

Figure 1



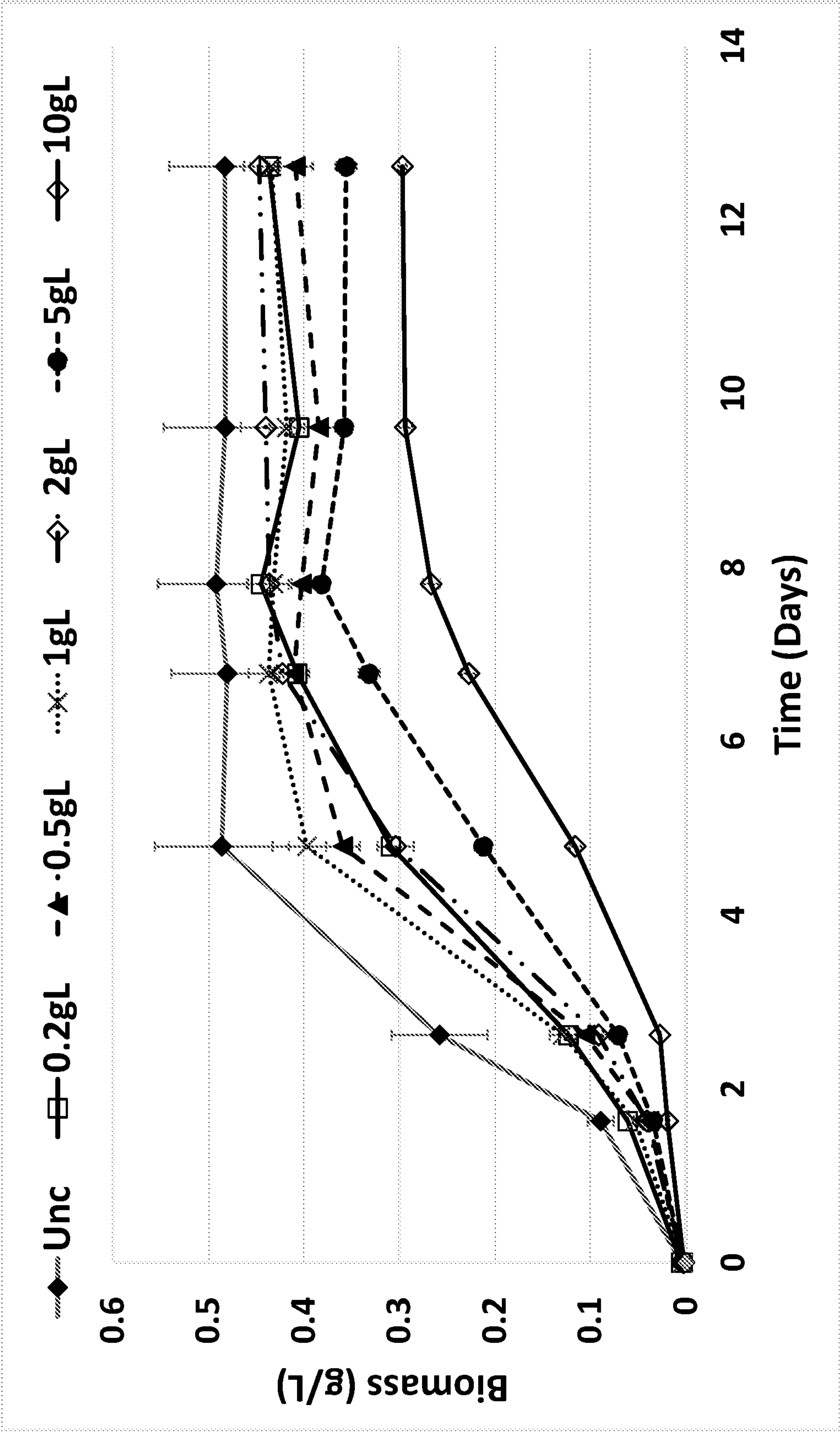


Figure 2A

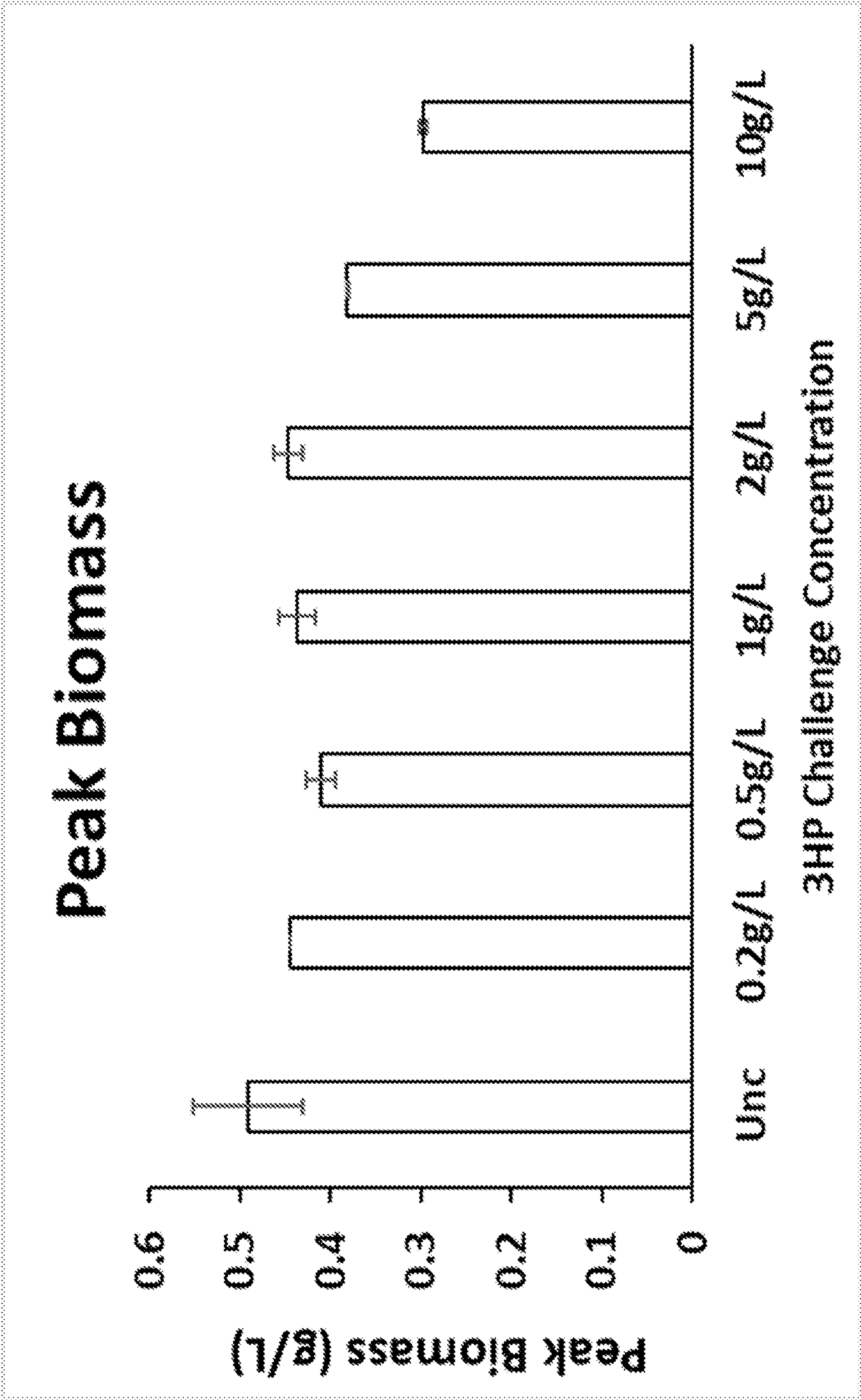


Figure 2B

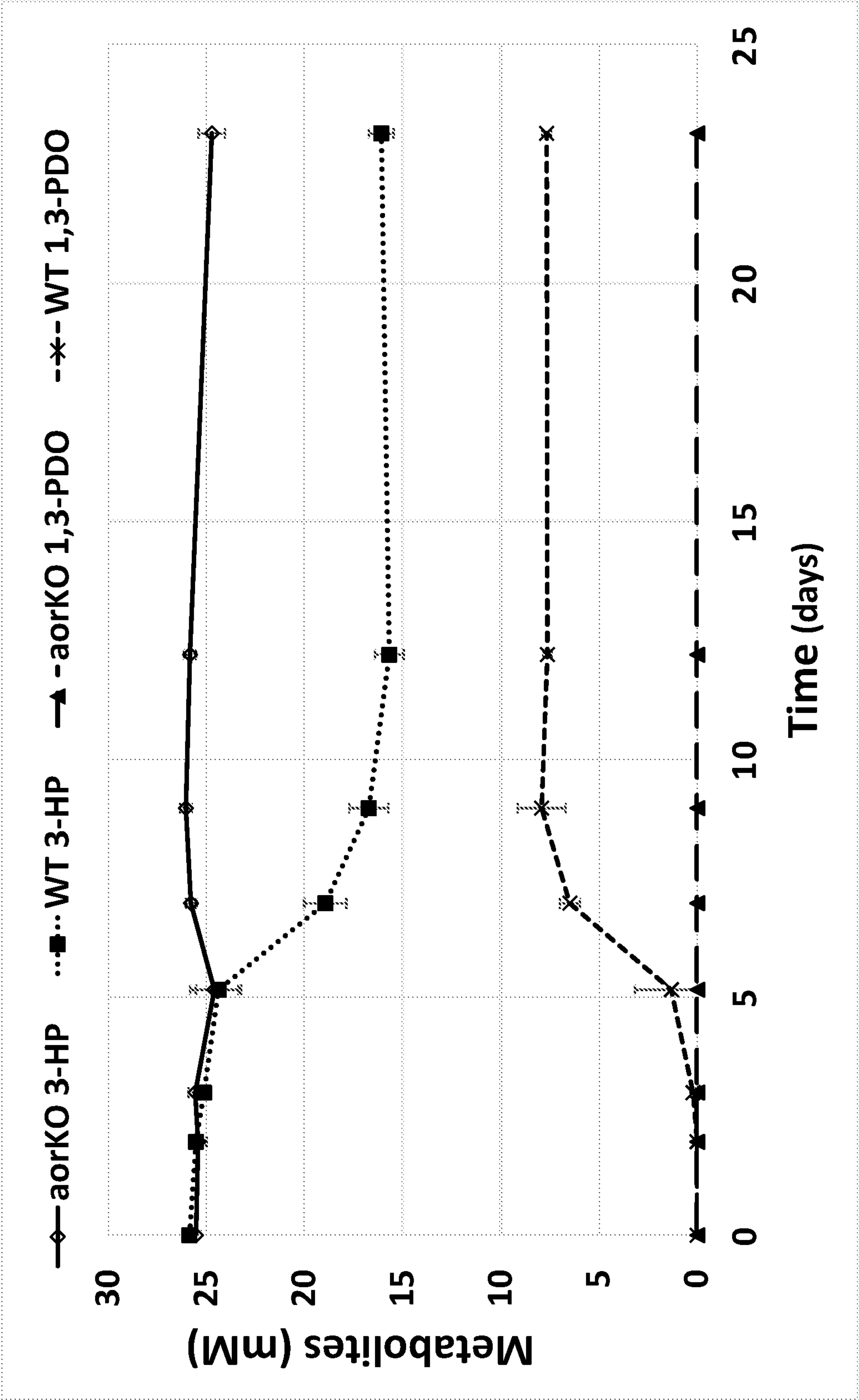


Figure 3

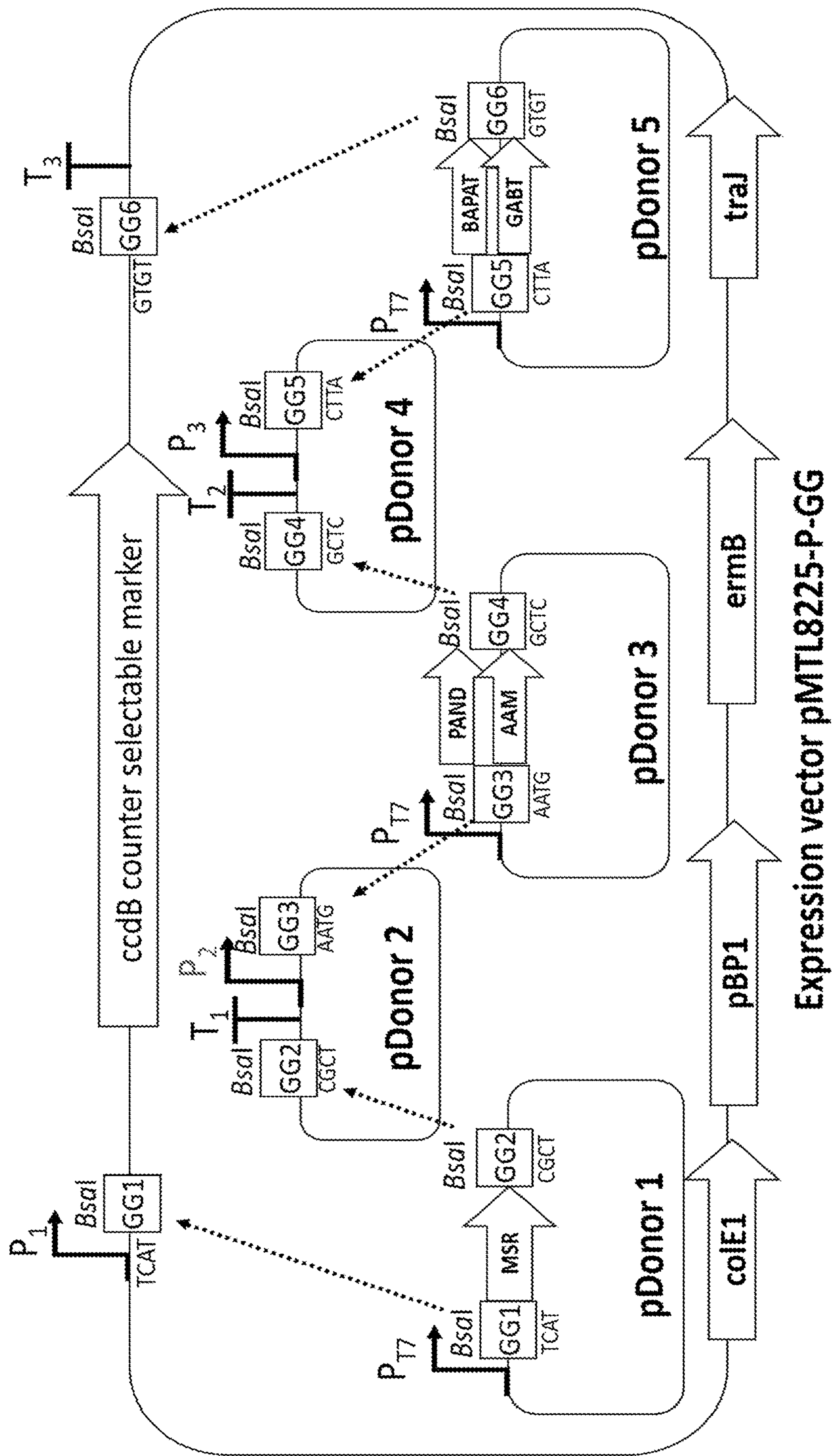


Figure 4



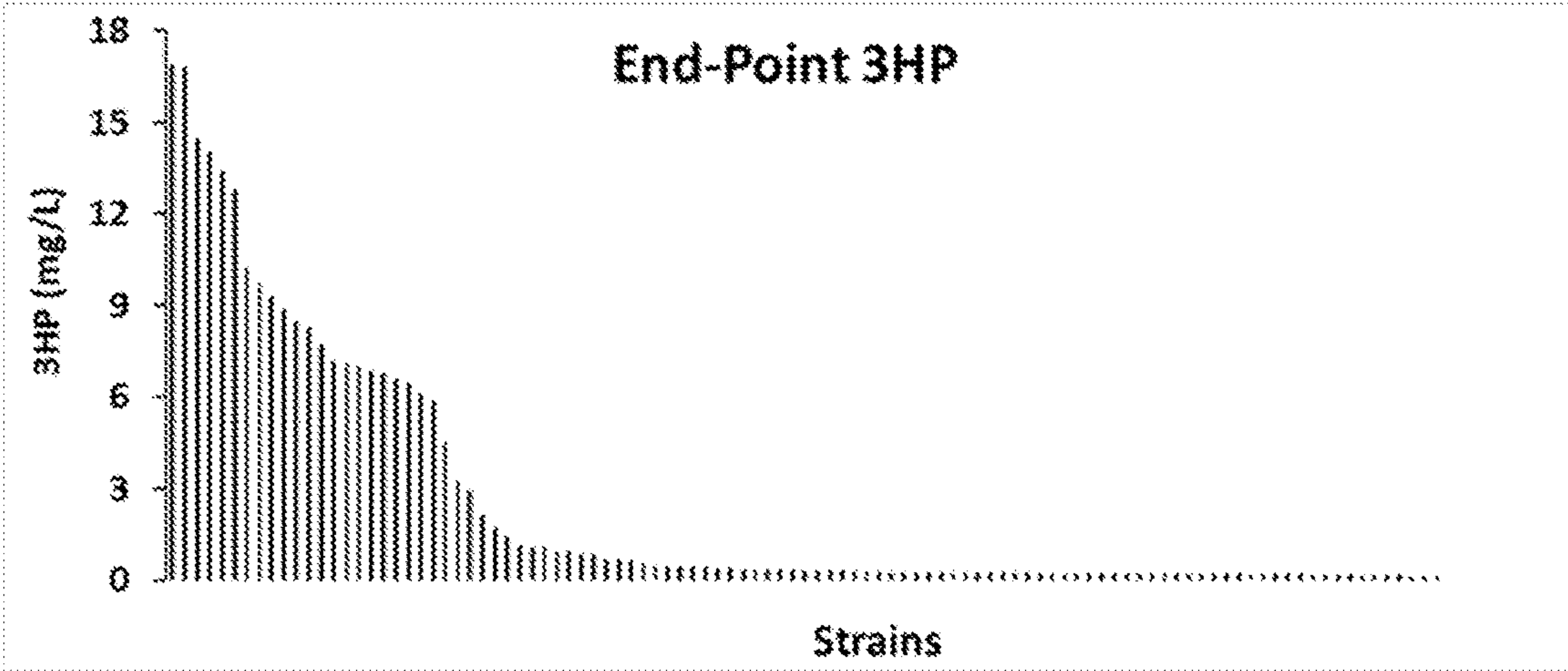


Figure 5A

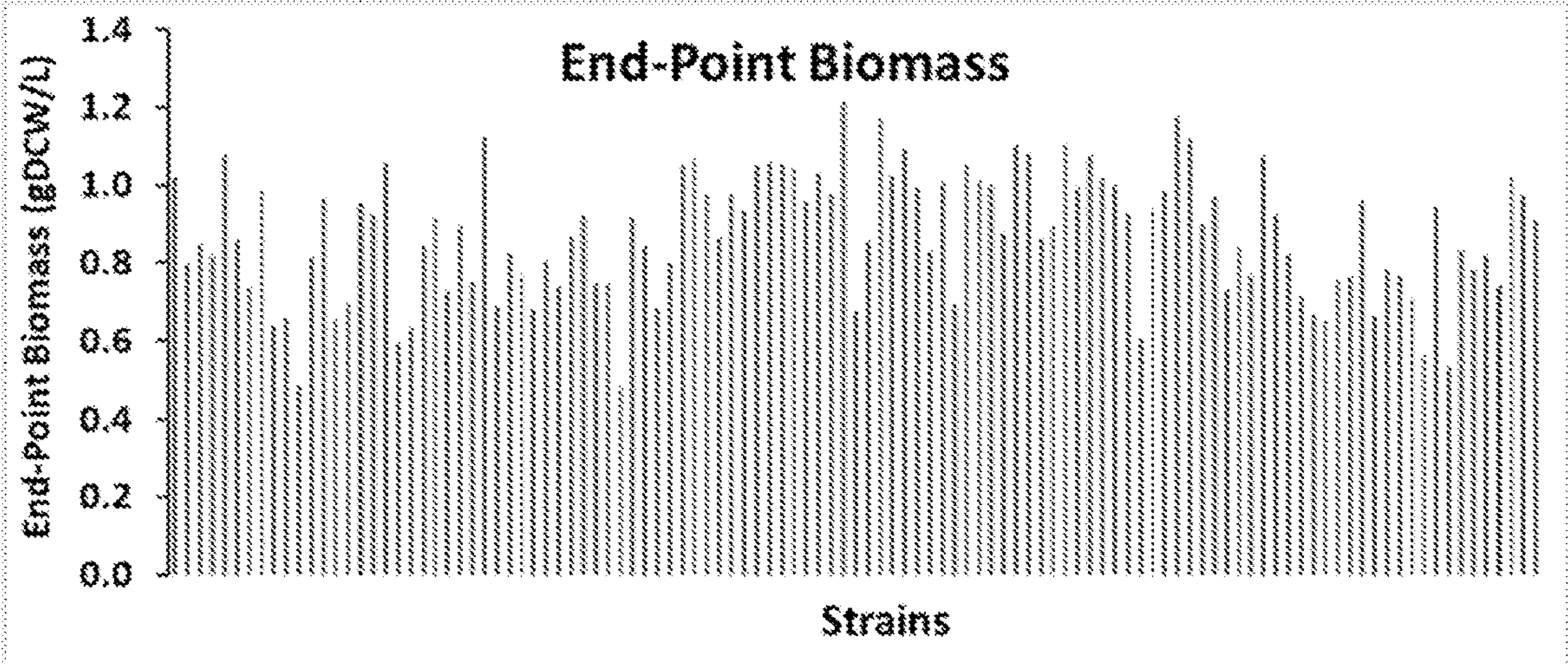


Figure 5B

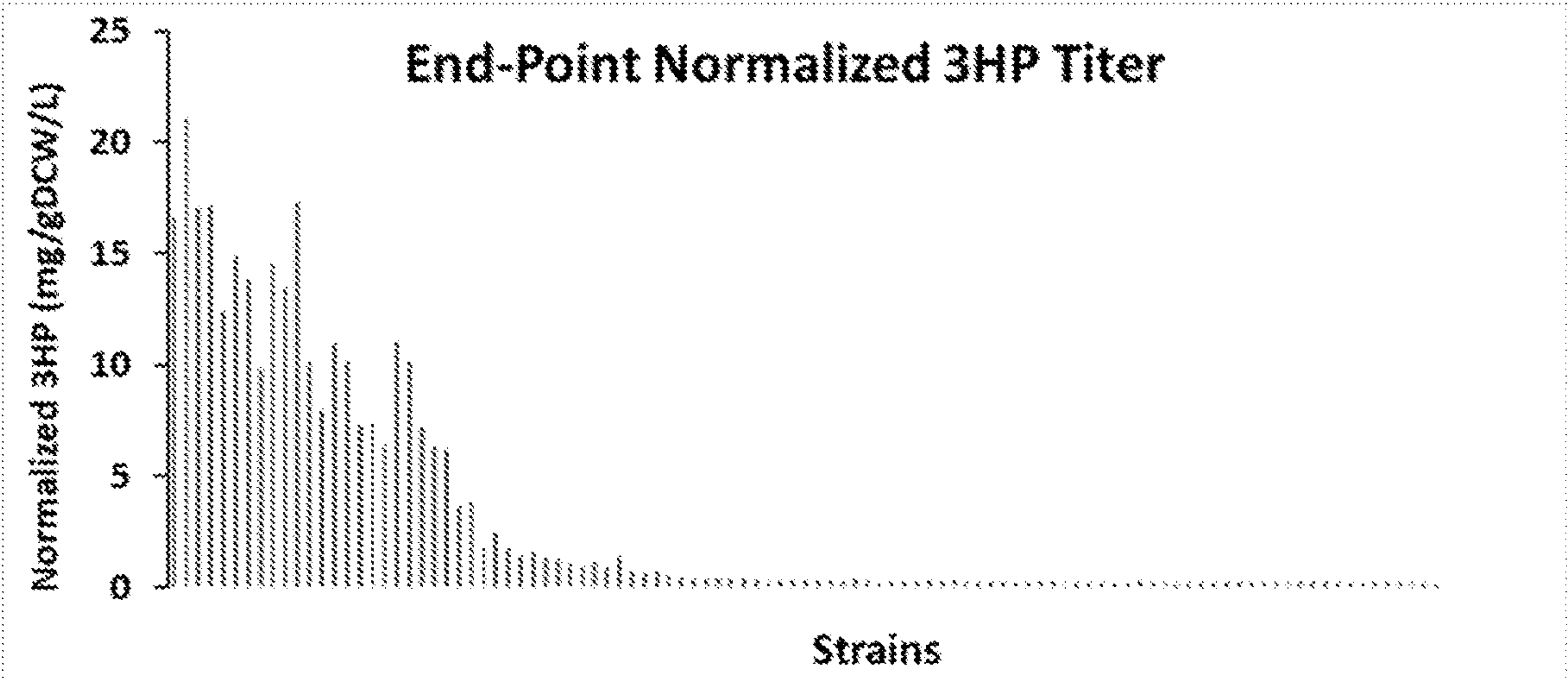


Figure 5C

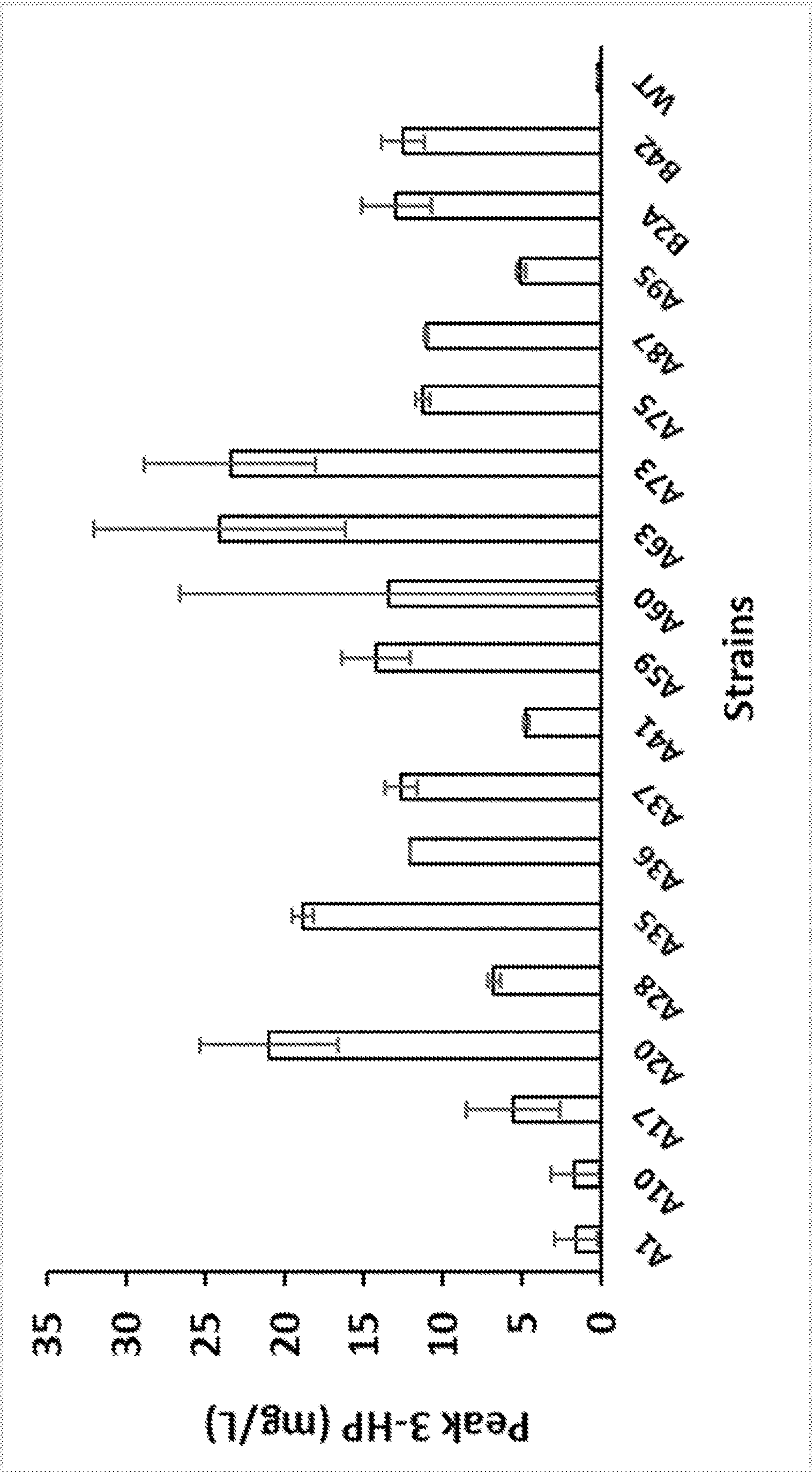


Figure 6



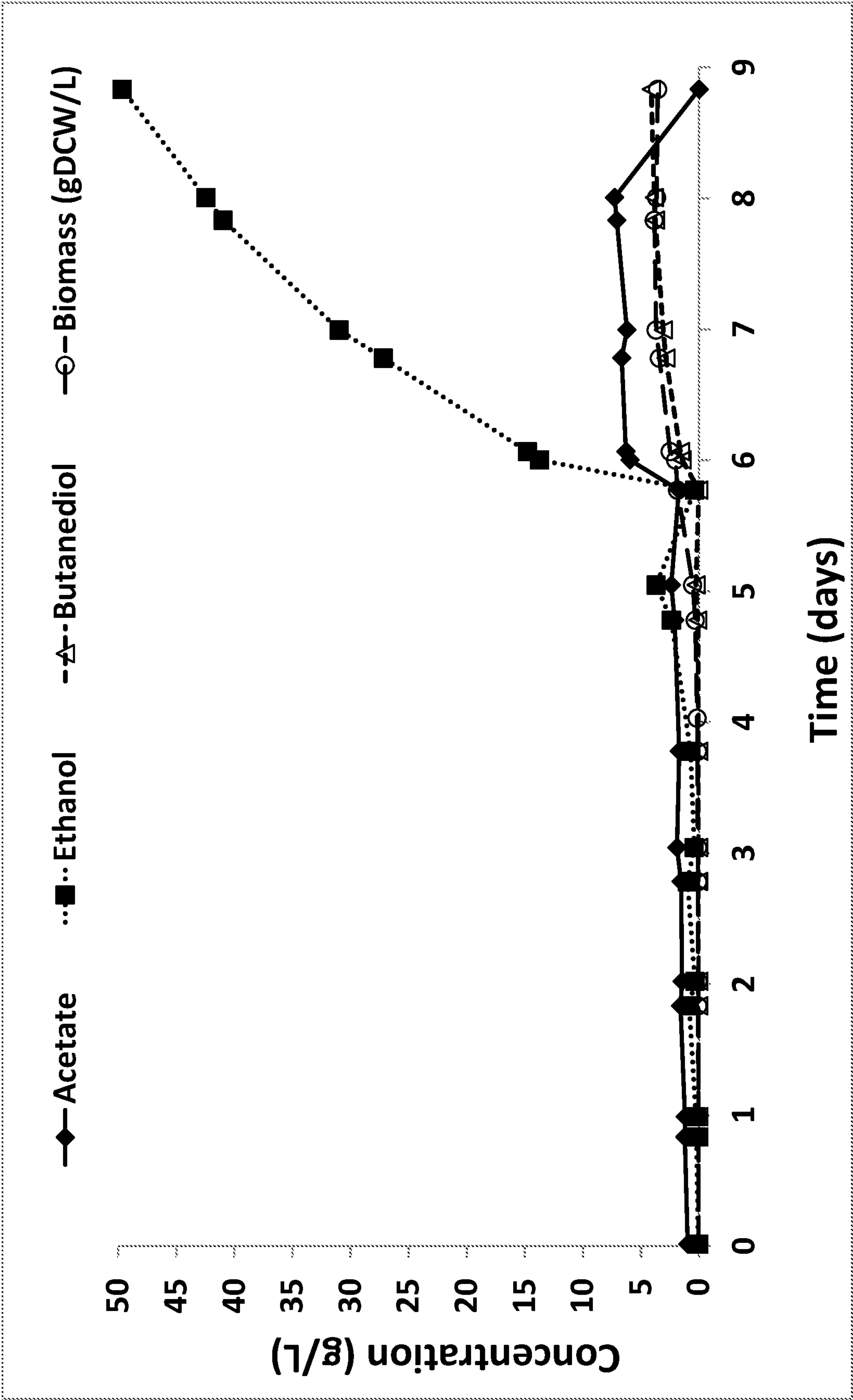


Figure 7A

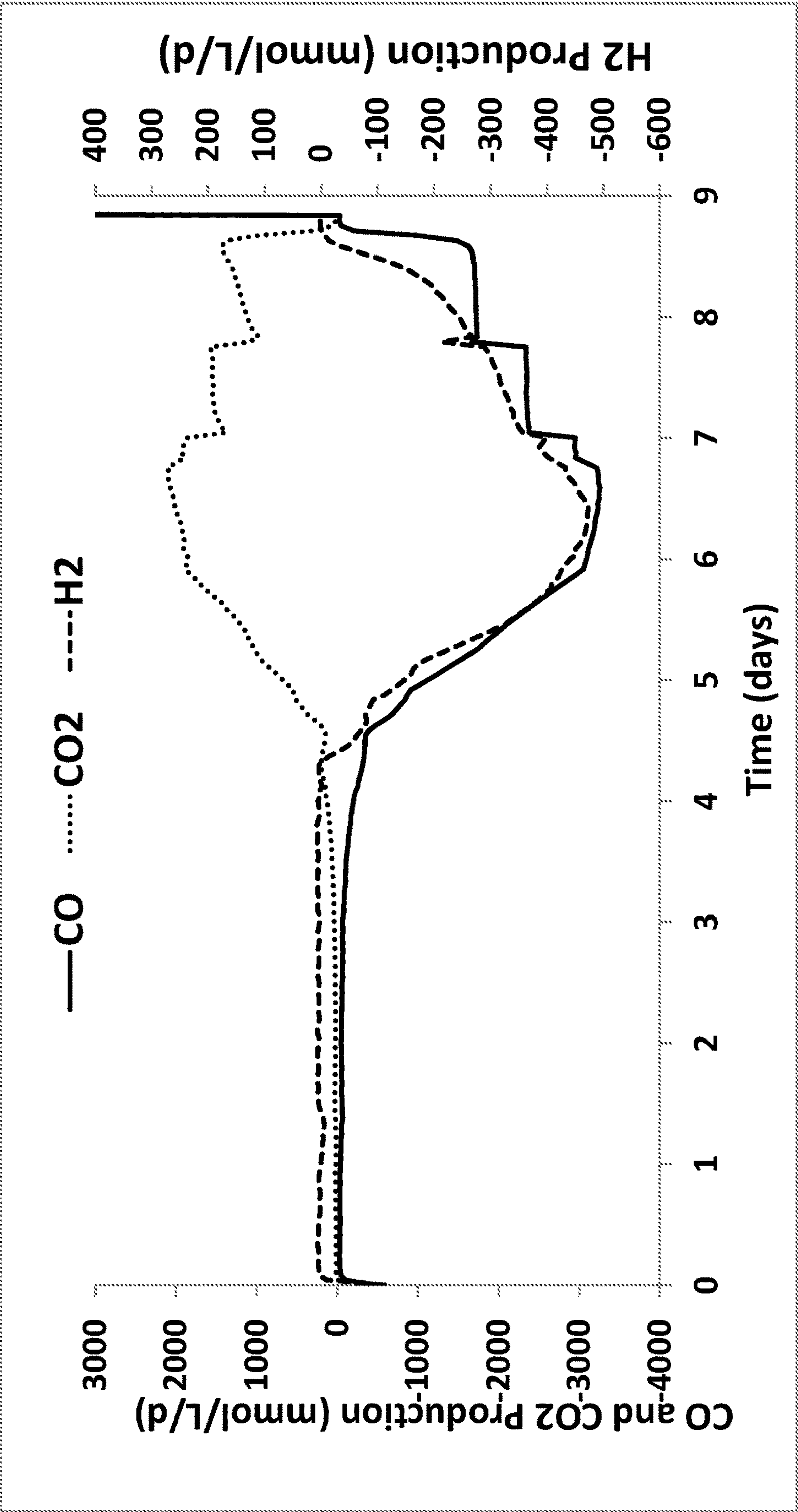


Figure 7B

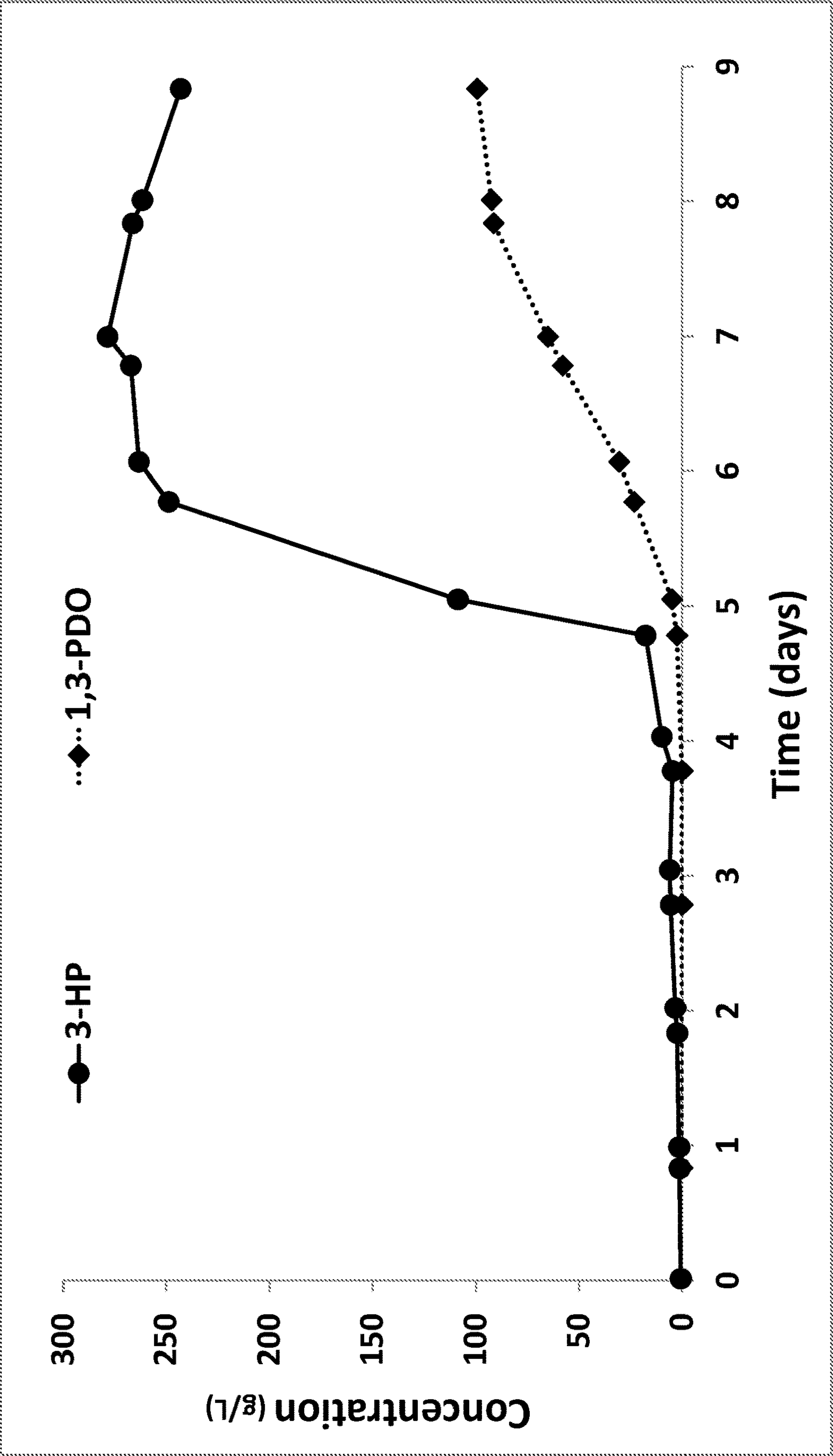


Figure 7C



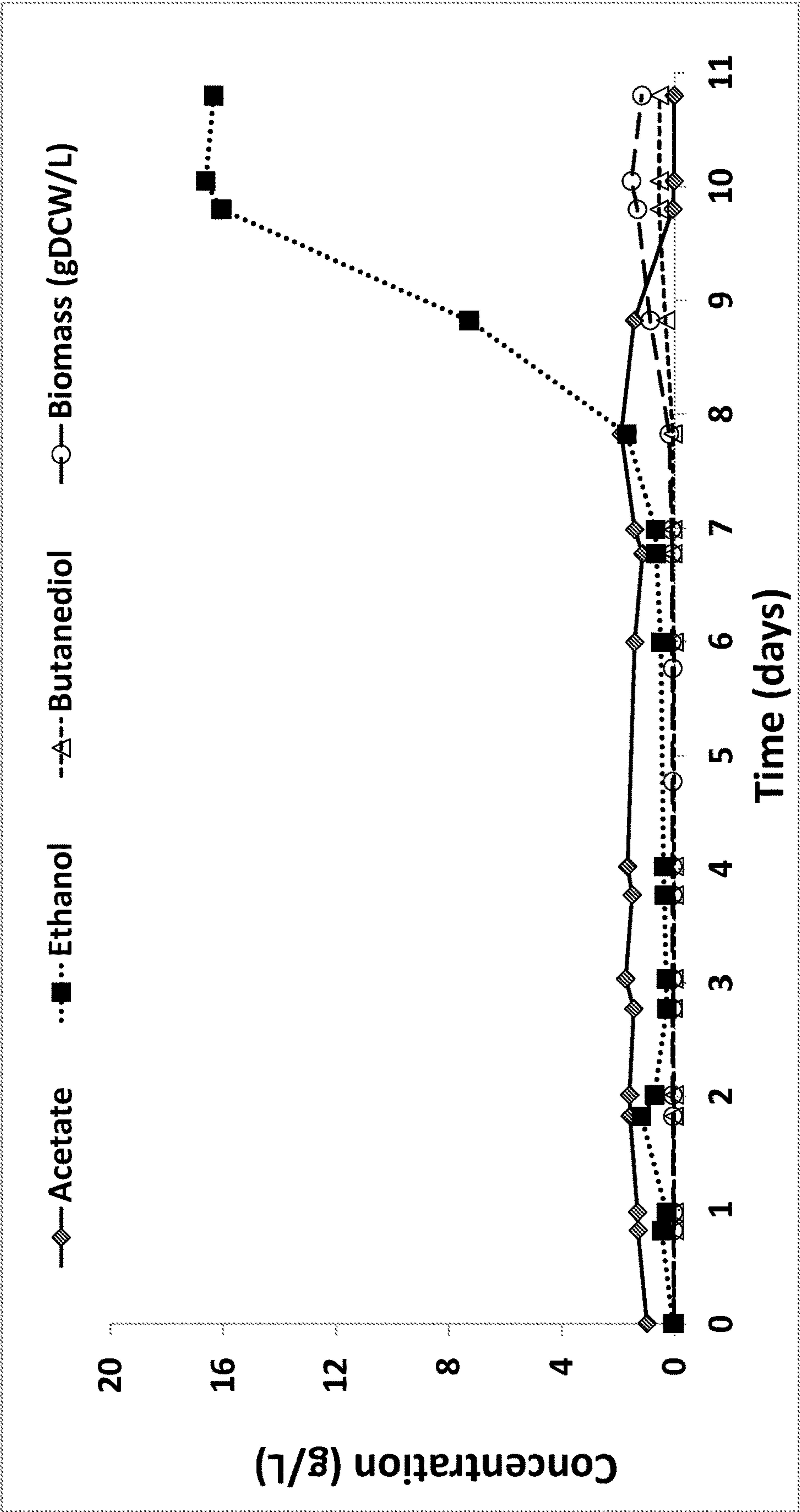


Figure 8A

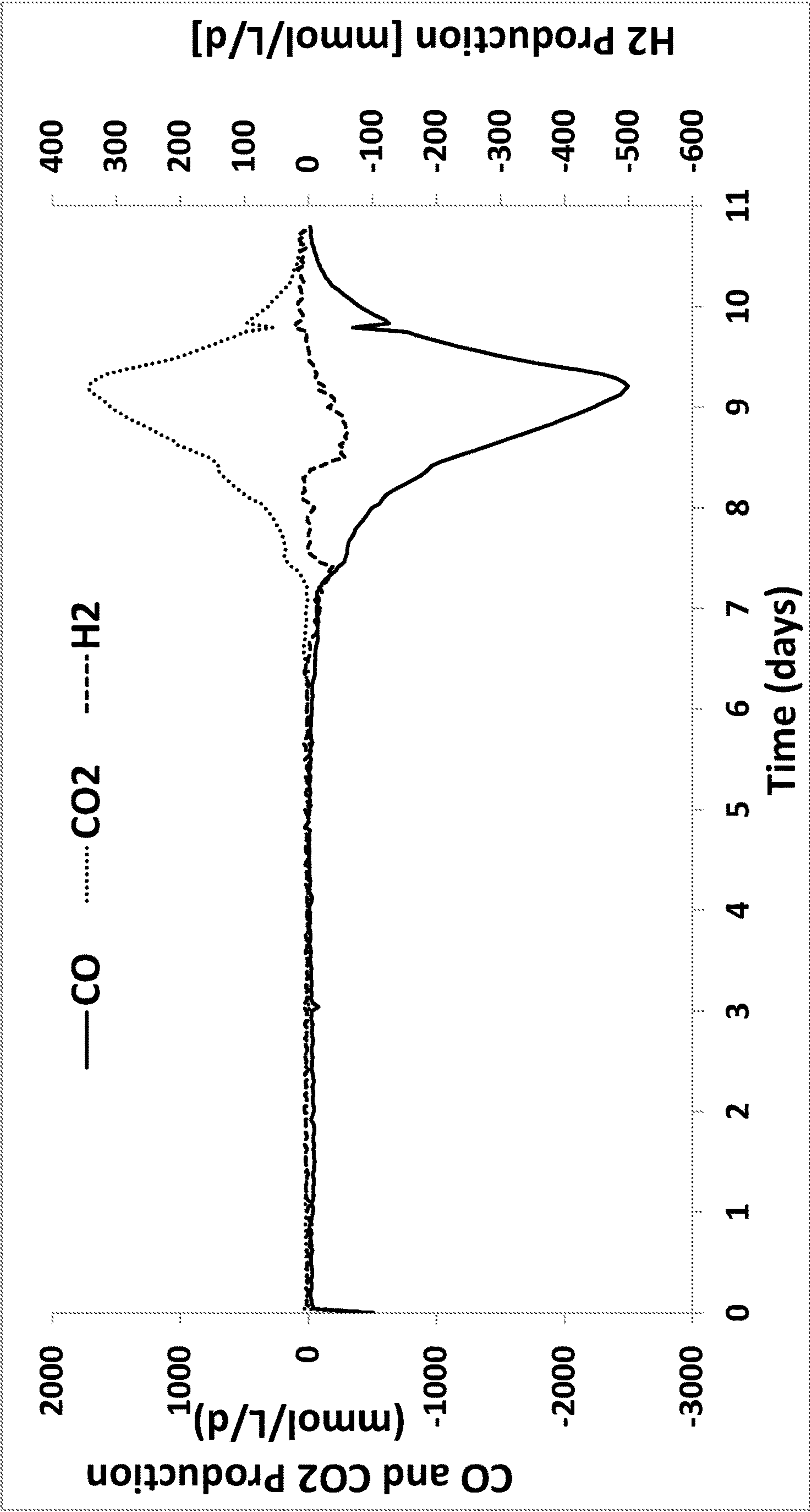


Figure 8B

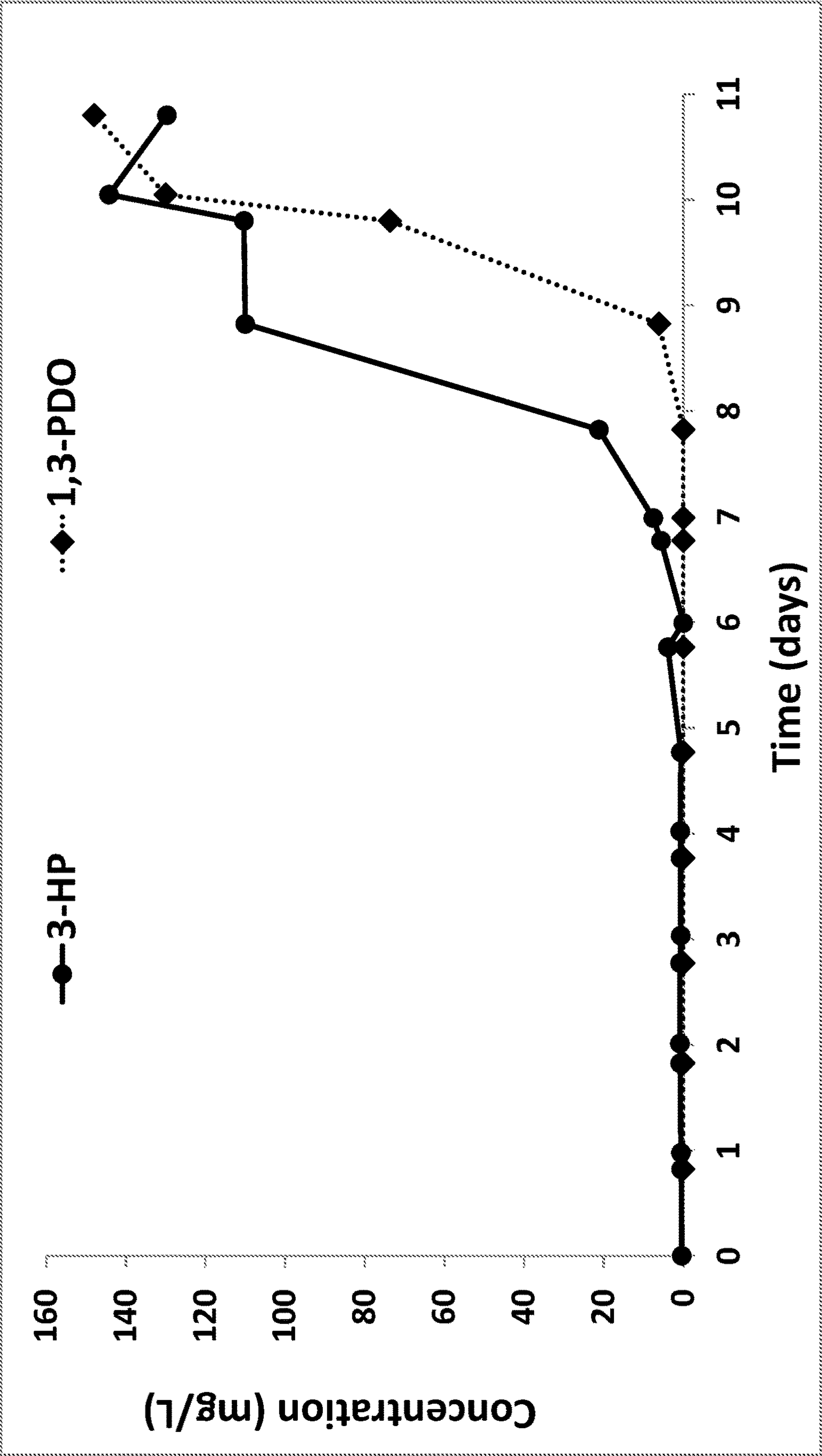


Figure 8C



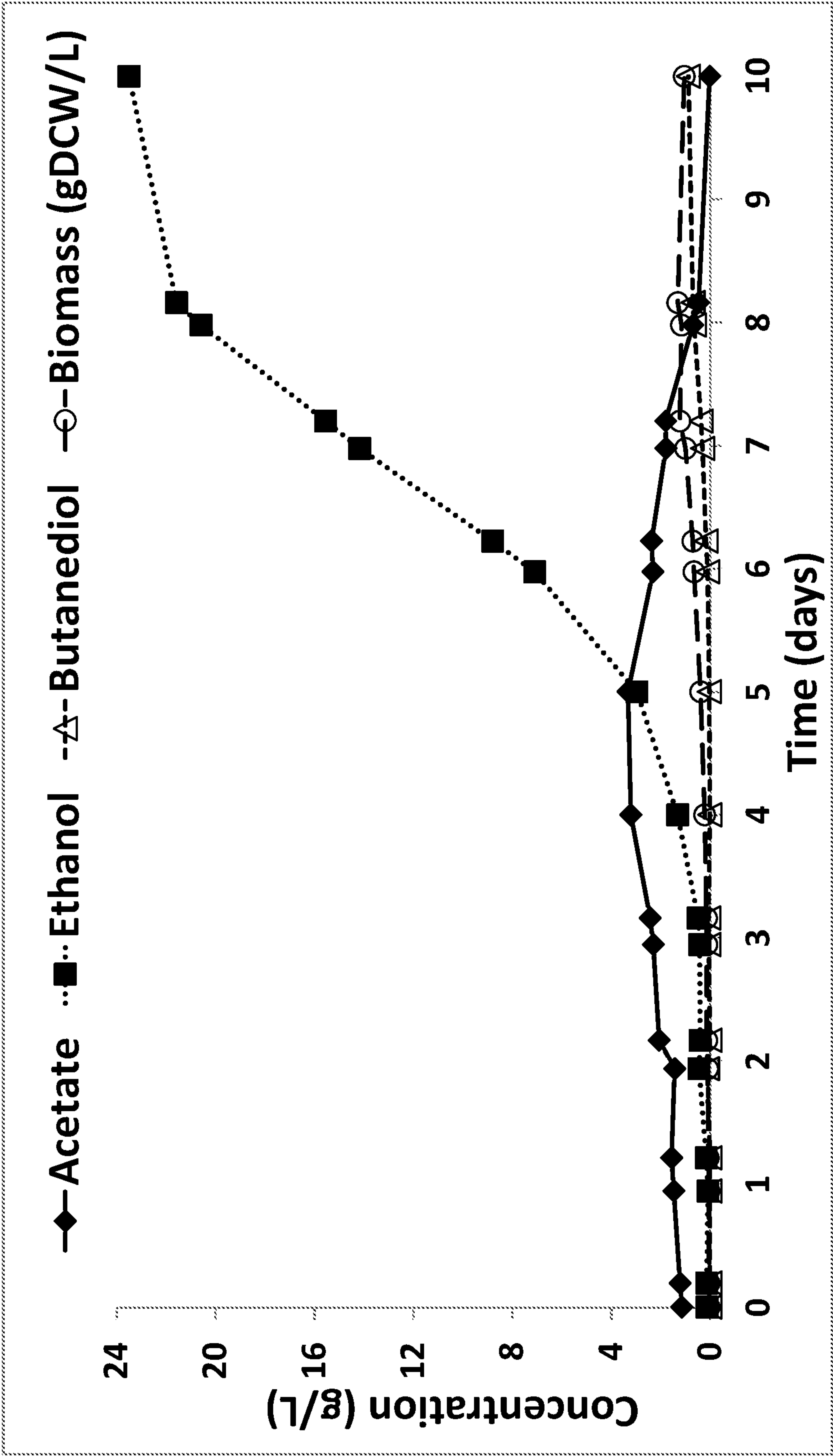


Figure 9A

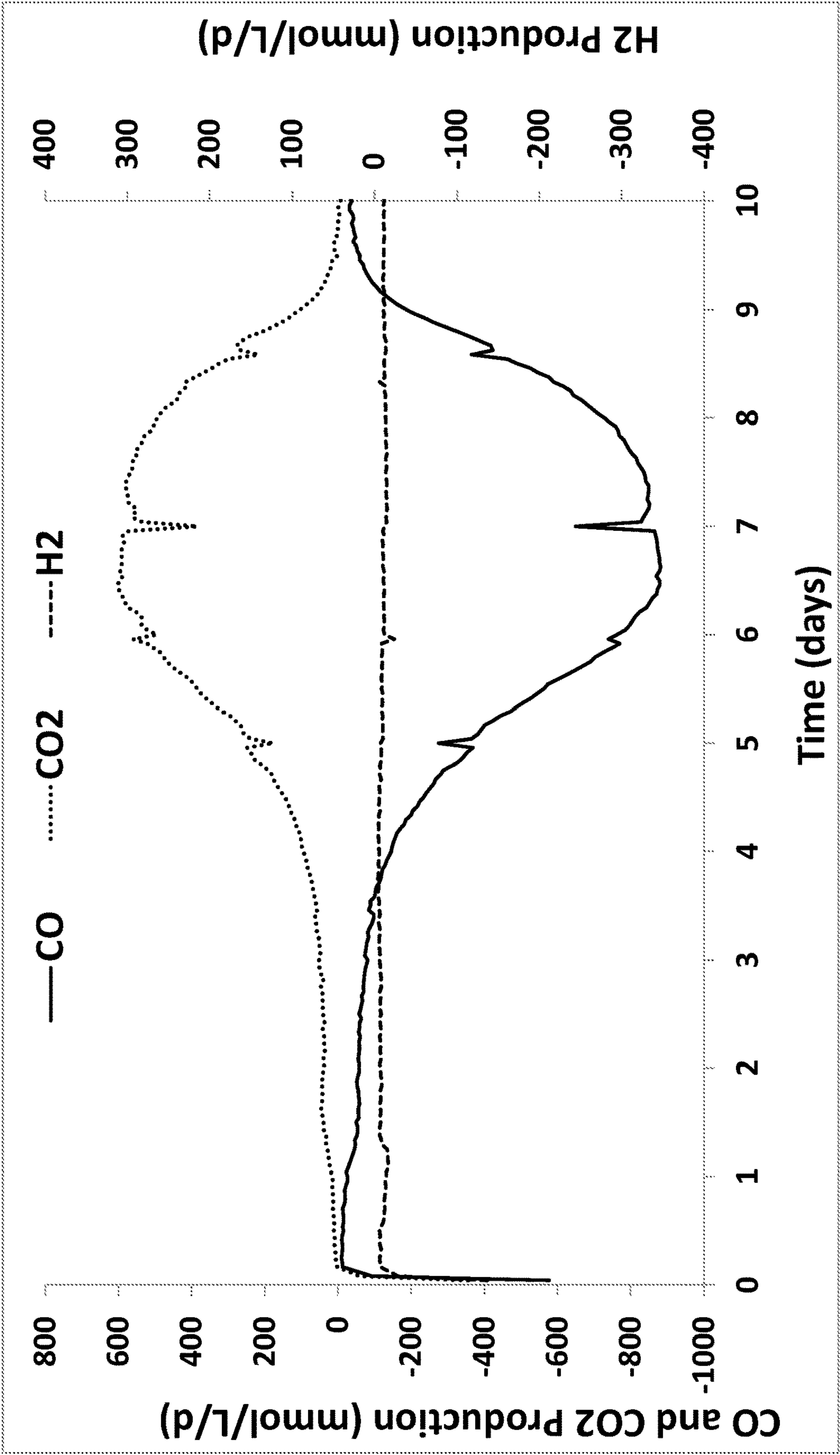


Figure 9B

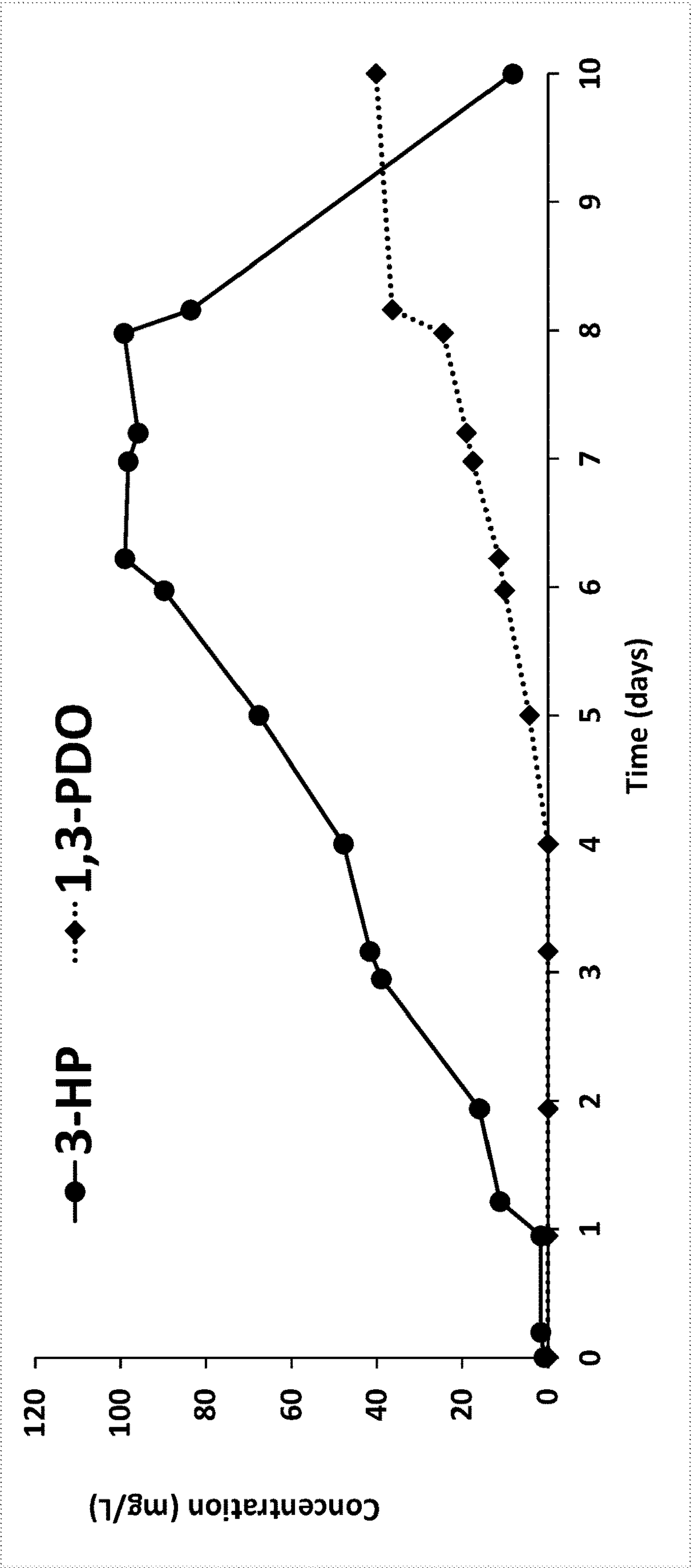


Figure 9C



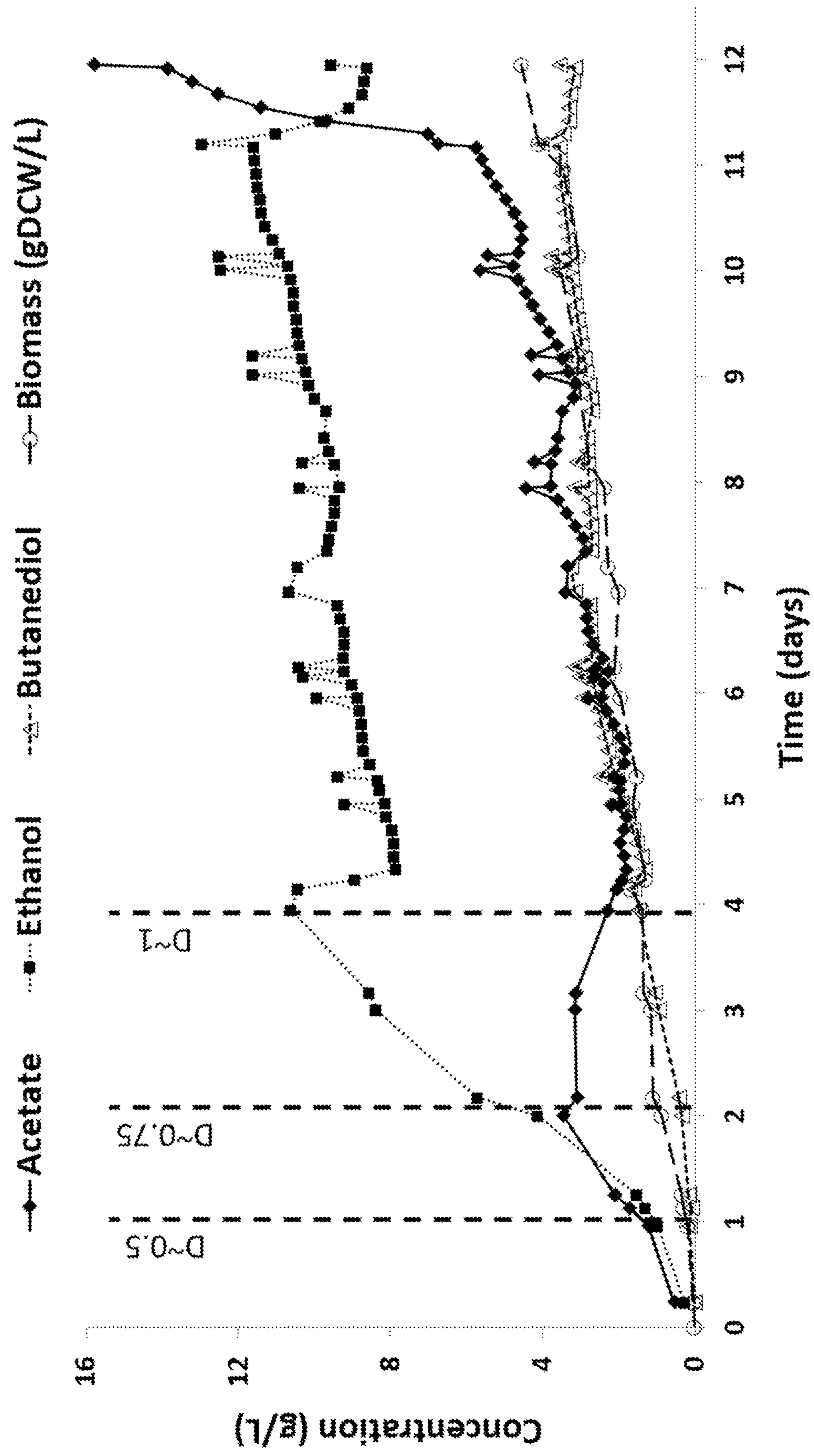


Figure 10A

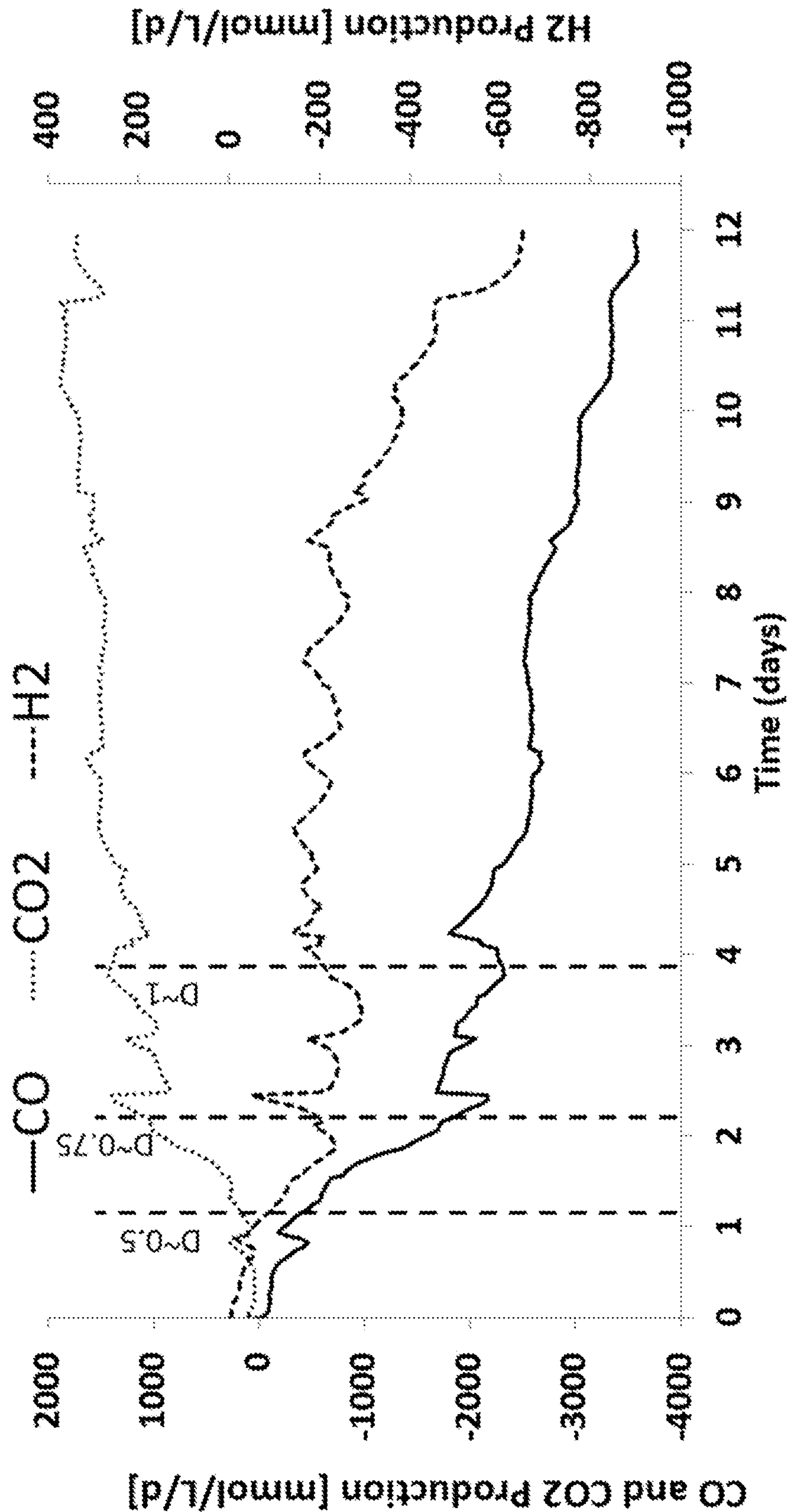


Figure 10B

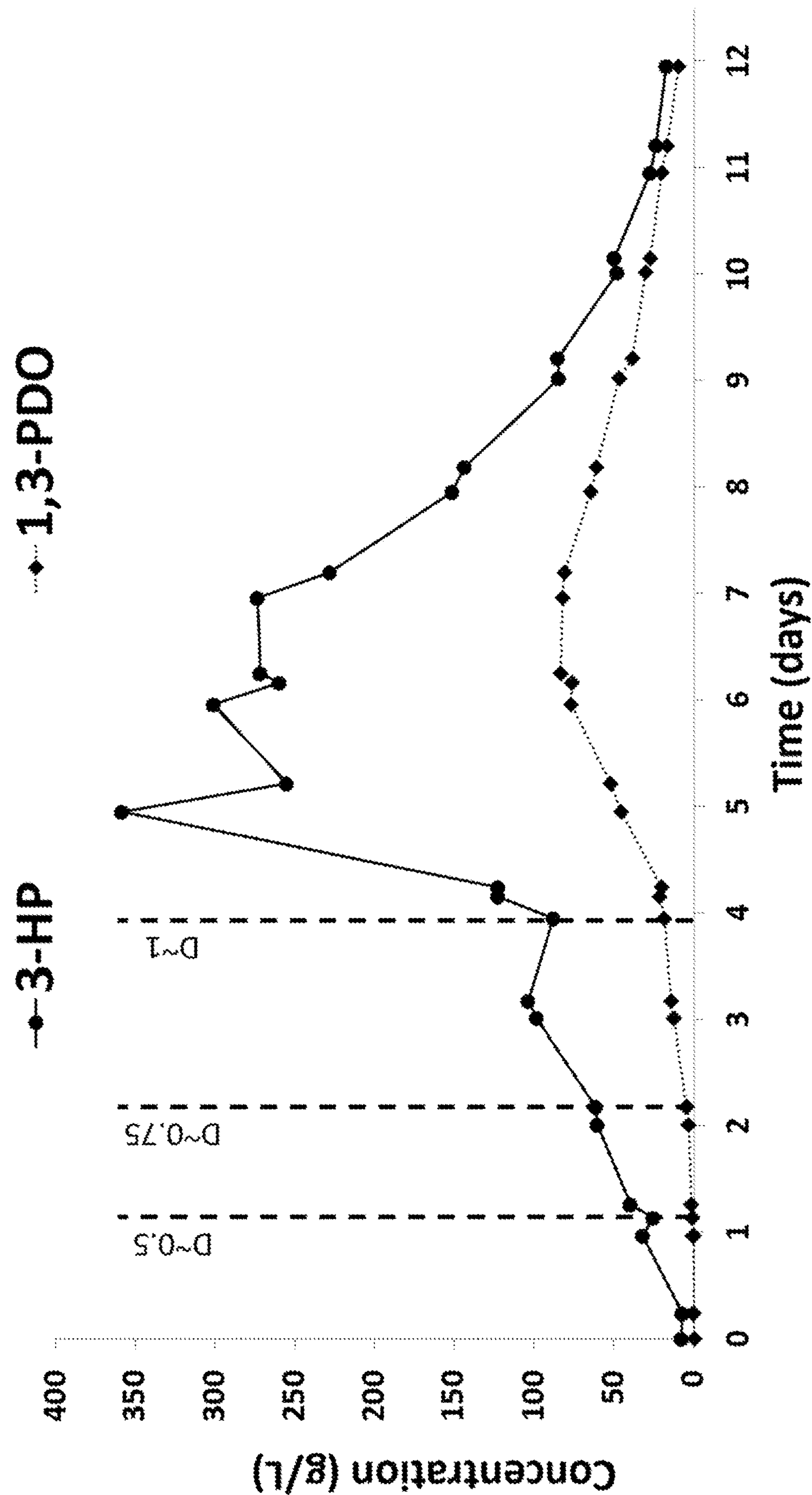


Figure 10C



## RECOMBINANT MICROORGANISMS AND USES THEREFOR

### CROSS REFERENCE TO RELATED APPLICATIONS

**[0001]** This application is a divisional of U.S. patent application Ser. No. 17/650,191, filed on Feb. 7, 2022, which claims the benefit of U.S. Provisional Patent Application No. 63/147,108, filed on Feb. 8, 2021, the entirety of which is incorporated herein by reference.

### GOVERNMENT RIGHTS

**[0002]** This disclosure was made with government support under Cooperative Agreement DE-SC0019090 awarded by the Department of Energy. The government has certain rights in the invention.

### REFERENCE TO A SEQUENCE LISTING

**[0003]** The application contains a Sequence Listing which has been submitted electronically in ST.26 Sequence listing XML format and is hereby incorporated by reference in its entirety. Said ST.26 Sequence listing XML, created on Aug. 21, 2023, is named LT198US2-Sequences.xml and is 43,051 bytes in size.

### FIELD OF THE DISCLOSURE

**[0004]** This disclosure relates to recombinant microorganisms and methods for the production of 3-Hydroxypropionate [3-HP] by microbial fermentation of a substrate comprising CO and/or CO<sub>2</sub>.

### BACKGROUND

**[0005]** 3-Hydroxypropionate [3-HP] is a platform chemical, acting as precursor for production of polymer materials and as chemical feedstock. Poly(3-hydroxypropionic acid) [P(3-HP)] is a biodegradable polymer with promising characteristics such as unusual high heat stability.

