahren nach Anspruch 1, wobei das erste Ausgangsmaterial einen nicht bevorzugten Zucker umfasst und wobei das zweite Ausgangsmaterial CO, CO_2, Carbonat, Bicarbonat, H_2, Glycerin, Methanol, Formiat, Harnstoff oder eine Kombination davon umfasst.

19. Mixotrophes Fermentationsverfahren, umfassend

(i) Bereitstellen eines isolierten natürlich acetogenen Organismus der Spezies Clostridium ljungdahlii, wobei der Organismus genetisch modifiziert wurde, um eine Thiolase, CoA-Transferase Untereinheit A, CoA-Transferase Untereinheit B, Acetoacetat-Decarboxylase und Sekundäralkoholdehydrogenase zu exprimieren;
(ii) Bereitstellen eines Fermentationsmediums umfassend ein erstes Ausgangsmaterial und ein zweites Ausgangsmaterial, wobei das erste Ausgangsmaterial einen Zucker umfasst und das zweite Ausgangsmaterial CO, CO_2 oder eine Kombination davon umfasst; und
(iii) Kultivieren des Organismus in dem Fermentationsmedium, wodurch beide Ausgangsmaterialen metabolisiert werden und eine Fermentationsbrühe gebildet wird, wobei die Fermentationsbrühe mindestens ein Bioprodukt ausgewählt aus Isopropanol, Aceton und Acetat umfasst;

wobei das Verfahren eine größere Menge des mindestens einen Bioprodukts ergibt als die kombinierten Mengen des mindestens einen Bioprodukts, das durch heterotrophe und autotrophe Fermentation mit dem gleichen Organismus unter den gleichen Bedingungen produziert wird; und wobei die Kohlenstoffausbeute, basierend auf der Gesamtmenge an Kohlenstoff in produzierten Bioprodukten dividiert durch die Gesamtmenge an Kohlenstoff, die aus dem ersten Ausgangsmaterial metabolisiert wird, mindestens 50 % beträgt.

20. Mixotrophes Fermentationsverfahren, umfassend

(i) Bereitstellen eines isolierten natürlich acetogenen Organismus der Spezies Clostridium ljungdahlii, wobei der Organismus genetisch modifiziert wurde, um die Expression einer Sekundäralkoholdehydrogenase zu reduzieren oder Sekundäralkoholdehydrogenase aus dem Genom zu deletieren, und der genetisch modifiziert wurde, um eine Thiolase, CoA-Transferase Untereinheit A, CoA-Transferase Untereinheit B und Acetoacetat-Decarboxylase zu exprimieren;
(ii) Bereitstellen eines Fermentationsmediums, umfassend ein erstes Ausgangsmaterial und ein zweites Ausgangsmaterial, wobei das erste Ausgangsmaterial einen Zucker umfasst, der durch die native Form des Organismus mit einer Rate von weniger als 0,01 g/h/g Zellmasse metabolisiert wird; und wobei das zweite Ausgangsmaterial CO, CO_2, H_2, oder Mischungen davon umfasst; und
(iii) Kultivieren des Organismus in dem Fermentationsmedium, wodurch beide Ausgangsmaterialien metabolisiert werden und eine Fermentationsbrühe gebildet wird, wobei die Brühe mindestens ein Bioprodukt umfassend Aceton umfasst,

wobei das Verfahren eine größere Menge des mindestens einen Bioprodukts ergibt als die kombinierten Mengen des mindestens einen Bioprodukts, das durch heterotrophe und autotrophe Fermentation mit dem gleichen Organismus unter den gleichen Bedingungen produziert wird; und wobei die Kohlenstoffausbeute, basierend auf der Gesamtmenge an Kohlenstoff in produzierten Bioprodukten dividiert durch die Gesamtmenge an Kohlenstoff, die aus dem ersten Ausgangsmaterial metabolisiert wird, mindestens 50 % beträgt.

