

CLAIMS

What is claimed is:

1. A biomanufacturing system for producing an organic product comprising:
at least one bioreactor culture vessel;
5 wherein the at least one bioreactor culture vessel contains an organic substrate culture solution,
wherein the organic substrate culture solution contains a first recombinant microorganism having an improved organic substrate producing ability,
wherein the first recombinant microorganism expresses at least one organic substrate
10 forming recombinant enzyme by expressing at least one non-native organic substrate forming enzyme nucleotide sequence,
wherein the first recombinant microorganism is capable of utilizing a carbon source to produce the organic substrate,
wherein the first recombinant microorganism produces at least one organic substrate
15 culture impurity, including oxygen, at least a byproduct in gas, a liquid, and a solid phase; and
wherein the at least one bioreactor culture vessel contains an organic product culture solution,
wherein the organic product culture solution contains a second recombinant microorganism having an improved organic product producing ability,
20 wherein the second recombinant organism expresses at least one organic product forming enzyme by expressing at least one non-native organic product forming enzyme nucleotide sequence,
wherein the second recombinant organism is capable of utilizing the organic substrate to produce the at least one organic product.
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2. The system of claim 1, wherein the at least one bioreactor culture vessel includes a first bioreactor culture vessel including a carbon source inlet, and a power source; and a second bioreactor culture vessel including a fluid flow path connected to and between the first bioreactor culture vessel and the second bioreactor culture vessel, and an organic product outlet;
30 wherein the first bioreactor culture vessel includes the organic substrate culture solution, and the second bioreactor culture vessel includes the organic product culture solution; or
wherein the first bioreactor culture vessel or the second bioreactor culture vessel further comprises a biomass collection port.

3. The system of claim 2, wherein the carbon source includes carbon dioxide, carbon monoxide, glycerol, glucose, fructose, sucrose, a monosaccharide, a disaccharide, a polysaccharide, glycogen, acetic acid, a fatty acid, or a combination thereof; or
wherein the power source includes sunlight, a solar power source, an electrical power
5 source, or a combination thereof; or
wherein the volatile gas includes oxygen, methane, or a combination thereof; or
wherein the at least one organic substrate includes alpha-ketoglutarate, sucrose, glucose, fructose, xylose, arabinose, galactose, glycerol, a monosaccharide, a disaccharide, a polysaccharide, glycogen, a fatty acid, or a combination thereof; or
10 wherein the at least one organic product includes an alcohol, methanol, ethanol, propanol, butanol, ethane diol, an organic acid, propionic acid, acetic acid, an aldehyde, formaldehyde, a long chain fatty acid, an n-alkane, a hydrocarbon, ethane, propene, butene, ethane, propane, butane, C₂-C₂₀ alkane, C₂-C₂₀ alkene, or a combination thereof; or
wherein the second bioreactor culture vessel further includes a carbon source inlet, a
15 volatile gas outlet, a power source, or a combination thereof.
4. The system of claim 1, wherein the organic substrate culture solution and the organic product culture solution are combined in one bioreactor culture vessel; or
the organic substrate culture solution and the organic product culture are separated by a
20 filter wherein the filter includes a pore size of from about 0.2 μm to about 10 μm or more; or
wherein the system further comprises a carbonation unit, an amine stripper, an amine scrubber, a catalytic converter, a condenser, a compressor, a caustic tower, a dryer, or combinations thereof.
5. The system of claim 1, wherein the organic substrate includes alpha-ketoglutarate (AKG),
25 wherein an amount of the at least one AKG forming enzyme produced by the first recombinant microorganism is greater than that produced relative to a control microorganism lacking a non-native AKG forming enzyme expressing nucleotide sequence; or
wherein the at least one organic product forming enzyme includes ethylene forming enzyme (EFE), and an amount of EFE produced by the second recombinant microorganism is
30 greater than that produced relative to a control microorganism lacking a non-native EFE expressing nucleotide sequence.
6. The system of claim 5, wherein the first recombinant microorganism expresses at least one alpha-ketoglutarate permease protein (AKGP) by expressing at least one non-native AKGP
35 forming nucleotide sequence.

7. The system of claim 5, wherein the at least one AKG forming enzyme includes an isocitrate dehydrogenase (ICD) protein, a glutamate dehydrogenase (GDH) protein, or a combination thereof.