**[0006]** 3-HP can be used to derive a number of valuable industrial chemicals including: acrylic acid which is used in the manufacture of paint, paper, adhesives, textiles, specialty coatings, ink, and superabsorbent polymer polyacrylates; 1,3-propanediol which is of use as a solvent, adhesive, cosmetic, or to make polytrimethylene terephthalate used in carpet and textiles; 3-hydroxypropionaldehyde which is used in the preparation of foods, as a feed additive, and as a preservative in the nutritional industry.

**[0007]** 3-HP is listed as third most important renewable chemical by the U.S. Department of Energy and a global market opening for 3-HP has been estimated to be 3.63 million tons per year (Sauer et al, 2008, Bozell et al, 2010, US DOE report: 48-49).

**[0008]** It is an object of the disclosure to provide recombinant microorganisms and a method for the production of 3-HP by microbial fermentation which may provide one or more advantages over known methods, or to at least to provide the public with a useful choice.

### SUMMARY

**[0009]** The disclosure generally provides, inter alia, methods for the production of 3-HP by microbial fermentation of a substrate comprising CO and/or CO<sub>2</sub>, and recombinant

microorganisms of use in such methods. It combines two different CO<sub>2</sub> fixation pathways to produce a single metabolic product.

**[0010]** In a first aspect, the disclosure provides an anaerobic acetogenic recombinant microorganism capable of producing 3-HP and optionally one or more other products by fermentation of a substrate comprising CO and/or CO<sub>2</sub>.

**[0011]** In one particular embodiment, the microorganism is adapted to express one or more enzymes (or one or more subunits thereof) in the 3-HP biosynthesis pathway which enzymes are not naturally present in a parental microorganism from which the recombinant microorganism is derived. In another embodiment, the microorganism is adapted to over-express one or more enzymes (or one or more subunits thereof) in the 3-HP biosynthesis pathway, which enzymes are naturally present in a parental microorganism from which the recombinant microorganism is derived. In one embodiment, the microorganism is adapted to express one or more enzymes (or one or more subunits thereof) in the 3-HP-biosynthesis pathway which are not naturally present in a parental microorganism and over-express one or more enzymes (or one or more subunits thereof) in the 3-HP biosynthesis pathway which are naturally present in a parental microorganism.