21. Mixotrophes Fermentationsverfahren, umfassend

(i) Bereitstellen

(a) eines isolierten natürlich acetogenen Organismus, ausgewählt aus Clostridium ljungdahlii und Clostridium autoethanogenum, wobei die native Form des Organismus eine Glukosemetabolismusrate von weniger als 0,01 g/h/g Zellmasse aufweist; oder
(b) eines acetogenen Organismus, ausgewählt aus Clostridium ljungdahlii, Clostridium autoethanogenum und Clostridium ragsdalei, der genetisch modifiziert wurde, um mindestens eine Komponente eines Phosphotransferasesystems zu exprimieren;

(ii) Bereitstellen eines ersten Ausgangsmaterials und eines zweiten Ausgangsmaterials, wobei das erste Ausgangsmaterial einen Zucker umfasst; und wobei das zweite Ausgangsmaterial CO, CO_2, H_2, oder eine Kombination davon umfasst; und
(iii) Kultivieren des Organismus in dem Fermentationsmedium, wodurch beide Ausgangsmaterialien metaboli-

siert werden und eine Fermentationsbrühe gebildet wird, wobei die Brühe mindestens ein Bioprodukt ausgewählt aus Aceton, Ethanol, 2,3-Butandiol, Lactat oder eine Kombination davon umfasst;

wobei das Verfahren eine größere Menge des mindestens einen Bioprodukts ergibt als die kombinierten Mengen des mindestens einen Bioprodukts, das durch heterotrophe und autotrophe Fermentation mit dem gleichen Organismus unter den gleichen Bedingungen produziert wird; und wobei die Kohlenstoffausbeute, basierend auf der Gesamtmenge an Kohlenstoff in produzierten Bioprodukten dividiert durch die Gesamtmenge an Kohlenstoff, die aus dem ersten Ausgangsmaterial metabolisiert wird, mindestens 50 % beträgt.

22. Mixotrophes Fermentationsverfahren, umfassend

(i) Bereitstellen eines isolierten natürlich acetogenen Organismus der Spezies Clostridium ljungdahlii, wobei der Organismus genetisch modifiziert wurde, um eine Thiolase, 3-Hydroxybutyryl-CoA-Dehydrogenase, 3-Hydroxybutyryl-CoA-Dehydratase und Acetaldehyd/Alkoholdehydrogenase zu exprimieren;
(ii) Bereitstellen eines Fermentationsmediums, umfassend ein erstes Ausgangsmaterial und ein zweites Ausgangsmaterial, wobei das erste Ausgangsmaterial einen Zucker umfasst, der durch die native Form des Organismus mit einer Rate von weniger als 0,01 g/h/g Zellmasse metabolisiert wird; und wobei das zweite Ausgangsmaterial CO, CO_2, H_2, oder Mischungen davon umfasst; und
(iii) Kultivieren des Organismus in dem Fermentationsmedium, wodurch beide Ausgangsmaterialien metabolisiert werden und eine Fermentationsbrühe gebildet wird, wobei die Brühe mindestens ein Bioprodukt umfassend Crotylalkohol umfasst,

wobei das Verfahren eine größere Menge des mindestens einen Bioprodukts ergibt als die kombinierten Mengen des mindestens einen Bioprodukts, das durch heterotrophe und autotrophe Fermentation mit dem gleichen Organismus unter den gleichen Bedingungen produziert wird; und wobei die Kohlenstoffausbeute, basierend auf der Gesamtmenge an Kohlenstoff in produzierten Bioprodukten dividiert durch die Gesamtmenge an Kohlenstoff, die aus dem ersten Ausgangsmaterial metabolisiert wird, mindestens 50 % beträgt.

Revendications

1. Procédé de fermentation mixotrophique comprenant

(i) la fourniture

(a) d'un organisme isolé naturellement acétogène sélectionné parmi *Clostridium ljungdahlii, Clostridium autoethanogenum* et *Clostridium ragsdalei* de type sauvage, la forme native de l'organisme ayant un taux de métabolisme du glucose inférieur à 0,01 g/h/g de masse cellulaire,
(b) un organisme acétogène sélectionné parmi *Clostridium ljungdahlii, Clostridium autoethanogenum* et *Clostridium ragsdalei* qui a été génétiquement modifié pour exprimer une thiolase, une sous-unité A de la CoA-transférase, une sous-unité B de la CoA-transférase, une acétoacétate décarboxylase et une alcool secondaire déshydrogénase, ou
(c) un organisme acétogène sélectionné parmi *Clostridium ljungdahlii, Clostridium autoethanogenum* et *Clostridium ragsdalei* qui a été génétiquement modifié pour réduire l'expression d'une alcool secondaire déshydrogénase ou pour supprimer du génome une alcool secondaire déshydrogénase, et qui a été génétiquement modifié pour exprimer une thiolase, une sous-unité A de la CoA-transférase, une sous-unité B de la CoA-transférase et une acétoacétate décarboxylase ;

