PRODUCTION AND SEPARATION OF 3-HYDROXYPROPIONIC ACID

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority under 35 U.S.C. §119(e) of U.S. Provisional Application No. 62/577,361, filed on October 26, 2017 and Provisional Application No. 62/594,318, filed on December 4, 2017, the entire contents of each of which are incorporated herein by reference.

TECHNICAL FIELD

This disclosure relates to the production of 3-hydroxypropionic acid, including its production from glycerol by recombinant strains, as well as its removal from an aqueous solution (e.g., aqueous broth), and use thereof for producing various chemicals.

BACKGROUND

3-Hydroxypropionic acid (3-HP) and 3-hydroxypropanoate (a salt of 3-HP) are used in the industrial production of various chemicals, such as acrylic acid, which can be used as a cross-linking agent for polymer coatings, metal lubricants and antistatic agents for textiles.

SUMMARY

The disclosure provides methods and apparatus for producing 3-HP or a salt thereof, for removing 3-HP from aqueous solution (e.g., aqueous broth), and for using it to make various chemicals.

In some ~~embodiment~~examples, this disclosure provides methods of producing 3-HP or a salt thereoff from glycerol using a genetically modified strain of *Pseudomonas denitrificans.*

In certain ~~embodiment~~examples, this disclosure provides methods to create *Pseudomonas* strains that can produce 3-HP or a salt thereof at a high titer without external supplementation of coenzyme B12.

In some ~~embodiment~~examples, this disclosure provides an expression system (e.g., an expression module or an expression construct) for use in *Pseudomonas* strains. Such an expression system can include, *inter alia,* a tandem promoter system which includes two

or more promoters operably linked to control expression of a downstream gene. Such a promoter can be an inducible promoter or a constitutive promoter. Optionally, an expression system includes at least one inducible promoter. An expression system can be used to produce target proteins/enzymes or to enhance metabolic pathways to produce target products at high titer, such as 3-HP or a salt thereof, by minimizing toxic intermediates, and/or increase vitamin B12 production, a cofactor in the first reaction in the 3-HP synthetic pathway.

In some ~~embodiment~~examples, this disclosure provides nucleic acids that encode these systems, the recombinant bacteria expressing these systems, and/or the methods of culturing the bacteria to produce 3-HP or a salt thereof. These bacteria have been modified to include one or more expression systems that respond to the target product levels, in this case 3-HP or a salt thereof in the media and express the genes used for the production of the target product, e.g., 3-HP or a salt thereof. In some cases, these bacteria have also been modified to include one or more expression systems to increase expression of the genes used for the production of B12. The conditions for culturing said bacterium, to increase 3-HP production (or a salt thereof), are also provided herein.

In one ~~aspect~~example, the disclosure provides for an expression system, comprising, consisting of, or consisting essentially of a first promoter that is inducible by a small molecule, a second promoter, a first gene encoding a protein involved in the synthesis of 3-HP (or a salt thereof) or B12, a modified UTR, and a native sequence encoding up to 20 amino acids from the N-terminus of a native *Pseudomonas denitrificans* protein, wherein the native sequence is operably linked to the 3'-terminus of the modified UTR and operably linked to the 5'-terminus of the first gene, and the first and second promoters are operably linked in tandem upstream of the modified UTR.

In some ~~embodiment~~examples ~~of all aspects~~, the expression system further comprises, consists of, or consists essentially of a third promoter; and a second gene encoding a transcriptional regulator configured to regulate expression of the first gene, wherein the third promoter is operably connected to the second gene. In some instances, the second promoter is inducible by the small molecule. In some cases, the native sequence is operably linked to the 5'-terminus of the first gene to define a fusion gene, the second promoter is a native promoter for the native *Pseudomonas denitrificans* protein, and the

second promoter is operably linked to the 5'-terminus of the fusion gene. In some ~~embodiment~~examples the first promoter is operably linked to the 5'-terminus of the second promoter, the second promoter is operably linked to the 5'-terminus of the modified UTR, and the modified UTR is operably linked to the 5'terminus of the fusion gene.

In one aspect, the disclosure provides a nucleic acid, comprising a first and a second promoter, wherein either the first is an inducible promoter and is inducible by a small molecule; the first and second promoters are operably linked to a first gene; and the first gene encodes a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP), a salt of 3-HP, or coenzyme B12, wherein at least the 10 codons at the 5'-terminus of the first gene are optimized for translation in Pseudomonas denitrificans, and wherein codon optimization comprises:

measuring a codon frequency of each amino acid in a gene encoding a native *Pseudomonas denitrificans* protein; and

using the codon frequency of the native protein to replace the 10 codons at the 5'-terminus of the first gene with an optimized 10 codons, wherein the codon for each amino acid of the optimized 10 codons is present at the same frequency as the codon frequency of the native protein,

wherein the gene encoding a native *Pseudomonas denitrificans* protein is mmsA.

In one ~~aspect~~example, the disclosure provides for a nucleic acid, comprising, consisting of, or consisting essentially of a first and a second promoter, wherein the first promoter is an inducible promoter and is inducible by a small molecule; the first and second promoters are operably linked to a first gene; and the first gene encodes a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP)(or a salt thereof) or coenzyme B12.

In some ~~embodiment~~examples ~~of all aspects~~, the first gene encodes a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP) (or a salt thereof) and is selected from the group consisting of a glycerol dehydratase, a glycerol dehydratase reactivase, and an aldehyde dehydrogenase. In some cases, the first gene comprises at least one gene selected from the group consisting of dhaB1, dhaB2, dhaB3, gdrA, gdrB, and kgsA. In some instances, the first gene comprises a dhaB1 gene, a dhaB2 gene, a dhaB3 gene, a gdrA gene, and a gdrB gene.

In some ~~embodiment~~examples, the first gene is dhaB1, and the sequence comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 1. In some cases, the first gene is dhaB2, and the sequence comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 2. In some instances, the first gene is dhaB3, and the sequence comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 3.

In some ~~embodiment~~examples ~~of all aspects~~, the first gene is gdrA, and the sequence comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 4. In some cases, the first gene is grdB and the sequence comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 5.

In some ~~embodiment~~examples ~~of all aspects~~, the first gene comprises, consists of, or consists essentially of an aldehyde dehydrogenase gene. In some instances, the aldehyde dehydrogenase is kgsA. In some cases, kgsA comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 6.

In some ~~embodiment~~examples ~~of all aspects~~, the small molecule is an acid or alcohol. In some cases, the small molecule acid or alcohol is selected from the group consisting of L-lactic acid (LAC), acetic acid (AcOH), propionic acid (PA), 3-hydroxypropionic acid (3-HP), 3-hydroxybutyrate (3-HB), 1,3-propanediol (1,3-PDO), 2,3-butanediol (2,3-BDO), L-valine (L-val), and 3-hydroxyisobutyrate (3-HIB), or salts thereof. In some instances, the small molecule acid or alcohol is selected from the group consisting of 3-hydroxypropionic acid (3-HP), 3-hydroxybutyrate (3-HB), L-valine (L-val), and 3-hydroxyisobutyrate (3-HIB). In some cases, the small molecule is 3-hydroxypropionic acid (3-HP) (or a salt thereof).

In some ~~embodiment~~examples ~~of all aspects~~, the nucleic acid comprises, consists of, or consists essentially of a sequence that is at least 85% identical to SEQ ID NO: 65. In some cases, the nucleic acid comprises, consists of, or consists essentially of SEQ ID NO: 65.

In some ~~embodiment~~examples ~~of all aspects~~, the second promoter is operably linked to the 3'-terminus of the first promoter and the first gene is operably downstream of the second promoter. In some ~~embodiment~~examples ~~of all aspects~~, the first promoter is operably linked to the 3'-terminus of the second promoter and the first gene is operably downstream of the second promoter.

In some ~~embodiment~~examples ~~of all aspects~~, the second promoter is a constitutive promoter. In some instances, the second promoter is a second inducible promoter. In some cases, the intergenic space between the first and the second promoter does not include a terminator sequence. In some instances, first promoter and the second promoter regulate expression of the first gene.

In some ~~embodiment~~examples ~~of all aspects~~, the first promoter is derived from a P_{mmsA} promoter, a P_{hbdH-1} promoter, a P_{hbdH-4} promoter, or a P_{hpdH} promoter. In some instances, the first promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 14 (PmmsA promoter). In some cases, the first promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 13 (PhbdH-1 promoter). In some instances, the first promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 11 (PhbdH-4 promoter). In some cases, the first promoter comprises,

consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 12 (PhpdH promoter). In some instances, the first promoter comprises, consists of, or consists essentially of a sequence that is selected from the group consisting of SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; and SEQ ID NO: 14.

In some ~~embodiment~~examples ~~of all aspects~~, the first promoter comprises, consists of, or consists essentially of a P_{mmsA} promoter and the nucleic acid further comprises an operator site operably linked to the 5'-terminus of the P_{mmsA} promoter. In some cases, the nucleic acid described here in comprises, consists of, or consists essentially of a sequence that is 95% identical to SEQ ID NO: 15. In some instances, the nucleic acid described herein comprises, consists of, or consists essentially of a sequence that is 95% identical to SEQ ID NO: 16 (O1 of MmsA operator) and a sequence that is 95% identical to SEQ ID NO: 17 (O2 of MmsA operator). In some ~~embodiment~~examples ~~of all aspects~~, the nucleic acid described herein comprises, consists of, or consists essentially of a sequence that is 95% identical to SEQ ID NO: 18 (PmmsA and operator Fig. 3). In some cases, the nucleic acid described herein comprises, consists of, or consists essentially of a sequence selected from the group consisting of SEQ ID NO: 15, 18, 19 (PmmsA2a), 20 (PmmsA2b), and 21 (PmmsA2ab).

In some ~~embodiment~~examples ~~of all aspects~~, the second promoter is derived from a P_{mmsA} promoter, a P_{hbdH-1} promoter, a P_{hbdH-4} promoter, a P_{hpdH} promoter, or a P_{zwf} promoter. In some cases, the second promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 11 (PhbdH-4 promoter). In some instances, the second promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 12 (PhpdH promoter). In some ~~embodiment~~examples ~~of all aspects~~, the second promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 14 (PmmsA promoter). In some cases, the second promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 13 (PhbdH-1 promoter). In some ~~embodiment~~examples ~~of all aspects~~, the second promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 7 (Pzwf). In some cases, the second promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 8 (Pzwf-1). In some

instances, the second promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 9 (Pzwf-7). In some ~~embodiment~~examples ~~of all aspects~~, the second promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 62 (Pzwf-7 shorter). In some cases, the second promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 10 (Pzwf-12). In some instances, the second promoter comprises, consists of, or consists essentially of a sequence selected from the group consisting of SEQ ID NO: 7, 8, 9, 10, 11, 12, 13, 14, 52-60, 61, 62, and 63 (e.g., Pzwf promoters).

In some ~~embodiment~~examples ~~of all aspects~~, the first promoter comprises, consists of, or consists essentially of the P_{mmsA} promoter and the second promoter comprises, consists of, or consists essentially of the P_{hbdH-4} promoter. In some cases, the first promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 14 (PmmsA promoter sequence), and the second promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 11 (PhbdH-4 promoter). In some instances, the first promoter comprises, consists of, or consists essentially of the PhbdH-1 promoter and the second promoter comprises, consists of, or consists essentially of the PhpdH promoter. In some cases, the first promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 13 (PhbdH-1 promoter sequence), and the second promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 12 (PhpdH promoter).

In some ~~embodiment~~examples ~~of all aspects~~, the second promoter comprises, consists of, or consists essentially of a PhbdH promoter and the nucleic acid further comprises, consists of, or consists essentially of an operator site operably linked to the N-terminus of the PhbdH promoter. In some cases, the operator site comprises, consists of, or consists essentially of one or more sequence that is at least 95% identical to SEQ ID NO: RBS-1, RBS-2, ABS-1, ABS-2, and ABS-3. In some instances, the operator site comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NOs: RBS-1 site and ABS-2. In some cases, the operator site comprises, consists of, or consists essentially of SEQ ID NOs: RBS-1 site and ABS-2.

In some ~~embodiment~~examples ~~of all aspects~~, the nucleic acid further comprises, consists of, or consists essentially of a gene encoding a transcriptional regulator that regulates expression of the first gene. In some instances, the transcriptional regulator binds to the first or second promoter. In some cases, the transcriptional regulator is a LysR-type transcriptional regulator (LTTR). In some ~~embodiment~~examples, the transcriptional regulator is MmsR or HpdR. In some cases, the transcriptional regulator is MmsR.

In some ~~embodiment~~examples ~~of all aspects~~, the transcriptional regulator binds to the first promoter. In some cases, the transcriptional regulator is derived from a mmsR protein and the first promoter is derived from a PmmsA promoter. In some cases, the transcriptional regulator comprises, consists of, or consists essentially of a mmsR protein and the first promoter comprises, consists of, or consists essentially of a PmmsA promoter.

In some ~~embodiment~~examples ~~of all aspects~~, the nucleic acid described herein further comprises, consists of, or consists essentially of an operator site, wherein the MmsR protein binds to the operator site. In some cases, the nucleic acid described herein comprises, consists of, or consists essentially of a sequence that is at least 95% identical to SEQ ID NO: 19 (PmmsA2a). In some instances, the nucleic acid described herein comprises, consists of, or consists essentially of a sequence that is selected from the group consisting of SEQ ID NO: 19-21 (PmmsA2a, A2b, A2ab). In some cases, the transcriptional regulator binds to the second promoter. In some ~~embodiment~~examples ~~of all aspects~~, the second promoter is a constitutive promoter. In some cases, the transcriptional regulator is a HpdR protein and the second promoter is derived from a hpdH promoter. In some instances, the second promoter comprises, consists of, or consists essentially of a sequence that is at least 95% identical to the sequence of P_{hpdH} SEQ ID NO: 12.

In some ~~embodiment~~examples ~~of all aspects~~, the transcriptional regulator binds to the first promoter and has enhanced binding to the first promoter in the presence of the small molecule. In some cases, the transcriptional regulator binds to the second promoter and has enhanced binding to the second promoter in the presence of the small molecule. In some instances, the transcriptional regulator is self-regulating. In some cases, the nucleic

acid described herein further comprises, consists of, or consists essentially of a third promoter that is operably linked to the gene encoding the first transcriptional regulator.

In some ~~embodiment~~examples ~~of all aspects~~, the third promoter comprises, consists of, or consists essentially of a sequence that is 95% identical to the sequence selected from the group consisting of SEQ ID NO: 7-10 and 52-63. In some cases, the second constitutive promoter is selected from the group consisting of SEQ ID NOs: 9, 10, 57-60, 62, and 63; the transcriptional regulator comprises, consists of, or consists essentially of a MmsR protein; and the first promoter comprises, consists of, or consists essentially of P_{mmsA}. In some instances, the second constitutive promoter is selected from the group consisting of SEQ ID NOs: 9, 10, 57-60, 62, and 63; the transcriptional regulator comprises, consists of, or consists essentially of a HpdR protein; and the first promoter comprises, consists of, or consists essentially of P_{hbdH}. In some cases, the third promoter comprises, consists of, or consists essentially of SEQ ID NO: 10. In some cases, the third promoter comprises, consists of, or consists essentially of SEQ ID NO: 62.