**[0012]** In one embodiment, the one or more enzymes are chosen from the group consisting of:  $\beta$ -alanine pyruvate aminotransferase (BAPAT) (EC 2.6.1.19); malonic semialdehyde reductase (MSR) (EC 1.1.1.59 and EC 1.1.1.295); Acetyl-CoA Carboxylase (ACC) (EC 6.4.1.2); and a functionally equivalent variant of any one thereof.

**[0013]** In one embodiment, the parental microorganism is capable of fermenting a substrate comprising CO and/or CO<sub>2</sub> to produce Acetyl-CoA but not of converting Acetyl-CoA to 3-HP and the recombinant microorganism is adapted to express one or more enzymes (or one or more subunits thereof) involved in the conversion of Acetyl-CoA to 3-HP.

**[0014]** In one embodiment, the microorganism comprises one or more exogenous nucleic acids adapted to increase expression of one or more nucleic acids native to the parental microorganism and which one or more nucleic acids encode one or more of the enzymes (or one or more subunits thereof) referred to herein before.

**[0015]** In one embodiment, the one or more exogenous nucleic acid adapted to increase expression is a regulatory element. In one embodiment, the regulatory element is a promoter.

**[0016]** In one embodiment, the promoter is a constitutive promoter. In one embodiment, the promoter is selected from the group comprising Wood-Ljungdahl gene cluster or Phosphotransacetylase/Acetate kinase operon promoters.

**[0017]** In one embodiment, the microorganism comprises one or more exogenous nucleic acids encoding and adapted to express one or more of the enzymes (or one or more subunits thereof) referred to herein before. In one embodiment, the microorganisms comprise one or more exogenous nucleic acid encoding and adapted to express at least two of the enzymes (or one or more subunits thereof).

**[0018]** In one embodiment, the one or more exogenous nucleic acid is a nucleic acid construct or vector, in one particular embodiment a plasmid, encoding one or more of the enzymes referred to hereinbefore in any combination.

**[0019]** In one embodiment, the exogenous nucleic acid is an expression plasmid.



[0020] In one embodiment, the parental microorganism is selected from the group of anaerobic acetogens.