(ii) la fourniture d'une première matière première et d'une deuxième matière première, dans lequel ladite première matière première comprend un sucre qui est métabolisé par la forme native de l'organisme à un taux inférieur à 0,01 g/h/g de masse cellulaire ; et dans lequel ladite deuxième matière première comprend du CO, du CO_2, du carbonate, du bicarbonate, du H_2, du glycérol, du méthanol, du formiate, de l'urée, ou une combinaison de ceux-ci ; et
(iii) la culture dudit organisme dans un milieu de fermentation, moyennant quoi les deux matières premières sont métabolisées et un bouillon de fermentation est formé, lequel bouillon comprend au moins un bioproduit sélectionné parmi l'acétone, l'isopropanol, l'acétate ou une combinaison de ceux-ci ;

dans lequel le procédé donne une plus grande quantité de l'au moins un bioproduit que les quantités combinées

de l'au moins un bioproduit produit par fermentation hétérotrophe et autotrophe avec le même organisme dans les mêmes conditions ; et
dans lequel le rendement en carbone, basé sur la quantité totale de carbone dans les bioproduits produits divisée par la quantité totale de carbone métabolisée à partir de ladite première matière première, est d'au moins 50 %.

2. Procédé selon la revendication 1, dans lequel le rapport isotopique $^{13}C/^{12}C$ du carbone présent dans le bioproduit est inférieur à celui du CO_2 atmosphérique.

3. Procédé selon la revendication 1, dans lequel ladite première matière première et ladite deuxième matière première sont présentes dans le milieu de fermentation en même temps.

4. Procédé selon la revendication 1, dans lequel ledit milieu de fermentation comprend au moins un élément parmi le CO, le CO_2 et l'hydrogène.

5. Procédé selon la revendication 1, dans lequel ledit milieu de fermentation comprend une composition de CO produite par une aciérie.

6. Procédé selon la revendication 1, dans lequel la culture est réalisée en totalité ou en partie à une pression super-atmosphérique.

7. Procédé selon la revendication 1, dans lequel ledit au moins un bioproduit est sélectionné parmi l'acétone, l'isopropanol, ou une combinaison de ceux-ci.

8. Procédé selon la revendication 1, dans lequel ledit au moins un bioproduit est d'origine non naturelle.

9. Procédé selon la revendication 1, dans lequel ladite deuxième matière première comprend du CO, du CO_2, du carbonate, du bicarbonate, du méthanol, ou une combinaison de ceux-ci, et dans lequel le rapport isotopique $^{13}C/^{12}C$ du carbone présent dans la deuxième matière première est inférieur à celui du CO_2 atmosphérique.

10. Procédé selon la revendication 1, comprenant la fourniture audit milieu de fermentation d'un mélange de CO_2 et d'hydrogène à un rapport molaire dans la plage allant de 1:0,1 à 1:5.

11. Procédé selon la revendication 10, comprenant en outre le reformage à la vapeur d'un hydrocarbure pour former ledit mélange de CO_2 et d'hydrogène.

12. Procédé selon la revendication 1, dans lequel la première matière première comprend un sucre sélectionné parmi le glucose et le sucrose, la deuxième matière première comprend au moins un élément parmi le H_2 et le méthanol, et l'organisme métabolise le CO_2 produit lors de la métabolisation du sucre.

13. Procédé selon la revendication 1, dans lequel au moins un bioproduit est l'acétone.

14. Procédé selon la revendication 1, dans lequel ledit au moins un bioproduit est l'acétate.

15. Procédé selon la revendication 1, dans lequel ledit au moins un bioproduit est l'isopropanol.

16. Procédé selon la revendication 1, 13 ou 15, dans lequel la première matière première comprend un sucre sélectionné parmi le glucose et le sucrose, et l'organisme métabolise le CO_2 produit lors de la métabolisation du sucre.