In some ~~embodiment~~examples ~~of all aspects~~, the first gene comprises, consists of, or consists essentially of a modified 5' UTR of the gene. In some instances, the first gene comprises, consists of, or consists essentially of a sequence selected from the group consisting of SEQ ID NO: 22-28 and 64. (UTR 0-6). In some cases, the 5' UTR comprises, consists of, or consists essentially of SEQ ID NO: 28 (UTR-6). In some instances, the first gene comprises, consists of, or consists essentially of a gene encoding kgsA and the 5'UTR comprises, consists of, or consists essentially of SEQ ID NO: 28 (UTR-6).

In some ~~embodiment~~examples ~~of all aspects~~, at least the 10 codons at the 5'terminus of the first gene are optimized for translation in *Pseudomonas denitrificans*. In some cases, up to 10 codons at the 5'terminus of the first gene are optimized for translation in *Pseudomonas denitrificans*. In some instances, codon optimization comprises, consists of, or consists essentially of: measuring a codon frequency of each amino acid in a gene encoding a native *Pseudomonas denitrificans* protein; using the codon frequency of the native protein to replace the 10 codons at the 5'-terminus of the first gene with an optimized 10 codons, wherein the codon for each amino acid of the optimized 10 codons is present at the same frequency as the codon frequency of the native protein. In some

cases, the codon frequency for each amino acid in the optimized second 10 codons is more than 0. In some instances, the codon frequency is measured of each amino acid in the 10 codons at the 5' terminus of the gene encoding the native *Pseudomonas denitrificans* protein.

In some ~~embodiment~~examples ~~of all aspects~~, the nucleic acid comprises, consists of, or consists essentially of a sequence that is 95% identical to a sequence selected from the group consisting of SEQ ID NOs: 29, 30, and 31. (Opt1-3). In some cases, the first gene comprises, consists of, or consists essentially of a gene encoding kgsA and comprises, consists of, or consists essentially of a sequence selected from the group consisting of SEQ ID NO: 29-31. In some instances, the first gene is fused at its 5'-terminus to a sequence encoding up to 20 amino acids that are derived from the 5'terminus of a second gene encoding a native *Pseudomonas denitrificans* protein, thereby creating a fusion gene. In some cases, the mRNA of the fusion gene has a higher stability than the mRNA of the first gene alone. In some instances, the fusion gene increases gene translation when compared to the first gene alone. In some ~~embodiment~~examples ~~of all aspects~~, the mRNA of the fusion gene is more resistant to ribonuclease degradation than the mRNA of the first gene alone. In some cases, the fusion gene increases gene translation in comparison to the first gene alone.

In some ~~embodiment~~examples ~~of all aspects~~, the first gene is fused at its 5'-terminus to a sequence encoding up to 20 (5, 10, 15, or 20) amino acids that are derived from the 5'terminus of a second gene encoding a native *Pseudomonas denitrificans* protein, thereby creating a fusion gene, and the second promoter is derived from the native promoter for the second gene. In some cases, the second promoter is derived from the P_{mmsA} promoter and the fusion gene comprises, consists of, or consists essentially of a sequence encoding at least 5, 10, 15, or 20 amino acids in the N-terminus of a native MmsA protein. In some instances, the second promoter comprises, consists of, or consists essentially of the P_{mmsA} promoter and the fusion gene comprises, consists of, or consists essentially of a sequence encoding five or more amino acids from the N-terminus of a native MmsA gene, and the fusion gene is operably linked to the 3' end of the P_{mmsA} promoter. In some cases, the nucleic acid comprises, consists of, or consists essentially of a sequence selected from the group consisting of SEQ ID NOs: 32-35.

In some ~~embodiment~~examples ~~of all aspect~~, the first gene is fused at its 5'-terminus to a sequence encoding up to 20 (5, 10, 15, or 20) amino acids that are derived from a sequence encoding the N-terminus of a native *Pseudomonas denitrificans* MmsA protein. In some cases, the nucleic acid described herein comprises, consists of, or consists essentially of a sequence selected from the group consisting of SEQ ID NOs: 32-35 (Pcm-mmsA(5)(10)(15)(20)).

In some ~~embodiment~~examples ~~of all aspect~~, the first gene encodes a protein involved in the synthesis of vitamin B12. In some cases, the first gene comprises, consists of, or consists essentially of a gene selected from the group consisting of cobJ, cobI, cobH, cobG, cobL, cobF, cobK, gst, xre,chlD, chlI, dahp, cobN, cobW, cbtBA, cobE, cobM, btuB, cobO, cob, cobR, cobD, cobC, cobQ, cobU, cobP, bgpM, and cobV. In some instances, the first gene comprises, consists of, or consists essentially of bgpM. In some cases, the first gene is derived from a *Pseudomonas* species selected from the group consisting of *P. denitrifcans; P. aeruginosa; P. entomophila; P. putida; P. syringae; P. fluorescens; P mendocina;* and *P. stutzeri.*

In another ~~aspect~~example, the disclosure provides for a nucleic acid comprising, consisting of, or consisting essentially of a first promoter operably linked to a first gene that encodes a protein involved in the synthesis of coenzyme B12. In some cases, the first promoter is a constitutive promoter. In some instances, the nucleic acid comprises, consists of, or consists essentially of a sequence selected from SEQ ID NOs: 7-10 and 52-63. In some ~~embodiment~~examples ~~of all aspects~~, the first gene comprises, consists of, or consists essentially of one or more genes selected from the group consisting of cobJ, cobI, cobH, cobG, cobL, cobF, cobK, gst, xre,chlD, chlI, dahp, cobN, cobW, cbtBA, cobE, cobM, btuB, cobO, cob, cobR, cobD, cobC, cobQ, cobU, cobP, bgpM, and cobV. In some cases, the first gene is from a *Pseudomonas* species selected from the group consisting of *P. denitrifcans; P. aeruginosa; P. entomophila; P. putida; P. syringae; P. fluorescens; P mendocina;* and *P. stutzeri.*

In some ~~embodiment~~examples ~~of all aspects~~, the nucleic acid described herein further comprises, consists of, or consists essentially of a second promoter operably linked to the first promoter. In some cases, the first and the second promoter regulate expression of

the first gene. In some instances, the first promoter is not a native promoter for the first gene.

In another ~~aspect~~example, the disclosure provides for a nucleic acid comprising a cobG gene, a P_{edd} promoter, a P_{sucA} promoter, and a cobL gene; wherein the P_{edd} promoter is operably linked to the cobG gene and the P_{sucA} promoter, and the P_{sucA} promoter is operably linked to the cobL gene. In some cases, the P_{edd} promoter comprises, consists of, or consists essentially of the sequence SEQ ID NO: 36; and the P_{sucA} promoter comprises, consists of, or consists essentially of the sequence SEQ ID NO: 37. In some ~~embodiment~~examples, the nucleic acid described herein comprises, consists of, or consists essentially of the sequence SEQ ID NO: 38.

In one ~~aspect~~example, the disclosure provides for a nucleic acid comprising, consisting of, or consisting essentially of a cobG gene, a P_{sp9} promoter, a P_{zwf} promoter, and a cobL gene; wherein the P_{sp9} promoter is operably linked to the cobG gene and the P_{zwf} promoter, and the P_{zwf} promoter is operably linked to the cobL gene. In some cases, the P_{sp9} promoter comprises, consists of, or consists essentially of SEQ ID NO: 39; and the P_{zwf} promoter comprises SEQ ID NO: 40. In some instances, the nucleic acid described herein comprises, consists of, or consists essentially of SEQ ID NO: 41.

In one ~~aspect~~example, the disclosure provides for a nucleic acid comprising, consisting of, or consisting essentially of a cobW gene, a P_{zwf} promoter, a P_{sp9} promoter, and a cbtB gene, wherein the P_{zwf} promoter is operably linked to the cobW gene and the P_{sp9} promoter, and the P_{sp9} promoter is operably linked to the cbtB gene. In some cases, the Pzwf promoter comprises SEQ ID NO: 42; and the Psp9 promoter comprises SEQ ID NO: 43. In some instance, the nucleic acid described herein comprises, consists of, or consists essentially of SEQ ID NO: 44.

In one ~~aspect~~example, the disclosure provides for a nucleic acid comprising, consisting of, or consisting essentially of a cobW gene, a P_{tkt} promoter, a P_{sp2} promoter, and a cbtB gene, wherein the P_{tkt} promoter is operably linked to the cobW gene and the P_{sp2} promoter, and the P_{sp2} promoter is operably linked to the cbtB gene. In some instances, the P_{tkt} promoter comprises, consists of, or consists essentially of SEQ ID NO: 45; and the Psp2 promoter comprises, consists of, or consists essentially of SEQ ID NO: 46. In

some cases, the nucleic acid described herein comprises, consists of, or consists essentially of SEQ ID NO: 47.

In one ~~aspect~~example, the disclosure provides for a nucleic acid comprising, consisting of, or consisting essentially of a P_{zwf} promoter operably linked to a tonB gene (e.g., a butB gene). In some cases, the Pzwf promoter comprises, consists of, or consists essentially of SEQ ID NO: 48. In some instances, the nucleic acid described herein comprises, consists of, or consists essentially of SEQ ID NO: 49.

In one ~~aspect~~example, the disclosure provides for a nucleic acid comprising, consisting of, or consisting essentially of a P_{sp9} promoter operably linked to a tonB gene (e.g., a butB gene). In some cases, the Psp9 promoter comprises, consists of, or consists essentially of SEQ ID NO: 50. In some instances, the nucleic acid described herein comprises, consists of, or consists essentially of SEQ ID NO: 51.

In some ~~embodiment~~examples ~~of all aspects~~, the nucleic acid comprises, consists of, or consists essentially of one or more sequences derived from *Pseudomonas denitrificans*. In some cases, the first or the second promoters are derived from *Pseudomonas denitrificans*. In some instances, the nucleic acid comprises, consists of, or consists essentially of one or more sequences derived from an Enterobacter, Lactobacillus, Pseudomonas, or Azospirillum bacterium. In some cases, the first gene is derived from an Enterobacter, Lactobacillus, Pseudomonas, or Azospirillum bacterium.

In one ~~aspect~~example, the disclosure provides for a nucleic acid comprising a DhaB expression module that comprises, consists of, or consists essentially of a sequence that is at least 85% identical to SEQ ID NO: 66 (DhaB expression module). In some cases, the sequence is at least 90% identical to SEQ ID NO: 66. In some instances, the nucleic acid described herein comprises, consists of, or consists essentially of SEQ ID NO: 66.

In one ~~aspect~~example, the disclosure provides for a nucleic acid comprising a DhaB expression module that comprises, consists of, or consists essentially of a sequence that is at least 85% identical to SEQ ID NO: 67 (kgsA expression module). In some cases, the sequence is at least 90% identical to SEQ ID NO: 67. In some instances, the sequence comprises, consists of, or consists essentially of SEQ ID NO: 67.

In one ~~aspect~~example, the disclosure provides for a nucleic acid comprising a DhaB expression module comprising, consisting of, or consisting essentially of a UTR of the

gdrA gene comprising, consisting of, or consisting essentially of SEQ ID NO: 68 and a UTR of the *gdrB* gene comprising, consisting of, or consisting essentially of SEQ ID NO: 69. In some cases, the nucleic acid comprises, consists of, or consists essentially of a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NO: 70-72. In some instances, the nucleic acid comprises, consists of, or consists essentially of a sequence selected from the group consisting of SEQ ID NO: 70-72.

In one ~~aspect~~example, the disclosure provides for a nucleic acid comprising a coenzyme B12 sensor comprising, consisting of, or consisting essentially of a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NO: 73 and 74. In some cases, the nucleic acid described herein comprises, consists of, or consists essentially of a sequence selected from the group consisting of SEQ ID NO: 73 and 74.

In one ~~aspect~~example, the disclosure provides for a nucleic acid comprising, consisting of, or consisting essentially of a coenzyme B12 expression module that comprises, consists of, or consists essentially of a first gene involved in the synthesis of coenzyme B12, a first promoter that is induced by a small molecule, and a second promoter, wherein the first and second promoters are operably linked in tandem upstream of the first gene such that they regulate expression of the first gene. In some cases, the nucleic acid further comprises, consists of, or consists essentially of a second gene involved in the synthesis of coenzyme B12, a third promoter that is induced by a small molecule, and a fourth promoter, wherein the third and fourth promoters are operably linked in tandem upstream of the second gene such that they regulate expression of the second gene. In some ~~embodiment~~examples ~~of all aspects~~, the nucleic acid further comprises, consists of, or consists essentially of a third gene involved in the synthesis of coenzyme B12, a fifth promoter that is induced by a small molecule, and a sixth promoter, wherein the fifth and sixth promoters are operably linked in tandem upstream of the third gene such that they regulate expression of the third gene. In some cases, the nucleic acid further comprises, consists of, or consists essentially of a fourth gene involved in the synthesis of coenzyme B12, a seventh promoter that is induced by a small molecule, and a eighth promoter, wherein the seventh and eighth promoters are operably linked in tandem upstream of the

fourth gene such that they regulate expression of the fourth gene. In some instances, the first, second, third, and fourth genes each comprise, consist of, or consist essentially of one or more genes selected from the group consisting of cobJ, cobI, cobH, cobG, cobL, cobF, cobK, gst, xre,chlD, chlI, dahp, cobN, cobW, cbtBA, cobE, cobM, btuB, cobO, cob, cobR, cobD, cobC, cobQ, cobU, cobP, bgpM, and cobV. In some cases, the first gene comprises, consists of, or consists essentially of bgpM.

In some ~~embodiment~~examples ~~of all aspects~~ the nucleic acid does not include any native riboswitches that regulate genes involved in the production of coenzyme B12. In some cases, the nucleic acid does not include SEQ ID NOs: 75 or 76.

In another ~~aspect~~example, the disclosure provides for a recombinant bacterium comprising, consisting of, or consisting essentially of the nucleic acid described herein. In some cases, the bacterium is a *Pseudomonas* species. In some instances, the bacterium is *Pseudomonas denitrificans*.

In some ~~embodiment~~examples ~~of all aspects~~, the nucleic acid is on an expression plasmid. In some cases, the nucleic acid is integrated into the bacterium's chromosome. In some instances, the nucleic acid is on an episome.

In another ~~aspect~~example, the disclosure provides for a recombinant bacterium comprising, consisting of, or consisting essentially of a first nucleic acid comprising, consisting of, or consisting essentially of a DhaB expression module and a second nucleic acid comprising, consisting of, or consisting essentially of a ALDH expression module. In some cases, the DhaB expression module comprises, consists of, or consists essentially of any one of the nucleic acids described herein. In some instances, the ALDH expression module comprises, consists of, or consists essentially of any one of the nucleic acids described herein.

In some ~~embodiment~~examples ~~of all aspects~~, the first and the second nucleic acids are on expression plasmids. In some cases, the first and the second nucleic acids are on episomes. In some instances, the first and the second nucleic acids are integrated into the bacterium's chromosome. In some cases, the first nucleic acid is integrated into the bacterium's chromosome and the second nucleic acid is on an expression plasmid. In some instances, the first nucleic acid is on an expression plasmid and the second nucleic acid is integrated into the bacterium's chromosome. In some ~~embodiment~~examples ~~of all aspects~~,

the first nucleic acid is integrated into the bacterium's chromosome and the second nucleic acid is on an episome. In some instances, the first nucleic acid is on an episome and the second nucleic acid is integrated into the bacterium's chromosome.