[0021] In one particular embodiment, the parental microorganism is selected from the group of carboxydophilic acetogenic bacteria, in one embodiment from the group comprising *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, *Clostridium ragsdalei*, *Clostridium carboxidivorans*, *Clostridium drakei*, *Clostridium scatologenes*, *Clostridium aceticum*, *Clostridium formicoaceticum*, *Clostridium magnum*, *Butyribacterium methylotrophicum*, *Acetobacterium woodii*, *Alkalibaculum bacchii*, *Blautia producta*, *Eubacterium limosum*, *Moorella thermoacetica*, *Moorella thermautotrophica*, *Sporomusa ovata*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, *Oxobacter pfennigii*, and *Thermoanaerobacter kivui*.

[0022] In one embodiment the parental microorganism is *Clostridium autoethanogenum* or *Clostridium ljungdahlii*. In one particular embodiment, the microorganism is *Clostridium autoethanogenum* DSM23693. In another particular embodiment, the microorganism is *Clostridium ljungdahlii* DSM13528 (or ATCC55383).

[0023] In one embodiment, the parental microorganism lacks one or more genes encoding  $\beta$ -alanine pyruvate aminotransferase and/or Acetyl-CoA carboxylase, or one or more subunits thereof.

[0024] In a second aspect, the disclosure provides a nucleic acid encoding one or more enzymes (or one or more subunits thereof) which when expressed in a microorganism allows the microorganism to produce 3-HP by fermentation of substrate comprising CO and/or CO<sub>2</sub>.

[0025] In one embodiment, the nucleic acid encodes two or more enzymes (or one or more subunits thereof) which when expressed in a microorganism allows the microorganism to produce 3-HP by fermentation of substrate comprising CO.

[0026] In one embodiment, the enzymes are chosen from  $\beta$ -alanine pyruvate aminotransferase and Acetyl CoA carboxylase, and a functionally equivalent variant of any one or more thereof.

[0027] In one embodiment, the nucleic acid comprises nucleic acid sequences encoding  $\beta$ -alanine pyruvate aminotransferase, Acetyl CoA carboxylase, or a functionally equivalent variant of any one or more thereof, in any order.

[0028] In one embodiment, the nucleic acid encoding  $\beta$ -alanine pyruvate aminotransferase has the sequence of SEQ ID NO: 13, or is a functionally equivalent variant thereof. In one embodiment, the nucleic acid encoding  $\beta$ -alanine pyruvate aminotransferase has the sequence of SEQ ID NO: 14, or is a functionally equivalent variant thereof. In one embodiment, the nucleic acid encoding  $\beta$ -alanine pyruvate aminotransferase has the sequence of SEQ ID NO: 15, or is a functionally equivalent variant thereof.

[0029] In one embodiment, the nucleic acids of the disclosure further comprise a promoter. In one embodiment, the promoter allows for constitutive expression of the genes under its control. In a particular embodiment a Wood-Ljungdahl cluster promoter is used. In another particular embodiment, a Phosphotransacetylase/Acetate kinase operon promoter is used. In one particular embodiment, the promoter is from *C. autoethanogenum*.

[0030] In a third aspect, the disclosure provides a nucleic acid construct or vector comprising one or more nucleic acid of the second aspect.

[0031] In one particular embodiment, the nucleic acid construct or vector is an expression construct or vector. In one particular embodiment, the expression construct or vector is a plasmid.

[0032] In a fourth aspect, the disclosure provides host organisms comprising any one or more of the nucleic acids of the seventh aspect or vectors or constructs of the third aspect.

[0033] In a fifth aspect, the disclosure provides a composition comprising an expression construct or vector as referred to in the third aspect of the disclosure and a methylation construct or vector.

[0034] Preferably, the composition is able to produce a recombinant microorganism according to the first aspect of the disclosure.

[0035] In one particular embodiment, the expression construct/vector and/or the methylation construct/vector is a plasmid.

[0036] In a sixth aspect, the disclosure provides a method for the production of 3-HP, and optionally one or more other products, by microbial fermentation comprising fermenting a substrate comprising CO and/or CO<sub>2</sub> using a recombinant microorganism of the first aspect of the disclosure.

[0037] In one embodiment the method comprises the steps of:

[0038] (a) providing a substrate comprising CO and/or CO<sub>2</sub> to a bioreactor containing a culture of one or more microorganism of the first aspect of the disclosure; and

[0039] (b) anaerobically fermenting the culture in the bioreactor to produce 3-HP.

[0040] In one embodiment the method comprises the steps of:

[0041] (a) capturing CO— and/or CO<sub>2</sub>-containing gas produced as a result of the industrial process, before the gas is released into the atmosphere;

[0042] (b) the anaerobic fermentation of the CO— and/or CO<sub>2</sub>-containing gas to produce at least 3-HP by a culture containing one or more microorganism of the first aspect of the disclosure.

[0043] In particular embodiments of the method aspects, the microorganism is maintained in an aqueous culture medium.

[0044] In particular embodiments of the method aspects, the fermentation of the substrate takes place in a bioreactor.

[0045] Preferably, the substrate comprising CO and/or CO<sub>2</sub> is a gaseous substrate comprising CO and/or CO<sub>2</sub>. In one embodiment, the substrate comprises an industrial waste gas. In certain embodiments, the gas is steel mill waste gas or syngas.

[0046] In a particular embodiment, the substrate is a substrate comprising CO.

[0047] In embodiments of the disclosure where the substrate comprises CO<sub>2</sub>, but no CO, the substrate preferably also comprises H<sub>2</sub>.

[0048] In one embodiment, the substrate comprises CO and CO<sub>2</sub>. In one embodiment, the substrate comprises CO<sub>2</sub> and H<sub>2</sub>. In another embodiment, the substrate comprises CO, CO<sub>2</sub>, and H<sub>2</sub>.

[0049] In one embodiment, the substrate will typically contain a major proportion of CO, such as at least about 20% to about 100% CO by volume, from 20% to 70% CO by volume, from 30% to 60% CO by volume, and from 40% to 55% CO by volume. In particular embodiments, the substrate comprises about 25%, or about 30%, or about 35%, or



about 40%, or about 45%, or about 50% CO, or about 55% CO, or about 60% CO by volume.

**[0050]** In certain embodiments the methods further comprise the step of recovering 3-HP and optionally one or more other products from the fermentation broth.

**[0051]** In a seventh aspect, the disclosure provides 3-HP when produced by the method of the sixth aspect.

**[0052]** In another aspect, the disclosure provides a method for the production of a microorganism of the first aspect of the disclosure comprising transforming a parental microorganism with one or more exogenous nucleic acid such that the microorganism is capable of producing 3-HP, and optionally one or more other products, by fermentation of a substrate comprising CO and/or CO<sub>2</sub>, wherein the parental microorganism is not capable of producing 3-HP by fermentation of a substrate comprising CO and/or CO<sub>2</sub>.

**[0053]** In one particular embodiment, a parental microorganism is transformed with one or more exogenous nucleic acid adapted to express one or more enzymes in the 3-HP biosynthesis pathway which are not naturally present in the parental microorganism. In another embodiment, a parental microorganism is transformed with one or more nucleic acid adapted to over-express one or more enzymes in the 3-HP biosynthesis pathway which are naturally present in the parental microorganism. In another embodiment, a parental microorganism is transformed with one or more exogenous nucleic acid adapted to express one or more enzymes in the 3-HP biosynthesis pathway which are not naturally present in the parental microorganism and over-express one or more enzymes in the 3-HP biosynthesis pathway which are naturally present in the parental microorganism.

**[0054]** In certain embodiments, the one or more enzymes are as herein before described.

**[0055]** In certain embodiment, the parental microorganism is as herein before described.

**[0056]** According to one embodiment a process is provided for converting CO or CO<sub>2</sub> into 3-hydroxypropionate (3-HP). A gaseous CO-containing and/or CO<sub>2</sub>-containing substrate is passed to a bioreactor containing a culture of carboxydutrophic, acetogenic bacteria in a culture medium such that the bacteria convert the CO and/or CO<sub>2</sub> to 3-HP. The carboxydutrophic acetogenic bacteria are genetically engineered to express a  $\beta$ -alanine pyruvate aminotransferase. They also express an acetyl-CoA carboxylase, whether native or exogenous. The 3-HP is recovered from the bioreactor.

**[0057]** According to another embodiment an isolated, genetically engineered, carboxydutrophic, acetogenic bacterium is provided that comprises a nucleic acid encoding a  $\beta$ -alanine pyruvate aminotransferase. The nucleic acid is exogenous to the host bacteria. The bacteria express the  $\beta$ -alanine pyruvate aminotransferase and the bacteria acquire the ability to generate malonic semialdehyde via transamination reaction with pyruvate and  $\beta$ -alanine. The  $\beta$ -alanine pyruvate aminotransferase is typically at least 85% identical to the amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 13. The  $\beta$ -alanine pyruvate aminotransferase is typically at least 85% identical to the amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 14. The  $\beta$ -alanine pyruvate aminotransferase is typically at least 85% identical to the amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 15. In one embodiment,  $\beta$ -alanine pyruvate aminotransferase is selected because pyruvate is a central

metabolite and a co-factor providing distinct advantages, such as driving enzymatic flux.

**[0058]** The bacteria may further comprise an exogenous nucleic acid encoding acetyl-Coenzyme A carboxylase. The nucleic acid may be operably linked to a promoter. The nucleic acid may have been codon optimized. The nucleic acid or the encoded carboxylase may be from a nonsulfur, photosynthetic bacterium. The bacteria may be selected from the group consisting of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, *Clostridium ragsdalei*, *Clostridium carboxidivorans*, *Clostridium drakei*, *Clostridium scatologenes*, *Clostridium aceticum*, *Clostridium formicoaceticum*, *Clostridium magnum*, *Butyribacterium methylotrophicum*, *Acetobacterium woodii*, *Alkalibaculum bacchii*, *Blautia producta*, *Eubacterium limosum*, *Moorella thermoacetica*, *Moorella thermautotrophica*, *Sporomusa ovata*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, *Oxobacter pfennigii*, and *Thermoanaerobacter kivui*. The donor bacterium of the exogenous nucleic acid may be a nonsulfur, photosynthetic bacterium such as, *Chloroflexus aurantiacus*, *Metallosphaera*, and *Sulfolobus* spp.

**[0059]** The genetically engineered bacteria may be cultured by growing in a medium comprising a gaseous carbon source. The carbon source may comprise CO and/or CO<sub>2</sub>, which may be used as either or both of an energy source or a carbon source. The bacteria may optionally be grown under strictly anaerobic conditions. The carbon source may comprise an industrial waste product or off-gas.

**[0060]** The disclosure may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, in any or all combinations of two or more of said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which the disclosure relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0061]** These and other aspects of the present disclosure, which should be considered in all its novel aspects, will become apparent from the following description, which is given by way of example only, with reference to the accompanying figures, in which:

**[0062]** FIG. 1 shows the metabolic pathway leading to the biosynthesis of 3-hydroxypropionic acid (3-HP) and 1,3-propanediol (1,3-PDO) by *Clostridium autoethanogenum* from gas fermentation. Enzymatic steps that require heterologous expression are shown in black while native reactions are shown in grey. Carbon and hydrogen is assimilated to acetyl-CoA by the Wood-Ljungdahl pathway (WLP) which is then converted to pyruvate by a pyruvate::ferredoxin oxidoreductase (PFOR). Through the actions of pyruvate carboxylase (PYC), malate dehydrogenase (MDH), fumarate hydratase (FH), aspartate ammonia-lyase (AAL), and aspartate aminotransferase (AAT), L-aspartate is formed. Aspartate-4-decarboxylase (ADC) then converts L-aspartate to L-alanine. The key pathway intermediate  $\beta$ -alanine can be synthesized from L-aspartate through heterologous expression of aspartate-1-decarboxylase (PAND), or from L-alanine via 2,3-alanine aminomutase (AAM). Alanine dehydrogenase (ALADH) can be heterologous expressed to convert pyruvate to L-alanine.  $\beta$ -alanine pyruvate aminotransferase (BAPAT) and/or  $\gamma$ -aminobutyrate transaminase (GABT) can then convert  $\beta$ -alanine to malonic



semialdehyde, which is then reduced to 3-HP via malonic semialdehyde (MSR). Alanine transaminase (AT) can be heterologously expressed to convert L-alanine to pyruvate for BAPAT reaction. Glutamate dehydrogenase (GDH) can be heterologously expressed to convert glutamate to  $\alpha$ -ketoglutarate for GABT reaction. From 3-HP, 1,3-PDO can be generated via the action of aldehyde::ferredoxin oxidoreductase (AOR) and alcohol dehydrogenase (ADH). NAD(P)H comprises NADPH and NADH.

[0063] FIGS. 2A-2B show autotrophic growth of *Clostridium autoethanogenum*  $\Delta$ aor strain in the presence of different amounts of 3-hydroxypropionic acid (at 0, 0.2, 0.5, 1, 2, 5 and 10 g/L) in Schott bottles. FIG. 2A: Growth profile; FIG. 2B: Peak Biomass. Unc=Unchallenged; N=2; Error bar=S.D.

[0064] FIG. 3 shows that *Clostridium autoethanogenum* is able to convert exogenous 3-hydroxypropionic acid to 1,3-propanediol during autotrophic growth in Schott bottles. The deletion of aldehyde::ferredoxin oxidoreductase (AOR) stops this native reaction from occurring in *C. autoethanogenum*. N=2; Error bar=S.D.

[0065] FIG. 4 shows combinatorial assembly of 3-hydroxypropionic acid pathway genes using the Golden Gate (GG) method.

[0066] FIGS. 5A-C show characterization of 111 3-hydroxypropionic acid (3-HP) combinatorial strains in 12-well plates with 200 kPa synthetic gas blend (50% CO, 10% H<sub>2</sub>, 30% CO<sub>2</sub>, and 10% N<sub>2</sub>). FIG. 5A: End-point 3-HP titer; FIG. 5B: Biomass concentration; FIG. 5C: 3-HP titer normalized by biomass concentration; Incubation duration=8 days.

[0067] FIG. 6 shows the peak 3-hydroxypropionic acid titer from combinatorial strains and WT *C. autoethanogenum* strain during autotrophic growth in Schott bottles. Incubation duration=14 days; N=2; Error bar=S.D.

[0068] FIGS. 7A-7C show the performance of combinatorial strain A59B in batch CSTR fermentation using a synthetic gas blend (50% CO, 10% H<sub>2</sub>, 30% CO<sub>2</sub> and 10% N<sub>2</sub>). FIG. 7A: Biomass and metabolite profile analyzed by HPLC; FIG. 7B: gas profile analyzed by GC-TCD (negative=uptake); FIG. 7C: 3-HP and 1,3-PDO profile analyzed by LC-MS and GC-MS, respectively.

[0069] FIGS. 8A-8C show the performance of combinatorial strain B2A in batch CSTR fermentation using a synthetic gas blend (50% CO, 10% H<sub>2</sub>, 30% CO<sub>2</sub> and 10% N<sub>2</sub>). FIG. 8A: Biomass and metabolite profile analyzed by HPLC; FIG. 8B: gas profile analyzed by GC-TCD (negative=uptake); FIG. 8C: 3-HP and 1,3-PDO profile analyzed by LC-MS and GC-MS, respectively.

[0070] FIGS. 9A-9C show the performance of combinatorial strain A63B in batch CSTR fermentation using a synthetic gas blend (50% CO, 10% H<sub>2</sub>, 30% CO<sub>2</sub> and 10% N<sub>2</sub>). FIG. 9A: Biomass and metabolite profile analyzed by HPLC; FIG. 9B: gas profile analyzed by GC-TCD (negative=uptake); FIG. 9C: 3-HP and 1,3-PDO profile analyzed by LC-MS and GC-MS, respectively.

[0071] FIG. 10A-10C shows the performance of combinatorial strain A59B in continuous CSTR fermentation using a synthetic gas blend (50% CO, 10% H<sub>2</sub>, 30% CO<sub>2</sub> and 10% N<sub>2</sub>). FIG. 10A: Biomass and metabolite profile analyzed by HPLC; FIG. 10B: gas profile analyzed by GC-TCD (negative=uptake); FIG. 10C: 3-HP and 1,3-PDO profile analyzed by LC-MS and GC-MS, respectively. D=dilution rate/day.

## DETAILED DESCRIPTION

[0072] The following description of embodiments is given in general terms. The disclosure is further elucidated from the disclosure given under the heading “Examples” herein below, which provides experimental data supporting the disclosure, specific examples of various aspects of the disclosure, and means of performing the disclosure.

[0073] The inventors have surprisingly been able to engineer a carboxydutrophic acetogenic microorganism to 3-Hydroxypropionate (3-HP) by fermentation of a substrate comprising CO and/or CO<sub>2</sub>. This offers an alternative means for the production of 3-HP which may have benefits over the current methods for the production of 3-HP. In addition, it offers a means of using carbon monoxide from industrial processes which would otherwise be released into the atmosphere and pollute the environment. In this disclosure, a microorganism expresses enzymes from the beta-alanine pathway, which resulted in significantly higher titer and productivity of 3-HP from a gaseous substrate.

[0074] In engineering the microorganisms of the disclosure, the inventors have surprisingly been able to combine two separate CO<sub>2</sub> fixation pathways, as illustrated in FIG. 1. This provides for sustainable fermentation to produce 3-HP using a substrate comprising CO and/or a substrate comprising CO<sub>2</sub>. Two pathways fixing CO<sub>2</sub> are thus linked to produce a desired product. In one embodiment, the disclosure describes fixation of three molecules of CO<sub>2</sub> into one molecule of 3-HP by combining two separate CO<sub>2</sub> fixation pathways (FIG. 1), the Wood-Ljungdahl pathways of acetogens that allows fixation of two molecules of CO<sub>2</sub>, and the initial carbon fixation steps of the 3-HP cycle allows fixation of another molecule of CO<sub>2</sub>. CO<sub>2</sub> could also be replaced with CO, as the key enzyme of the Wood-Ljungdahl pathway, a CO dehydrogenase (CODH) is able to convert CO into CO<sub>2</sub> and energy in a biological water gas shift reaction ( $\text{CO} + \text{H}_2\text{O} \rightleftharpoons \text{CO}_2 + \text{H}_2$ ). Any mixture of CO and CO<sub>2</sub> can be used. When CO<sub>2</sub> alone is used, energy in form of hydrogen or electricity may need to be supplied, while CO can serve as both carbon and energy source.

[0075] While the inventors have demonstrated the efficacy of the disclosure in *Clostridium autoethanogenum*, the disclosure is applicable to the wider group of anaerobic acetogenic microorganisms and fermentation on substrates comprising CO and/or CO<sub>2</sub>, as discussed above and further herein.

[0076] Unless otherwise defined, the following terms as used throughout this specification are defined as follows:

[0077] The terms “increasing the efficiency,” “increased efficiency,” and the like, when used in relation to a fermentation process, include, but are not limited to, increasing one or more of the rate of growth of microorganisms catalyzing the fermentation, the growth and/or product production rate at elevated product concentrations, increasing the volume of desired product produced per volume of substrate consumed, increasing the rate of production or level of production of the desired product, increasing the relative proportion of the desired product produced compared with other by-products of the fermentation, decreasing the amount of water consumed by the process, and decreasing the amount of energy utilized by the process.

[0078] The term “fermentation” should be interpreted as a metabolic process that produces chemical changes in a substrate. For example, a fermentation process receives one or more substrates and produces one or more products



through utilization of one or more microorganisms. The term “fermentation,” “gas fermentation” and the like should be interpreted as the process which receives one or more substrate, such as syngas produced by gasification and produces one or more product through the utilization of one or more C1-fixing microorganism. Preferably the fermentation process includes the use of one or more bioreactor. The fermentation process may be described as either “batch” or “continuous”. “Batch fermentation” is used to describe a fermentation process where the bioreactor is filled with raw material, e.g. the carbon source, along with microorganisms, where the products remain in the bioreactor until fermentation is completed. In a “batch” process, after fermentation is completed, the products are extracted, and the bioreactor is cleaned before the next “batch” is started. “Continuous fermentation” is used to describe a fermentation process where the fermentation process is extended for longer periods of time, and product and/or metabolite is extracted during fermentation. Preferably the fermentation process is continuous.

**[0079]** The term “non-naturally occurring” when used in reference to a microorganism is intended to mean that the microorganism has at least one genetic modification not found in a naturally occurring strain of the referenced species, including wild-type strains of the referenced species. Non-naturally occurring microorganisms are typically developed in a laboratory or research facility.

**[0080]** The terms “genetic modification,” “genetic alteration,” or “genetic engineering” broadly refer to manipulation of the genome or nucleic acids of a microorganism by the hand of man. Likewise, the terms “genetically modified,” “genetically altered,” or “genetically engineered” refers to a microorganism containing such a genetic modification, genetic alteration, or genetic engineering. These terms may be used to differentiate a lab-generated microorganism from a naturally-occurring microorganism. Methods of genetic modification of include, for example, heterologous gene expression, gene or promoter insertion or deletion, nucleic acid mutation, altered gene expression or inactivation, enzyme engineering, directed evolution, knowledge-based design, random mutagenesis methods, gene shuffling, and codon optimization.

**[0081]** Metabolic engineering of microorganisms, such as Clostridia, can tremendously expand their ability to produce many important fuel and chemical molecules other than native metabolites, such as ethanol. However, until recently, Clostridia were considered genetically intractable and therefore generally off limits to extensive metabolic engineering efforts. In recent years several different methods for genome engineering for Clostridia have been developed including intron-based methods (ClosTron) (Kuehne, *Strain Eng: Methods and Protocols*, 389-407, 2011), allelic exchange methods (ACE) (Heap, *Nucl Acids Res*, 40: e59, 2012; Ng, *PLoS One*, 8: e56051, 2013), Triple Cross (Liew, *Frontiers Microbiol*, 7: 694, 2016), methods mediated through I-SceI (Zhang, *Journal Microbiol Methods*, 108: 49-60, 2015), MazF (Al-Hinai, *Appl Environ Microbiol*, 78: 8112-8121, 2012), or others (Argyros, *Appl Environ Microbiol*, 77: 8288-8294, 2011), Cre-Lox (Ueki, *mBio*, 5: e01636-01614, 2014), and CRISPR/Cas9 (Nagaraju, *Biotechnol Biofuels*, 9: 219, 2016). However, it remains extremely challenging to iteratively introduce more than a few genetic changes, due to slow and laborious cycling times and limitations on the transferability of these genetic techniques across species.

Furthermore, we do not yet sufficiently understand C1 metabolism in Clostridia to reliably predict modifications that will maximize C1 uptake, conversion, and carbon/energy/redox flows towards product synthesis. Accordingly, introduction of target pathways in Clostridia remains a tedious and time-consuming process.

**[0082]** “Recombinant” indicates that a nucleic acid, protein, or microorganism is the product of genetic modification, engineering, or recombination. Generally, the term “recombinant” refers to a nucleic acid, protein, or microorganism that contains or is encoded by genetic material derived from multiple sources, such as two or more different strains or species of microorganisms.

**[0083]** “Wild type” refers to the typical form of an organism, strain, gene, or characteristic as it occurs in nature, as distinguished from mutant or variant forms.

**[0084]** “Endogenous” refers to a nucleic acid or protein that is present or expressed in the wild-type or parental microorganism from which the microorganism of the disclosure is derived. For example, an endogenous gene is a gene that is natively present in the wild-type or parental microorganism from which the microorganism of the disclosure is derived. In one embodiment, the expression of an endogenous gene may be controlled by an exogenous regulatory element, such as an exogenous promoter.

**[0085]** “Exogenous” refers to a nucleic acid or protein that originates outside the microorganism of the disclosure. For example, an exogenous gene or enzyme may be artificially or recombinantly created and introduced to or expressed in the microorganism of the disclosure. An exogenous gene or enzyme may also be isolated from a heterologous microorganism and introduced to or expressed in the microorganism of the disclosure. Exogenous nucleic acids may be adapted to integrate into the genome of the microorganism of the disclosure or to remain in an extra-chromosomal state in the microorganism of the disclosure, for example, in a plasmid.

**[0086]** “Heterologous” refers to a nucleic acid or protein that is not present in the wild-type or parental microorganism from which the microorganism of the disclosure is derived. For example, a heterologous gene or enzyme may be derived from a different strain or species and introduced to or expressed in the microorganism of the disclosure. The heterologous gene or enzyme may be introduced to or expressed in the microorganism of the disclosure in the form in which it occurs in the different strain or species. Alternatively, the heterologous gene or enzyme may be modified in some way, e.g., by codon-optimizing it for expression in the microorganism of the disclosure or by engineering it to alter function, such as to reverse the direction of enzyme activity or to alter substrate specificity.

**[0087]** The terms “polynucleotide,” “nucleotide,” “nucleotide sequence,” “nucleic acid,” and “oligonucleotide” are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, short interfering RNA (siRNA), short-hairpin RNA (shRNA), microRNA (miRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated



DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise one or more modified nucleotides, such as methylated nucleotides or nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component.

**[0088]** As used herein, “expression” refers to the process by which a polynucleotide is transcribed from a DNA template (such as into and mRNA or other RNA transcript) and/or the process by which a transcribed mRNA is subsequently translated into peptides, polypeptides, or proteins. Transcripts and encoded polypeptides may be collectively referred to as “gene products.”

**[0089]** The terms “polypeptide”, “peptide,” and “protein” are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component. As used herein, the term “amino acid” includes natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics.

**[0090]** “Enzyme activity,” or simply “activity,” refers broadly to enzymatic activity, including, but not limited, to the activity of an enzyme, the amount of an enzyme, or the availability of an enzyme to catalyze a reaction. Accordingly, “increasing” enzyme activity includes increasing the activity of an enzyme, increasing the amount of an enzyme, or increasing the availability of an enzyme to catalyze a reaction. Similarly, “decreasing” enzyme activity includes decreasing the activity of an enzyme, decreasing the amount of an enzyme, or decreasing the availability of an enzyme to catalyze a reaction.

**[0091]** “Mutated” refers to a nucleic acid or protein that has been modified in the microorganism of the disclosure compared to the wild-type or parental microorganism from which the microorganism of the disclosure is derived. In one embodiment, the mutation may be a deletion, insertion, or substitution in a gene encoding an enzyme. In another embodiment, the mutation may be a deletion, insertion, or substitution of one or more amino acids in an enzyme.

**[0092]** In particular, a “disruptive mutation” is a mutation that reduces or eliminates (i.e., “disrupts”) the expression or activity of a gene or enzyme. The disruptive mutation may partially inactivate, fully inactivate, or delete the gene or enzyme. The disruptive mutation may be any mutation that reduces, prevents, or blocks the biosynthesis of a product produced by an enzyme. The disruptive mutation may be a knockout (KO) mutation. The disruption may also be a knockdown (KD) mutation that reduces, but does not entirely eliminate, the expression or activity of a gene, protein, or enzyme. While KOs are generally effective in increasing product yields, they sometimes come with the penalty of growth defects or genetic instabilities that outweigh the benefits, particularly for non-growth coupled products. The disruptive mutation may include, for example, a mutation in a gene encoding an enzyme, a mutation in a

genetic regulatory element involved in the expression of a gene encoding an enzyme, the introduction of a nucleic acid which produces a protein that reduces or inhibits the activity of an enzyme, or the introduction of a nucleic acid (e.g., antisense RNA, siRNA, CRISPR) or protein which inhibits the expression of an enzyme. The disruptive mutation may be introduced using any method known in the art.