17. Procédé selon la revendication 1, dans lequel la métabolisation de la première matière première n'inhibe pas la métabolisation de la deuxième matière première.

18. Procédé selon la revendication 1, dans lequel la première matière première comprend un sucre non préféré et dans lequel ladite deuxième matière première comprend du CO, du CO_2, du carbonate, du bicarbonate, du H_2, du glycérol, du méthanol, du formiate, de l'urée ou une combinaison de ceux-ci.

19. Procédé de fermentation mixotrophique comprenant

(i) la fourniture d'un organisme isolé naturellement acétogène de l'espèce *Clostridium ljungdahlii,* dans lequel

l'organisme a été génétiquement modifié pour exprimer une thiolase, une sous-unité A de la CoA-transférase, une sous-unité B de la CoA-transférase, une acétoacétate décarboxylase et une alcool secondaire déshydrogénase,
(ii) la fourniture d'un milieu de fermentation comprenant une première matière première et une deuxième matière première, dans lequel ladite première matière première comprend un sucre et ladite deuxième matière première comprend du CO, du CO_2, ou une combinaison de ceux-ci ; et
(iii) la culture dudit organisme dans ledit milieu de fermentation, moyennant quoi les deux matières premières sont métabolisées et un bouillon de fermentation est formé, lequel bouillon de fermentation comprend au moins un bioproduit sélectionné parmi l'isopropanol, l'acétone et l'acétate ;

dans lequel le procédé donne une plus grande quantité de l'au moins un bioproduit que les quantités combinées de l'au moins un bioproduit produit par fermentation hétérotrophe et autotrophe avec le même organisme dans les mêmes conditions ; et
dans lequel le rendement en carbone, basé sur la quantité totale de carbone dans les bioproduits produits divisée par la quantité totale de carbone métabolisée à partir de ladite première matière première, est d'au moins 50 %.

20. Procédé de fermentation mixotrophique comprenant

(i) la fourniture d'un organisme isolé naturellement acétogène de l'espèce *Clostridium ljungdahlii,* dans lequel l'organisme a été génétiquement modifié pour réduire l'expression d'une alcool secondaire déshydrogénase ou pour supprimer du génome une alcool secondaire déshydrogénase, et génétiquement modifié pour exprimer une thiolase, une sous-unité A de la CoA-transférase, une sous-unité B de la CoA-transférase, et une acétoacétate décarboxylase ;
(ii) la fourniture d'un milieu de fermentation comprenant une première matière première et une deuxième matière première, dans lequel ladite première matière première comprend un sucre qui est métabolisé par la forme native de l'organisme à un taux inférieur à 0,01 g/h/g de masse cellulaire ; et dans lequel ladite deuxième matière première comprend du CO, du CO_2, du H_2, ou des mélanges de ceux-ci ; et
(iii) la culture dudit organisme dans ledit milieu de fermentation, moyennant quoi les deux matières premières sont métabolisées et un bouillon de fermentation est formé, lequel bouillon comprend au moins un bioproduit comprenant de l'acétone ;

dans lequel le procédé donne une plus grande quantité de l'au moins un bioproduit que les quantités combinées de l'au moins un bioproduit produit par fermentation hétérotrophe et autotrophe avec le même organisme dans les mêmes conditions ; et
dans lequel le rendement en carbone, basé sur la quantité totale de carbone dans les bioproduits produits divisée par la quantité totale de carbone métabolisée à partir de ladite première matière première, est d'au moins 50 %.