In some ~~embodiment~~examples ~~of all aspects~~, the first nucleic acid is integrated into the bacterium's chromosome at a first position and the second nucleic acid is integrated into the bacterium's chromosome at a second position; and the first and second positions are within 2500 kilo base pairs of each other. In some cases, the first and second positions are within 100 kilo base pairs of each other. In some instances, the first and second positions are within 50 kilo base pairs of each other. In some cases, the first and second positions are within 1000 base pairs of each other.

In some ~~embodiment~~examples ~~of all aspects~~, the first nucleic acid is integrated into the bacterium's chromosome at a first position that is within 4000 base pairs of the origin of replication. In some cases, the first position is within 1000 base pairs of the origin of replication.

In another ~~aspect~~example, the disclosure provides for a recombinant bacterium comprising, consisting of, or consisting essentially of a coenzyme B12 expression module that comprises, consists of, or consists essentially of a first gene involved in the synthesis of coenzyme B12, a first promoter that is induced by a small molecule, and a second promoter, wherein the first and second promoters are operably linked in tandem upstream of the first gene.

In some ~~embodiment~~examples ~~of all aspects~~, the bacterium is a *Pseudomonas denitrificans* bacterium. In some cases, the bacterium comprises, consists of, or consists essentially of a deletion at a first position of a first riboswitch which regulates expression of the first gene, and the first and second promoters are integrated into the bacterium's chromosome at the first position such that they regulate expression of the first gene. In some instances, the bacterium further comprises, consists of, or consists essentially of a second deletion at a second position of a second riboswitch which regulates expression of a second gene involved in the synthesis of coenzyme B12, a third deletion at a third position of a third riboswitch which regulates expression of a third gene involved in the synthesis of coenzyme B12, and a fourth deletion at a fourth position of a fourth riboswitch which regulates expression of a fourth gene involved in the synthesis of coenzyme B12. In

some cases, the recombinant bacterium further comprises, consists of, or consists essentially of a third promoter and a fourth promoter which are operably linked in tandem and integrated into the chromosome at the second position such that they regulate expression of the second gene; a fifth promoter and a sixth promoter which are operably linked in tandem and integrated into the chromosome at the third position such that they regulate expression of the third gene; and a seventh and eighth promoter which are operably linked in tandem and integrated into the chromosome at the fourth position such that they regulate expression of the fourth gene. In some ~~embodiment~~example~~s of all aspects~~, the bacterium produces more coenzyme B12 than native *Pseudomonas denitrificans*. In some ~~embodiment~~example~~s of all aspects~~, the recombinant bacterium described herein further comprises, consists of, or consists essentially of a coenzyme B12 expression module.

In another ~~aspect~~example, the disclosure provides for a method of producing 3-HP or a salt thereof, comprising, consisting of, or consisting essentially of culturing a recombinant bacterium described herein under conditions sufficient to produce 3-HP or a salt thereof. In some cases, the conditions are sufficient to produce at least 85 g/L of 3-HP or a salt thereof. In some instances, the conditions are sufficient to produce at least 90 g/L of 3-HP or a salt thereof. In some ~~embodiment~~example~~s of all aspects~~, the conditions are sufficient to produce at least 95 g/L of 3-HP or a salt thereof. In some cases, the conditions are sufficient to produce at least 100 g/L of 3-HP or a salt thereof.

In some ~~embodiment~~example~~s of all aspects~~, culturing the bacterium does not include the addition of external coenzyme B12. In some cases, culturing the bacterium comprises addition external coenzyme B12 to the culture.

In some cases, culturing comprises, consists of, or consists essentially of growing the bacterium in a media comprising a carbon source selected from the group consisting of glycerol, glucose, gluconate, glutamate, and citric acid. In some instances culturing comprising, consisting of, or consisting essentially of inducing the bacterium to produce 3-HP or a salt thereof by adding glycerol. In some cases, the glyercol is add mid-log phase.

In some ~~embodiment~~example~~s of all aspects~~, culturing comprises, consists of, or consists essentially of growing the bacterium in a media comprising a nitrogen source selected

from the group consisting of yeast extract, corn steep liquor powder, and corn steep liquor paste.

In some ~~embodiment~~examples ~~of all aspects~~, culturing comprises, consists of, or consists essentially of growing the bacterium at a pH between 6.8 and 7.8. In some cases, culturing comprises, consists of, or consists essentially of adding one or more bases selected from the group consisting of NaOH, KOH, $NaHCO_3$, NH_4HCO_3, NH_4OH, $(NH4)_2CO_3$, and Na_2CO_3. In some instances, culturing comprises, consists of, or consists essentially of adding at least one base selected from the group consisting of NaOH, KOH, $NaHCO_3$, NH_4HCO_3, NH_4OH, $(NH_4)_2CO_3$, and Na_2CO_3. In some cases, culturing comprises, consists of, or consists essentially of diluting the base concentration and increasing the acid concentration (see FIG. 46 and FIG. 49).

In some ~~embodiment~~examples ~~of all aspects~~, culturing comprises, consists of, or consists essentially of growing the bacterium at a temperature between 28 and 40 degrees Celsius. In some cases, culturing comprises, consists of, or consists essentially of growing the bacterium at 33 degrees Celsius.

In another ~~aspect~~example, the disclosure provides for a method of producing 3-HP or a salt thereof, the method comprising, consisting of, or consisting essentially of: providing the recombinant bacterium described herein in a culture media suitable for growth of the bacterium and production of 3-HP or a salt thereof. In some cases the method described herein comprises, consists of, or consists essentially of separating 3-HP or a salt thereof from the bacterium or culture media.

In some instances, the culture media comprises, consists of, or consists essentially of a dissolved oxygen concentration that is approximately 2-20% dissolved oxygen. In some ~~embodiment~~examples ~~of all aspects~~ the dissolved oxygen concentration is no more than 20%. In some cases, the dissolved oxygen concentration is at least 2%.

Further, this disclosure relates to methods of providing and reacting 3-hydroxypropionic acid.

The disclosure provides methods for removing 3-hydroxypropionic acid (3-HP) from an aqueous solution. The methods can be relatively simple and inexpensive to perform. The methods can provide relatively high yields of 3-HP.

The disclosure also provides methods for making acrylic acid. The methods can be relatively simple and inexpensive to perform. The methods can be performed with little, or no, acrylic acid polymerization taking place. The methods can provide relatively high yields of acrylic acid.

In one ~~aspect~~example, the present disclosure provides a method removing 3-HP from an aqueous solution without using a counter-current liquid flow.

In another ~~aspect~~example, the present disclosure provides a method that includes evaporating a first solvent, condensing the evaporated first solvent, and directing a flow of the first solvent to remove 3-HP from the aqueous solution.

In another ~~aspect~~example, the present disclosure provides a method including using a water-immiscible solvent to remove 3-HP from an aqueous solution with a yield of at least 50%.

In another ~~aspect~~example, the present disclosure provides a method including using an organic liquid containing at least two solvents to remove 3-hydroxypropionic acid (3-HP) from an aqueous solution.

In another ~~aspect~~example, the present disclosure provides a method including using a liquid to remove 3-HP from an aqueous solution, wherein the liquid includes a two different solvents (e.g., a water-miscible solvent and a water-immiscible solvent).

In another ~~aspect~~example, the present disclosure provides a method including removing 3-HP from an aqueous solution, wherein pH of the aqueous solution is at least about 3.

In another ~~aspect~~example, the present disclosure provides a method that includes reacting 3-HP to form acrylic acid at a pressure of less than about one atmosphere.

In another ~~aspect~~example, the present disclosure provides a method that includes reacting 3-HP to form acrylic acid in a reaction mixture, and removing gaseous acrylic acid from the reaction mixture.

In another ~~aspect~~example, the present disclosure provides a method that includes reacting a liquid containing 3-HP to form acrylic acid.

In another ~~aspect~~example, the present disclosure provides a method that includes reacting 3-HP to form acrylic acid with a yield of at least about 70%.

~~Table 2: Fragments for EMSA used in this study.~~

Fragments	Sequence (5' – 3')[a]	Source
F_{12}	GTCAGCCTCAGCGCACCTCG**AATGTGCAA**AAACGCAGACCATAC**TTGCACATC**ACCGCATTGAGT ACATCA*AAAAATGCAC*TGTTAGGATCGA*TCCAGACA*ACAAAAAAGCCACAGGCTGGGAGAATCCCG	This study
F_{12M}	GTCAGCCTCAGCGCACCTCG**AATGTGCAA**AAACGCAGACCATAC**TTGCACATC**ACCGCATTGAGT ACATCA*AATCCTTGCT*TGTTAGGATCGCGCCC*AGGCACAA*AAAAAGCCACAGGCTGGGAGAATCCCG	This study
F_{1M2}	GTCAGCCTCAGCGCACCTCG**ATCCTTGCT**AAACGCAGACCATAC**CGCCCAGGC**ACCGCATTGAGT ACATCA*AAAAATGCAC*TGTTAGGATCGATCC*AGACAACA*AAAAAGCCACAGGCTGGGAGAATCCCG	This study
F_{1M2M}	GTCAGCCTCAGCGCACCTCG**ATCCTTGCT**AAACGCAGACCATAC**CGCCCAGGC**ACCGCATTGAGT ACATCA*AATCCTTGCT*TGTTAGGATCGCGCC*CAGGCACAAA*AAAGCCACAGGCTGGGAGAATCCCG	This study
DNaseI footprinting fragment	CCGCAGGTTGTCCCAGTCCATGTCAGCCTCAGCGCACCTCG**AATGTGCAA**AAACGCAGACCATAC **TTGCACATC**ACCGCATTGAGTACATCA*AAAAATGCAC*TGTTAGGATCGATCC*AGACAACA*AAAAAG CCACAGGCTGGGAGAATCCCGATGACCGCAACTGCCCCG	This study

[a]~~Bold letter indicated the half sites of O1 operator; italic letter indicated the half sites of O2 operator; underlined letter indicated site-directed mutagenesis region.~~

Table 2: Fragments for EMSA used in this study.

Fragments	Sequence (5' – 3')	Source
F_{12}	GTCAGCCTCAGCGCACCTCG**AATGTGCAA**AAACGCAGACCATAC**TTGCACATC**ACCGC ATTGAGTACATCA*AAAAATGCAC*TGTTAGGATCG*ATCCAGACA*ACAAAAAAGCCACAGG CTGGGAGAATCCCG	This study
F_{12M}	GTCAGCCTCAGCGCACCTCG**AATGTGCAA**AAACGCAGACCATAC**TTGCACATC**ACCGCAT TGAGTACATCA*ATCCTTGCT*TGTTAGGAT*CGCGCCCAGGCACAA*AAAAAGCCACAGGCTGG GAGAATCCCG	This study
F_{1M2}	GTCAGCCTCAGCGCACCTCG**ATCCTTGCT**AAACGCAGACCATAC**CGCCCAGGC**ACCGCAT TGAGTACATCA*AAAAATGCAC*TGTTAGGATCGATCC*AGACAACA*AAAAAGCCACAGGCTGG GAGAATCCCG	This study
F_{1M2M}	GTCAGCCTCAGCGCACCTCG**ATCCTTGCT**AAACGCAGACCATAC**CGCCCAGGC**ACCGCAT TGAGTACATCA*ATCCTTGCT*TGTTAGGATCGCGCC*CAGGCACAAA*AAAGCCACAGGCTGG GAGAATCCCG	This study
DNaseI footprinting fragment	CCGCAGGTTGTCCCAGTCCATGTCAGCCTCAGCGCACCTCG**AATGTGCAA**AAACGCAG ACCATAC**TTGCACATC**ACCGCATTGAGTACATCA*AAAAATGCAC*TGTTAGGATCGATCC*A GACAACAA*AAAAGCCACAGGCTGGGAGAATCCCGATGACCGCAACTGCCCCG	This study

[a]Bold letter indicated the half sites of O1 operator; italic letter indicated the half sites of O2 operator; underlined letter indicated site-directed mutagenesis region.

To analyze the MmsR binding regions *in vitro*, DNase I foot-printing analysis was performed by capillary electrophoresis (Fig. 7B). A longer 169 bp DNA fragment that includes the complete 130 bp intergenic region (used in the EMSA experiments described above), was used as probe (Table 2). The concentration of DNA was fixed at a specific concentration, while MmsR concentration varied at 0 – 1.2 μM. The foot-printing results clearly exhibit the presence of two protected regions by MmsR, which should correspond to operator O_1 (centered at position -81) and O_2 (centered at position -33), respectively. Protection became more evident as MmsR concentration increased. However, it was difficult to pinpoint the exact regions of protection at the base-pair level of the DNA sequence.

The EMSA experiments were repeated at a low DNA concentration (0.4 nM) to estimate the dissociation constant, K_D, between MmsR and the PmmsA promoter (Fig. 8). Three DNA fragments, one containing both the O_1 and O_2 operator sites (F_{12}) and the other two having the same length as F_{12} but mutated (randomized) in the palindromic regions of the O_1(F_{1M2}) or O_2 (F_{12M}) operator site, were employed. Among the three DNA

form of *cobG* (first 35 bp of *cobG* fused to the gfp) exhibited a 4.2-fold higher florescence, compared to its unfused gfp construct (Fig. 27, Table 6). However, we observed no significant difference in the florescence levels produced by other promoters systems (intergenic segments from other B12 operons) when the first 35 of *cobG* was fused to gfp, suggesting that they are not controlled at the translational initiation level. These results further suggest that P_{cobG}-RS1, tightly controls the expression of genes both at the transcription and translation initiation levels. The stringent regulation at transcription and translation signifies the importance of controlled expression of genes in the *cobGHIJ* operon.

The estimation of the strength of native promoters is crucial when overexpressing any pathways involving several genes, such as the vitamin B_{12} pathway, or pathways involving several membrane proteins. The overexpression of pathways with many genes can be deleterious to cell growth, and imbalanced expression of the pathway genes/operons might lead to the accumulation of toxic pathway intermediates. The analysis and quantification of native promoter strength can assist us in redesigning the operons with synthetic promoters without overexpressing the pathway enzymes during engineering. Table 5 shows the estimated promoter strengths (in relative florescence units) we observed for various native *cob* promoters in the operon. The promoter P_{cbtB} that controls the expression of cobalt transporter genes has the highest strength, and the P_{cobL} promoter has the lowest strength of this group. The genes encoding for methyltransferase enzymes appeared to usually be by weak promoter systems.

~~**Table 6. Characterization of *cob* regulon intergenic regions in *P. denitrificans*.**~~

Intergenic regions	Promoters	R/S control	Promoter strength (AU/OD_{600})
IR1	P_{cobG}	o	2200
IR1	P_{cobL}	x	1800
IR3	P_{cobW}	o	3,000
IR3	P_{cbtB}	o	5,000
IR4	P_{btuB}	o	2,800

Table 6: Characterization of *cob* regulon intergenic regions in *P. denitrificans*.