**[0093]** Introduction of a disruptive mutation results in a microorganism of the disclosure that produces no target product or substantially no target product or a reduced amount of target product compared to the parental microorganism from which the microorganism of the disclosure is derived. For example, the microorganism of the disclosure may produce no target product or at least about 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% less target product than the parental microorganism. For example, the microorganism of the disclosure may produce less than about 0.001, 0.01, 0.10, 0.30, 0.50, or 1.0 g/L target product.

**[0094]** “Codon optimization” refers to the mutation of a nucleic acid, such as a gene, for optimized or improved translation of the nucleic acid in a particular strain or species. Codon optimization may result in faster translation rates or translation accuracy. In an embodiment, the genes of the disclosure are codon optimized for expression in *Clostridium*, particularly *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, or *Clostridium ragsdalei*. In a further embodiment, the genes of the disclosure are codon optimized for expression in *Clostridium autoethanogenum* LZ1561, which is deposited under DSMZ accession number DSM23693.

**[0095]** “Overexpressed” refers to an increase in expression of a nucleic acid or protein in the microorganism of the disclosure compared to the wild-type or parental microorganism from which the microorganism of the disclosure is derived. Overexpression may be achieved by any means known in the art, including modifying gene copy number, gene transcription rate, gene translation rate, or enzyme degradation rate.

**[0096]** The term “variants” includes nucleic acids and proteins whose sequence varies from the sequence of a reference nucleic acid and protein, such as a sequence of a reference nucleic acid and protein disclosed in the prior art or exemplified herein. The disclosure may be practiced using variant nucleic acids or proteins that perform substantially the same function as the reference nucleic acid or protein. For example, a variant protein may perform substantially the same function or catalyze substantially the same reaction as a reference protein. A variant gene may encode the same or substantially the same protein as a reference gene. A variant promoter may have substantially the same ability to promote the expression of one or more genes as a reference promoter.

**[0097]** Such nucleic acids or proteins may be referred to herein as “functionally equivalent variants.” By way of example, functionally equivalent variants of a nucleic acid may include allelic variants, fragments of a gene, mutated genes, polymorphisms, and the like. Homologous genes from other microorganisms are also examples of functionally equivalent variants. These include homologous genes in species such as *Clostridium acetobutylicum*, *Clostridium beijerinckii*, or *Clostridium ljungdahlii*, the details of which are publicly available on websites such as Genbank or NCBI. Functionally equivalent variants also include nucleic acids whose sequence varies as a result of codon optimiza-



tion for a particular microorganism. A functionally equivalent variant of a nucleic acid will preferably have at least approximately 70%, approximately 80%, approximately 85%, approximately 90%, approximately 95%, approximately 98%, or greater nucleic acid sequence identity (percent homology) with the referenced nucleic acid. A functionally equivalent variant of a protein will preferably have at least approximately 70%, approximately 80%, approximately 85%, approximately 90%, approximately 95%, approximately 98%, or greater amino acid identity (percent homology) with the referenced protein. The functional equivalence of a variant nucleic acid or protein may be evaluated using any method known in the art.

**[0098]** “Complementarity” refers to the ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. A percent complementarity indicates the percentage of residues in a nucleic acid molecule which can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). “Perfectly complementary” means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. “Substantially complementary” as used herein refers to a degree of complementarity that is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, or more nucleotides, or refers to two nucleic acids that hybridize under stringent conditions.

**[0099]** As used herein, “stringent conditions” for hybridization refer to conditions under which a nucleic acid having complementarity to a target sequence predominantly hybridizes with the target sequence, and substantially does not hybridize to non-target sequences. Stringent conditions are generally sequence-dependent and vary depending on a number of factors. In general, the longer the sequence, the higher the temperature at which the sequence specifically hybridizes to its target sequence. Non-limiting examples of stringent conditions are well known in the art (e.g., Tijssen, Laboratory techniques in biochemistry and molecular biology-hybridization with nucleic acid probes, Second Chapter “Overview of principles of hybridization and the strategy of nucleic acid probe assay,” Elsevier, N.Y., 1993).

**[0100]** “Hybridization” refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson Crick base pairing, Hoogsteen binding, or in any other sequence specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of PCR, or the cleavage of a polynucleotide by an enzyme. A sequence capable of hybridizing with a given sequence is referred to as the “complement” of the given sequence.

**[0101]** Nucleic acids may be delivered to a microorganism of the disclosure using any method known in the art. For example, nucleic acids may be delivered as naked nucleic acids or may be formulated with one or more agents, such as liposomes. The nucleic acids may be DNA, RNA, cDNA,

or combinations thereof, as is appropriate. Restriction inhibitors may be used in certain embodiments. Additional vectors may include plasmids, viruses, bacteriophages, cosmids, and artificial chromosomes. In an embodiment, nucleic acids are delivered to the microorganism of the disclosure using a plasmid. By way of example, transformation (including transduction or transfection) may be achieved by electroporation, ultrasonication, polyethylene glycol-mediated transformation, chemical or natural competence, protoplast transformation, prophage induction, or conjugation. In certain embodiments having active restriction enzyme systems, it may be necessary to methylate a nucleic acid before introduction of the nucleic acid into a microorganism.

**[0102]** Furthermore, nucleic acids may be designed to comprise a regulatory element, such as a promoter, to increase or otherwise control expression of a particular nucleic acid. The promoter may be a constitutive promoter or an inducible promoter. Ideally, the promoter is a Wood-Ljungdahl pathway promoter, a ferredoxin promoter, a pyruvate:ferredoxin oxidoreductase promoter, an Rnf complex operon promoter, an ATP synthase operon promoter, or a phosphotransacetylase/acetate kinase operon promoter.

**[0103]** A “microorganism” is a microscopic organism, especially a bacterium, archaeon, virus, or fungus. The microorganism of the disclosure is typically a bacterium. As used herein, recitation of “microorganism” should be taken to encompass “bacterium.”

**[0104]** A “parental microorganism” is a microorganism used to generate a microorganism of the disclosure. The parental microorganism may be a naturally-occurring microorganism (i.e., a wild-type microorganism) or a microorganism that has been previously modified (i.e., a mutant or recombinant microorganism). The microorganism of the disclosure may be modified to express or overexpress one or more enzymes that were not expressed or overexpressed in the parental microorganism. Similarly, the microorganism of the disclosure may be modified to contain one or more genes that were not contained by the parental microorganism. The microorganism of the disclosure may also be modified to not express or to express lower amounts of one or more enzymes that were expressed in the parental microorganism. In one embodiment, the parental microorganism is *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, or *Clostridium ragsdalei*. In an embodiment, the parental microorganism is *Clostridium autoethanogenum* LZ1561, which was deposited with Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) located at Inhoffenstraße 7B, D-38124 Braunschweig, Germany on Jun. 7, 2010, under the terms of the Budapest Treaty and accorded accession number DSM23693. This strain is described in International Patent Application No. PCT/NZ2011/000144, which published as WO 2012/015317.

**[0105]** The term “derived from” indicates that a nucleic acid, protein, or microorganism is modified or adapted from a different (e.g., a parental or wild-type) nucleic acid, protein, or microorganism, so as to produce a new nucleic acid, protein, or microorganism. Such modifications or adaptations typically include insertion, deletion, mutation, or substitution of nucleic acids or genes. Generally, the microorganism of the disclosure is derived from a parental microorganism. In one embodiment, the microorganism of the disclosure is derived from *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, or *Clostridium ragsdalei*. In



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

[0106] The microorganism of the disclosure may be further classified based on functional characteristics. For example, the microorganism of the disclosure may be or may be derived from a C1-fixing microorganism, an anaerobe, an acetogen, an ethanologen, a carboxydotroph, and/or a methanotroph. Table 1 provides a representative list of microorganisms and identifies their functional characteristics.

source. Typically, the microorganism of the disclosure is a C1-fixing bacterium. In an embodiment, the microorganism of the disclosure is derived from a C1-fixing microorganism identified in Table 1.

[0109] An “anaerobe” is a microorganism that does not require oxygen for growth. An anaerobe may react negatively or even die if oxygen is present above a certain threshold. However, some anaerobes are capable of tolerating low levels of oxygen (e.g., 0.000001-5% oxygen). Typically, the microorganism of the disclosure is an anaer-

TABLE 1

	Wood-Ljungdahl	C1-fixing	Anaerobe	Acetogen	Ethanologen	Autotroph	Carboxydotroph
<i>Acetobacterium woodii</i>	+	+	+	+	+/- <sup>1</sup>	+	-
<i>Alkalibaculum bacchii</i>	+	+	+	+	+	+	+
<i>Blautia producta</i>	+	+	+	+	-	+	+
<i>Butyribacterium methylotrophicum</i>	+	+	+	+	+	+	+
<i>Clostridium aceticum</i>	+	+	+	+	-	+	+
<i>Clostridium autoethanogenum</i>	+	+	+	+	+	+	+
<i>Clostridium carboxidivorans</i>	+	+	+	+	+	+	+
<i>Clostridium coskatii</i>	+	+	+	+	+	+	+
<i>Clostridium drakei</i>	+	+	+	+	-	+	+
<i>Clostridium formicoaceticum</i>	+	+	+	+	-	+	+
<i>Clostridium ljungdahlii</i>	+	+	+	+	+	+	+
<i>Clostridium magnum</i>	+	+	+	+	-	+	+/- <sup>2</sup>
<i>Clostridium ragsdalei</i>	+	+	+	+	+	+	+
<i>Clostridium scatologenes</i>	+	+	+	+	-	+	+
<i>Eubacterium limosum</i>	+	+	+	+	-	+	+
<i>Moorella thermoautotrophica</i>	+	+	+	+	+	+	+
<i>Moorella thermoacetica</i> (formerly <i>Clostridium thermoaceticum</i> )	+	+	+	+	- <sup>3</sup>	+	+
<i>Oxobacter pfennigii</i>	+	+	+	+	-	+	+
<i>Sporomusa ovata</i>	+	+	+	+	-	+	+/- <sup>4</sup>
<i>Sporomusa silvacetica</i>	+	+	+	+	-	+	+/- <sup>5</sup>
<i>Sporomusa sphaeroides</i>	+	+	+	+	-	+	+/- <sup>6</sup>
<i>Thermoanaerobacter kivui</i>	+	+	+	+	-	+	-

<sup>1</sup> *Acetobacterium woodii* can produce ethanol from fructose, but not from gas.

<sup>2</sup> It has not been investigated whether *Clostridium magnum* can grow on CO.

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

<sup>4</sup> It has not been investigated whether *Sporomusa ovata* can grow on CO.

<sup>5</sup> It has not been investigated whether *Sporomusa silvacetica* can grow on CO.

<sup>6</sup> It has not been investigated whether *Sporomusa sphaeroides* can grow on CO.

[0107] “Wood-Ljungdahl” refers to the Wood-Ljungdahl pathway of carbon fixation as described, e.g., by Ragsdale, *Biochim Biophys Acta*, 1784: 1873-1898, 2008. “Wood-Ljungdahl microorganisms” refers, predictably, to microorganisms containing the Wood-Ljungdahl pathway. Generally, the microorganism of the disclosure contains a native Wood-Ljungdahl pathway. Herein, a Wood-Ljungdahl pathway may be a native, unmodified Wood-Ljungdahl pathway or it may be a Wood-Ljungdahl pathway with some degree of genetic modification (e.g., overexpression, heterologous expression, knockout, etc.) so long as it still functions to convert CO, CO<sub>2</sub>, and/or H<sub>2</sub> to acetyl-CoA.

[0108] “C1” refers to a one-carbon molecule, for example, CO, CO<sub>2</sub>, CH<sub>4</sub>, or CH<sub>3</sub>OH. “C1-oxygenate” refers to a one-carbon molecule that also comprises at least one oxygen atom, for example, CO, CO<sub>2</sub>, or CH<sub>3</sub>OH. “C1-carbon source” refers a one carbon-molecule that serves as a partial or sole carbon source for the microorganism of the disclosure. For example, a C1-carbon source may comprise one or more of CO, CO<sub>2</sub>, CH<sub>4</sub>, CH<sub>3</sub>OH, or CH<sub>2</sub>O<sub>2</sub>. Preferably, the C1-carbon source comprises one or both of CO and CO<sub>2</sub>. A “C1-fixing microorganism” is a microorganism that has the ability to produce one or more products from a C1 carbon

obe. In an embodiment, the microorganism of the disclosure is derived from an anaerobe identified in Table 1.

[0110] “Acetogens” are obligately anaerobic bacteria that use the Wood-Ljungdahl pathway as their main mechanism for energy conservation and for synthesis of acetyl-CoA and acetyl-CoA-derived products, such as acetate (Ragsdale, *Biochim Biophys Acta*, 1784: 1873-1898, 2008). In particular, acetogens use the Wood-Ljungdahl pathway as a (1) mechanism for the reductive synthesis of acetyl-CoA from CO<sub>2</sub>, (2) terminal electron-accepting, energy conserving process, (3) mechanism for the fixation (assimilation) of CO<sub>2</sub> in the synthesis of cell carbon (Drake, *Acetogenic Prokaryotes*, In: *The Prokaryotes*, 3rd edition, p. 354, New York, NY, 2006). All naturally occurring acetogens are C1-fixing, anaerobic, autotrophic, and non-methanotrophic. Typically, the microorganism of the disclosure is an acetogen. In an embodiment, the microorganism of the disclosure is derived from an acetogen identified in Table 1.

[0111] An “ethanologen” is a microorganism that produces or is capable of producing ethanol. Typically, the microorganism of the disclosure is an ethanologen. In an embodiment, the microorganism of the disclosure is derived from an ethanologen identified in Table 1.



[0112] An “autotroph” is a microorganism capable of growing in the absence of organic carbon. Instead, autotrophs use inorganic carbon sources, such as CO and/or CO<sub>2</sub>. Typically, the microorganism of the disclosure is an autotroph. In an embodiment, the microorganism of the disclosure is derived from an autotroph identified in Table 1.

[0113] A “carboxydutroph” is a microorganism capable of utilizing CO as a sole source of carbon and energy. Typically, the microorganism of the disclosure is a carboxydutroph. In an embodiment, the microorganism of the disclosure is derived from a carboxydutroph identified in Table 1.

[0114] A “methanotroph” is a microorganism capable of utilizing methane as a sole source of carbon and energy. In certain embodiments, the microorganism of the disclosure is a methanotroph or is derived from a methanotroph. In other embodiments, the microorganism of the disclosure is not a methanotroph or is not derived from a methanotroph.

[0115] More broadly, the microorganism of the disclosure may be derived from any genus or species identified in Table 1. For example, the microorganism may be a member of a genus selected from the group consisting of *Acetobacterium*, *Alkalibaculum*, *Blautia*, *Butyribacterium*, *Clostridium*, *Eubacterium*, *Moorella*, *Oxobacter*, *Sporomusa*, and *Thermoanaerobacter*. In particular, the microorganism may be derived from a parental bacterium selected from the group consisting of *Acetobacterium woodii*, *Alkalibaculum bacchii*, *Blautia producta*, *Butyribacterium methylotrophicum*, *Clostridium aceticum*, *Clostridium autoethanogenum*, *Clostridium carboxidivorans*, *Clostridium coskatii*, *Clostridium drakei*, *Clostridium formicoaceticum*, *Clostridium ljungdahlii*, *Clostridium magnum*, *Clostridium ragsdalei*, *Clostridium scatologenes*, *Eubacterium limosum*, *Moorella thermoautotrophica*, *Moorella thermoacetica*, *Oxobacter pfennigii*, *Sporomusa ovata*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, and *Thermoanaerobacter kivui*.

[0116] In an embodiment, the microorganism of the disclosure is derived from the cluster of Clostridia comprising the species *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, and *Clostridium ragsdalei*. These species were first reported and characterized by Abrini, Arch Microbiol, 161: 345-351, 1994 (*Clostridium autoethanogenum*), Tanner, Int J System Bacteriol, 43: 232-236, 1993 (*Clostridium ljungdahlii*), and Huhnke, WO 2008/028055 (*Clostridium ragsdalei*).

[0117] These three species have many similarities. In particular, these species are all C1 fixing, anaerobic, acetogenic, ethanologenic, and carboxydutrophic members of the genus *Clostridium*. These species have similar genotypes and phenotypes and modes of energy conservation and fermentative metabolism. Moreover, these species are clustered in clostridial rRNA homology group I with 16S rRNA DNA that is more than 99% identical, have a DNA G+C content of about 22-30 mol %, are gram-positive, have similar morphology and size (logarithmic growing cells between 0.5-0.7×3-5 μm), are mesophilic (grow optimally at 30-37° C.), have similar pH ranges of about 4-7.5 (with an optimal pH of about 5.5-6), lack cytochromes, and conserve energy via an Rnf complex. Also, reduction of carboxylic acids into their corresponding alcohols has been shown in these species (Perez, Biotechnol Bioeng, 110:1066-1077, 2012). Importantly, these species also all show strong autotrophic growth on CO-containing gases, produce ethanol

and acetate (or acetic acid) as main fermentation products, and produce small amounts of 2,3-butanediol and lactic acid under certain conditions.

[0118] However, these three species also have a number of differences. These species were isolated from different sources: *Clostridium autoethanogenum* from rabbit gut, *Clostridium ljungdahlii* from chicken yard waste, and *Clostridium ragsdalei* from freshwater sediment. These species differ in utilization of various sugars (e.g., rhamnose, arabinose), acids (e.g., gluconate, citrate), amino acids (e.g., arginine, histidine), and other substrates (e.g., betaine, butanol). Moreover, these species differ in auxotrophy to certain vitamins (e.g., thiamine, biotin). These species have differences in nucleic and amino acid sequences of Wood-Ljungdahl pathway genes and proteins, although the general organization and number of these genes and proteins has been found to be the same in all species (Köpke, Curr Opin Biotechnol, 22: 320-325, 2011).

[0119] Thus, in summary, many of the characteristics of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, or *Clostridium ragsdalei* are not specific to that species, but are rather general characteristics for this cluster of C1 fixing, anaerobic, acetogenic, ethanologenic, and carboxydutrophic members of the genus *Clostridium*. However, since these species are, in fact, distinct, the genetic modification or manipulation of one of these species may not have an identical effect in another of these species. For instance, differences in growth, performance, or product production may be observed.

[0120] The microorganism of the disclosure may also be derived from an isolate or mutant of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, or *Clostridium ragsdalei*. Isolates and mutants of *Clostridium autoethanogenum* include JA1-1 (DSM10061) (Abrini, Arch Microbiol, 161: 345-351, 1994), LZ1560 (DSM19630) (WO 2009/064200), and LZ1561 (DSM23693) (WO 2012/015317). Isolates and mutants of *Clostridium ljungdahlii* include ATCC 49587 (Tanner, Int J Syst Bacteriol, 43: 232-236, 1993), PETCT (DSM13528, ATCC 55383), ERI-2 (ATCC 55380) (U.S. Pat. No. 5,593,886), C-01 (ATCC 55988) (U.S. Pat. No. 6,368,819), O-52 (ATCC 55989) (U.S. Pat. No. 6,368,819), and OTA-1 (Tirado-Acevedo, Production of bioethanol from synthesis gas using *Clostridium ljungdahlii*, PhD thesis, North Carolina State University, 2010). Isolates and mutants of *Clostridium ragsdalei* include PI 1 (ATCC BAA-622, ATCC PTA-7826) (WO 2008/028055).

[0121] “Substrate” refers to a carbon and/or energy source for the microorganism of the disclosure. Typically, the substrate is gaseous and comprises a C1-carbon source, for example, CO, CO<sub>2</sub>, and/or CH<sub>4</sub>. Preferably, the substrate comprises a C1-carbon source of CO or CO+CO<sub>2</sub>. The substrate may further comprise other non-carbon components, such as H<sub>2</sub>, N<sub>2</sub>, or electrons.

[0122] The substrate generally comprises at least some amount of CO, such as about 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 mol % CO. The substrate may comprise a range of CO, such as about 20-80, 30-70, or 40-60 mol % CO. Preferably, the substrate comprises about 40-70 mol % CO (e.g., steel mill or blast furnace gas), about 20-30 mol % CO (e.g., basic oxygen furnace gas), or about 15-45 mol % CO (e.g., syngas). In some embodiments, the substrate may comprise a relatively low amount of CO, such as about 1-10 or 1-20 mol % CO. The microorganism of the disclosure typically converts at least a portion of the CO in the substrate



to a product. In some embodiments, the substrate comprises no or substantially no (<1 mol %) CO.

**[0123]** The substrate may comprise some amount of H<sub>2</sub>. For example, the substrate may comprise about 1, 2, 5, 10, 15, 20, or 30 mol % H<sub>2</sub>. In some embodiments, the substrate may comprise a relatively high amount of H<sub>2</sub>, such as about 60, 70, 80, or 90 mol % H<sub>2</sub>. In further embodiments, the substrate comprises no or substantially no (<1 mol %) H<sub>2</sub>.

**[0124]** The substrate may comprise some amount of CO<sub>2</sub>. For example, the substrate may comprise about 1-80 or 1-30 mol % CO<sub>2</sub>. In some embodiments, the substrate may comprise less than about 20, 15, 10, or 5 mol % CO<sub>2</sub>. In another embodiment, the substrate comprises no or substantially no (<1 mol %) CO<sub>2</sub>.

**[0125]** Although the substrate is typically gaseous, the substrate may also be provided in alternative forms. For example, the substrate may be dissolved in a liquid saturated with a CO-containing gas using a microbubble dispersion generator. By way of further example, the substrate may be adsorbed onto a solid support.

**[0126]** The substrate and/or C1-carbon source may be a waste gas obtained as a byproduct of an industrial process or from some other source, such as from automobile exhaust fumes or biomass gasification. In certain embodiments, the industrial process is selected from the group consisting of ferrous metal products manufacturing, such as a steel mill manufacturing, non-ferrous products manufacturing, petroleum refining, coal gasification, electric power production, carbon black production, ammonia production, methanol production, and coke manufacturing. In these embodiments, the substrate and/or C1-carbon source may be captured from the industrial process before it is emitted into the atmosphere, using any convenient method.

**[0127]** The substrate and/or C1-carbon source may be syngas, such as syngas obtained by gasification of coal or refinery residues, gasification of biomass or lignocellulosic material, or reforming of natural gas. In another embodiment, the syngas may be obtained from the gasification of municipal solid waste or industrial solid waste. The substrate and/or C1-carbon source may be derived from pyrolysis with or without subsequent partial oxidation of the pyrolysis oil.

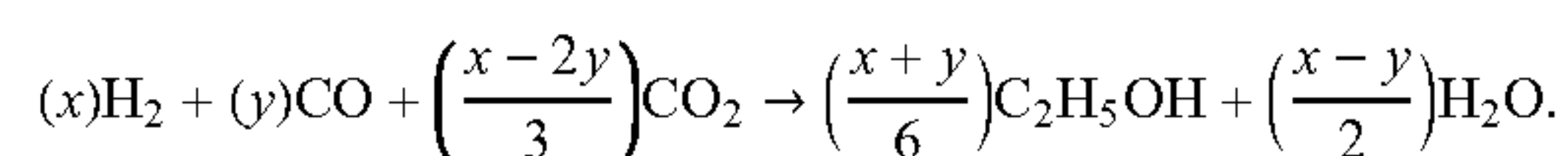
**[0128]** The composition of the substrate may have a significant impact on the efficiency and/or cost of the reaction. For example, the presence of oxygen (O<sub>2</sub>) may reduce the efficiency of an anaerobic fermentation process. Depending on the composition of the substrate, it may be desirable to treat, scrub, or filter the substrate to remove any undesired impurities, such as toxins, undesired components, or dust particles, and/or increase the concentration of desirable components.

**[0129]** In particular embodiments, the presence of hydrogen results in an improved overall efficiency of the fermentation process.