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8. The system of claim 7, wherein the first recombinant microorganism expresses an ICD protein having an amino acid sequence at least 95% identical to SEQ ID NO: 1 by expressing a non-native ICD protein nucleotide sequence having a nucleotide sequence at least 95% identical to SEQ ID NO: 2; or

10 the first recombinant microorganism expresses a GDH protein having an amino acid sequence at least 95% identical to SEQ ID NO: 5 by expressing a non-native GDH protein nucleotide sequence having a nucleotide sequence at least 95% identical to SEQ ID NO: 6, or a combination thereof; or wherein the second recombinant microorganism expresses an EFE protein having an amino acid sequence at least 95% identical to SEQ ID NO: 7 by
15 expressing a non-native EFE protein nucleotide sequence having a nucleotide sequence at least 95% identical to SEQ ID NO: 8.

9. The system of claim 1, wherein the first recombinant microorganism includes a microorganism selected from the group consisting of a photosynthetic bacterium, a

20 *Cyanobacteria*, a *Synechococcus*, *Synechococcus elongatus*, *Synechococcus leopoliensis*, *Synechocystis*, *Anabaena*, a *Pseudomonas*, *Pseudomonas syringae*, *Pseudomonas savastanoi*, *Chlamydomonas*, and *Chlamydomonas reinhardtii*; or

wherein the second recombinant microorganism includes a microorganism selected from the group consisting of *Escherichia*, *Escherichia coli*, *Geobacteria*, *Arthrobacter paraffineus*,
25 *Pseudomonas fluorescens*, *Pseudomonas Putida*, a *Pseudomonas*, *Pseudomonas syringae*, *Pseudomonas savastanoi*, *Serratia marcescens*, *Bacillus metatherium*, *Candida paludigena*, *Pichia inositovora*, *Torulopsis glabrata*, *Candida lipolytica*, *Yarrowia lipolytica*, *Saccharomyces cerevisiae*., *Aspergillus sp.*, *Bacillus subtilis*, and *Lactobacillus sp.*

30 10. The system of claim 1, wherein the first recombinant microorganism includes a delta-gluc mutant microorganism lacking expression of a glucose-1-phosphate adenylyltransferase protein; or wherein the first recombinant microorganism expresses a sucrose synthase protein having an amino acid sequence at least 95% identical to SEQ ID NO: 9 by expressing a non-native sucrose synthase protein nucleotide sequence having a nucleotide sequence at least 95% identical to SEQ
35 ID NO: 10; or wherein the first recombinant microorganism expresses a sucrose phosphate

synthase protein having an amino acid sequence at least 95% identical to SEQ ID NO: 11 by expressing a non-native sucrose phosphate synthase nucleotide sequence having a nucleotide sequence at least 95% identical to SEQ ID NO: 12.

- 5 11 The system of claim 5, wherein an amount of at least one AKG forming enzyme produced by the first recombinant microorganism is from about 5% to about 200% or more greater than that produced relative to the control microorganism lacking the non-native AKG forming enzyme expressing nucleotide sequence; or
- wherein an amount of EFE protein produced by the second recombinant microorganism is
- 10 from about 5% to about 200% or more greater than that produced relative to the control microorganism lacking the non-native EFE expressing nucleotide sequence; or
- wherein an amount of EFE protein produced by the second recombinant microorganism is from about 20 grams to about 100 grams per liter or more of organic product culture solution.
- 15 12. The system of claim 5, wherein the second recombinant microorganism includes *E. Coli*, and an amount of EFE protein produced by the second recombinant microorganism is from about 30% to about 80% or more of a total cellular amount of protein of the second recombinant microorganism; or
- a production rate of the at least one organic product produced by the second recombinant
- 20 microorganism is from about 100 million pounds/year to about 1 billion pounds/year or more; or wherein a cell population concentration of the second recombinant microorganism ranges from about 10^7 to about 10^{13} cells per milliliter, or a dry cell weight per liter of organic product culture solution of from about 100 grams to about 300 grams dry cell weight per liter.
- 25 13. The system of claim 5, wherein the non-native AKG forming enzyme expressing nucleotide sequence or the non-native EFE expressing nucleotide sequence is inserted into a microbial expression vector, wherein the microbial expression vector includes a bacterial vector plasmid, a nucleotide guide of a homologous recombination system, an antibiotic-resistant system, an aid system for protein purification and detection, a CRISPR CAS system, a phage
- 30 display system, or a combination thereof.
14. The system of claim 5, wherein the EFE expressing nucleotide sequence has a copy number in the microbial expression vector of from about 2 to about 500; or wherein the microbial expression vector includes at least one microbial expression promoter.