Intergenic regions	Promoters	R/S control	Promoter strength (AU/OD_{600})
IR1	P_{cobG}	O	2200
IR1	P_{cobL}	x	1800
IR3	P_{cobW}	O	3000
IR3	P_{cbtB}	O	5000
IR4	P_{btuB}	O	2800

Engineering coenzyme B12 production pathway

Rational engineering of B12 promoters requires quantification of promoter strengths along with their UTR structures (fused and unfused versions). Therefore, the native promoter strengths of cob operon, encoding coenzyme B12 synthetic pathway enzymes were meticulously quantified (Fig. 27, Table 6&10). Further, to increase the expression levels of these (cob) genes (by 3- and 5-folds relative to their native expression). The expression of cob operons were modified by replacing with suitable constitutive promoters (Fig. 57). The constitutive promoters were selected by screening and identifying their strengths of several native *Pseudomonas* promoters (Table 10). For instance, to develop Pedd-IR13-PsucA recombinant strain with three fold improved promoter strengths, PcobG and PcobL were replaced with constitutive promoters, Pedd and PsucA respectively (See Table 8, for more details).

Table 10. Strategy for B12 promoter replacement

Promoter	Native strength	Recombinant strength	
		3-fold	5-fold
P_{cobG}	2200	6600(P_{edd})	11000 (P_{sp9})
P_{cobL}	1800	5400 (P_{sucA})	9000(P_{zwf})
P_{cobW}	3000	9000 (P_{zwf})	15000(P_{tkt})
P_{cbtB}	5000	15000 (P_{sp9})	25000 (P_{sp2})
P_{btuB}	2800		14000 (P_{sp9})

regions in riboswitches 1, 2, and 3 that were randomly selected for mutation were not sufficient to eliminate function, therefore we did not see any alteration in coenzyme B_{12} production in the ΔRS1 and ΔRS2/RS3 mutant strains. On the other hand, the mutation in ΔRS4 likely affects protein expression, as the riboswitch was overlapping with the coding region. To overcome this limitation, instead of developing a deletion mutant, we modified riboswitch regions where the secondary structures in mRNA are mutationally neutralized, and at the same time the protein sequence is maintained by codon optimization.

~~Table 7. Transcription levels of B_{12} synthetic genes in the presence and absence of 25 mg/L $CoCl_2$.~~

Genes	WT	WT ($CoCl_2$)	ΔRS1	ΔRS1 ($CoCl_2$)	ΔRS2,3	ΔRS2,3 ($CoCl_2$)	ΔRS4	ΔRS4 ($CoCl_2$)
rpoD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
cobG	0.26	0.05	0.28	0.05	0.30	0.05	0.28	0.18
cobW	0.28	0.07	0.32	0.08	0.35	0.09	0.28	0.26
cbtB	0.94	0.16	0.88	0.19	0.82	0.22	0.92	0.63
btuB	0.17	0.04	0.17	0.04	0.17	0.04	0.13	0.13

Table 7: Transcription levels of B_{12} synthetic genes in the presence and absence of 25 mg/L $CoCl_2$.

Genes	WT	WT ($CoCl_2$)	ΔRS1	ΔRS1 ($CoCl_2$)	ΔRS2,3	ΔRS2,3 ($CoCl_2$)	ΔRS4	ΔRS4 ($CoCl_2$)
rpoD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
cobG	0.26	0.05	0.28	0.05	0.30	0.05	0.28	0.18
cobW	0.28	0.07	0.32	0.08	0.35	0.09	0.28	0.26
cbtB	0.94	0.16	0.88	0.19	0.82	0.22	0.92	0.63
btuB	0.17	0.04	0.17	0.04	0.17	0.04	0.13	0.13

We designed the sequences using software, and the nucleic acids were chemically synthesized and replaced in the genome to avoid secondary structure formation in the cob mRNA, which encodes enzymes of the coenzyme B_{12} synthetic pathway. We developed seven recombinant strains by changing each riboswitch, or a combination of riboswitches. Interestingly we noticed that when riboswitch 1 was deleted (ΔRS1 strain), a there was marginal decrease in cell growth and coenzyme B_{12} production was noticed. This could possibly be due to the accumulation of some toxic intermediate. However, deletion of riboswitches 2 and 3 (ΔRS2/RS3) or riboswitch 4 (ΔRS4) did not improve coenzyme B_{12} production. This could be due to a limitation in the upper pathway carbon flux. However, when riboswitch 1 was deleted either with riboswitches 2 and 3 (ΔRS1 ΔRS2/RS3) or with riboswitch 4 (ΔRS1 ΔRS4), there was marginal improvement in coenzyme B_{12} production. Moreover, a mutant strain lacking all the riboswitches showed comparably higher coenzyme B_{12} production (Table 8).

This study showed that coenzyme B_{12} production by *P. denitrificans* can be improved by removing the secondary structures from the cob mRNA. When the mutant

CLAIMS

What is claimed is:

1. An expression system, comprising a first promoter that is inducible by a small molecule, a second promoter, a first gene encoding a protein involved in the synthesis of 3-HP, a salt of 3-HP, or B12, a modified UTR, and a native sequence encoding up to 20 amino acids from the N-terminus of a native *Pseudomonas denitrificans* protein, wherein the native sequence is operably linked to the 3' terminus of the modified UTR and operably linked to the 5' terminus of the first gene, and the first and second promoters are operably linked in tandem upstream of the modified UTR.

2. The expression system of claim 1, further comprising a third promoter; and a second gene encoding a transcriptional regulator configured to regulate expression of the first gene, wherein the third promoter is operably connected to the second gene.

3. The expression system of claim 1 or claim 2, wherein the second promoter is inducible by the small molecule.

4. The expression system of any one of claims 1-3, wherein the native sequence is operably linked to the 5'-terminus of the first gene to define a fusion gene, the second promoter is a native promoter for the native *Pseudomonas denitrificans* protein, and the second promoter is operably linked to the 5' terminus of the fusion gene.

5. The expression system of any one of claims 1-4, wherein the first promoter is operably linked to the 5'-terminus of the second promoter, the second promoter is operably linked to the 5'-terminus of the modified UTR, and the modified UTR is operably linked to the 5'terminus of the fusion gene.

6. A nucleic acid, comprising a first and a second promoter, wherein the first promoter is an inducible promoter and is inducible by a small molecule; the first and

second promoters are operably linked to a first gene; and the first gene encodes a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP), a salt of 3-HP, or coenzyme B12.

7. The nucleic acid of claim 6, wherein the first gene encodes a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP) and is selected from the group consisting of a glycerol dehydratase, a glycerol dehydratase reactivase, and an aldehyde dehydrogenase.

8. The nucleic acid of claim 6 or claim 7, wherein the first gene comprises at least one gene selected from the group consisting of dhaB1, dhaB2, dhaB3, gdrA, gdrB, and kgsA.

9. The nucleic acid of any one of claims 6-8, wherein the first gene comprises a dhaB1 gene, a dhaB2 gene, a dhaB3 gene, a gdrA gene, and a gdrB gene.

10. The nucleic acid of any one of claims 6-8, wherein the first gene is dhaB1, and the sequence comprises a sequence that is at least 95% identical to SEQ ID NO: 1.

11. The nucleic acid of any one of claims 6-8, wherein the first gene is dhaB2, and the sequence comprises a sequence that is at least 95% identical to SEQ ID NO: 2.

12. The nucleic acid of any one of claims 6-8, wherein the first gene is dhaB3, and the sequence comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

13. The nucleic acid of any one of claims 6-8, wherein the first gene is gdrA, and the sequence comprises a sequence that is at least 95% identical to SEQ ID NO: 4.

14. The nucleic acid of any one of claims 6-8, wherein the first gene is grdB and the sequence comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

15. The nucleic acid of claim 7, wherein the first gene comprises an aldehyde dehydrogenase gene.

16. The nucleic acid of claim 15, wherein the aldehyde dehydrogenase is kgsA.

17. The nucleic acid of claim 16, wherein kgsA comprises a sequence that is at least 95% identical to SEQ ID NO: 6.

18. The nucleic acid of any one of claims 6-17, wherein the small molecule is an acid or alcohol.

19. The nucleic acid of claim 18, wherein the small molecule acid or alcohol is selected from the group consisting of L-lactic acid (LAC), acetic acid (AcOH), propionic acid (PA), 3-hydroxypropionic acid (3-HP), 3-hydroxybutyrate (3-HB), 1,3-propanediol (1,3-PDO), 2,3-butanediol (2,3-BDO), L-valine (L-val), and 3-hydroxyisobutyrate (3-HIB).

20. The nucleic acid of claim 19, wherein the small molecule acid or alcohol is selected from the group consisting of 3-hydroxypropionic acid (3-HP), 3-hydroxybutyrate (3-HB), L-valine (L-val), and 3-hydroxyisobutyrate (3-HIB).

21. The nucleic acid of claim 20, wherein the small molecule is 3-hydroxypropionic acid (3-HP).

22. The nucleic acid of any one of claims 6-21, wherein the second promoter is operably linked to the 3'-terminus of the first promoter and the first gene is operably downstream of the second promoter.

23. The nucleic acid of any one of claims 6-22, wherein the nucleic acid comprises a sequence that is at least 85% identical to SEQ ID NO: 65.

~~24. The nucleic acid of any one of claims 6-22, wherein the nucleic acid comprises SEQ ID NO: 65.~~

~~25. The nucleic acid of any one of claims 6-21, wherein the first promoter is operably linked to the 3'-terminus of the second promoter and the first gene is operably downstream of the second promoter.~~

~~26. The nucleic acid of any one of claims 6-25, wherein the second promoter is a constitutive promoter.~~

~~27. The nucleic acid of any one of claims 6-25, wherein the second promoter is a second inducible promoter.~~

~~28. The nucleic acid of any one of claims 6-27, wherein the intergenic space between the first and the second promoter does not include a terminator sequence.~~

~~29. The nucleic acid of any one of claims 6-28, wherein the first promoter and the second promoter regulate expression of the first gene.~~

~~30. The nucleic acid of any one of claims 6-28, wherein the first promoter is derived from a P_{mmsA} promoter, a P_{hbdH-1} promoter, a P_{hbdH-4} promoter, or a P_{hpdH} promoter.~~

~~31. The nucleic acid of any one of claims 6-28, wherein the first promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 14 (PmmsA promoter).~~

~~32. The nucleic acid of any one of claims 6-28, wherein the first promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 13 (PhbdH-1 promoter).~~

~~33. The nucleic acid of any one of claims 6-28, wherein the first promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 11 (PhbdH-4 promoter).~~

~~34. The nucleic acid of any one of claims 6-28, wherein the first promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 12 (PhpdH promoter).~~

~~35. The nucleic acid of claim 30, wherein the first promoter comprises a sequence that is selected from the group consisting of SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; and SEQ ID NO: 14.~~

~~36. The nucleic acid of any one of claims 6-35, wherein the first promoter comprises a P_{mmsA} promoter and the nucleic acid further comprises an operator site operably linked to the 5' terminus of the P_{mmsA} promoter.~~

~~37. The nucleic acid of claim 36, comprising a sequence that is 95% identical to SEQ ID NO: 15 (PmmsA sequence Δ2 FIG. 4).~~

~~38. The nucleic acid of claim 36, comprising a sequence that is 95% identical to SEQ ID NO: 16 (O1 of MmsA operator) and a sequence that is 95% identical to SEQ ID NO: 17 (O2 of MmsA operator).~~

~~39. The nucleic acid of claim 36, comprising a sequence that is 95% identical to SEQ ID NO: 18 (PmmsA and operator Fig. 3).~~

~~40. The nucleic acid of claim 36, comprising a sequence selected from the group consisting of SEQ ID NO: 15, 18, 19 (PmmsA2a), 20 (PmmsA2b), and 21 (PmmsA2ab).~~

41. The nucleic acid of any one of claims 6-40, wherein the second promoter is derived from a P_{mmsA} promoter, a P_{hbdH-1} promoter, a P_{hbdH-4} promoter, a P_{hpdH} promoter, or a P_{zwf} promoter.

42. The nucleic acid of any one of claims 6-40, wherein the second promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 11 (PhbdH-4 promoter).

43. The nucleic acid of any one of claims 6-40, wherein the second promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 12 (PhpdH promoter).

44. The nucleic acid of any one of claims 6-40, wherein the second promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 14 (PmmsA promoter).

45. The nucleic acid of any one of claims 6-40, wherein the second promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 13 (PhbdH-1 promoter).

46. The nucleic acid of any one of claims 6-40, wherein the second promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 7 (Pzwf).

47. The nucleic acid of any one of claims 6-40, wherein the second promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 8 (Pzwf-1).

48. The nucleic acid of any one of claims 6-40, wherein the second promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 9 (Pzwf-7).

49. The nucleic acid of any one of claims 6-40, wherein the second promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 62 (Pzwf-7-shorter).

~~50. The nucleic acid of any one of claims 6-40, wherein the second promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 10 (Pzwf-12).~~

~~51. The nucleic acid of any one of claims 6-40, wherein the second promoter comprises a sequence selected from the group consisting of SEQ ID NO: 7, 8, 9, 10, 11, 12, 13, 14, 52-60, 61, 62, and 63. (the Pzwf promoters and the other promoters).~~

~~52. The nucleic acid of any one of claims 6-29, wherein the first promoter comprises the P_{mmsA} promoter and the second promoter comprises the P_{hbdH-4} promoter.~~

~~53. The nucleic acid of any one of claims 6-29, wherein the first promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 14 (PmmsA promoter sequence), and the second promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 11 (PhbdH-4 promoter).~~

~~54. The nucleic acid of any one of claims 6-29, wherein the first promoter comprises the PhbdH-1 promoter and the second promoter comprises the PhpdH promoter.~~

~~55. The nucleic acid of any one of claims 6-29, wherein the first promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 13 (PhbdH-1 promoter sequence), and the second promoter comprises a sequence that is at least 95% identical to SEQ ID NO: 12 (PhpdH promoter).~~

~~56. The nucleic acid of any one of claims 6-29, wherein the second promoter comprises a PhbdH promoter and the nucleic acid further comprises an operator site operably linked to the N-terminus of the PhbdH promoter.~~

~~57. The nucleic acid of claim 56, wherein the operator site comprises one or more sequence that is at least 95% identical to SEQ ID NO: RBS-1, RBS-2, ABS-1, ABS-2, and ABS-3.~~

~~58. The nucleic acid of claim 57, wherein the operator site comprises a sequence that is at least 95% identical to SEQ ID NOs: RBS-1 site and ABS-2.~~

~~59. The nucleic acid of claim 58, wherein the operator site comprises SEQ ID NOs: RBS-1 site and ABS-2.~~

~~60. The nucleic acid of any one of claims 6-59, wherein the nucleic acid further comprises a gene encoding a transcriptional regulator that regulates expression of the first gene.~~

~~61. The nucleic acid of claim 60, wherein the transcriptional regulator binds to the first or second promoter.~~

~~62. The nucleic acid of claims 60 or 61, wherein the transcriptional regulator is a LysR-type transcriptional regulator (LTTR).~~

~~63. The nucleic acid of claim 62, wherein the transcriptional regulator is MmsR or HpdR.~~

~~64. The nucleic acid of claim 63, wherein the transcriptional regulator is MmsR.~~

~~65. The nucleic acid of claim 61, wherein the transcriptional regulator binds to the first promoter.~~

~~66. The nucleic acid of claim 65, wherein the transcriptional regulator is derived from a mmsR protein and the first promoter is derived from a PmmsA promoter.~~

~~67. The nucleic acid of claim 65, wherein the transcriptional regulator comprises a mmsR protein and the first promoter comprises a PmmsA promoter.~~

68. The nucleic acid of claim 66, further comprising an operator site, wherein the MmsR protein binds to the operator site.