**[0130]** Syngas composition can be improved to provide a desired or optimum H<sub>2</sub>:CO:CO<sub>2</sub> ratio. The syngas composition may be improved by adjusting the feedstock being fed to the gasification process. The desired H<sub>2</sub>:CO:CO<sub>2</sub> ratio is dependent on the desired fermentation product of the fermentation process. For ethanol, the optimum H<sub>2</sub>:CO:CO<sub>2</sub> ratio would be:

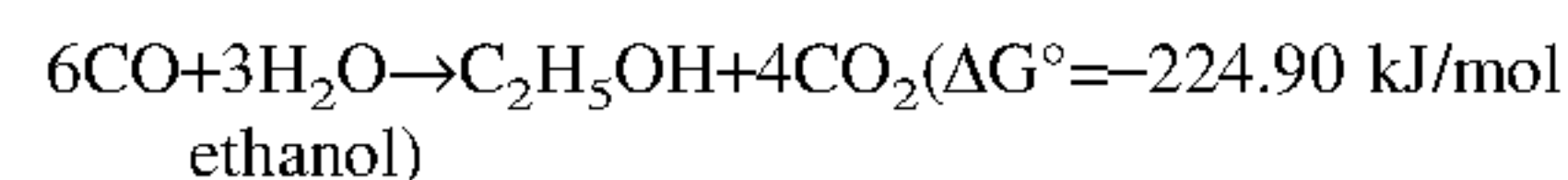
$$(x):(y):\left(\frac{x-2y}{3}\right),$$

where  $x > 2y$ , in order to satisfy the stoichiometry for ethanol production

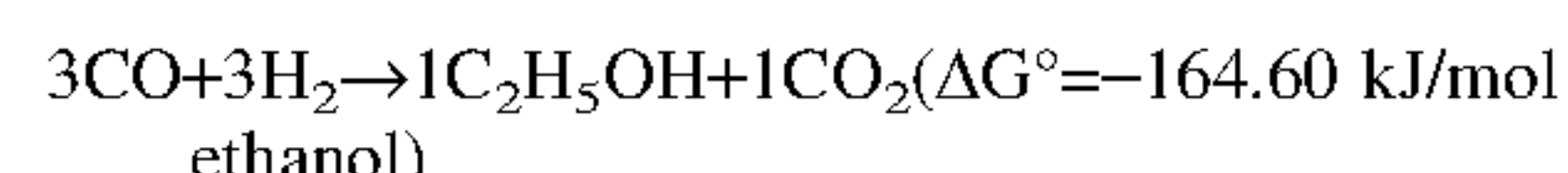
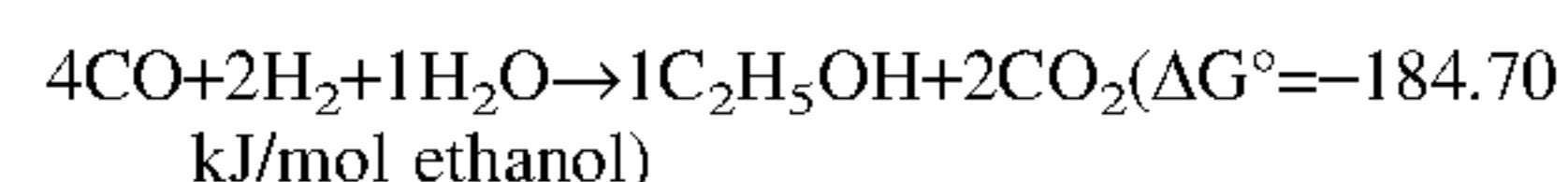
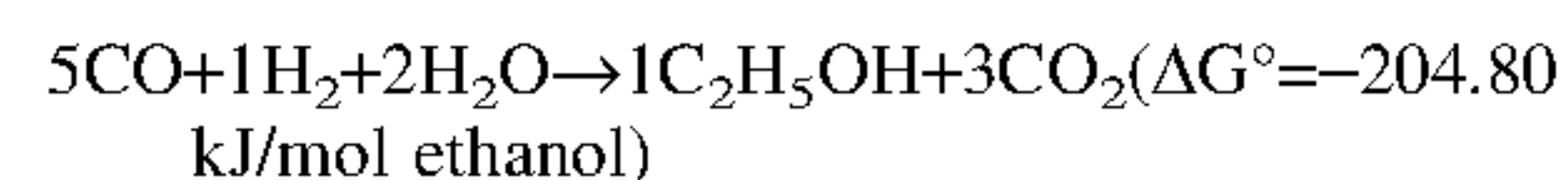


**[0131]** Operating the fermentation process in the presence of hydrogen has the added benefit of reducing the amount of CO<sub>2</sub> produced by the fermentation process. For example, a gaseous substrate comprising minimal H<sub>2</sub> will typically produce ethanol and CO<sub>2</sub> by the following stoichiometry [ $6\text{CO} + 3\text{H}_2\text{O} \rightarrow \text{C}_2\text{H}_5\text{OH} + 4\text{CO}_2$ ]. As the amount of hydrogen utilized by the C1-fixing bacterium increases, the amount of CO<sub>2</sub> produced decreases [e.g.,  $2\text{CO} + 4\text{H}_2 \rightarrow \text{C}_2\text{H}_5\text{OH} + \text{H}_2\text{O}$ ].

**[0132]** When CO is the sole carbon and energy source for ethanol production, a portion of the carbon is lost to CO<sub>2</sub> as follows:



**[0133]** As the amount of H<sub>2</sub> available in the substrate increases, the amount of CO<sub>2</sub> produced decreases. At a stoichiometric ratio of 2:1 (H<sub>2</sub>:CO), CO<sub>2</sub> production is completely avoided.



**[0134]** “Stream” refers to any substrate which is capable of being passed, for example, from one process to another, from one module to another, and/or from one process to a carbon capture means.

**[0135]** “Reactants” as used herein refer to a substance that takes part in and undergoes change during a chemical reaction. In particular embodiments, the reactants include but are not limited to CO and/or H<sub>2</sub>.

**[0136]** “Microbe inhibitors” as used herein refer to one or more constituent that slows down or prevents a particular chemical reaction or another process including the microbe. In particular embodiments, the microbe inhibitors include, but are not limited to, oxygen (O<sub>2</sub>), hydrogen cyanide (HCN), acetylene (C<sub>2</sub>H<sub>2</sub>), and BTEX (benzene, toluene, ethylbenzene, xylene).

**[0137]** “Catalyst inhibitor”, “adsorbent inhibitor”, and the like, as used herein, refer to one or more substance that decreases the rate of, or prevents, a chemical reaction. In particular embodiments, the catalyst and/or adsorbent inhibitors may include but are not limited to, hydrogen sulfide (H<sub>2</sub>S) and carbonyl sulfide (COS).

**[0138]** “Removal process”, “removal module”, “clean-up module”, and the like includes technologies that are capable of either converting and/or removing microbe inhibitors and/or catalyst inhibitors from the gas stream. In particular embodiments, catalyst inhibitors must be removed by an



upstream removal module in order to prevent inhibition of one or more catalyst in a downstream removal module.

**[0139]** The term “constituents”, “contaminants”, and the like, as used herein, refers to the microbe inhibitors, and/or catalyst inhibitors that may be found in the gas stream. In particular embodiments, the constituents include, but are not limited to, sulphur compounds, aromatic compounds, alkynes, alkenes, alkanes, olefins, nitrogen compounds, phosphorous-containing compounds, particulate matter, solids, oxygen, halogenated compounds, silicon-containing compounds, carbonyls, metals, alcohols, esters, ketones, peroxides, aldehydes, ethers, and tars.

**[0140]** The term “treated gas”, “treated stream” and the like refers to the gas stream that has been passed through at least one removal module and has had one or more constituent removed and/or converted.

**[0141]** The term “desired composition” is used to refer to the desired level and types of components in a substance, such as, for example, of a gas stream, including but not limited to syngas. More particularly, a gas is considered to have a “desired composition” if it contains a particular component (e.g. CO, H<sub>2</sub>, and/or CO<sub>2</sub>) and/or contains a particular component at a particular proportion and/or does not contain a particular component (e.g. a contaminant harmful to the microorganisms) and/or does not contain a particular component at a particular proportion. More than one component may be considered when determining whether a gas stream has a desired composition.

**[0142]** The composition of the substrate may have a significant impact on the efficiency and/or cost of the reaction. For example, the presence of oxygen (O<sub>2</sub>) may reduce the efficiency of an anaerobic fermentation process. Depending on the composition of the substrate, it may be desirable to treat, scrub, or filter the substrate to remove any undesired impurities, such as toxins, undesired components, or dust particles, and/or increase the concentration of desirable components.

**[0143]** The term “carbon capture” as used herein refers to the sequestration of carbon compounds including CO<sub>2</sub> and/or CO from a stream comprising CO<sub>2</sub> and/or CO and either:

**[0144]** converting the CO<sub>2</sub> and/or CO into products; or

**[0145]** converting the CO<sub>2</sub> and/or CO into substances suitable for long-term storage; or

**[0146]** trapping the CO<sub>2</sub> and/or CO in substances suitable for long-term storage;

**[0147]** or a combination of these processes.

**[0148]** In certain embodiments, the fermentation is performed in the absence of carbohydrate substrates, such as sugar, starch, lignin, cellulose, or hemicellulose.

**[0149]** The microorganism of the disclosure may be cultured with the gaseous substrate to produce one or more products. For instance, the microorganism of the disclosure may produce or may be engineered to produce ethanol (WO 2007/117157), acetate (WO 2007/117157), 1-butanol (WO 2008/115080, WO 2012/053905, and WO 2017/066498), butyrate (WO 2008/115080), 2,3-butanediol (WO 2009/151342 and WO 2016/094334), lactate (WO 2011/112103), butene (WO 2012/024522), butadiene (WO 2012/024522), methyl ethyl ketone (2-butanone) (WO 2012/024522 and WO 2013/185123), ethylene (WO 2012/026833), acetone (WO 2012/115527), isopropanol (WO 2012/115527), lipids (WO 2013/036147), 3-hydroxypropionate (3-HP) (WO 2013/180581), terpenes, including isoprene (WO 2013/180584), fatty acids (WO 2013/191567), 2-butanol (WO

2013/185123), 1,2-propanediol (WO 2014/036152), 1-propanol (WO 2017/066498), 1-hexanol (WO 2017/066498), 1-octanol (WO 2017/066498), chorismate-derived products (WO 2016/191625), 3-hydroxybutyrate (WO 2017/066498), 1,3-butanediol (WO 2017/066498), 2-hydroxyisobutyrate or 2-hydroxyisobutyric acid (WO 2017/066498), isobutylene (WO 2017/066498), adipic acid (WO 2017/066498), 1,3-hexanediol (WO 2017/066498), 3-methyl-2-butanol (WO 2017/066498), 2-buten-1-ol (WO 2017/066498), isovalerate (WO 2017/066498), isoamyl alcohol (WO 2017/066498), and/or monoethylene glycol (WO 2019/126400) in addition to 2-phenylethanol. In certain embodiments, microbial biomass itself may be considered a product. These products may be further converted to produce at least one component of diesel, jet fuel, and/or gasoline. In certain embodiments, 2-phenylethanol may be used as an ingredient in fragrances, essential oils, flavors, and soaps. Additionally, the microbial biomass may be further processed to produce a single cell protein (SCP).

**[0150]** A “single cell protein” (SCP) refers to a microbial biomass that may be used in protein-rich human and/or animal feeds, often replacing conventional sources of protein supplementation such as soymeal or fishmeal. To produce a single cell protein or other product, the process may comprise additional separation, processing, or treatments steps. For example, the method may comprise sterilizing the microbial biomass, centrifuging the microbial biomass, and/or drying the microbial biomass. In certain embodiments, the microbial biomass is dried using spray drying or paddle drying. The method may also comprise reducing the nucleic acid content of the microbial biomass using any method known in the art, since intake of a diet high in nucleic acid content may result in the accumulation of nucleic acid degradation products and/or gastrointestinal distress. The single cell protein may be suitable for feeding to animals, such as livestock or pets. In particular, the animal feed may be suitable for feeding to one or more beef cattle, dairy cattle, pigs, sheep, goats, horses, mules, donkeys, deer, buffalo/bison, llamas, alpacas, reindeer, camels, bantengs, gayals, yaks, chickens, turkeys, ducks, geese, quail, guinea fowl, squabs/pigeons, fish, shrimp, crustaceans, cats, dogs, and rodents. The composition of the animal feed may be tailored to the nutritional requirements of different animals. Furthermore, the process may comprise blending or combining the microbial biomass with one or more excipients.

**[0151]** An “excipient” may refer to any substance that may be added to the microbial biomass to enhance or alter the form, properties, or nutritional content of the animal feed. For example, the excipient may comprise one or more of a carbohydrate, fiber, fat, protein, vitamin, mineral, water, flavor, sweetener, antioxidant, enzyme, preservative, probiotic, or antibiotic. In some embodiments, the excipient may be hay, straw, silage, grains, oils or fats, or other plant material. The excipient may be any feed ingredient identified in Chiba, Section 18: Diet Formulation and Common Feed Ingredients, Animal Nutrition Handbook, 3rd revision, pages 575-633, 2014.

**[0152]** A “native product” is a product produced by a genetically unmodified microorganism. For example, ethanol, acetate, and 2,3-butanediol are native products of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, and *Clostridium ragsdalei*. A “non-native product” is a product that is produced by a genetically modified microorganism



but is not produced by a genetically unmodified microorganism from which the genetically modified microorganism is derived.

**[0153]** “Selectivity” refers to the ratio of the production of a target product to the production of all fermentation products produced by a microorganism. The microorganism of the disclosure may be engineered to produce products at a certain selectivity or at a minimum selectivity. In one embodiment, a target product account for at least about 5%, 10%, 15%, 20%, 30%, 50%, or 75% of all fermentation products produced by the microorganism of the disclosure. In one embodiment, the target product accounts for at least 10% of all fermentation products produced by the microorganism of the disclosure, such that the microorganism of the disclosure has a selectivity for the target product of at least 10%. In another embodiment, the target product accounts for at least 30% of all fermentation products produced by the microorganism of the disclosure, such that the microorganism of the disclosure has a selectivity for the target product of at least 30%.

**[0154]** “Increasing the efficiency,” “increased efficiency,” and the like include, but are not limited to, increasing growth rate, product production rate or volume, product volume per volume of substrate consumed, or product selectivity. Efficiency may be measured relative to the performance of parental microorganism from which the microorganism of the disclosure is derived.

**[0155]** Typically, the culture is performed in a bioreactor. The term “bioreactor” includes a culture/fermentation device consisting of one or more vessels, towers, or piping arrangements, such as a continuous stirred tank reactor (CSTR), immobilized cell reactor (ICR), trickle bed reactor (TBR), bubble column, gas lift fermenter, static mixer, or other vessel or other device suitable for gas-liquid contact. In some embodiments, the bioreactor may comprise a first growth reactor and a second culture/fermentation reactor. The substrate may be provided to one or both of these reactors. As used herein, the terms “culture” and “fermentation” are used interchangeably. These terms encompass both the growth phase and product biosynthesis phase of the culture/fermentation process.

**[0156]** The culture is generally maintained in an aqueous culture medium that contains nutrients, vitamins, and/or minerals sufficient to permit growth of the microorganism. Preferably the aqueous culture medium is an anaerobic microbial growth medium, such as a minimal anaerobic microbial growth medium. Suitable media are well known in the art.

**[0157]** The culture/fermentation should desirably be carried out under appropriate conditions for production of the target product. Typically, the culture/fermentation is performed under anaerobic conditions. Reaction conditions to consider include pressure (or partial pressure), temperature, gas flow rate, liquid flow rate, media pH, media redox potential, agitation rate (if using a continuous stirred tank reactor), inoculum level, maximum gas substrate concentrations to ensure that gas in the liquid phase does not become limiting, and maximum product concentrations to avoid product inhibition. In particular, the rate of introduction of the substrate may be controlled to ensure that the concentration of gas in the liquid phase does not become limiting, since products may be consumed by the culture under gas-limited conditions.

**[0158]** Operating a bioreactor at elevated pressures allows for an increased rate of gas mass transfer from the gas phase to the liquid phase. Accordingly, it is generally preferable to perform the culture/fermentation at pressures higher than atmospheric pressure. Also, since a given gas conversion rate is, in part, a function of the substrate retention time and retention time dictates the required volume of a bioreactor, the use of pressurized systems can greatly reduce the volume of the bioreactor required and, consequently, the capital cost of the culture/fermentation equipment. This, in turn, means that the retention time, defined as the liquid volume in the bioreactor divided by the input gas flow rate, can be reduced when bioreactors are maintained at elevated pressure rather than atmospheric pressure. The optimum reaction conditions will depend partly on the particular microorganism used. However, in general, it is preferable to operate the fermentation at a pressure higher than atmospheric pressure. Also, since a given gas conversion rate is in part a function of substrate retention time and achieving a desired retention time in turn dictates the required volume of a bioreactor, the use of pressurized systems can greatly reduce the volume of the bioreactor required, and consequently the capital cost of the fermentation equipment.

**[0159]** In certain embodiments, the fermentation is performed in the absence of light or in the presence of an amount of light insufficient to meet the energetic requirements of photosynthetic microorganisms. In certain embodiments, the microorganism of the disclosure is a non-photosynthetic microorganism.

**[0160]** As used herein, the terms “fermentation broth” or “broth” refer to the mixture of components in a bioreactor, which includes cells and nutrient media. As used herein, a “separator” is a module that is adapted to receive fermentation broth from a bioreactor and pass the broth through a filter to yield a “retentate” and a “permeate.” The filter may be a membrane, e.g. a cross-flow membrane or a hollow fibre membrane. The term “permeate” is used to refer to substantially soluble components of the broth that pass through the separator. The permeate will typically contain soluble fermentation products, byproducts, and nutrients. The retentate will typically contain cells. As used herein, the term “broth bleed” is used to refer to a portion of the fermentation broth that is removed from a bioreactor and not passed to a separator.

**[0161]** Target products may be separated or purified from a fermentation broth using any method or combination of methods known in the art, including, for example, fractional distillation, evaporation, pervaporation, gas stripping, phase separation, and extractive fermentation, including for example, liquid-liquid extraction. In certain embodiments, target products are recovered from the fermentation broth by continuously removing a portion of the broth from the bioreactor, separating microbial cells from the broth (conveniently by filtration), and recovering one or more target products from the broth. Alcohols and/or acetone may be recovered, for example, by distillation. Acids may be recovered, for example, by adsorption on activated charcoal. Separated microbial cells are preferably recycled back to the bioreactor. The cell-free permeate remaining after target products have been removed is also preferably returned to the bioreactor. Additional nutrients may be added to the cell-free permeate to replenish the medium before it is returned to the bioreactor.



**[0162]** As referred to herein, a “shuttle microorganism” is a microorganism in which a methyltransferase enzyme is expressed and is distinct from the destination microorganism.

**[0163]** As referred to herein, a “destination microorganism” is a microorganism in which the genes included on an expression construct/vector are expressed and is distinct from the shuttle microorganism. This is also called a host microorganism.

**[0164]** The term “main fermentation product” is intended to mean the one fermentation product which is produced in the highest concentration and/or yield. There may be one or more fermentation products. The most prevalent may or may not be the most commercially valuable.

**[0165]** The phrase “substrate comprising carbon monoxide” and like terms should be understood to include any substrate in which carbon monoxide is available to one or more strains of bacteria for growth and/or fermentation, for example.

**[0166]** The phrase “gaseous substrate comprising carbon monoxide” and like phrases and terms includes any gas which contains a level of carbon monoxide. In certain embodiments the substrate contains at least about 20% to about 100% CO by volume, from 20% to 70% CO by volume, from 30% to 60% CO by volume, and from 40% to 55% CO by volume. In particular embodiments, the substrate comprises about 25%, or about 30%, or about 35%, or about 40%, or about 45%, or about 50% CO, or about 55% CO, or about 60% CO by volume.

**[0167]** While it is not necessary for a substrate comprising CO to contain any hydrogen, the presence of H<sub>2</sub> should not be detrimental to product formation in accordance with methods of the disclosure. In particular embodiments, the presence of hydrogen results in an improved overall efficiency of alcohol production. For example, in particular embodiments, the substrate may comprise an approx. 2:1, or 1:1, or 1:2 ratio of H<sub>2</sub>:CO. In one embodiment the substrate comprises about 30% or less H<sub>2</sub> by volume, 20% or less H<sub>2</sub> by volume, about 15% or less H<sub>2</sub> by volume or about 10% or less H<sub>2</sub> by volume. In other embodiments, the substrate stream comprises low concentrations of H<sub>2</sub>, for example, less than 5%, or less than 4%, or less than 3%, or less than 2%, or less than 1%, or is substantially hydrogen free. The substrate may also contain some CO<sub>2</sub> for example, such as about 1% to about 80% CO<sub>2</sub> by volume, or 1% to about 30% CO<sub>2</sub> by volume. In one embodiment the substrate comprises less than or equal to about 20% CO<sub>2</sub> by volume. In particular embodiments the substrate comprises less than or equal to about 15% CO<sub>2</sub> by volume, less than or equal to about 10% CO<sub>2</sub> by volume, less than or equal to about 5% CO<sub>2</sub> by volume or substantially no CO<sub>2</sub>.

**[0168]** The phrase “substrate comprising carbon dioxide” and like terms should be understood to include any substrate in which carbon dioxide is available to one or more strains of bacteria for growth and/or fermentation, for example. Substrates comprising carbon dioxide may further comprise hydrogen and/or carbon monoxide.

**[0169]** The phrase “gaseous substrate comprising carbon dioxide” and like phrases and terms includes any gas which contains a level of carbon dioxide. In certain embodiments the substrate contains at least about 10% to about 60% CO<sub>2</sub> by volume, from 20% to 50% CO<sub>2</sub> by volume, from 30% to 60% CO<sub>2</sub> by volume, and from 40% to 55% CO<sub>2</sub> by volume. In particular embodiments, the substrate comprises about

20%, or about 25%, or about 30%, or about 35%, or about 40%, or about 45%, or about 50% CO, or about 55% CO, or about 60% CO<sub>2</sub> by volume.

**[0170]** Preferably, a substrate comprising CO<sub>2</sub> will also contain a level of CO or H<sub>2</sub>. In particular embodiments, the substrate comprises a CO<sub>2</sub>:H<sub>2</sub> ratio of at least about 1:1, or at least about 1:2, or at least about 1:3, or at least about 1:4, or at least about 1:5.

**[0171]** In the description which follows, embodiments of the disclosure are described in terms of delivering and fermenting a “gaseous substrate containing CO and/or CO<sub>2</sub>.” However, it should be appreciated that the gaseous substrate may be provided in alternative forms. For example, the gaseous substrate containing CO and/or CO<sub>2</sub> may be provided dissolved in a liquid. Essentially, a liquid is saturated with a carbon monoxide containing gas and then that liquid is added to the bioreactor. This may be achieved using standard methodology. By way of example, a microbubble dispersion generator (Hensirisak et. al. Scale-up of microbubble dispersion generator for aerobic fermentation; Applied Biochemistry and Biotechnology Volume 101, Number 3/October 2002) could be used. By way of further example, the gaseous substrate containing CO may be adsorbed onto a solid support. Such alternative methods are encompassed by use of the term “substrate containing CO and/or CO<sub>2</sub>” and the like.

**[0172]** In particular embodiments of the disclosure, the CO-containing gaseous substrate (or a gaseous substrate comprising CO<sub>2</sub>, or CO and CO<sub>2</sub>, or CO<sub>2</sub> and H<sub>2</sub> and CO) is an industrial off or waste gas. “Industrial waste or off gases” should be taken broadly to include any gases comprising CO and/or CO<sub>2</sub> produced by an industrial process and include gases produced as a result of ferrous metal products manufacturing, non-ferrous products manufacturing, petroleum refining processes, gasification of coal, gasification of biomass, electric power production, carbon black production, and coke manufacturing. Further examples may be provided elsewhere herein.

**[0173]** Unless the context requires otherwise, the phrases “fermenting”, “fermentation process” or “fermentation reaction” and the like, as used herein, are intended to encompass both the growth phase and product biosynthesis phase of the process. As will be described further herein, in some embodiments the bioreactor may comprise a first growth reactor and a second fermentation reactor. As such, the addition of metals or compositions to a fermentation reaction should be understood to include addition to either or both of these reactors.

**[0174]** The term “bioreactor” includes a fermentation device consisting of one or more vessels and/or towers or piping arrangement, which includes the Continuous Stirred Tank Reactor (CSTR), Immobilized Cell Reactor (ICR), Trickle Bed Reactor (TBR), Bubble Column, Gas Lift Fermenter, Static Mixer, or other vessel or other device suitable for gas-liquid contact. In some embodiments the bioreactor may comprise a first growth reactor and a second fermentation reactor. As such, when referring to the addition of substrate to the bioreactor or fermentation reaction it should be understood to include addition to either or both of these reactors where appropriate.

**[0175]** It should be appreciated that the disclosure may be practiced using nucleic acids whose sequence varies from the sequences specifically exemplified herein provided they perform substantially the same function. For nucleic acid



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

[0176] These include homologous genes in species such as *Clostridium ljungdahlii*, *Chloroflexus aurantiacus*, *Metallosphaera* or *Sulfolobus* spp, details of which are publicly available on websites such as Genbank or NCBI. The phrase “functionally equivalent variants” should also be taken to include nucleic acids whose sequence varies as a result of codon optimisation for a particular organism. “Functionally equivalent variants” of a nucleic acid herein will preferably have at least approximately 70%, preferably approximately 80%, more preferably approximately 85%, preferably approximately 90%, preferably approximately 95% or greater nucleic acid sequence identity with the nucleic acid identified.

[0177] It should also be appreciated that the disclosure may be practiced using polypeptides whose sequence varies from the amino acid sequences specifically exemplified herein. These variants may be referred to herein as “functionally equivalent variants.” A functionally equivalent variant of a protein or a peptide includes those proteins or peptides that share at least 40%, preferably 50%, preferably 60%, preferably 70%, preferably 75%, preferably 80%, preferably 85%, preferably 90%, preferably 95% or greater amino acid identity with the protein or peptide identified and has substantially the same function as the peptide or protein of interest. Such variants include within their scope fragments of a protein or peptide wherein the fragment comprises a truncated form of the polypeptide wherein deletions may be from 1 to 5, to 10, to 15, to 20, to 25 amino acids, and may extend from residue 1 through 25 at either terminus of the polypeptide, and wherein deletions may be of any length within the region; or may be at an internal location. Functionally equivalent variants of the specific polypeptides herein should also be taken to include polypeptides expressed by homologous genes in other species of bacteria, for example as exemplified in the previous paragraph.

[0178] The microorganisms of the disclosure may be prepared from a parental microorganism and one or more exogenous nucleic acids using any number of techniques known in the art for producing recombinant microorganisms. By way of example only, transformation (including transduction or transfection) may be achieved by electroporation, ultrasonication, polyethylene glycol-mediated transformation, chemical or natural competence, or conjugation. Suitable transformation techniques are described for example in, Sambrook J, Fritsch E F, Maniatis T: Molecular Cloning: A laboratory Manual, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, 1989.

[0179] In certain embodiments, due to the restriction systems which are active in the microorganism to be transformed, it is necessary to methylate the nucleic acid to be

introduced into the microorganism. This can be done using a variety of techniques, including those described below, and further exemplified in the Examples section herein after.

[0180] By way of example, in one embodiment, a recombinant microorganism of the disclosure is produced by a method comprises the following steps: introduction into a shuttle microorganism of (i) of an expression construct/vector as described herein and (ii) a methylation construct/vector comprising a methyltransferase gene; expression of the methyltransferase gene; isolation of one or more constructs/vectors from the shuttle microorganism; and, introduction of the one or more construct/vector into a destination microorganism.

[0181] In one embodiment, the methyltransferase gene of step B is expressed constitutively. In another embodiment, expression of the methyltransferase gene of step B is induced.

[0182] The shuttle microorganism is a microorganism, preferably a restriction negative microorganism that facilitates the methylation of the nucleic acid sequences that make up the expression construct/vector. In a particular embodiment, the shuttle microorganism is a restriction negative *E. coli*, *Bacillus subtilis*, or *Lactococcus lactis*.

[0183] The methylation construct/vector comprises a nucleic acid sequence encoding a methyltransferase.

[0184] Once the expression construct/vector and the methylation construct/vector are introduced into the shuttle microorganism, the methyltransferase gene present on the methylation construct/vector is induced. Induction may be by any suitable promoter system although in one particular embodiment of the disclosure, the methylation construct/vector comprises an inducible lac promoter and is induced by addition of lactose or an analogue thereof, more preferably isopropyl- $\beta$ -D-thio-galactoside (IPTG). Other suitable promoters include the ara, tet, or T7 system. In a further embodiment of the disclosure, the methylation construct/vector promoter is a constitutive promoter.

[0185] In a particular embodiment, the methylation construct/vector has an origin of replication specific to the identity of the shuttle microorganism so that any genes present on the methylation construct/vector are expressed in the shuttle microorganism. Preferably, the expression construct/vector has an origin of replication specific to the identity of the destination microorganism so that any genes present on the expression construct/vector are expressed in the destination microorganism.