21. Procédé de fermentation mixotrophique comprenant

(i) la fourniture

(a) d'un organisme isolé naturellement acétogène sélectionné parmi *Clostridium ljungdahlii, Clostridium autoethanogenum,* la forme native de l'organisme ayant un taux de métabolisme du glucose inférieur à 0,01 g/h/g de masse cellulaire, ou
(b) d'un organisme acétogène sélectionné parmi *Clostridium ljungdahlii, Clostridium autoethanogenum,* et *Clostridium ragsdalei* qui a été génétiquement modifié pour exprimer au moins un composant d'un système de phosphotransférase ;

(ii) la fourniture d'une première matière première et une deuxième matière première, dans lequel ladite première matière première comprend un sucre ; et dans lequel ladite deuxième matière première comprend du CO, du CO_2, du H_2, ou une combinaison de ceux-ci ; et
(iii) la culture dudit organisme dans un milieu de fermentation, moyennant quoi les deux matières premières sont métabolisées et un bouillon de fermentation est formé, lequel bouillon comprend au moins un bioproduit sélectionné parmi l'acétone, l'éthanol, le 2,3-butanediol, le lactate ou une combinaison de ceux-ci ;

dans lequel le procédé donne une plus grande quantité de l'au moins un bioproduit que les quantités combinées de l'au moins un bioproduit produit par fermentation hétérotrophe et autotrophe avec le même organisme dans les mêmes conditions ; et dans lequel le rendement en carbone, basé sur la quantité totale de carbone dans les bioproduits produits divisée par la quantité totale de carbone métabolisée à partir de ladite première matière première, est d'au moins 50 %.

22. Procédé de fermentation mixotrophique comprenant

(i) la fourniture d'un organisme isolé naturellement acétogène de l'espèce *Clostridium ljungdahlii,* dans lequel l'organisme a été génétiquement modifié pour exprimer une thiolase, une 3-hydroxybutyryl-CoA déshydrogénase, une 3-hydroxybutyryl-CoA déshydratase, et une acétaldéhyde/alcool déshydrogénase ;
(ii) la fourniture d'un milieu de fermentation comprenant une première matière première et une deuxième matière première, dans lequel ladite première matière première comprend un sucre qui est métabolisé par la forme native de l'organisme à un taux inférieur à 0,01 g/h/g de masse cellulaire ; et dans lequel ladite deuxième matière première comprend du CO, du CO_2, du H_2, ou des mélanges de ceux-ci ; et
(iii) la culture dudit organisme dans ledit milieu de fermentation, moyennant quoi les deux matières premières sont métabolisées et un bouillon de fermentation est formé, lequel bouillon comprend au moins un bioproduit comprenant de l'alcool crotylique ;

dans lequel le procédé donne une plus grande quantité de l'au moins un bioproduit que les quantités combinées de l'au moins un bioproduit produit par fermentation hétérotrophe et autotrophe avec le même organisme dans les mêmes conditions ; et
dans lequel le rendement en carbone, basé sur la quantité totale de carbone dans les bioproduits produits divisée par la quantité totale de carbone métabolisée à partir de ladite première matière première, est d'au moins 50 %.

Figure 1

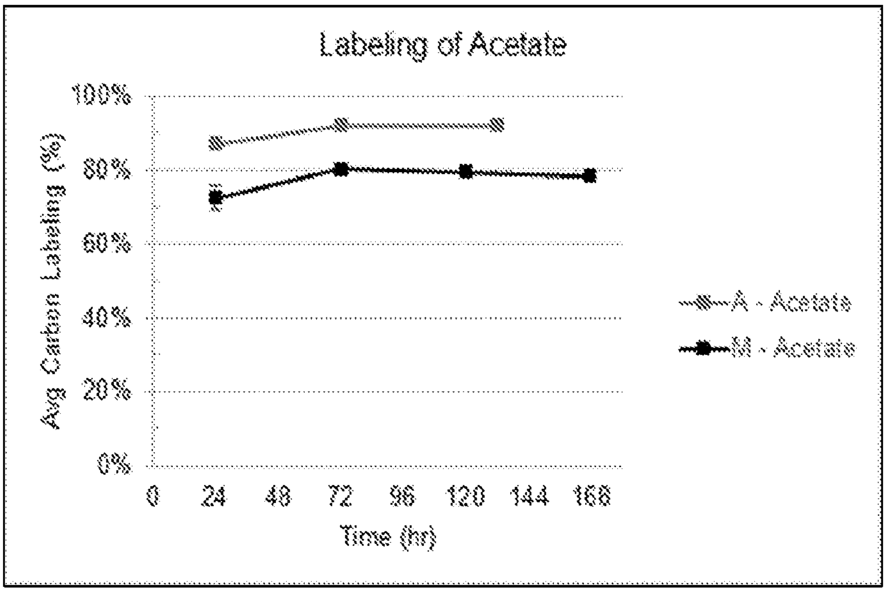

Labeling of Acetate
100%
80%
60%
40%
20%
0%
Avg Carbon Labeling (%)
0
24
48
72
96
120
144
168
Time (hr)
A - Acetate
M - Acetate