69. The nucleic acid of claim 68, comprising a sequence that is at least 95% identical to SEQ ID NO: 19 (PmmsA2a).

70. The nucleic acid of claim 68, comprising a sequence that is selected from the group consisting of SEQ ID NO: 19-21 (PmmsA2a, A2b, A2ab).

71. The nucleic acid of claim 60, wherein the transcriptional regulator binds to the second promoter.

72. The nucleic acid of claim 71, wherein the second promoter is a constitutive promoter.

73. The nucleic acid of claim 71, wherein the transcriptional regulator is a HpdR protein and the second promoter is derived from a hpdH promoter.

74. The nucleic acid of claim 73, wherein the second promoter comprises a sequence that is at least 95% identical to the sequence of P_{hpdH} SEQ ID NO: 12.

75. The nucleic acid of claim 60, wherein the transcriptional regulator binds to the first promoter and has enhanced binding to the first promoter in the presence of the small molecule.

76. The nucleic acid of claim 60, wherein the transcriptional regulator binds to the second promoter and has enhanced binding to the second promoter in the presence of the small molecule.

77. The nucleic acid of claim 60, wherein the transcriptional regulator is self-regulating.

78. The nucleic acid of claim 60, further comprising a third promoter that is operably linked to the gene encoding the first transcriptional regulator.

79. The nucleic acid of claim 78, wherein the third promoter comprises a sequence that is 95% identical to the sequence selected from the group consisting of SEQ ID NO: 7-10 and 52-63.

80. The nucleic acid of claim 78, wherein:

the second constitutive promoter is selected from the group consisting of SEQ ID NOs: 9, 10, 57-60, 62, and 63;

the transcriptional regulator comprises a MmsR protein; and

the first promoter comprises P_{mmsA}.

81. The nucleic acid of claim 78, wherein the

the second constitutive promoter is selected from the group consisting of SEQ ID NOs: 9, 10, 57-60, 62, and 63;

the transcriptional regulator comprises HpdR protein; and

the first promoter comprises P_{hbdH}.

82. The nucleic acid of claim 80 or 81, wherein the third promoter comprises SEQ ID NO: 10.

83. The nucleic acid of claim 80 or 81, wherein the third promoter comprises SEQ ID NO: 62.

84. The nucleic acid of any one of claims 6-83, wherein the first gene comprises a modified 5' UTR of the gene.

~~85. The nucleic acid of claim 84, wherein the first gene comprises a sequence selected from the group consisting of SEQ ID NO: 22-28 and 64. (UTR 0-6)~~

~~86. The nucleic acid of claim 85, wherein the 5' UTR comprises SEQ ID NO: 28 (UTR-6).~~

~~87. The nucleic acid of claim 84, wherein the first gene comprises a gene encoding kgsA and the 5'UTR comprises SEQ ID NO: 28 (UTR-6).~~

~~88. The nucleic acid of any one of claims 6-87, wherein at least the 10 codons at the 5'terminus of the first gene are optimized for translation in *Pseudomonas denitrificans*.~~

~~89. The nucleic acid of any one of claims 6-88, wherein up to 10 codons at the 5'terminus of the first gene are optimized for translation in *Pseudomonas denitrificans*.~~

~~90. The nucleic acid of claims 88 or 89, wherein codon optimization comprises:~~

~~measuring a codon frequency of each amino acid in a gene encoding a native *Pseudomonas denitrificans* protein;~~

~~using the codon frequency of the native protein to replace the 10 codons at the 5'-terminus of the first gene with an optimized 10 codons, wherein the codon for each amino acid of the optimized 10 codons is present at the same frequency as the codon frequency of the native protein.~~

~~91. The nucleic acid of claim 90, wherein the codon frequency for each amino acid in the optimized second 10 codons is more than 0.~~

~~92. The nucleic acid of claim 90, wherein the codon frequency is measured of each amino acid in the 10 codons at the 5' terminus of the gene encoding the native *Pseudomonas denitrificans* protein.~~

~~93. The nucleic acid of claim 88, wherein the nucleic acid comprises a sequence that is 95% identical to a sequence selected from the group consisting of SEQ ID NOs: 29, 30, and 31. (Opt1-3).~~

~~94. The nucleic acid of any one of claims 6-93, wherein the first gene comprises a gene encoding kgsA and comprises a sequence selected from the group consisting of SEQ ID NO: 29-31.~~

~~95. The nucleic acid of any one of claims 6-93, wherein the first gene is fused at its 5'-terminus to a sequence encoding up to 20 amino acids that are derived from the 5'terminus of a second gene encoding a native *Pseudomonas denitrificans* protein, thereby creating a fusion gene.~~

~~96. The nucleic acid of claim 95, wherein the mRNA of the fusion gene has a higher stability than the mRNA of the first gene alone.~~

~~97. The nucleic acid of claim 95, wherein the fusion gene increases gene translation when compared to the first gene alone.~~

~~98. The nucleic acid of claim 95, wherein the mRNA of the fusion gene is more resistant to ribonuclease degradation than the mRNA of the first gene alone.~~

~~99. The nucleic acid of claim 95, wherein the fusion gene increases gene translation in comparison to the first gene alone.~~

~~100. The nucleic acid of any one of claims 6-29, wherein the first gene is fused at its 5'-terminus to a sequence encoding up to 20 (5, 10, 15, or 20) amino acids that are derived from the 5'terminus of a second gene encoding a native *Pseudomonas denitrificans* protein, thereby creating a fusion gene, and the second promoter is derived from the native promoter for the second gene.~~

~~101. The nucleic acid of claim 100, wherein the second promoter is derived from the P_{mmsA} promoter and the fusion gene comprises a sequence encoding at least 5, 10, 15, or 20 amino acids in the N-terminus of a native MmsA protein.~~

~~102. The nucleic acid of any one of claims 6-29, wherein the second promoter comprises the P_{mmsA} promoter and the fusion gene comprises a sequence encoding five or more amino acids from the N-terminus of a native MmsA gene, and the fusion gene is operably linked to the 3' end of the P_{mmsA} promoter.~~

~~103. The nucleic acid of claim 102, wherein the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs: 32-35.~~

~~104. The nucleic acid of any one of claims 6-29, wherein the first gene is fused at its 5'-terminus to a sequence encoding up to 20 (5, 10, 15, or 20) amino acids that are derived from a sequence encoding the N-terminus of a native *Pseudomonas denitrificans* MmsA protein.~~

~~105. The nucleic acid of claim 104, comprising a sequence selected from the group consisting of SEQ ID NOs: 32-35 (Pcm-mmsA(5)(10)(15)(20)).~~

~~106. The nucleic acid of any one of claims 6-29, wherein the first gene encodes a protein involved in the synthesis of vitamin B12.~~

~~107. The nucleic acid of claim 106, wherein the first gene comprises a gene selected from the group consisting of cobJ, cobI, cobH, cobG, cobL, cobF, cobK, gst, xre,chlD, chlI, dahp, cobN, cobW, cbtBA, cobE, cobM, btuB, cobO, cob, cobR, cobD, cobC, cobQ, cobU, cobP, bgpM, and cobV.~~

~~108. The nucleic acid of claim 107, wherein the first gene comprises bgpM.~~

~~109. The nucleic acid of claim 106, wherein the first gene is derived from a *Pseudomonas* species selected from the group consisting of *P. denitrifcans; P. aeruginosa; P. entomophila; P. putida; P. syringae; P. fluorescens; P mendocina;* and *P. stutzeri.*~~

~~110. A nucleic acid comprising a first promoter operably linked to a first gene that encodes a protein involved in the synthesis of coenzyme B12.~~

~~111. The nucleic acid of claim 110, wherein the first promoter is a constitutive promoter.~~

~~112. The nucleic acid of claims 110 or 111, wherein the nucleic acid comprises a sequence selected from SEQ ID NOs: 7-10 and 52-63.~~

~~113. The nucleic acid of claims 110 or 111, wherein the first gene comprises one or more genes selected from the group consisting of cobJ, cobI, cobH, cobG, cobL, cobF, cobK, gst, xre,chlD, chlI, dahp, cobN, cobW, cbtBA, cobE, cobM, btuB, cobO, cob, cobR, cobD, cobC, cobQ, cobU, cobP, bgpM, and cobV.~~

~~114. The nucleic acid of claims 110 or 111, wherein the first gene is from a *Pseudomonas* species selected from the group consisting of *P. denitrifcans; P. aeruginosa; P. entomophila; P. putida; P. syringae; P. fluorescens; P mendocina;* and *P. stutzeri.*~~

~~115. The nucleic acid of any one of claims 110-114, further comprising a second promoter operably linked to the first promoter.~~

~~116. The nucleic acid of claim 115, wherein the first and the second promoter regulate expression of the first gene.~~

~~117. The nucleic acid of any one of claims 110-116, wherein the first promoter is not a native promoter for the first gene.~~

~~118. A nucleic acid comprising a cobG gene, a P_{edd} promoter, a P_{sucA} promoter, and a cobL gene; wherein the P_{edd} promoter is operably linked to the cobG gene and the P_{sucA} promoter, and the P_{sucA} promoter is operably linked to the cobL gene.~~

~~119. The nucleic acid of claim 118, wherein the P_{edd} promoter comprises the sequence SEQ ID NO: 36; and the P_{sucA} promoter comprises the sequence SEQ ID NO: 37.~~

~~120. The nucleic acid of claim 118, comprising the sequence SEQ ID NO: 38.~~

~~121. A nucleic acid comprising a cobG gene, a P_{sp9} promoter, a P_{zwf} promoter, and a cobL gene; wherein the P_{sp9} promoter is operably linked to the cobG gene and the P_{zwf} promoter, and the P_{zwf} promoter is operably linked to the cobL gene.~~

~~122. The nucleic acid of claim 121, wherein the P_{sp9} promoter comprises SEQ ID NO: 39; and the P_{zwf} promoter comprises SEQ ID NO: 40.~~

~~123. The nucleic acid of claim 121, comprising SEQ ID NO: 41.~~

~~124. A nucleic acid comprising a cobW gene, a P_{zwf} promoter, a P_{sp9} promoter, and a cbtB gene, wherein the P_{zwf} promoter is operably linked to the cobW gene and the P_{sp9} promoter, and the P_{sp9} promoter is operably linked to the cbtB gene.~~

~~125. The nucleic acid of claim 124, wherein the Pzwf promoter comprises SEQ ID NO: 42; and the Psp9 promoter comprises SEQ ID NO: 43.~~

~~126. The nucleic acid of claim 124, comprising SEQ ID NO: 44.~~

~~127. A nucleic acid comprising a cobW gene, a P_{tkt} promoter, a P_{sp2} promoter, and a cbtB gene, wherein the P_{tkt} promoter is operably linked to the cobW gene and the P_{sp2} promoter, and the P_{sp2} promoter is operably linked to the cbtB gene.~~

~~128. The nucleic acid of claim 127, wherein the P_{tkt} promoter comprises SEQ ID NO: 45; and the Psp2 promoter comprises SEQ ID NO: 46.~~

~~129. The nucleic acid of claim 127, comprising SEQ ID NO: 47.~~

~~130. A nucleic acid comprising a P_{zwf} promoter operably linked to a tonB gene (e.g., a butB gene).~~

~~131. The nucleic acid of claim 130, wherein the Pzwf promoter comprises SEQ ID NO: 48.~~

~~132. The nucleic acid of claim 130, comprising SEQ ID NO: 49.~~

~~133. A nucleic acid comprising a P_{sp9} promoter operably linked to a tonB gene (e.g., a butB gene).~~

~~134. The nucleic acid of claim 133, wherein the Psp9 promoter comprises SEQ ID NO: 50.~~

~~135. The nucleic acid of claim 133, comprising SEQ ID NO: 51.~~

~~136. The nucleic acid of any of the preceding claims, wherein the nucleic acid comprises one or more sequences derived from *Pseudomonas denitrificans*.~~

~~137. The nucleic acid of claim 136, wherein the first or the second promoters are derived from *Pseudomonas denitrificans*.~~

~~138. The nucleic acid of any of claims 1-137, wherein the nucleic acid comprises one or more sequences derived from an Enterobacter, Lactobacillus, Pseudomonas, or Azospirillum bacterium.~~

~~139. The nucleic acid of claim 138, wherein the first gene is derived from an Enterobacter, Lactobacillus, Pseudomonas, or Azospirillum bacterium.~~

~~140. A nucleic acid comprising a DhaB expression module that comprises a sequence that is at least 85% identical to SEQ ID NO: 66 (DhaB expression module).~~

~~141. The nucleic acid of claim 140, wherein the sequence is at least 90% identical to SEQ ID NO: 66.~~

~~142. The nucleic acid of claim 140, wherein the sequence comprises SEQ ID NO: 66.~~

~~143. A nucleic acid comprising a DhaB expression module that comprises a sequence that is at least 85% identical to SEQ ID NO: 67 (kgsA expression module).~~

~~144. The nucleic acid of claim 143, wherein the sequence is at least 90% identical to SEQ ID NO: 67.~~

~~145. The nucleic acid of claim 143, wherein the sequence comprises SEQ ID NO: 67.~~

~~146. A nucleic acid comprising a DhaB expression module comprising a UTR of the *gdrA* gene comprising SEQ ID NO: 68 and a UTR of the *gdrB* gene comprising SEQ ID NO: 69.~~

~~147. The nucleic acid of any one of claims 6-29, wherein the nucleic acid comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NO: 70-72.~~

~~148. The nucleic acid of any one of claims 6-29, wherein the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO: 70-72.~~

~~149. A nucleic acid comprising a coenzyme B12 sensor comprising a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NO: 73 and 74.~~

~~150. The nucleic acid of claim 149, comprising a sequence selected from the group consisting of SEQ ID NO: 73 and 74.~~

~~151. A nucleic acid comprising a coenzyme B12 expression module that comprises a first gene involved in the synthesis of coenzyme B12, a first promoter that is induced by a small molecule, and a second promoter, wherein the first and second promoters are operably linked in tandem upstream of the first gene such that they regulate expression of the first gene.~~

~~152. The nucleic acid of claim 151, further comprising a second gene involved in the synthesis of coenzyme B12, a third promoter that is induced by a small molecule, and a fourth promoter, wherein the third and fourth promoters are operably linked in tandem upstream of the second gene such that they regulate expression of the second gene.~~

~~153. The nucleic acid of claim 152, further comprising a third gene involved in the synthesis of coenzyme B12, a fifth promoter that is induced by a small molecule, and a sixth promoter, wherein the fifth and sixth promoters are operably linked in tandem upstream of the third gene such that they regulate expression of the third gene.~~

~~154. The nucleic acid of claim 153, further comprising a fourth gene involved in the synthesis of coenzyme B12, a seventh promoter that is induced by a small molecule, and a eighth promoter, wherein the seventh and eighth promoters are operably linked in tandem upstream of the fourth gene such that they regulate expression of the fourth gene.~~