[0186] Expression of the methyltransferase enzyme results in methylation of the genes present on the expression construct/vector. The expression construct/vector may then be isolated from the shuttle microorganism according to any one of a number of known methods. By way of example only, the methodology described in the Examples section described hereinafter may be used to isolate the expression construct/vector.

[0187] In one particular embodiment, both construct/vector are concurrently isolated.

[0188] The expression construct/vector may be introduced into the destination microorganism using any number of known methods. However, by way of example, the methodology described in the Examples section hereinafter may be used. Since the expression construct/vector is methylated, the nucleic acid sequences present on the expression construct/vector are able to be incorporated into the destination microorganism and successfully expressed.



[0189] It is envisaged that a methyltransferase gene may be introduced into a shuttle microorganism and over-expressed. Thus, in one embodiment, the resulting methyltransferase enzyme may be collected using known methods and used in vitro to methylate an expression plasmid. The expression construct/vector may then be introduced into the destination microorganism for expression. In another embodiment, the methyltransferase gene is introduced into the genome of the shuttle microorganism followed by introduction of the expression construct/vector into the shuttle microorganism, isolation of one or more constructs/vectors from the shuttle microorganism and then introduction of the expression construct/vector into the destination microorganism.

[0190] It is envisaged that the expression construct/vector and the methylation construct/vector as defined above may be combined to provide a composition of matter. Such a composition has particular utility in circumventing restriction barrier mechanisms to produce the recombinant microorganisms of the disclosure.

[0191] In one particular embodiment, the expression construct/vector and/or the methylation construct/vector are plasmids.

[0192] Persons of ordinary skill in the art will appreciate a number of suitable methyltransferases of use in producing the microorganisms of the disclosure. However, by way of example the *Bacillus subtilis* phage  $\Phi$ T1 methyltransferase and the methyltransferase described in the Examples herein after may be used. Nucleic acids encoding suitable methyltransferases will be readily appreciated having regard to the sequence of the desired methyltransferase and the genetic code.

[0193] Any number of constructs/vectors adapted to allow expression of a methyltransferase gene may be used to generate the methylation construct/vector.

[0194] The disclosure provides a method for the production of 3-HP and optionally one or more other products by microbial fermentation comprising fermenting a substrate comprising CO and/or CO<sub>2</sub> using a recombinant microorganism of the disclosure. Preferably, 3-HP is the main fermentation product. The methods of the disclosure may be used to reduce the total atmospheric carbon emissions from an industrial process.

[0195] Preferably, the fermentation comprises the steps of anaerobically fermenting a substrate in a bioreactor to produce at least 3-HP using a recombinant microorganism of the disclosure.

[0196] In one embodiment the method comprises the steps of:

[0197] (a) providing a substrate comprising CO and/or CO<sub>2</sub> to a bioreactor containing a culture of one or more microorganism of the disclosure; and

[0198] (b) anaerobically fermenting the culture in the bioreactor to produce at least 3-HP.

[0199] In one embodiment the method comprises the steps of:

[0200] (a) capturing CO— and/or CO<sub>2</sub>-containing gas produced as a result of the industrial process, before the gas is released into the atmosphere;

[0201] (b) the anaerobic fermentation of the CO— and/or CO<sub>2</sub>-containing gas to produce the at least 3-HP by a culture containing one or more microorganism of the disclosure.

[0202] In one embodiment, the substrate comprises CO. In one embodiment, the substrate comprises CO<sub>2</sub> and CO. In another embodiment, the substrate comprises CO<sub>2</sub> and H<sub>2</sub>. In another embodiment, the substrate comprises CO<sub>2</sub> and CO and H<sub>2</sub>.

[0203] In one particular embodiment of the disclosure, the gaseous substrate fermented by the microorganism is a gaseous substrate containing CO. The gaseous substrate may be a CO-containing waste gas obtained as a by-product of an industrial process, or from some other source such as from automobile exhaust fumes. In certain embodiments, the industrial process is selected from the group consisting of ferrous metal products manufacturing, such as a steel mill, non-ferrous products manufacturing, petroleum refining processes, gasification of coal, electric power production, carbon black production, ammonia production, methanol production and coke manufacturing. In these embodiments, the CO-containing gas may be captured from the industrial process before it is emitted into the atmosphere, using any convenient method. The CO may be a component of syngas (gas comprising carbon monoxide and hydrogen). The CO produced from industrial processes is normally flared off to produce CO<sub>2</sub> and therefore the disclosure has particular utility in reducing CO<sub>2</sub> greenhouse gas emissions and producing butanol for use as a biofuel. Depending on the composition of the gaseous CO-containing substrate, it may also be desirable to treat it to remove any undesired impurities, such as dust particles before introducing it to the fermentation. For example, the gaseous substrate may be filtered or scrubbed using known methods.

[0204] In particular embodiments of the disclosure, the gaseous substrate fermented by the microorganisms a gaseous substrate comprising CO<sub>2</sub> and H<sub>2</sub>. The CO<sub>2</sub>/H<sub>2</sub> containing substrate may be a waste gas obtained as a by-product of an industrial process. In certain embodiments the industrial process is selected from the group consisting of hydrogen production. In certain embodiments the gaseous substrate comprising CO<sub>2</sub> and H<sub>2</sub> may be a blended gas stream, wherein at least a portion of the gas stream is derived from one or more industrial process is blended with at least a portion of CO<sub>2</sub> or H<sub>2</sub> to optimise the CO<sub>2</sub>:H<sub>2</sub> ratio of the gaseous substrate. This may be particularly beneficial for industrial gas streams rich in either CO<sub>2</sub> or H<sub>2</sub>. Examples of industrial process which produce by-product gas streams which can be used as a source for a CO<sub>2</sub> and H<sub>2</sub> substrate, or a CO<sub>2</sub> and H<sub>2</sub> blended substrate include coke manufacturing, refinery processes, ammonia production processes, methanol production processes, acetic acid production, natural gas refineries and power plants.

[0205] It will be appreciated that for growth of the bacteria and conversion of gas to products comprising 3-HP to occur, a suitable liquid nutrient medium in addition to the CO— and/or CO<sub>2</sub>-containing substrate gas will need to be fed to the bioreactor. The substrate and media may be fed to the bioreactor in a continuous, batch or batch fed fashion. A nutrient medium will contain vitamins and minerals sufficient to permit growth of the micro-organism used. Anaerobic media suitable for fermentation to produce one or more products using CO and/or CO<sub>2</sub> are known in the art. For example, suitable media are described Biebel (2001). In one embodiment of the disclosure the media is as described in the Examples section herein after.

[0206] The fermentation should desirably be carried out under appropriate conditions for the fermentation supporting



the conversion of the gas to products comprising 3-HP to occur. Reaction conditions that should be considered include pressure, temperature, gas flow rate, liquid flow rate, media pH, media redox potential, agitation rate (if using a continuous stirred tank reactor), inoculum level, maximum gas substrate concentrations to ensure that CO and/or CO<sub>2</sub> in the liquid phase does not become limiting, and maximum product concentrations to avoid product inhibition.

[0207] In addition, it is often desirable to increase the CO and/or CO<sub>2</sub> concentration of a substrate stream (or CO and/or CO<sub>2</sub> partial pressure in a gaseous substrate) and thus increase the efficiency of fermentation reactions where CO and/or CO<sub>2</sub> is a substrate. Operating at increased pressures allows a significant increase in the rate of CO and/or CO<sub>2</sub> transfer from the gas phase to the liquid phase where it can be taken up by the micro-organism as a carbon source to make products comprising 3-HP. This in turn means that the retention time (defined as the liquid volume in the bioreactor divided by the input gas flow rate) can be reduced when bioreactors are maintained at elevated pressure rather than atmospheric pressure. The optimum reaction conditions will depend partly on the particular micro-organism of the disclosure used. However, in general, it is preferred that the fermentation be performed at pressure higher than ambient pressure. Also, since a given CO— and/or CO<sub>2</sub>-to-at least 3-HP conversion rate is in part a function of the substrate retention time, and achieving a desired retention time in turn dictates the required volume of a bioreactor, the use of pressurized systems can greatly reduce the volume of the bioreactor required, and consequently the capital cost of the fermentation equipment. According to examples given in U.S. Pat. No. 5,593,886, reactor volume can be reduced in linear proportion to increases in reactor operating pressure, i.e. bioreactors operated at 10 atmospheres of pressure need only be one tenth the volume of those operated at 1 atmosphere of pressure.

[0208] By way of example, the benefits of conducting a gas-to-ethanol fermentation at elevated pressures has been described. For example, WO 02/08438 describes gas-to-ethanol fermentations performed under pressures of 30 psig and 75 psig, giving ethanol productivities of 150 g/l/day and 369 g/l/day respectively. However, example fermentations performed using similar media and input gas compositions at atmospheric pressure were found to produce between 10 and 20 times less ethanol per litre per day.

[0209] It is also desirable that the rate of introduction of the CO and/or CO<sub>2</sub>-containing gaseous substrate is such as to ensure that the concentration of CO and/or CO<sub>2</sub> in the liquid phase does not become limiting. This is because a consequence of CO— and/or CO<sub>2</sub>-limited conditions may be that one or more product is consumed by the culture.

[0210] The composition of gas streams used to feed a fermentation reaction can have a significant impact on the efficiency and/or costs of that reaction. For example, O<sub>2</sub> may reduce the efficiency of an anaerobic fermentation process. Processing of unwanted or unnecessary gases in stages of a fermentation process before or after fermentation can increase the burden on such stages (to products comprising where the gas stream is compressed before entering a bioreactor, unnecessary energy may be used to compress gases that are not needed in the fermentation). Accordingly, it may be desirable to treat substrate streams, particularly

substrate streams derived from industrial sources, to remove unwanted components and increase the concentration of desirable components.

[0211] In certain embodiments a culture of a bacterium of the disclosure is maintained in an aqueous culture medium. Preferably the aqueous culture medium is a minimal anaerobic microbial growth medium. Suitable media are known in the art and described for example in U.S. Pat. Nos. 5,173,429 and 5,593,886 and WO 02/08438, and as described in the Examples section herein after.

[0212] 3-HP, or a mixed stream containing 3-HP and/or one or more other products, may be recovered from the fermentation broth by methods known in the art, such as fractional distillation or evaporation, pervaporation, gas stripping and extractive fermentation, including for example, liquid-liquid extraction.

[0213] In certain embodiments of the disclosure, 3-HP and one or more products are recovered from the fermentation broth by continuously removing a portion of the broth from the bioreactor, separating microbial cells from the broth (conveniently by filtration), and recovering one or more products from the broth. Alcohols may conveniently be recovered for example by distillation. Acetone may be recovered for example by distillation. Any acids produced may be recovered for example by adsorption on activated charcoal. The separated microbial cells are preferably returned to the fermentation bioreactor. The cell free permeate remaining after any alcohol(s) and acid(s) have been removed is also preferably returned to the fermentation bioreactor. Additional nutrients (such as B vitamins) may be added to the cell free permeate to replenish the nutrient medium before it is returned to the bioreactor.

[0214] Also, if the pH of the broth was adjusted as described above to enhance adsorption of acetic acid to the activated charcoal, the pH should be re-adjusted to a similar pH to that of the broth in the fermentation bioreactor, before being returned to the bioreactor.

[0215] 3-HP may be recovered following fermentation using any appropriate methodology including but not limited to pervaporation, reverse osmosis, and liquid extraction techniques.

[0216] One embodiment is directed to a recombinant C1-fixing bacteria capable of producing 3-hydroxypropionate (3-HP) from a carbon source comprising a nucleic acid encoding a group of exogenous enzymes comprising  $\beta$ -alanine pyruvate aminotransferase and malonic semialdehyde reductase.

[0217] The bacteria according to an embodiment, further comprising a nucleic acid encoding a group of exogenous enzymes comprising pyruvate carboxylase, aspartate aminotransferase, and aspartate decarboxylase, wherein the nucleic acid is operably linked to a promoter.

[0218] The bacteria according to an embodiment, further comprising a nucleic acid encoding a group of exogenous enzymes comprising alanine dehydrogenase and 2,3-alanine aminomutase, wherein the nucleic acid is operably linked to a promoter.

[0219] The bacteria according to an embodiment, which are selected from the group consisting of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, *Clostridium ragsdalei*, *Clostridium carboxidivorans*, *Clostridium drakei*, *Clostridium scatologenes*, *Clostridium aceticum*, *Clostridium formicoaceticum*, *Clostridium magnum*, *Butyrivibrio methylotrophicum*, *Acetobacterium woodii*, *Alka-*



*libaculum bacchii*, *Blautia producta*, *Eubacterium limosum*, *Moorella thermoacetica*, *Moorella thermautotrophica*, *Sporomusa ovata*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, *Oxobacter pfennigii*, and *Thermoanaerobacter kivui*.

[0220] The bacteria according to an embodiment, which are a *Clostridium* species selected from the group consisting of *C. ljungdahlii*, and *C. autoethanogenum*.

[0221] The bacteria according to an embodiment, further comprising a nucleic acid encoding acetyl-Coenzyme A carboxylase, wherein the nucleic acid is codon optimized for expression in the bacteria.

[0222] The bacteria according to an embodiment, wherein the acetyl-Coenzyme A carboxylase is derived from a member of the genus *Clostridium*, *Metallosphaera*, *Sulfolobus*, or *Chloroflexus*.

[0223] The bacteria according to an embodiment, wherein the nucleic acid encoding a group of exogenous enzymes comprising  $\beta$ -alanine pyruvate aminotransferase and malonic semialdehyde reductase is codon optimized for expression in the bacteria.

[0224] The bacteria according to an embodiment, further comprising a nucleic acid encoding a group of exogenous enzymes comprising aldehyde:ferredoxin oxidoreductase and alcohol dehydrogenase, wherein the nucleic acid is operably linked to a promoter.

[0225] The bacteria according to an embodiment, wherein the nucleic acid encoding a group of exogenous enzymes enable production of 1,3-propanediol.

[0226] The bacteria according to an embodiment, further comprises at least one disruptive mutation in a gene encoding an enzyme selected from aldehyde:ferredoxin oxidoreductase, alcohol dehydrogenase, or any combination thereof.

[0227] The bacteria according to an embodiment, wherein 3-HP is converted into a material selected from the group consisting of acrylic acid, acrylonitrile, paint, paper, adhesives, textiles, specialty coatings, ink, superabsorbent polymer polyacrylates, 1,3-propanediol, solvents, adhesives, cosmetics, polytrimethylene terephthalate, 3-hydroxypropionaldehyde, foods, feed additives, and any combination thereof.

[0228] A method of converting CO and/or CO<sub>2</sub> into 3-hydroxypropionate (3-HP), the process comprising: passing a gaseous CO-containing and/or CO<sub>2</sub>-containing substrate to a bioreactor containing a culture of a recombinant C1-fixing bacteria according to claim 1, in a culture medium such that the bacteria convert the CO and/or CO<sub>2</sub> to 3-HP; and recovering the 3-HP from the bioreactor.

[0229] A method of culturing the bacteria according to an embodiment, comprising growing the bacteria in a medium comprising a gaseous carbon source, wherein the carbon source comprises CO and/or CO<sub>2</sub>.

[0230] A method of culturing the bacteria according to an embodiment, comprising growing the bacteria in a medium comprising an energy source, wherein the energy source comprises CO and/or CO<sub>2</sub>.

[0231] The method according to an embodiment, wherein the culturing is strictly anaerobic.

[0232] The method according to an embodiment, wherein the carbon source comprises an industrial waste product or off-gas.

[0233] The method according to an embodiment, wherein the energy source comprises an industrial waste product or off-gas.

[0234] The method according to an embodiment, wherein 3-HP is converted into a material selected from the group consisting of acrylic acid, acrylonitrile, paint, paper, adhesives, textiles, specialty coatings, ink, superabsorbent polymer polyacrylates, 1,3-propanediol, solvents, adhesives, cosmetics, polytrimethylene terephthalate, 3-hydroxypropionaldehyde, foods, feed additives, and any combination thereof.

[0235] The method according to an embodiment, wherein 3-HP is converted into a material selected from the group consisting of acrylic acid, acrylonitrile, paint, paper, adhesives, textiles, specialty coatings, ink, superabsorbent polymer polyacrylates, 1,3-propanediol, solvents, adhesives, cosmetics, polytrimethylene terephthalate, 3-hydroxypropionaldehyde, foods, feed additives, and any combination thereof.

## EXAMPLES

[0236] The following examples further illustrate the methods and compositions of the disclosure but should not be construed to limit its scope in any way.

[0237] In this work, combinatorial analysis of the aspartate-1-decarboxylase (PAND),  $\beta$ -alanine pyruvate aminotransferase (BAPAT),  $\gamma$ -aminobutyrate transaminase (GABT), malonic semialdehyde reductase (MSR), 2,3-alanine aminomutase (AAM), alanine transaminase (AT), and alanine dehydrogenase (ALADH) was carried out in C1-fixing bacteria to improve 3-HP selectivity and production. The 3-HP biosynthesis pathway and the genes that were heterologously expressed in the following Examples are highlighted in FIG. 1.

### Example 1. 3-HP Tolerance of *C. autoethanogenum* in Schott Bottles

[0238] 3-HP has been shown to be toxic to microorganisms such as *Synechococcus elongatus* with a minimal inhibitory concentration of 180 mg/L (Begemann et al., PLoS One. 2013; 8:e76594). To study the toxicity of 3-HP on autotrophic growth of *C. autoethanogenum*, a 3-HP challenge experiment (at 0, 0.2, 0.5, 1, 2, 5 and 10 g/L) was conducted in Schott bottles with synthetic gas blend (50% CO, 10% H<sub>2</sub>, 30% CO<sub>2</sub> and 10% N<sub>2</sub>) using a strain with aldehyde:ferredoxin oxidoreductase (AOR) enzymes deleted (FIG. 2). In comparison to the unchallenged culture, 5 g/L of 3-HP reduced the growth rate and reduced peak OD<sub>600 nm</sub> by 22%. At 10 g/L 3-HP, a decrease in peak OD<sub>600 nm</sub> of 40% was observed and accompanied by a more pronounced decrease in growth rate. FIG. 2A shows growth profile; FIG. 2B shows peak biomass. Unc=Unchallenged; N=2; Error bar=S.D.

### Example 2. Conversion of Exogenously Added 3-HP to 1,3-Propanediol (1,3-PDO) in *C. autoethanogenum*

[0239] The genome of *C. autoethanogenum* encodes two AOR enzyme variants and they were shown to be reduce a range of carboxylic acids (including propanoic acid and butyric acid) to the corresponding aldehydes, followed by native alcohol dehydrogenase activities that result in alcohols (Liew et al., Metabolic Engineering. 2017: 104-114.). To test if *C. autoethanogenum* is able to convert 3-HP to 1,3-propanediol (1,3-PDO) via 3-hydroxypropionaldehyde, exogenous 3-HP was added to growth medium of *C. auto-*



*ethanogenum* at 25 mM (2.25 g/L) concentration and subjected to autotrophic growth in Schott bottles. As shown in FIG. 3, the *C. autoethanogenum* wild-type (WT) strain is able to convert some of the added 3-HP to 1,3-PDO at similar molar concentrations. In contrast, the *aor* deletion strain was unable to generate any 1,3-PDO under the same growth conditions.

### Example 3. Combinatorial Analysis of 3-HP Pathway

**[0240]** In order to determine the optimum flux and gene variants for the biosynthesis of 3-HP, a combinatorial assembly of the 3-HP pathway genes and promoters using the Golden Gate (GG) method was first performed in *Escherichia coli* (FIG. 4) before transformation into C1-utilizing microorganisms. To facilitate the screening of assembly, a *ccdB* toxin-antitoxin counter-selectable marker flanked by Golden Gate site GG1 and GG6 was first cloned into *Clostridium-E. coli* shuttle vector pMTL8225 (Heap, J Microbiol Methods, 78: 79-85, 2009). These shuttle vectors

have a pre-cloned clostridial promoter (shown as P<sub>1</sub> in FIG. 4) and terminator (shown as T<sub>3</sub> in FIG. 4). The genes that encode aspartate-1-decarboxylase (PAND),  $\beta$ -alanine pyruvate aminotransferase (BAPAT),  $\gamma$ -aminobutyrate transaminase (GABT), malonic semialdehyde reductase (MSR), and 2,3-alanine aminomutase (AAM), together with two donor vectors (pDonor 2 and pDonor 4, which provide the terminators and promoters), were added to the shuttle vectors for GG assembly. The promoter sequences, Golden Gate sites, and assembly workflow are described in Karim et al. Synthetic Biology. 2020; 5(1): ysaa019. The resulting combinatorial plasmids with *ermB* antibiotic selectable marker have a promoter and terminator to express each of MSR, PAND/AAM and BAPAT/GABT in an insulated manner. The gene variants (7 $\times$  MSR (SEQ ID NOs: 1-7), 5 $\times$  PAND (SEQ ID NOs: 8-12), 4 $\times$  BAPAT/GABT (SEQ ID NOs: 13-16), and 2 $\times$  AAM (SEQ ID NOs: 17-18) that were included in the combinatorial analysis are shown in Table 2. When combined with a choice of three clostridial promoters per gene, the total permutation of this combinatorial library is 5292 (from 3 $\times$ 7 $\times$ 3 $\times$ 7 $\times$ 3 $\times$ 4).

TABLE 2

3-HP pathway gene variants employed for combinatorial analysis.			
Enzyme	Gene [SEQ ID NO]	Description	Reference
Malonic Semialdehyde Reductase (MSR) (E.C. 1.1.1.59 or 1.1.1.295)	bcMSR [1]	<i>Bacillus cereus</i>	Yao et al. Applied Biochemistry and Biotechnology. 2010; 160: 694-703
	pdMSR [2]	<i>Pseudomonas denitrificans</i>	Zhou et al. PLOS ONE. 2013; 8(5): e62666
	nmMSR [3]	<i>Nitrosopumilus maritimus</i>	Otte et al. Applied and Environmental Microbiology. 2015; 81(5): 1700-1707
	ecMSR [4]	<i>Escherichia coli</i>	Fujisawa et al. Biochimica et Biophysica Acta - Proteins and Proteomics. 2003; 1645(1): 89-94
	scMSR [5]	<i>Saccharomyces cerevisiae</i>	Fujisawa et al. Biochimica et Biophysica Acta - Proteins and Proteomics. 2003; 1645(1): 89-94
	msMSR [6]	<i>Metallosphaera sedula</i>	Kockelkorn et al. Journal of Bacteriology. 2009; 191(20): 6352-6362
	caMSR-N [7]	N-terminus of <i>Chloroflexus aurantiacus</i> malonyl-CoA reductase	Liu et al. PLOS ONE. 2013; 8(9): e75554
Aspartate Decarboxylase (PAND) (E.C. 4.1.1.11)	cgPAND [8]	<i>Corynebacterium glutamicum</i>	Dusch et al. Applied and Environmental Microbiology. 1999; 65(4): 1530-1539
	caPAND [9]	<i>Clostridium acetobutylicum</i>	Annan et al. Applied Microbiology and Biotechnology. 2019; 103(11): 4633-4648
	ecPAND [10]	<i>Escherichia coli</i>	Borodina et al. Metabolic Engineering. 2015; 27: 57-64
	tcPAND [11]	<i>Tribolium castaneum</i>	Borodina et al. Metabolic Engineering. 2015; 27: 57-64
	mjPAND [12]	<i>Methanocaldococcus jannaschii</i>	Wang et al. Journal of Bacteriology. 2014; 196(15): 2869-2875
	paBAPAT [13]	<i>Pseudomonas aeruginosa</i>	Song et al. ACS Synthetic Biology. 2016; 5(11): 1256-1263
$\beta$ -Alanine Aminotransferase (BAPAT) or $\gamma$ -aminobutyrate transaminase (GABT) (E.C. 2.6.1.19)	bcBAPAT [14]	<i>Bacillus cereus</i> YHXA gene	Borodina et al. Metabolic Engineering. 2015; 27: 57-64
	ppBAPAT [15]	<i>Pseudomonas putida</i>	Song et al. ACS Synthetic Biology. 2016; 5(11): 1256-1263
	skGABT [16]	<i>Saccharomyces kluyveri</i>	Andersen et al. FEBS Journal. 2007; 274 (7): 1804-1817
	pgAAM [17]	<i>Porphyromonas gingivalis</i>	U.S. Pat. No. 7,655,451 B2
2,3-Alanine Aminomutase (AAM) (E.C. 5.4.3.a)	bsAAM [18]	<i>Bacillus subtilis</i>	U.S. Pat. No. 7,655,451 B2



**[0241]** To determine the assembly efficiency of combinatorial plasmids in *E. coli*, and to investigate the promoter and gene variant combinations, 192 plasmids were extracted from transformed *E. coli* strain NEB10-beta and subjected to sequencing. Analysis of the sequencing results showed an assembly rate of 99%, and all the promoters and gene variants employed were represented without significant bias. Following transformation of these sequence-verified combinatorial plasmids into *C. autoethanogenum*, a total of 111 combinatorial strains were subjected to autotrophic growth in 12-well plates.

**[0242]** Growth experiments were conducted in 12-well plates with 2 mL minimal media and 200 kPa of synthetic gas mix (50% CO, 10% H<sub>2</sub>, 30% CO<sub>2</sub>, and 10% N<sub>2</sub>) at 37° C. for 8 days (FIGS. 5A-C). Broth samples were then taken for biomass measurement and LC-MS analysis to determine 3-HP titer.

**[0243]** Following 8 days of incubation, the 3-HP combinatorial strains reached biomass concentration of 0.5-1.2 gDCW/L (FIG. 5B). Out of these 111 combinatorial strains, 33 strains produced >1 mg/L 3-HP. Seven strains produced more than 10 mg/L 3-HP (17 mg/L being highest) (FIG. 5A). The genotype of the top 20 3-HP-producing strains is shown in Table 3. The 3-HP titer normalized by biomass concentration is shown in FIG. 5C.

mode to compare their 3-HP production to WT *C. autoethanogenum*. Actively growing (early exponential) culture from Schott bottles was used as inoculum for 2L CSTRs with a synthetic gas blend (50% CO, 10% H<sub>2</sub>, 30% CO<sub>2</sub>, and 10% N<sub>2</sub>) at atmospheric pressure. 3-HP combinatorial strain “A59B” (Table 3) was also characterized in CSTR under continuous mode.

**[0246]** Strain A59B achieved a peak biomass concentration of 3.8 gDCW/L (FIG. 7A) and reached a peak CO uptake of 3260 mmol/L/d (FIG. 7B). In addition to a peak ethanol concentration of 50 g/L (FIG. 7A), this strain reached a peak 3-HP titer of 278.5 mg/L and peak 1,3-PDO titer of 99.5 mg/L (FIG. 7C).