Figure 2

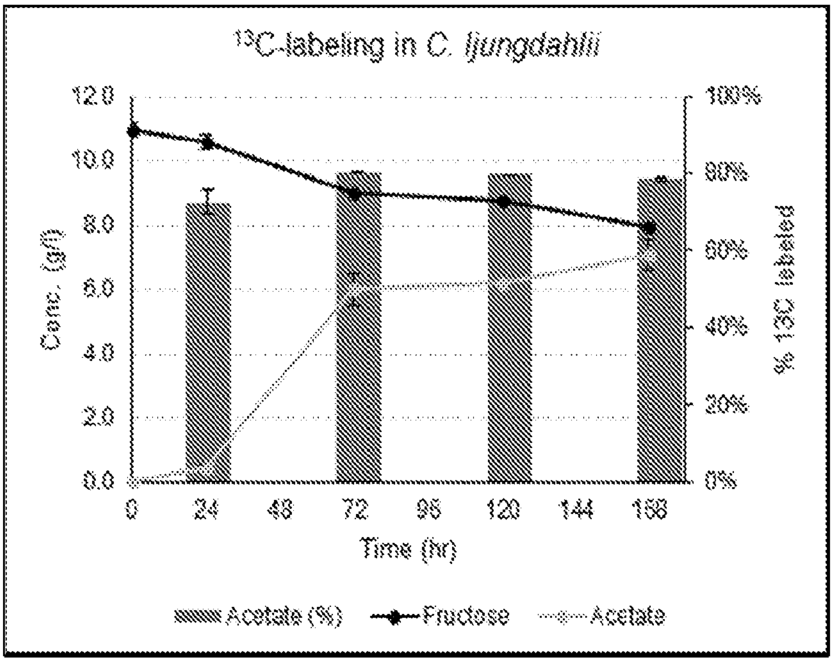
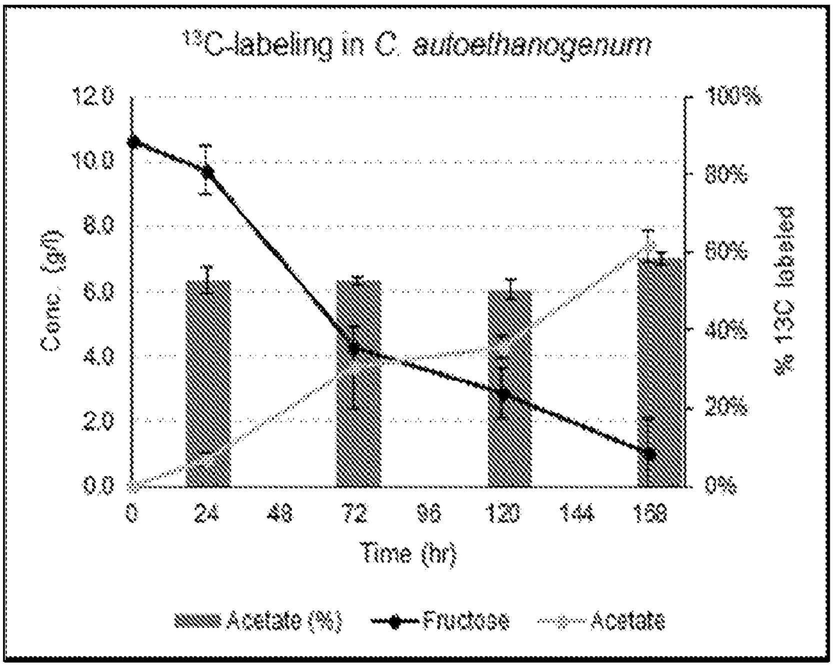