~~155. The nucleic acid of any one of claims 151-154, wherein the first, second, third, and fourth genes each comprise one or more genes selected from the group consisting of cobJ, cobI, cobH, cobG, cobL, cobF, cobK, gst, xre,chlD, chlI, dahp, cobN, cobW, cbtBA, cobE, cobM, btuB, cobO, cob, cobR, cobD, cobC, cobQ, cobU, cobP, bgpM, and cobV.~~

~~156. The nucleic acid of claim 155, wherein the first gene comprises bgpM.~~

~~157. The nucleic acid of claim 151, wherein the nucleic acid does not include any native riboswitches that regulate genes involved in the production of coenzyme B12.~~

~~158. The nucleic acid of claim 151, wherein the nucleic acid does not include SEQ ID NOs: 75 or 76.~~

~~159. A recombinant bacterium comprising the nucleic acid of any one of claims 1-158.~~

~~160. The recombinant bacterium of claim 159, wherein the bacterium is a *Pseudomonas* species.~~

~~161. The recombinant bacterium of claim 160, wherein the bacterium is *Pseudomonas denitrificans*.~~

~~162. The recombinant bacterium of claim 159, wherein the nucleic acid is on an expression plasmid.~~

~~163. The recombinant bacterium of claim 159, wherein the nucleic acid is integrated into the bacterium's chromosome.~~

~~164. The recombinant bacterium of claim 159, wherein the nucleic acid is on an episome.~~

~~165. A recombinant bacterium comprising a first nucleic acid comprising a DhaB expression module and a second nucleic acid comprising a ALDH expression module.~~

~~166. The recombinant bacterium of claim 165, wherein the DhaB expression module comprises the nucleic acid of any one of claims 1-14 and 18-142.~~

~~167. The recombinant bacterium of claim 165, wherein the ALDH expression module comprises the nucleic acid of any one of claims 1-8 and 15-142.~~

~~168. The recombinant bacterium of claim 165, wherein the first and the second nucleic acids are on expression plasmids.~~

~~169. The recombinant bacterium of claim 165, wherein the first and the second nucleic acids are on episomes.~~

~~170. The recombinant bacterium of claim 165, wherein the first and the second nucleic acids are integrated into the bacterium's chromosome.~~

~~171. The recombinant bacterium of claim 165, wherein the first nucleic acid is integrated into the bacterium's chromosome and the second nucleic acid is on an expression plasmid.~~

~~172. The recombinant bacterium of claim 165, wherein the first nucleic acid is on an expression plasmid and the second nucleic acid is integrated into the bacterium's chromosome.~~

~~173. The recombinant bacterium of claim 165, wherein the first nucleic acid is integrated into the bacterium's chromosome and the second nucleic acid is on an episome.~~

~~174. The recombinant bacterium of claim 165, wherein the first nucleic acid is on an episome and the second nucleic acid is integrated into the bacterium's chromosome.~~

~~175. The recombinant bacterium of claim 170, wherein the first nucleic acid is integrated into the bacterium's chromosome at a first position and the second nucleic acid is integrated into the bacterium's chromosome at a second position; and the first and second positions are within 2500 kilo base pairs of each other.~~

~~176. The recombinant bacterium of claim 175, wherein the first and second positions are within 100 kilo base pairs of each other.~~

~~177. The recombinant bacterium of claim 175, wherein the first and second positions are within 50 kilo base pairs of each other.~~

~~178. The recombinant bacterium of claim 175, wherein the first and second positions are within 1000 base pairs of each other.~~

~~179. The recombinant bacterium of claim 170, wherein the first nucleic acid is integrated into the bacterium's chromosome at a first position that is within 4000 base pairs of the origin of replication.~~

~~180. The recombinant bacterium of claim 179, wherein the first position is within 1000 base pairs of the origin of replication.~~

~~181. A recombinant bacterium comprising a coenzyme B12 expression module that comprises a first gene involved in the synthesis of coenzyme B12, a first promoter that is induced by a small molecule, and a second promoter, wherein the first and second promoters are operably linked in tandem upstream of the first gene.~~

~~182. The recombinant bacterium of claim 181, wherein the bacterium is a *Pseudomonas denitrificans* bacterium.~~

~~183. The recombinant bacterium of claim 182, wherein the bacterium comprises a deletion at a first position of a first riboswitch which regulates expression of the first gene, and the first and second promoters are integrated into the bacterium's chromosome at the first position such that they regulate expression of the first gene.~~

~~184. The recombinant bacterium of claim 183, wherein the bacterium further comprises a second deletion at a second position of a second riboswitch which regulates expression of a second gene involved in the synthesis of coenzyme B12, a third deletion at a third position of a third riboswitch which regulates expression of a third gene involved in the synthesis of coenzyme B12, and a fourth deletion at a fourth position of a fourth riboswitch which regulates expression of a fourth gene involved in the synthesis of coenzyme B12.~~

~~185. The recombinant bacterium of claim 184, further comprising a third promoter and a fourth promoter which are operably linked in tandem and integrated into the chromosome at the second position such that they regulate expression of the second gene; a fifth promoter and a sixth promoter which are operably linked in tandem and integrated into the chromosome at the third position such that they regulate expression of the third gene; and a seventh and eighth promoter which are operably linked in tandem and integrated into the chromosome at the fourth position such that they regulate expression of the fourth gene.~~

~~186. The recombinant bacterium of claim 181, wherein the bacterium produces more coenzyme B12 than native *Pseudomonas denitrificans*.~~

~~187. The recombinant bacterium of claim 165, further comprising a coenzyme B12 expression module.~~

~~188. A method of producing 3-HP or a salt thereof, comprising culturing the recombinant bacterium of any one of claims 159-187 under conditions sufficient to produce 3-HP or a salt thereof.~~

~~189. The method of claim 188, wherein the conditions are sufficient to produce at least 85 g/L of 3-HP or a salt thereof.~~

~~190. The method of claim 189, wherein the conditions are sufficient to produce at least 90 g/L of 3-HP or a salt thereof.~~

~~191. The method of claim 189, wherein the conditions are sufficient to produce at least 95 g/L of 3-HP or a salt thereof.~~

~~192. The method of claim 189, wherein the conditions are sufficient to produce at least 100 g/L of 3-HP or a salt thereof.~~

~~193. The method of claim 188, wherein culturing the bacterium does not include the addition of external coenzyme B12.~~

~~194. The method of claim 188, wherein culturing the bacterium comprises addition external coenzyme B12 to the culture.~~

~~195. The method of claim 188, wherein culturing comprises growing the bacterium in a media comprising a carbon source selected from the group consisting of glycerol, glucose, gluconate, glutamate, and citric acid.~~

~~196. The method of claim 188, wherein culturing comprising inducing the bacterium to produce 3-HP by adding glycerol.~~

~~197. The method of claim 196, wherein the glycercol is add mid-log phase.~~

~~198. The method of claim 188, wherein culturing comprises growing the bacterium in a media comprising a nitrogen source selected from the group consisting of yeast extract, corn steep liquor powder, and corn steep liquor paste.~~

~~199. The method of claim 188, wherein culturing comprises growing the bacterium at a pH between 6.8 and 7.8~~

~~200. The method of claim 199, wherein culturing comprises adding one or more bases selected from the group consisting of NaOH, KOH, $NaHCO_3$, NH_4HCO_3, NH_4OH, $(NH4)_2CO_3$, and Na_2CO_3.~~

~~201. The method of claim 199, wherein culturing comprises adding at least one base selected from the group consisting of NaOH, KOH, $NaHCO_3$, NH_4HCO_3, NH_4OH, $(NH_4)_2CO_3$, and Na_2CO_3.~~

~~202. The method of claim 188, wherein culturing comprises diluting the base concentration and increasing the acid concentration.~~

~~203. The method of claim 188, wherein culturing comprises growing the bacterium at a temperature between 28 and 40 degrees Celsius.~~

~~204. The method of claim 203, wherein culturing comprises growing the bacterium at 33 degrees Celsius.~~

~~205. A method of producing 3-HP or a salt thereof, the method comprising:~~

providing the recombinant bacterium of any one of claims 159-187 in a culture media suitable for growth of the bacterium and production of 3-HP or a salt thereof.

206. The method of claim 205, further comprising separating 3-HP from the bacterium or culture media.

207. The method of claim 205, wherein the culture media comprises a dissolved oxygen concentration that is approximately 2-20% dissolved oxygen.

208. The method of claim 207, wherein the dissolved oxygen concentration is approximately 5-10%.

209. The method of claim 207, wherein the dissolved oxygen concentration is no more than 20%.

210. The method of claim 207, wherein the dissolved oxygen concentration is at least 2%.

211. A method, comprising:

removing 3-hydroxypropionic acid (3-HP) from an aqueous solution without using a counter-current liquid flow.

212. The method of claim 211, comprising using a solvent to remove 3-HP from the aqueous solution.

213. The method of claim 212, comprising, before using the solvent to remove 3-HP from the aqueous solution:

evaporating the solvent; and

condensing the evaporated solvent.

~~214. The method of claim 212 or claim 213, wherein the solvent comprises a water-immiscible solvent.~~

~~215. The method of claim 212 or claim 213, wherein the solvent comprises a water-miscible solvent.~~

~~216. The method of any of claims 1-5, wherein the solvent has density of less than about 1 g/mL.~~

~~217. The method of any of claims 211-215, wherein the solvent has density of greater than about 1 g/mL.~~

~~218. The method of claim 212 or claim 213, wherein the solvent comprises at least one solvent selected from the group consisting of a C_{1-6} alkyl acetate, a C_{4-6} alcohol, and a C_{1-6} alkyl ether.~~

~~219. The method of claim 212 or claim 213, wherein the solvent comprises at least one solvent selected from the group consisting of methyl acetate, ethyl acetate, n-propyl acetate, isopropyl acetate, n-butyl acetate, isobutyl acetate, sec-butyl acetate, tert-butyl acetate, n-pentyl acetate, and n-hexyl acetate.~~

~~220. The method of claim 212 or claim 213, wherein the solvent comprises ethyl acetate.~~

~~221. The method of claim 212 or claim 213, wherein the solvent comprises at least one solvent selected from the group consisting of n-butyl alcohol, isobutyl alcohol, tert-butyl alcohol, n-pentyl alcohol and n-hexyl alcohol.~~

~~222. The method of claim 212 or claim 213, wherein the solvent comprises isobutyl alcohol.~~

~~223. The method of claim 212 or claim 213, wherein the solvent comprises at least one solvent selected from the group consisting of diethyl ether, dipropyl ether, ethyl propyl ether, methyl tert-butyl ether, and methyl hexyl ether.~~

~~224. The method of claim 212 or claim 213, wherein the solvent comprises at least one solvent selected from the group consisting of chloroform, methylene chloride and carbon tetrachloride.~~

~~225. The method of any of claims 212, 213 or 215, comprising adding the solvent to the aqueous solution.~~

~~226. The method of any of claims 212, 213, 215 or 225, wherein the solvent comprises a C_{1-3} alcohol.~~

~~227. The method of any of claims 212, 213, 215 or 225, wherein the solvent comprises at least one solvent selected from the group consisting of methanol, ethanol, n-propanol, and isopropanol.~~

~~228. The method of any of claims 212, 213, 215 or 225, wherein the solvent comprises methanol.~~

~~229. The method of any of claims 212, 213 or 225-228, wherein an amount of the solvent is from about 5 v/v% to about 50 v/v% of an amount of the aqueous solution.~~

~~230. The method of any of claims 212, 213 or 225-228, wherein an amount of the solvent is about 25 v/v% of an amount of the aqueous solution.~~

~~231. The method of any of claims 212, 213 or 225-228, comprising using a water-miscible solvent and a water-immiscible solvent to remove 3-HP from the aqueous solution, wherein a ratio of 1) a combined volume of the water-miscible solvent and the~~

~~water-immiscible solvent to 2) a volume of the aqueous solution is in from about 1:1 to about 2:1.~~

~~232. The method of any of claims 211-231, wherein 3-HP is removed from the aqueous solution with a yield of at least 50%.~~

~~233. The method of any of claims 211-231, wherein 3-HP is removed from the aqueous solution with a yield of at least 60%.~~

~~234. The method of any of claims 211-231, wherein 3-HP is removed from the aqueous solution with a yield of at least 70%.~~

~~235. The method of any of claims 211-231, wherein 3-HP is removed from the aqueous solution with a yield of at least 80%.~~

~~236. The method of any of claims 211-231, wherein 3-HP is removed from the aqueous solution with a yield of at least 90%.~~

~~237. The method of any of claims 211-231, wherein 3-HP is removed from the aqueous solution with a yield of at least 95%.~~

~~238. The method of any of claims 211-231, wherein 3-HP is removed from the aqueous solution with a yield of at least 99%.~~

~~239. A method, comprising:~~

~~evaporating a first solvent;~~

~~condensing the evaporated first solvent; and~~

~~directing a flow of the first solvent to remove 3-HP from the aqueous solution.~~

~~240. The method of claim 233, wherein the first solvent has density of less than about 1 g/mL.~~

~~241. The method of claim 233, wherein the first solvent has density of greater than about 1 g/mL.~~

~~242. The method of claim 239, wherein the first solvent comprises a water-immiscible solvent.~~

~~243. The method of claim 239, wherein the first solvent comprises at least one solvent selected from the group consisting of a C_{1-6} alkyl acetate, a C_{4-6} alcohol, and a C_{1-6} alkyl ether.~~

~~244. The method of claim 239, wherein the first solvent comprises at least one solvent selected from the group consisting of methyl acetate, ethyl acetate, n-propyl acetate, isopropyl acetate, n-butyl acetate, isobutyl acetate, sec-butyl acetate, tert-butyl acetate, n-pentyl acetate, and n-hexyl acetate.~~

~~245. The method of claim 239, wherein the first solvent comprises ethyl acetate.~~

~~246. The method of claim 239, wherein the first solvent comprises at least one solvent selected from the group consisting of n-butyl alcohol, isobutyl alcohol, tert-butyl alcohol, n-pentyl alcohol and n-hexyl alcohol.~~

~~247. The method of claim 239, wherein the first solvent comprises isobutyl alcohol.~~

~~248. The method of claim 239, wherein the first solvent comprises at least one solvent selected from the group consisting of diethyl ether, dipropyl ether, ethyl propyl ether, methyl tert-butyl ether, and methyl hexyl ether.~~

~~249. The method of claim 239, wherein the first solvent comprises at least one solvent selected from the group consisting of chloroform, methylene chloride and carbon tetrachloride.~~

~~250. The method of any of claims 239-249, comprising using a second solvent to remove 3-HP from the aqueous solution.~~

~~251. The method of claim 250, wherein the second solvent comprises a water-miscible solvent.~~

~~252. The method of claim 250, wherein the first solvent comprises a water-immiscible solvent, and the second solvent comprises a water-miscible solvent.~~

~~253. The method of any of claims 250-252, comprising adding the second solvent to the aqueous solution.~~

~~254. The method of any of claims 250-253, wherein the second solvent comprises a C_{1-3} alcohol.~~

~~255. The method of any of claims 250-253, wherein the second solvent comprises at least one solvent selected from the group consisting of methanol, ethanol, n-propanol, and isopropanol.~~

~~256. The method of any of claims 250-253, wherein the second solvent comprises methanol.~~

~~257. The method of any of claims 250-256, wherein an amount of the second solvent is from about 5 v/v% to about 50 v/v% of an amount of the aqueous solution.~~