**[0247]** Strain B2A grew to a peak biomass concentration of 2.7 gDCW/L (FIG. 8A) and achieved a peak CO uptake of 2497 mmol/L/d (FIG. 8B) while generating a maximum ethanol titer of 16.6 g/L (FIG. 8A). In the stationary growth phase, a peak 3-HP titer of 144.2 mg/L and peak 1,3-PDO titer of 148 mg/L were measured using LC-MS and GC-MS, respectively (FIG. 8C).

**[0248]** Strain A63B reached a peak biomass concentration of 1.3 gDCW/L (FIG. 9A) and reached a peak CO uptake of 880 mmol/L/d (FIG. 9B). Despite the low peak CO gas uptake, this strain was able to produce a maximum ethanol titer of 23.5 g/L (FIG. 9A), peak 3-HP titer of 99.2 mg/L and peak 1,3-PDO concentration of 40.2 mg/L (FIG. 9C).

TABLE 3

Genotype of top 20 3-HP-producing combinatorial strains in the 12-well plate growth assay.									
Strain Name	pro-moter 1	gene 1	pro-moter 2	gene 2	pro-moter 3	gene 3	3HP (mg/L)	Biomass (gDCW/L)	Normalized 3HP (mg/gDCW/L)
A59B	Pwl	pdMSR	Ppfor	caPAND	Pfer	paBAPAT	16.93	1.02	16.61
A63B	Pfer	scMSR	Pfer	caPAND	Pwl	skGABT	16.85	0.80	21.15
A63C	Pfer	scMSR	Pfer	caPAND	Pwl	skGABT	14.46	0.85	17.08
A63A	Pfer	scMSR	Pfer	caPAND	Pwl	skGABT	14.05	0.82	17.13
A35A	Pwl	pdMSR	Pfer	cgPAND	Pfer	paBAPAT	13.40	1.08	12.42
A59A	Pwl	pdMSR	Ppfor	caPAND	Pfer	paBAPAT	12.82	0.86	14.91
A59C	Pwl	pdMSR	Ppfor	caPAND	Pfer	paBAPAT	10.24	0.74	13.87
A10A	Ppfor	nmMSR	Pwl	mjPAND	Pwl	ppBAPAT	9.71	0.98	9.87
A95B	Pfer	ecMSR	Pwl	cgPAND	Ppfor	paBAPAT	9.29	0.64	14.55
A36B	Pwl	nmMSR	Pfer	cgPAND	Pwl	bcBAPAT	8.87	0.66	13.51
A37B	Pwl	nmMSR	Pfer	cgPAND	Pfer	bcBAPAT	8.44	0.49	17.34
A28B	Pfer	msMSR	Pfer	mjPAND	Ppfor	paBAPAT	8.28	0.82	10.15
A20B	Pfer	scMSR	Pfer	mjPAND	Pw	skGABT	7.70	0.97	7.97
A83A	Pfer	caMCR-N	Ppfor	cgPAND	Ppfor	paBAPAT	7.19	0.66	10.98
A37A	Pwl	nmMSR	Pfer	cgPAND	Pfer	bcBAPAT	7.07	0.69	10.20
A20A	Pfer	scMSR	Pfer	mjPAND	Pwl	skGABT	6.97	0.96	7.28
B02A	Pwl	scMSR	Pfer	cgPAND	Ppfor	bcBAPAT	6.84	0.92	7.41
A17A	Pwl	nmMSR	Pfer	cgPAND	Pfer	bcBAPAT	6.77	1.06	6.42
A75A	Pwl	caMCR-N	Pwl	cgPAND	Pwl	paBAPAT	6.56	0.60	11.03
A95A	Pfer	ecMSR	Pwl	cgPAND	Ppfor	paBAPAT	6.43	0.63	10.18

**[0244]** Eighteen 3-HP combinatorial strains were further subjected to autotrophic growth in 250 mL Schott bottle with 10 mL minimal media in the presence of 150 kPa synthetic gas mix (50% CO, 10% H<sub>2</sub>, 30% CO<sub>2</sub>, and 10% N<sub>2</sub>) at 37° C. for 14 days with regular samplings for biomass measurement and 3-HP analysis. Up to 24.09±7.95 mg/L 3-HP was detected in these combinatorial strains, compared to only 0.23±0.02 mg/L 3-HP in the WT control (FIG. 6).

Example 4. 3-HP and 1,3-PDO Production from Syngas Fermentation in CSTR

**[0245]** 3-HP combinatorial strains “A59B”, “B2A” and “A63B” (Table 3) were characterized in CSTR under batch

**[0249]** Under continuous CSTR conditions using strain A59B, a dilution (D) rate of 0.5/day was initiated on day 1.2, before increasing to D of 0.75/day on day 2.2, eventually reaching D of 1/day between day 3.9 and day 12. Biomass concentration accumulated to a peak concentration of 4.6 gDCW/L (FIG. 10A) and CO gas uptake reached a peak of 3574 mmol/L/d (FIG. 10B). Following the switch to D of 1/day, this strain achieved peak productivity of 358 mg/L/d 3-HP on day 5 and 82.2 mg/L/d 1,3-PDO on day 7 (FIG. 10C).

**[0250]** All references, including publications, patent applications, and patents, cited herein are hereby incorporated by



reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein. The reference to any prior art in this specification is not, and should not be taken as, an acknowledgement that that prior art forms part of the common general knowledge in the field of endeavor in any country.

[0251] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the disclosure (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to”) unless otherwise noted. The term “consisting essentially of” limits the scope of a composition, process, or method to the specified materials or steps, or to those that do not materially affect the basic and novel characteristics of the composition, process, or method. The use of the alternative (e.g., “or”) should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the term “about” means  $\pm 20\%$  of the indicated range, value, or structure, unless otherwise indicated.

[0252] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is

incorporated into the specification as if it were individually recited herein. For example, any concentration range, percentage range, ratio range, integer range, size range, or thickness range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated.

[0253] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the disclosure and does not pose a limitation on the scope of the disclosure unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the disclosure.

[0254] Embodiments of this disclosure are described herein. Variations of those embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the disclosure to be practiced otherwise than as specifically described herein. Accordingly, this disclosure includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the disclosure unless otherwise indicated herein or otherwise clearly contradicted by context.

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tcacatgagc	cagcaccatg	tgtaatagaa	gtagatgatg	aaagagtatt	aaat	1260
gcagatgaaa	taactggtac	tatcgttata	gcacaaagat	tagctagata	ttggcagagc	1320
cagagactta	ccccaggggc	aagggcaaga	ggaccaagag	taatattcct	ttcaaatgga	1380
gcagatcaga	acggtaaatg	ttatggaagg	attcaatcag	ctgcaatagg	tcagcttata	1440
agagtatgga	gacatgaagc	agagttggat	tatcaaaggg	cttcagcagc	tgggtgatcac	1500
gtacttcccc	ctgtatgggc	aaaccaaata	gttagatttg	caaacagatc	tttagaagga	1560
ttagaatttg	cttgtgcctg	gacagctcaa	ttgttgcatt	ctcaaagaca	tataaatgaa	1620
ataactttaa	atattcccgc	caacatctaa				1650
SEQ ID NO: 8	moltype = DNA length = 411					
FEATURE	Location/Qualifiers					
misc_feature	1..411					
	note = LZ1561 codon adapted nucleotide sequence of cgPAND					
source	1..411					
	mol_type = other DNA					
	organism = synthetic construct					
SEQUENCE: 8						
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gattatgtag	gaagtgttac	tatagatgca	gatttgggttc	atgctgctgg	gcttatagaa	120
ggagaaaaag	ttgctattgt	ggatattact	aatgggtgcta	gactggaaac	ttatgtaata	180
gtaggagatg	caggaacagg	aaatatatgt	attaatgggtg	ctgctgcaca	tcttattaat	240
ccaggagatc	ttgtaataat	aatgtcctac	cttcaggcaa	cagatgcaga	agcaaaggca	300
tacgaaccta	aaatagttca	tgtagatgcc	gataatagaa	tagtagcatt	gggaaatgat	360
ttagctgaag	cactaccagg	aagtggcctc	cttacttcaa	ggagtatcta	a	411
SEQ ID NO: 9	moltype = DNA length = 384					
FEATURE	Location/Qualifiers					
misc_feature	1..384					
	note = LZ1561 codon adapted nucleotide sequence of caPAND					
source	1..384					
	mol_type = other DNA					
	organism = synthetic construct					
SEQUENCE: 9						
atgcatttaa	atatgttaaa	aagtaaaata	cacagagcaa	cggtagtaca	agcagatcta	60
aaactatgtag	gaagtataac	tatagataga	aatccttatgg	ataaagcaaa	tatattggaa	120
tatgaaaaag	tagaaattgc	aaacattaac	aatggagcaa	gatttgagac	ttatgtaata	180
gctggagaag	caggctcagg	aataatatgc	ttaaattgggtg	cagcagcccg	ctgtgcacag	240
gctgggggata	aagttattat	aatgtgttat	tgttctttaa	caccagaaga	ggcttcagaa	300
catagaccaa	aagtagtatt	tgtaaacgat	gataacagta	tttctaattgt	aacagaatat	360
gaaaagcatg	gcactatagg	ataa				384
SEQ ID NO: 10	moltype = DNA length = 381					
FEATURE	Location/Qualifiers					
misc_feature	1..381					
	note = LZ1561 codon adapted nucleotide sequence of ecPAND					
source	1..381					
	mol_type = other DNA					
	organism = synthetic construct					
SEQUENCE: 10						
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cattatgaag	gaagctgtgc	aattgatcag	gatttcctag	atgccgctgg	tatacttgaa	120
aatgaagcaa	tagatatttg	gaatgtaaca	aacggaaaaa	gatttagtac	ctatgctatt	180
gctgctgaaa	gaggaagcag	aataatatct	gtaaatgggtg	ctgctgcgca	ttgtgcaagt	240
gttgagagata	ttgtttataat	tgcattcattt	gttactatgc	ctgatgaaga	ggctagaacc	300
tggcgacctc	acgtagctta	ttttgaaggt	gataatgaaa	tgaaacgcac	ggcaaaaagca	360
attccagtac	aagttgccta	a				381
SEQ ID NO: 11	moltype = DNA length = 1623					
FEATURE	Location/Qualifiers					
misc_feature	1..1623					
	note = LZ1561 codon adapted nucleotide sequence of tcPAND					
source	1..1623					
	mol_type = other DNA					
	organism = synthetic construct					
SEQUENCE: 11						
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tttagtgacg	cagtactaag	ctctgatgaa	gaacttttcc	accagaaatg	tcctaaacct	120
gctcctattt	atagtccagt	aagtaaacct	gtatcctttg	aatcattacc	aaacagaaga	180
ttgcatgaag	agttccttag	aagcagtggt	gatgtacttc	tccaagaggc	tgtatttgaa	240
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gcagtagatc	cttatggatt	agtagcacag	tgggctacag	atgcgttaaa	tccatctgta	480
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aaaaggattac	attcattacc	aagacttgta	ttattttacaa	gtgaagatgc	tcattattct	720
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gacccaatatg	aaaaaattgc	ggatgtttgc	caaaaatata	agttatggct	tcatgttgat	960
gctgcatggg	gcggcgggcg	acttgtatca	gcaaaacata	gacatctttt	gaagggaata	1020
gaacgtgcag	attctgtaac	ttggaatcca	cataaattac	ttacagctcc	tcagcaatgc	1080
tcaactttat	tgttaagaca	tgagggagta	cttgctgagg	ctcattctac	taatgcagct	1140
tatcttttcc	aaaaagataa	attttatgat	acaaaatacg	atacaggaga	taagcatatc	1200
caatgtggaa	gaagagcaga	tgtacttaaa	ttctgggttc	tgtggaaagc	aaaaggaaca	1260
tctggacttg	aaaaacatgt	agataaagta	tttgaaaatg	ctagattctt	tacggattgt	1320
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ttacataaag	tagcaccaag	aataaaggag	agaatgatga	aagagggaag	tatgatggta	1500
acttatcagg	ctcaaaaggg	acacccaaac	ttcttttagaa	tagtatttca	aaatagtgga	1560
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ttaa						1623

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SEQ ID NO: 12                   moltype = DNA   length = 1191  
FEATURE                       Location/Qualifiers  
misc\_feature                 1..1191  
                              note = LZ1561 codon adapted nucleotide sequence of mjPAND  
source                        1..1191  
                              mol\_type = other DNA  
                              organism = synthetic construct

SEQUENCE: 12

atgagaaata	tgcaagaaaa	gggagtatca	gaaaaagaaa	tattggaaga	attaaaaaaa	60
tacagaagtt	tggatttgaa	gtatgaagat	ggaaatatat	ttggaagtat	gtgtagtaat	120
gttcttccaa	taacacgtaa	aatagtagat	atatttttag	aaacaaatct	tggtgatcca	180
ggactattca	aaggaactaa	gttactggaa	gaaaaggcag	tagcgttgct	tggttcctta	240
ttaaataata	aagatgccta	tggacatata	gtttctggcg	gcactgaagc	aaatttgatg	300
gcacttagat	gtataaaaaa	tatctggaga	gaaaagagaa	gaaagggatt	aagtaaaaaa	360
gaacatccta	aaataatagt	tcctataact	gcacattttt	catttgaaaa	aggaagagaa	420
atgatggact	tagaatacat	ttatgcaccg	atcaaagaag	attataccat	agatgaaaaa	480
tttgttaaag	atgccgtaga	agattatgat	gttgacggta	ttataggtat	agcaggcaca	540
actgaacttg	gaactataga	caatatagag	gaattatcca	aaatcgctaa	agaaaataat	600
atatatatcc	atgtagatgc	tgcatttggc	ggcttagtaa	taccattcct	tgatgacaaa	660
tacaagaaaa	agggagttaa	ctataaattt	gacttttcac	ttggagtaga	ttctataacc	720
atagatccac	acaagatggg	acattgtcct	attccatcag	gcggcatatt	gttcaaagat	780
atagggtata	aaagatatct	ggatgttgat	gctccttatc	tcactgagac	tagacaggct	840
actatttttag	gaacacgtgt	aggttttggc	ggcgcttgta	cctatgcagt	tcttagatat	900
cttggaagag	aaggacagag	aaaaatagtt	aatgaatgta	tggagaatac	actatatctc	960
tacaaaaaac	ttaaagaaaa	taatttcaaa	ccagttatag	aacctatatt	aaatatagtt	1020
gctatagaag	atgaggatta	taaagaagtt	tgcaaaaagt	taagagatcg	tggaatatat	1080
gtaagtgttt	gtaattgtgt	aaaggcttta	aggattgttg	taatgcctca	catcaagaga	1140
gaacatattg	ataatttcat	tgaaatctta	aattccataa	aaagggacta	g	1191

SEQ ID NO: 13                   moltype = DNA   length = 1347  
FEATURE                       Location/Qualifiers  
misc\_feature                 1..1347  
                              note = LZ1561 codon adapted nucleotide sequence of paBAPAT  
source                        1..1347  
                              mol\_type = other DNA  
                              organism = synthetic construct

SEQUENCE: 13

atgaatcagc	cactaaatgt	tgccccacca	gtttcatcag	aacttaattt	aagagcccat	60
tgatgccaat	tttcagccaa	tagaaacttt	caaaaagatc	caaggattat	agtagcagct	120
gaagggttctt	ggcttactga	tgacaagggt	agaaaagtat	acgattcact	ttcaggacta	180
tgacatgctg	gtgctggcca	ttcaagaaaa	gaaatacagg	aagcagttgc	tagacagctt	240
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tcagaatgtg	cagataactt	aataaaaaatg	gcaagggtct	attggagact	caaaggctcag	420
cctcaaaaaa	ctaaacttat	aggacgtgcc	agaggatata	atgggtgtaaa	tgtggcagga	480
acatctttag	gcggcatagg	cggcaataga	aaaatgttcg	gtcaattaat	ggatgtagat	540
catttaccac	atacacttca	gccagggtat	gcatttacta	gaggaatggc	acagacaggc	600
ggcgtagaat	tagctaata	attattgaaa	ttgatagaac	tccatgatgc	ttctaacata	660
gcagctgtca	ttgtagaacc	tatgtctggg	tcagctgggg	tccttggttc	tccagtagga	720
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gtaataactg	cttttggaag	gctaggaact	tattcaggag	cagagtactt	tggaagtaact	840
cctgatctta	tgaatgtagc	aaaacaagta	acgaatgggtg	ctgtacctat	gggtgtctgtc	900
atagcatcat	cagaaattta	cgatacattc	atgaaccaag	cactgcctga	acatgcagta	960
gaattttcac	atggttatag	ttatagtggc	catccagtag	catgtgcagc	tggaactggca	1020
gcattagata	ttcttgcaag	ggacaatctt	gtacaacaaa	gtgctgaatt	agctcctcac	1080
tttgaagagg	gacttcatgg	acttcaaggt	gcaaaaaatg	tcatagatata	aagaaactgt	1140
ggacttgctg	gtgctataca	aattgcacca	agagatgggtg	accctacagt	aagacctttt	1200
gaagcaggaa	tgaaactttg	gcaacaggga	ttctatgtaa	gatttggcgg	cgatacttta	1260
caatttggac	ctacatttaa	tgctagacca	gaggaattag	atagattatt	tgatgcagtt	1320
ggagaggcat	taaatgggtat	agcctaa				1347

SEQ ID NO: 14                   moltype = DNA   length = 1356  
FEATURE                       Location/Qualifiers  
misc\_feature                 1..1356  
                              note = LZ1561 codon adapted nucleotide sequence of bcBAPAT  
source                        1..1356  
                              mol\_type = other DNA  
                              organism = synthetic construct

SEQUENCE: 14

atggaattaa	tgatagtaca	ggtaacagaa	cagactcaat	cacttaaaaa	gacagacgaa	60
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aaggctgaag	gtgcgtgggt	tacggatatt	gacggaaaca	gatatttaga	tggaatgagt	180
ggactttggg	gcgtaaatgt	tggttatgga	agaaaggaat	tggttagagc	agcattcgaa	240
cagttagaag	aaatgcctta	ttttcctctt	actcaatctc	atgtacctgc	tataaaatta	300
gctgaaaaat	tgaatgaatg	gcttgatgat	gaatatgtaa	tattcttttc	aaattcaggt	360
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catggaagat	ataaat	tatat	aagcagatat	agggcttata	atggtaattc	tatgggagcc	480
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cttcacgttg	ctcctcctga	tacttataga	aatcctgaag	atgttcacac	acttgcgtct		600
gctgaagaaa	tcgacagagt	tatgacttgg	gaattatctc	aaacagttgc	tgggtgttatt		660
atggaaccta	taataacagg	cggcggcatt	ttaatgccac	cagatggtta	tatggaaaaa		720
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tttggcgga	atcctgctgc	atgtgcttta	gctttaaaaa	atttagaaat	tatggaaaac		1020
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gtaaaagaac	acccaaatgt	tggggatggt	agaggaaaag	gacttttgct	aggtatagaa		1140
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aatgcgtgca	aagaaaaagg	gcttataatt	ggtaaaaatg	gtgacactgt	tgctggatat		1260
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gttaagacaa	tgaaagagtg	ccttgcacag	ctctaa				1356
SEQ ID NO: 15							
FEATURE							
misc_feature							
1..1347							
note = LZ1561 codon adapted nucleotide sequence of ppBAPAT							
source							
1..1347							
mol_type = other DNA							
organism = synthetic construct							
SEQUENCE: 15							
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FEATURE							
misc_feature							
1..1428							
note = LZ1561 codon adapted nucleotide sequence of skGABT							
source							
1..1428							
mol_type = other DNA							
organism = synthetic construct							
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atgccaaagt	acagtgttgc	agaactatat	tatccagatg	aaccaacaga	acaaaaaata		60
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ggactttctta	aagctgcacc	acaaggacaa	gataagattt	ggactgcttt	atctggaagt		420
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tccactacta	gaagtaaacc	aattcacaaa	ttagacatac	ctgctttcaa	ctggcctaaa		660
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gaatcaagtt	gtatagaaaa	attttctcaa	atagttcaag	aatggcaagg	taaaatagca		780
gctgttataa	tagagcctat	acagagtga	ggcggcgata	atcatgcac	aagtgatttt		840
tttcaaaaag	ttagagaaat	tacaattgaa	aatggtatat	tgatgatagt	agacgaagtt		900
caaaccggtg	taggagcaac	aggtaaaatg	tgggctcatg	agcactggaa	tctttcaaac		960
ccccagatt	tagttacctt	ttcaaaaaaa	ttccaagctg	ctggttttta	ctatcatgat		1020
cctaaattac	aacctgatca	gcctttcaga	caatttaata	cctggtgtgg	tgacccatca		1080
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acagctgaag taggaaatta tctattttaat aggttagaaa agttatttga aggaaagaac 1200
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tcatctgaaa gggattcttt cctatcaaga ttaagatgta acgggtgctaa tgtagctggg 1320
tgtggagatt cagctgtaag attgaggcct tcacttacct ttgaagaaaa gcatgcagat 1380
gttttagttt ctatatttga taaaactctt agacaattgt atggataa 1428

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SEQ ID NO: 17      moltype = DNA   length = 1251
FEATURE            Location/Qualifiers
misc_feature       1..1251
                   note = LZ1561 codon adapted nucleotide sequence of pgAAM
source             1..1251
                   mol_type = other DNA
                   organism = synthetic construct

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SEQUENCE: 17
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ttaactgcag aagaagaaga aggagtaaaa gaaagcccta aagtactcag gatggctata 180
actccttatt atctatcatt aatagatcca gaaaacccaa attgtccaat tagaaagcaa 240
gcaataccta ctcagcaaga acttgtagct gccctgaag atcaagtaga tcctctaagt 300
gaagatgacg attcaccagt accaggggta actcatagat atcctgata ggtactgttc 360
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caaaaagatg cttcttcacc aagtgaagg atagatagat gcatagatta tatagcaa 480
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aaaaagtatc atccagtatg gcttaatact cactttaacc acccaaatga agtaacagaa 720
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source             1..1416
                   mol_type = other DNA
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1. A recombinant C1-fixing bacteria for producing 3-hydroxypropionate (3-HP) from a carbon source comprising a nucleic acid encoding a group of exogenous enzymes comprising  $\beta$ -alanine pyruvate aminotransferase or  $\gamma$ -aminobutyrate transaminase; and malonic semialdehyde reductase.

2. The bacteria according to claim 1, further comprising a nucleic acid encoding a group of exogenous enzymes comprising pyruvate carboxylase, aspartate aminotransferase, and aspartate decarboxylase, wherein the nucleic acid is operably linked to a promoter.

3. The bacteria according to claim 1, further comprising a nucleic acid encoding a group of exogenous enzymes comprising alanine dehydrogenase and 2,3-alanine aminomutase, wherein the nucleic acid is operably linked to a promoter.

4. The bacteria according to claim 1, which are selected from the group consisting of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, *Clostridium ragsdalei*, *Clostridium carboxidivorans*, *Clostridium drakei*, *Clostridium scatologenes*, *Clostridium aceticum*, *Clostridium formicoaceticum*, *Clostridium magnum*, *Butyrivibrio methylotrophicum*, *Acetobacterium woodii*, *Alkalibaculum bacchii*, *Blautia producta*, *Eubacterium limosum*, *Moorella thermoacetica*, *Moorella thermautotrophica*, *Sporomusa ovata*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, *Oxobacter pfennigii*, and *Thermoanaerobacter kivui*.

5. The bacteria according to claim 4, which are a *Clostridium* species selected from the group consisting of *C. ljungdahlii*, and *C. autoethanogenum*.

6. The bacteria according to claim 1, further comprising a nucleic acid encoding acetyl-Coenzyme A carboxylase, wherein the nucleic acid is codon optimized for expression in the bacteria.

7. The bacteria according to claim 6, wherein the acetyl-Coenzyme A carboxylase is derived from a member of the genus *Clostridium*, *Metallosphaera*, *Sulfolobus*, or *Chloroflexus*.

8. The bacteria according to claim 1, wherein the nucleic acid encoding a group of exogenous enzymes comprising  $\beta$ -alanine pyruvate aminotransferase,  $\gamma$ -aminobutyrate transaminase, and malonic semialdehyde reductase is codon optimized for expression in the bacteria.

9. The bacteria according to claim 1, further comprising a nucleic acid encoding a group of exogenous enzymes comprising aldehyde:ferredoxin oxidoreductase and alcohol dehydrogenase, wherein the nucleic acid is operably linked to a promoter.

10. The bacteria according to claim 9, wherein the nucleic acid encoding a group of exogenous enzymes enable production of 1,3-propanediol.

11. The bacteria according to claim 1, wherein 3-HP is converted into a material selected from the group consisting of acrylic acid, acrylonitrile, paint, paper, adhesives, textiles, specialty coatings, ink, superabsorbent polymer polyacrylates, 1,3-propanediol, solvents, adhesives, cosmetics, polytrimethylene terephthalate, 3-hydroxypropionaldehyde, foods, feed additives, and any combination thereof.

12. A method of converting CO and/or CO<sub>2</sub> into 3-hydroxypropionate (3-HP), the process comprising: passing a gaseous CO-containing and/or CO<sub>2</sub>-containing substrate to a bioreactor containing a culture of a recombinant C1-fixing bacteria according to claim 1, in a culture medium such that the bacteria convert the CO and/or CO<sub>2</sub> to 3-HP; and recovering the 3-HP from the bioreactor.

13. A method of culturing the bacteria according to claim 1, comprising growing the bacteria in a medium comprising a gaseous carbon source, wherein the carbon source comprises CO and/or CO<sub>2</sub>.

14. A method of culturing the bacteria according to claim 1, comprising growing the bacteria in a medium comprising an energy source, wherein the energy source comprises CO and/or CO<sub>2</sub>.

15. The method according to claim 13, wherein the culturing is strictly anaerobic.

16. The method according to claim 14, wherein the culturing is strictly anaerobic.

17. The method according to claim 13, wherein the carbon source comprises an industrial waste product or off-gas.

18. The method according to claim 14, wherein the energy source comprises an industrial waste product or off-gas.

19. The method according to claim 13, wherein 3-HP is converted into a material selected from the group consisting of acrylic acid, acrylonitrile, paint, paper, adhesives, textiles, specialty coatings, ink, superabsorbent polymer polyacrylates, 1,3-propanediol, solvents, adhesives, cosmetics, polytrimethylene terephthalate, 3-hydroxypropionaldehyde, foods, feed additives, and any combination thereof.

20. The method according to claim 14, wherein 3-HP is converted into a material selected from the group consisting of acrylic acid, acrylonitrile, paint, paper, adhesives, textiles, specialty coatings, ink, superabsorbent polymer polyacrylates, 1,3-propanediol, solvents, adhesives, cosmetics, polytrimethylene terephthalate, 3-hydroxypropionaldehyde, foods, feed additives, and any combination thereof.

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