13C-labeling in C. ljungdahlii
Conc. (g/l)
% 13C labeled
12.0
10.0
8.0
6.0
4.0
2.0
0.0
100%
80%
60%
40%
20%
0%
0
24
48
72
96
120
144
168
Time (hr)
Acetate (%)
Fructose
Acetate

Figure 3



^{13}C-labeling in *C. autoethanogenum*
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Conc. (g/l)
100%
80%
60%
40%
20%
0%
% 13C labeled
0
24
48
72
96
120
144
168
Time (hr)
Acetate (%)
Fructose
Acetate

Figure 4

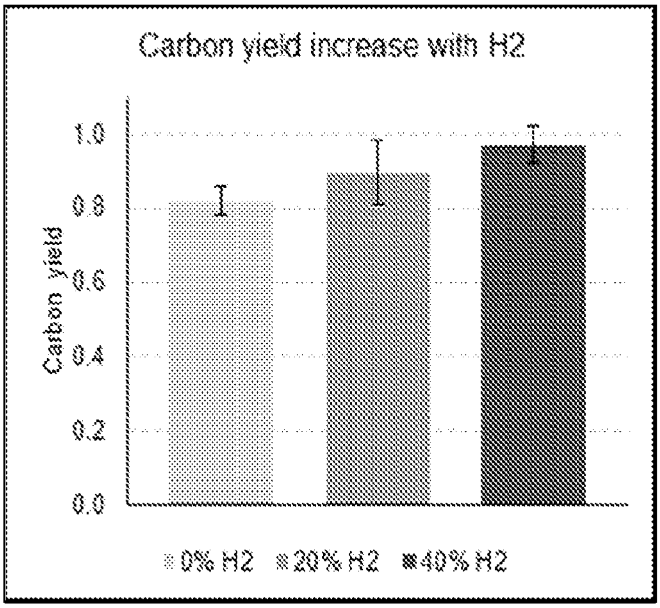
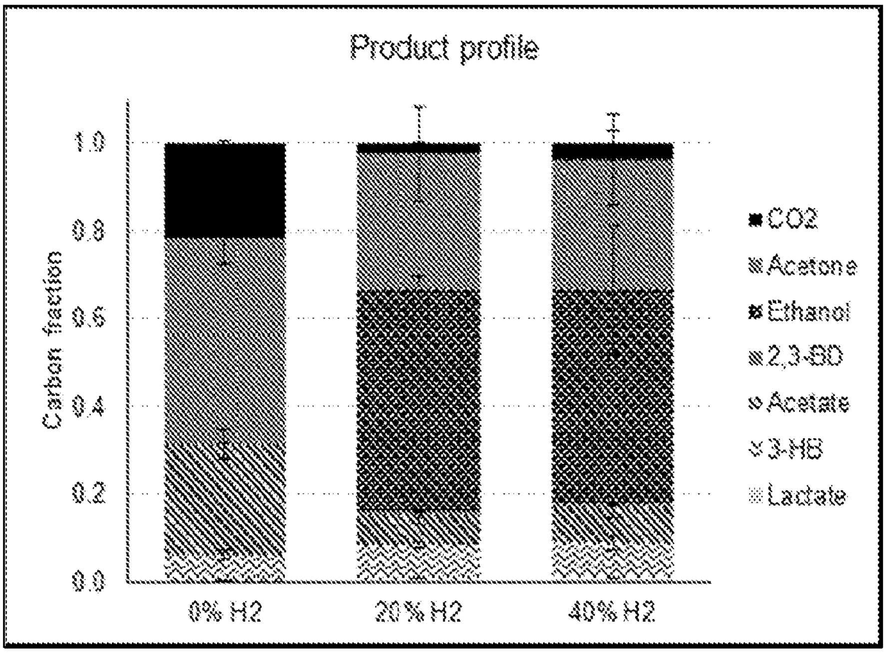

Carbon yield increase with H2
Carbon yield
1.0
0.8
0.6
0.4
0.2
0.0
0% H2
20% H2
40% H2

Figure 5


Product profile
Carbon fraction
1.0
0.8
0.6
0.4
0.2
0.0
0% H2
20% H2
40% H2
CO2
Acetone
Ethanol
2,3-BD
Acetate
3-HB
Lactate

REFERENCES CITED IN THE DESCRIPTION

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