~~258. The method of any of claims 250-256, wherein an amount of the second solvent is about 25 v/v% of an amount of the aqueous solution.~~

~~259. The method of any of claims 250-258, wherein a ratio of 1) a combined volume of the first and second solvents to 2) a volume of the aqueous solution is in from 1:1 to about 2:1.~~

~~260. The method of any of claims 239-259, wherein 3-HP is removed from the aqueous solution with a yield of at least 50%.~~

~~261. The method of any of claims 239-259, wherein 3-HP is removed from the aqueous solution with a yield of at least 60%.~~

~~262. The method of any of claims 239-259, wherein 3-HP is removed from the aqueous solution with a yield of at least 70%.~~

~~263. The method of any of claims 239-259, wherein 3-HP is removed from the aqueous solution with a yield of at least 80%.~~

~~264. The method of any of claims 239-259, wherein 3-HP is removed from the aqueous solution with a yield of at least 90%.~~

~~265. The method of any of claims 239-259, wherein 3-HP is removed from the aqueous solution with a yield of at least 95%.~~

~~266. The method of any of claims 239-259, wherein 3-HP is removed from the aqueous solution with a yield of at least 99%.~~

~~267. A method, comprising:~~

~~using a water-immiscible solvent to remove 3-hydroxypropionic acid (3-HP) from an aqueous solution with a yield of at least 50%.~~

~~268. The method of claim 267, wherein the yield of removal of 3-HP from the aqueous solution is at least 60%.~~

~~269. The method of claim 267, wherein the yield of removal of 3-HP from the aqueous solution is at least 70%.~~

~~270. The method of claim 267, wherein the yield of removal of 3-HP from the aqueous solution is at least 80%.~~

~~271. The method of claim 267, wherein the yield of removal of 3-HP from the aqueous solution is at least 90%.~~

~~272. The method of claim 267, wherein the yield of removal of 3-HP from the aqueous solution is at least 95%.~~

~~273. The method of claim 265, wherein the yield of removal of 3-HP from the aqueous solution is at least 99%.~~

~~274. The method of any of claims 267-273, wherein the water-immiscible solvent comprises at least one C_{1-6}-alkyl acetate.~~

~~275. The method of any of claims 267-273, wherein the water-immiscible solvent comprises at least one solvent selected from the group consisting of methyl acetate, ethyl acetate, n-propyl acetate, isopropyl acetate, n-butyl acetate, isobutyl acetate, sec-butyl acetate, tert-butyl acetate, n-pentyl acetate, and n-hexyl acetate.~~

~~276. The method of any of claims 267-273, wherein the water-immiscible solvent comprises ethyl acetate.~~

~~277. The method of any of claims 267-276, comprising using counter-current liquid flow.~~

278. The method of any of claims 267-276, comprising before using the water-immiscible solvent to remove 3-HP from the aqueous solution:

evaporating the water-immiscible solvent; and

condensing the evaporated water-immiscible solvent.

279. The method of any of claims 267-278, comprising using a water-miscible solvent to remove 3-HP from the aqueous solution.

280. The method of claim 279, comprising adding the water-miscible solvent to the aqueous solution.

281. The method of claim 279 or claim 280, wherein the water-miscible solvent comprises a C_{1-3} alcohol.

282. The method of claim 279 or claim 280, wherein the water-miscible solvent comprises at least one solvent selected from the group consisting of methanol, ethanol, n-propanol, and isopropanol.

283. The method of claim 279 or claim 280, wherein the water-miscible solvent comprises methanol.

284. The method of any of claims 279-283, wherein an amount of the water-miscible solvent is from about 5 v/v% to about 50 v/v% of an amount of the aqueous solution.

285. The method of any of claims 279-283, wherein an amount of the water-miscible solvent is about 25 v/v% of an amount of the aqueous solution.

286. The method of any of claims 279-283, wherein a ratio of 1) a combined volume of the water-miscible solvent and the water-immiscible solvent to 2) a volume of the aqueous solution is in from about 1:1 to about 2:1.

287. A method, comprising:

using a liquid to remove 3-hydroxypropionic acid (3-HP) from an aqueous solution,

wherein the liquid comprises first and second solvents.

288. The method of claim 287, wherein the first solvent comprises an organic solvent.

289. The method of claim 287 or claim 288, wherein the second solvent comprises an organic solvent.

290. The method of any of claims 287-289, wherein the first solvent is water-miscible.

291. The method of any of claims 287-289, wherein the first solvent is water-miscible, and the second solvent is water-immiscible.

292. The method of any of claims 287-291, wherein the method is performed without using counter-current liquid flow.

293. The method of any of claims 287-292, further comprising before using the liquid to remove 3-HP:

evaporating the second solvent; and

condensing the evaporated second solvent.

294. The method of any of claims 287-293, wherein the second solvent has density of less than about 1 g/mL.

295. The method of any of claims 287-294, wherein the second solvent comprises at least one solvent selected from the group consisting of a C_{1-6} alkyl acetate, a C_{4-6} alcohol, and a C_{1-6} alkyl ether.

296. The method of any of claims 287-294, wherein the second solvent comprises at least one solvent selected from the group consisting of methyl acetate, ethyl acetate, n-propyl acetate, isopropyl acetate, n-butyl acetate, isobutyl acetate, sec-butyl acetate, tert-butyl acetate, n-pentyl acetate, and n-hexyl acetate.

297. The method of any of claims 287-294, wherein the second solvent comprises ethyl acetate.

298. The method of any of claims 287-294, wherein the second solvent comprises at least one solvent selected from the group consisting of n-butyl alcohol, isobutyl alcohol, tert-butyl alcohol, n-pentyl alcohol and n-hexyl alcohol.

299. The method of any of claims 287-294, wherein the second solvent is isobutyl alcohol.

300. The method of any of claims 287-294, wherein the second solvent comprises at least one solvent selected from the group consisting of diethyl ether, dipropyl ether, ethyl propyl ether, methyl tert-butyl ether, and methyl hexyl ether.

301. The method of any of claims 287-300, wherein the second solvent has density of greater than about 1 g/mL.

302. The method of any of claims 287-300, wherein the first solvent comprises at least one solvent selected from the group consisting of chloroform, methylene chloride and carbon tetrachloride.

303. The method of any of claims 287-300, wherein the first solvent comprises a C_{1-3} alcohol.

304. The method of any of claims 287-300, wherein the first solvent comprises at least one solvent selected from the group consisting of methanol, ethanol, n-propanol, and isopropanol.

305. The method of any of claims 287-300, wherein the first solvent comprises methanol.

306. The method of any of claims 287-305, wherein an amount of the first solvent is from about 5 v/v% to about 50 v/v% of an amount of the aqueous solution.

307. The method of any of claims 287-305, wherein an amount of the first solvent is about 25 v/v% of an amount of the aqueous solution.

308. The method of any of claims 287-307, wherein a ratio of 1) a combined volume of the first and second solvents to 2) a volume of the aqueous solution is in from 1:1 to about 2:1.

309. The method of any of claims 287-308, wherein 3-HP is removed from the aqueous solution with a yield of at least 50%.

310. The method of any of claims 287-308, wherein 3-HP is removed from the aqueous solution with a yield of at least 60%.

311. The method of any of claims 287-308, wherein 3-HP is removed from the aqueous solution with a yield of at least 70%.

312. The method of any of claims 287-308, wherein 3-HP is removed from the aqueous solution with a yield of at least 80%.

313. The method of any of claims 287-308, wherein 3-HP is removed from the aqueous solution with a yield of at least 90%.

314. The method of any of claims 287-308, wherein 3-HP is removed from the aqueous solution with a yield of at least 95%.

315. The method of any of claims 287-308, wherein 3-HP is removed from the aqueous solution with a yield of at least 99%.

316. A method, comprising:

using a water-miscible solvent and a water-immiscible solvent to remove 3-hydroxypropionic acid (3-HP) from an aqueous solution.

317. The method of claim 316, wherein the method is performed without using counter-current liquid flow.

318. The method of claim 316 or claim 317, comprising before using the water-immiscible solvent to remove 3-HP from the aqueous solution:

evaporating the water-immiscible solvent; and

condensing the evaporated water-immiscible solvent.

319. The method of any of claims 316-318, comprising before the water-miscible solvent to remove 3-HP from the aqueous solution:

evaporating the water-miscible solvent; and

condensing the evaporated water-miscible solvent.

320. The method of any of claims 316-319, wherein the water-immiscible solvent has density of less than about 1 g/mL.

~~321. The method of any of claims 316-320, wherein the water-immiscible solvent comprises at least one solvent selected from the group consisting of a C_{1-6} alkyl acetate, a C_{4-6} alcohol, and a C_{1-6} alkyl ether.~~

~~322. The method of any of claims 316-320, wherein water-immiscible solvent comprises at least one solvent selected from the group consisting of methyl acetate, ethyl acetate, n-propyl acetate, isopropyl acetate, n-butyl acetate, isobutyl acetate, sec-butyl acetate, tert-butyl acetate, n-pentyl acetate, and n-hexyl acetate.~~

~~323. The method of any of claims 316-320, wherein the water-immiscible solvent comprises ethyl acetate.~~

~~324. The method of any of claims 316-320, wherein water-immiscible solvent comprises at least one solvent selected from the group consisting of n-butyl alcohol, isobutyl alcohol, tert-butyl alcohol, n-pentyl alcohol and n-hexyl alcohol.~~

~~325. The method of any of claims 316-320, wherein the water-immiscible solvent comprises isobutyl alcohol.~~

~~326. The method of any of claims 316-320, wherein water-immiscible solvent comprises at least one solvent selected from the group consisting of diethyl ether, dipropyl ether, ethyl propyl ether, methyl tert-butyl ether, and methyl hexyl ether.~~

~~327. The method of any of claims 316-326, wherein the water-immiscible solvent has density of greater than about 1 g/mL.~~

~~328. The method of any of claims 316-327, wherein the water-immiscible solvent comprises at least one member selected from the group consisting of chloroform, methylene chloride and carbon tetrachloride.~~

~~329. The method of any of claims 316-327, wherein the water-miscible solvent comprises a C_{1-3} alcohol.~~

~~330. The method of any of claims 316-327, wherein the water-miscible solvent comprises at least one solvent selected from the group consisting of methanol, ethanol, n-propanol, and isopropanol.~~

~~331. The method of any of claims 316-327, wherein the water-miscible solvent comprises methanol.~~

~~332. The method of any of claims 316-331, wherein an amount of the water-miscible solvent is from about 5 v/v% to about 50 v/v% of an amount of the aqueous solution.~~

~~333. The method of any of claims 316-331, wherein an amount of the water-miscible solvent is about 25 v/v% of an amount of the aqueous solution.~~

~~334. The method of any of claims 316-333, wherein a ratio of 1) a combined volume of the water-miscible solvent and the water-immiscible solvent to 2) a volume of the aqueous solution is from about 1:1 to about 2:1.~~

~~335. The method of any of claims 316-334, wherein 3-HP is removed from the aqueous solution with a yield of at least 50%.~~

~~336. The method of any of claims 316-334, wherein 3-HP is removed from the aqueous solution with a yield of at least 60%.~~

~~337. The method of any of claims 316-334, wherein 3-HP is removed from the aqueous solution with a yield of at least 70%.~~

~~338. The method of any of claims 316-334, wherein 3-HP is removed from the aqueous solution with a yield of at least 80%.~~

~~339. The method of any of claims 316-334, wherein 3-HP is removed from the aqueous solution with a yield of at least 90%.~~

~~340. The method of any of claims 316-334, wherein 3-HP is removed from the aqueous solution with a yield of at least 95%.~~

~~341. The method of any of claims 316-334, wherein 3-HP is removed from the aqueous solution with a yield of at least 99%.~~

~~342. The method of any of claims 211-341, wherein the pH of the aqueous solution is at least about 3.~~

~~343. The method of any of claims 211-341, wherein the pH of the aqueous solution is at least about 4.~~

~~344. The method of any of claims 211-341, wherein the pH of the aqueous solution is from about 3 to about 7.~~

~~345. The method of any of claims 211-341, wherein the pH of the aqueous solution is from about 4 to about 6.~~

~~346. The method of any of claims 211-341, wherein the pH the aqueous solution is from about 4.2 to about 4.7.~~

~~347. A method, comprising:~~

~~removing 3-hydroxypropionic acid (3-HP) from an aqueous solution,~~

~~wherein the pH of the aqueous solution is at least about 3.~~

~~348. The method of claim 347, wherein the pH of the aqueous solution is at least about 4.~~

~~349. The method of claim 347 or claim 348, wherein pH of the aqueous solution is at most about 7.~~

~~350. The method of claim 347, wherein pH of the aqueous solution is from about 4 to about 6.~~

~~351. The method of claim 347, wherein pH of the aqueous solution is from about 4 to about 5.~~

~~352. The method of claim 347, wherein pH of the aqueous solution is from about 4.2 to about 4.8.~~

~~353. The method of claim 347, wherein pH of the aqueous solution is from about 4.3 to about 4.6~~

~~354. The method of claim 347, wherein pH of the aqueous solution is about 4.4.~~

~~355. The method of any of claims 347-354, wherein the method is performed without using counter-current liquid flow.~~

~~356. The method of any of claims 347-355, comprising using a water-immiscible solvent to remove 3-HP from the aqueous solution.~~

~~357. The method of claim 356, comprising before using the water-immiscible solvent to remove the 3-HP:~~

~~evaporating the water-immiscible solvent; and~~

~~condensing the evaporated water-immiscible solvent.~~

~~358. The method of claim 356 or claim 357, wherein the water-immiscible solvent has density of less than about 1 g/mL.~~

~~359. The method of any of claims 356-358, wherein the water-immiscible solvent comprises at least one solvent selected from the group consisting of a C_{1-6} alkyl acetate, a C_{4-6} alcohol, and a C_{1-6} alkyl ether.~~

~~360. The method of any of claims 356-358, wherein the water-immiscible solvent comprises at least one solvent selected from the group consisting of methyl acetate, ethyl acetate, n-propyl acetate, isopropyl acetate, n-butyl acetate, isobutyl acetate, sec-butyl acetate, tert-butyl acetate, n-pentyl acetate, and n-hexyl acetate.~~

~~361. The method of any of claims 356-358, wherein the water-immiscible solvent comprises ethyl acetate.~~

~~362. The method of any of claims 356-358, wherein the water-immiscible solvent comprises at least one solvent selected from the group consisting of n-butyl alcohol, isobutyl alcohol, tert-butyl alcohol, n-pentyl alcohol and n-hexyl alcohol.~~

~~363. The method of any of claims 356-358, wherein the water-immiscible solvent comprises isobutyl alcohol.~~

~~364. The method of any of claims 356-358, wherein the water-immiscible solvent comprises at least one solvent selected from the group consisting of diethyl ether, dipropyl ether, ethyl propyl ether, methyl tert-butyl ether, and methyl hexyl ether.~~

~~365. The method of any of claims 355-364, wherein the water-immiscible solvent has density of greater than about 1 g/mL.~~

366. The method of any of claims 356-365, wherein the water-immiscible solvent comprises at least one solvent selected from the group consisting of chloroform, methylene chloride and carbon tetrachloride.