15. The system of claim 14, wherein the at least one microbial expression promoter includes a light sensitive promoter, a chemical sensitive promoter, a temperature sensitive promoter, a *Lac* promoter, a T7 promoter, a CspA promoter, a lambda PL promoter, a lambda CL promoter, a continuously producing promoter, a psbA promoter, or a combination thereof.

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16. A method of producing an organic product comprising:
providing a biomanufacturing system as in claim 2;
culturing the first recombinant microorganism in the first bioreactor culture vessel under conditions sufficient to produce an amount of the at least one organic substrate in the first
10 bioreactor culture vessel; and
culturing the second recombinant microorganism in the second bioreactor culture vessel under conditions sufficient to produce an amount of the at least one organic product in the second bioreactor culture vessel.

15 17. The method of claim 16, further comprising:
removing an amount of the at least one volatile gas from the organic substrate culture solution through the volatile gas outlet;
removing an amount of the at least one organic product from the organic product culture solution through the organic product outlet;
20 provided the carbon source includes carbon dioxide, feeding an amount of the carbon dioxide from a carbon dioxide source into the organic substrate culture solution through the carbon source inlet;
maintaining a pH level of the organic substrate culture solution and the organic product culture solution from about 5.0 to about 8.5;
25 maintaining the organic substrate culture solution and the organic product culture solution at a temperature of from about 25 degrees Celsius to about 70 degrees Celsius;
provided the first bioreactor culture vessel or the second bioreactor culture vessel includes a biomass collection port, collecting an amount of biomass produced by the first recombinant microorganism or the second recombinant microorganism through the biomass
30 collection port; or
maintaining an amount of volatile gas in the second bioreactor culture vessel of from about 10% by volume to about 1% by volume or less, based on a total internal volume of the second bioreactor culture vessel.

18. The method of claim 16, wherein the non-native organic substrate forming recombinant enzyme expressing nucleotide sequence or the non-native organic product expressing nucleotide sequence is inserted into a microbial expression vector, wherein the at least one microbial expression vector includes at least one microbial expression promoter, further comprising:

5 controlling the amount of the at least one organic substrate or the amount of the at least one organic product produced by adding at least one promoter inducer to the organic substrate culture solution or the organic product culture solution.

19. The method of claim 18, wherein the at least one microbial expression promoter includes
10 a light sensitive promoter, a chemical sensitive promoter, a temperature sensitive promoter, a Lac promoter, a T7 promoter, a CspA promoter, a lambda PL promoter, a lambda CL promoter, a continuously producing promoter, a psbA promoter, or a combination thereof; and the at least one promoter inducer includes lactose, xylose, IPTG, cold shock, heat shock, or a combination thereof.

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20. The method of claim 16, further comprising:

provided the at least one organic substrate culture impurity includes at least one volatile gas, removing the at least one volatile gas through the volatile gas outlet; or

recovering an amount of at least one organic product produced at a rate of from about 100
20 million pounds/year to about 1 billion pounds/year or more; or

wherein the amount of the at least one organic product produced contains an amount of volatile gas of about 1 mole percent or less.

21. A method of producing an organic product comprising:

25 providing a bioremediation system as in claim 1, wherein the organic substrate culture solution and the organic product culture solution are combined in one bioreactor culture vessel, wherein the non-native organic substrate forming recombinant enzyme expressing nucleotide sequence is inserted into a first microbial expression vector, wherein the non-native organic product forming enzyme expressing nucleotide sequence is inserted into a second microbial
30 expression vector, wherein the first and second microbial expression vector each includes at least one microbial expression promoter,

providing a carbon source connected to the carbon source inlet;

35 culturing the first recombinant microorganism in the first bioreactor culture vessel under conditions sufficient to produce an amount of the at least one organic substrate in the first bioreactor culture vessel;

producing the amount of the at least one organic substrate by adding at least one promoter inducer to the organic substrate culture solution at a first time point;

5 culturing the second recombinant microorganism in the second bioreactor culture vessel under conditions sufficient to produce an amount of the at least one organic product in the second bioreactor culture vessel; and

producing the amount of the at least one organic product by adding at least one promoter inducer to the organic product culture solution at a second time point.

22. The method of claim 21, further comprising lowering an amount of oxygen in the
10 second bioreactor culture vessel to from about 10% by volume to about 1% by volume or less, based on a total internal volume of the second bioreactor culture vessel, before the second time point.