367. The method of any of claims 347-365, comprising using a water-miscible solvent for removing 3-HP from the aqueous solution.

368. The method of claim 367, wherein the using of the water-miscible solvent comprises adding the water-miscible solvent to the aqueous solution.

369. The method of claim 367 or claim 368, wherein the water-miscible solvent is a C_{1-3} alcohol.

370. The method of claim 367 or claim 368, wherein water-miscible solvent comprises at least one solvent selected from the group consisting of methanol, ethanol, n-propanol, and isopropanol.

371. The method of claim 367 or claim 366, wherein the water-miscible solvent comprises methanol.

372. The method of any of claims 367-371, wherein an amount of the water-miscible solvent is from about 5 v/v% to about 50 v/v% of an amount of the aqueous solution.

373. The method of any of claims 367-371, wherein an amount of the water-miscible solvent is about 25 v/v% of an amount of the aqueous solution.

374. The method of any of claims 357-373, wherein a ratio of 1) a combined volume of the water-miscible solvent and the water-immiscible solvent to 2) a volume of the aqueous solution is from about 1:1 to about 2:1.

~~375. The method of any of claims 347-374, wherein 3-HP is removed from the aqueous solution with a yield of at least 50%.~~

~~376. The method of any of claims 347-374, wherein 3-HP is removed from the aqueous solution with a yield of at least 60%.~~

~~377. The method of any of claims 347-374, wherein 3-HP is removed from the aqueous solution with a yield of at least 70%.~~

~~378. The method of any of claims 347-374, wherein 3-HP is removed from the aqueous solution with a yield of at least 80%.~~

~~379. The method of any of claims 347-374, wherein 3-HP is removed from the aqueous solution with a yield of at least 90%.~~

~~380. The method of any of claims 347-374, wherein 3-HP is removed from the aqueous solution with a yield of at least 95%.~~

~~381. The method of any of claims 347-369, wherein 3-HP is removed from the aqueous solution with a yield of at least 99%.~~

~~382. The method of any of claims 211-381, wherein the aqueous solution of 3-HP comprises a decellularized fermentation broth.~~

~~383. The method of any of claims 211-382, wherein a concentration of 3-HP in the aqueous solution is from about 50 g/L to about 100 g/L.~~

~~384. The method of any of claims 211-382, wherein a concentration of 3-HP in the aqueous solution is from about 60 g/L to about 80 g/L.~~

~~385. The method of any of claims 211-384, wherein the aqueous solution comprises an acid.~~

~~386. The method of any of claims 211-384, wherein the acid comprises at least one acid selected from the group consisting of sulfuric acid, hydrochloric acid, acetic acid, and oxalic acid.~~

~~387. The method of any of claims 211-386, wherein a temperature of the aqueous solution is from about 15 °C to about 50 °C.~~

~~388. The method of any of claims 211-386, wherein a temperature of the aqueous solution is from about 15 °C to about 40 °C.~~

~~389. The method of any of claims 211-386, wherein a temperature of the aqueous solution is from about 20 °C to about 30 °C.~~

~~390. The method of any of claims 211-386, wherein the aqueous solution is at about room temperature.~~

~~391. The method of any of claims 211-390, further comprising, after removing 3-HP from the aqueous solution, reacting the 3-HP to make acrylic acid.~~

~~392. The method of 391, comprising reacting the 3-HP at a pressure that is less than about 700 mbar.~~

~~393. The method of claim 391 or 392, comprising reacting 3-HP is performed in an aqueous solution.~~

~~394. The method of claim 393, comprising contacting the aqueous solution with a zeolite.~~

~~395. The method of claim 393 or 394, wherein the aqueous solution comprises an acid.~~

~~396. The method of any of claims 391-395, wherein reacting the 3-HP comprises heating the 3-HP to a temperature of at least about 50 °C.~~

~~397. The method of any of claims 391-396, wherein the reacting is performed in the presence of a polymerization inhibitor.~~

~~398. A method, comprising:~~

~~reacting 3-hydroxypropionic acid (3-HP) to form acrylic acid at a pressure of less than about one atmosphere to provide acrylic acid.~~

~~399. The method of claim 398, wherein the pressure is less than about 700 mbar.~~

~~400. The method of claim 398, wherein the pressure is less than about 500 mbar.~~

~~401. The method of claim 398, wherein the pressure is less than about 200 mbar.~~

~~402. The method of claim 398, wherein the pressure is less than about 100 mbar.~~

~~403. The method of claim 398, wherein the pressure is in a range of about 70 to about 100 mbar.~~

~~404. The method of any of claims 398-403, wherein 3-HP is performed in an aqueous solution.~~

~~405. The method of claim 404, comprising contacting the aqueous solution with a zeolite.~~

~~406. The method of claim 405, wherein the zeolite comprises a 4A molecular sieve.~~

~~407. The method of claim 405 or claim 406, wherein an amount of zeolite is from about 1 wt.% to about 5 wt.% based on an amount of the aqueous solution.~~

~~408. The method of claim 405 or claim 406, wherein an amount of zeolite is from about 2 wt.% to about 4 wt.% based on an amount of the aqueous solution.~~

~~409. The method of any of claims 398-408, wherein reacting 3-HP comprises heating 3-HP to a temperature of at least about 50 °C.~~

~~410. The method of any of claims 398-408, wherein reacting 3-HP comprises heating 3-HP to a temperature of at least about 80 °C.~~

~~411. The method of any of claims 398-408, wherein reacting 3-HP comprises heating 3-HP to a temperature of at most about 180 °C.~~

~~412. The method of any of claim 404-411, wherein the aqueous solution comprises an acid.~~

~~413. The method of claim 412, wherein the acid comprises at least one acid selected from the group consisting of oxalic acid and polyphosphoric acid.~~

~~414. The method of claim 412 or claim 413, wherein an amount of the acid is from about 2 wt.% to about 20 wt.% based on an amount of 3-HP.~~

~~415. The method of any of claims 398-414, comprising reacting the 3-HP in the presence of a polymerization inhibitor.~~

~~416. The method of claim 415, wherein the polymerization inhibitor comprises at least one polymerization inhibitor selected from the group consisting of phenothiazine and hydroquinone.~~

~~417. The method of claim 415 or claim 416, wherein an amount of the polymerization inhibitor is from about 5 wt.% to about 10 wt.% based on an amount of 3-HP.~~

~~418. A method, comprising:~~

~~reacting 3-hydroxypropionic acid (3-HP) to form acrylic acid in a reaction mixture; and~~

~~removing gaseous acrylic acid from the reaction mixture.~~

~~419. The method of claim 418, wherein the reaction mixture comprises a liquid.~~

~~420. The method of claim 419, wherein the liquid is aqueous.~~

~~421. The method of any of claims 418-420, comprising contacting the reaction mixture with a zeolite.~~

~~422. The method of claim 421, wherein the zeolite comprises a 4A molecular sieve.~~

~~423. The method of claim 421 or claim 422, wherein an amount of zeolite is from about 1 wt.% to about 5 wt.% based on an amount of the aqueous solution.~~

~~424. The method of claim 421 or claim 422, wherein an amount of zeolite is from about 2 wt.% to about 4 wt.% based on an amount of the aqueous solution.~~

~~425. The method of any of claims 418-424, wherein removing the gaseous acrylic acid is performed at a pressure of less than about one atmosphere.~~

~~426. The method of claim 425, wherein the pressure is less than about 700 mbar.~~

~~427. The method of claim 425, wherein the pressure is less than about 500 mbar.~~

~~428. The method of claim 425, wherein the pressure is less than about 200 mbar.~~

~~429. The method of claim 425, wherein the pressure is less than about 100 mbar.~~

~~430. The method of claim 425, wherein the pressure is in a range of about 70 to about 100 mbar.~~

~~431. The method of any of claims 418-430, wherein reacting 3-HP comprises heating 3-HP to a temperature of at least about 50 °C.~~

~~432. The method of any of claims 418-430, wherein reacting 3-HP comprises heating 3-HP to a temperature of at least about 80 °C.~~

~~433. The method of any of claims 418-430, wherein reacting 3-HP comprises heating 3-HP to a temperature of at most about 180 °C.~~

~~434. The method of any of claim 418-423, wherein the reaction mixture comprises an acid.~~

~~435. The method of claim 434, wherein the acid comprises at least one acid selected from the group consisting of oxalic acid and polyphosphoric acid.~~

~~436. The method of claim 434 or claim 435, wherein an amount of the acid is from about 2 wt.% to about 20 wt.% based on an amount of 3-HP.~~

~~437. The method of any of claims 418-436, wherein the reaction mixture comprises a polymerization inhibitor.~~

~~438. The method of claim 437, wherein the polymerization inhibitor comprises a polymerization inhibitor selected from the group consisting of phenothiazine and hydroquinone.~~

~~439. The method of claim 437 or claim 438, wherein an amount of the polymerization inhibitor is from about 5 wt.% to about 10 wt.% based on an amount of 3-HP.~~

~~440. A method, comprising:~~

~~reacting a liquid comprising 3-hydroxypropionic acid (3-HP) to form acrylic acid.~~

~~441. The method of claim 440, wherein the liquid comprises an aqueous solution.~~

~~442. The method of claim 440 or claim 441, comprising forming gaseous acrylic acid.~~

~~443. The method of any of claims 440-442, comprising removing the acrylic acid from the liquid.~~

~~444. The method of claim 443, wherein removing is performed at a pressure of less than about one atmosphere.~~

~~445. The method of claim 444, wherein the pressure is less than about 700 mbar.~~

~~446. The method of claim 444, wherein the pressure is less than about 500 mbar.~~

~~447. The method of claim 444, wherein the pressure is less than about 200 mbar.~~

~~448. The method of claim 444, wherein the pressure is less than about 100 mbar.~~

~~449. The method of claim 444, wherein the pressure is in a range of about 70 to about 100 mbar.~~

~~450. The method of any of claims 440-449, comprising contacting the liquid with a zeolite.~~

~~451. The method of claim 450, wherein the zeolite comprises a 4A molecular sieve.~~

~~452. The method of claim 440 or claim 441, wherein an amount of zeolite is from about 1 wt.% to about 5 wt.% based on an amount of the aqueous solution.~~

~~453. The method of claim 440 or claim 441, wherein an amount of zeolite is from about 2 wt.% to about 4 wt.% based on an amount of the aqueous solution.~~

~~454. The method of any of claims 440-453, wherein reacting 3-HP comprises heating 3-HP to a temperature of at least about 50 °C.~~

455. The method of any of claims 440-453, wherein reacting 3-HP comprises heating 3-HP to a temperature of at least about 80 °C.

456. The method of any of claims 440-453, wherein reacting 3-HP comprises heating 3-HP to a temperature of at most about 180 °C.

457. The method of any of claim 440-456, wherein the liquid comprises an acid.

458. The method of claim 457, wherein the acid comprises at least one acid selected from the group consisting of oxalic acid and polyphosphoric acid.

459. The method of claim 247 or claim 248, wherein an amount of the acid is from about 2 wt.% to about 20 wt.% based on an amount of 3-HP.

460. The method of any of claims 440-459, wherein reacting is performed in the presence of a polymerization inhibitor.

461. The method of claim 460, wherein the polymerization inhibitor comprises at least one polymerization inhibitor selected from the group consisting of phenothiazine and hydroquinone.

462. The method of claim 460 or claim 461, wherein an amount of the polymerization inhibitor is from about 5 wt.% to about 10 wt.% based on an amount of 3-HP.

463. The method of any of claims 391-462, wherein the acrylic acid is formed with a yield of at least about 70%.

464. The method of any of claims 391-462, wherein the acrylic acid is formed with a yield of at least about 80%.

~~465. The method of any of claims 391-462, wherein the acrylic acid is formed with a yield of at least about 90%.~~

~~466. The method of any of claims 391-462, wherein the acrylic acid is formed with a yield of at least about 95%.~~

~~467. The method of any of claims 391-462, wherein the acrylic acid is formed with a yield of at least about 99%.~~

~~468. A method, comprising:~~

~~reacting 3-hydroxypropionic acid (3-HP) to form acrylic acid with a yield of at least about 70%.~~

~~469. The method of claim 468, wherein the acrylic acid is formed with a yield of at least about 80%.~~

~~470. The method of claim 468, wherein the acrylic acid is formed with a yield of at least about 90%.~~

~~471. The method of claim 468, wherein the acrylic acid is formed with a yield of at least about 95%.~~

~~472. The method of claim 468, wherein the acrylic acid is formed with a yield of at least about 99%.~~

~~473. The method of any of claims 468-472, wherein the 3-HP is performed in an aqueous solution.~~

~~474. The method of any of claims 468-473, comprising forming gaseous acrylic acid.~~

~~475. The method of any of claims 468-474, comprising removing the acrylic acid from the liquid.~~

~~476. The method of claim 475, wherein the removing is performed at a pressure of less than about one atmosphere.~~

~~477. The method of claim 476, wherein the pressure is less than about 700 mbar.~~

~~478. The method of claim 476, wherein the pressure is less than about 500 mbar.~~

~~479. The method of claim 476, wherein the pressure is less than about 200 mbar.~~

~~480. The method of claim 476, wherein the pressure is less than about 100 mbar.~~

~~481. The method of claim 476, wherein the pressure is in a range of about 70 to about 100 mbar.~~

~~482. The method of any of claims 468-481, comprising contacting the aqueous solution with a zeolite.~~

~~483. The method of claim 482, wherein the zeolite is a 4A molecular sieve.~~

~~484. The method of claim 482 or claim 483, wherein an amount of zeolite is from about 1 wt.% to about 5 wt.% based on an amount of the aqueous solution.~~

485. The method of claim 482 or claim 483, wherein an amount of zeolite is from about 2 wt.% to about 4 wt.% based on an amount of the aqueous solution.

486. The method of any of claim 468-485, wherein the aqueous solution comprises an acid.

487. The method of claim 486, wherein the acid comprises an acid selected from the group consisting of oxalic acid and polyphosphoric acid.

488. The method of claim 486 or claim 487, wherein an amount of the acid is from about 2 wt.% to about 20 wt.% based on an amount of 3-HP.

489. The method of any of claims 468-488, wherein the reacting is performed in the presence of a polymerization inhibitor.

490. The method of claim 489, wherein the polymerization inhibitor comprises a polymerization inhibitor selected from the group consisting of phenothiazine and hydroquinone.

491. The method of claim 489 or claim 490, wherein an amount of the polymerization inhibitor is from about 5 wt.% to about 10 wt.% based on an amount of 3-HP.

492. The method of any of claims 468-491, wherein reacting 3-HP comprises heating 3-HP to a temperature of at least about 50 °C.

493. The method of any of claims 468-491, wherein reacting 3-HP comprises heating 3-HP to a temperature of at least about 80 °C.

494. The method of any of claims 468-491, wherein reacting 3-HP comprises heating 3-HP to a temperature of at most about 180 °C.

1. A nucleic acid, comprising a first and a second promoter, wherein either the first is an inducible promoter and is inducible by a small molecule; the first and second promoters are operably linked to a first gene; and the first gene encodes a protein involved in the synthesis of 3-hydroxypropionic acid (3-HP), a salt of 3-HP, or coenzyme B12, wherein at least the 10 codons at the 5'-terminus of the first gene are optimized for translation in Pseudomonas denitrificans, and wherein codon optimization comprises:

measuring a codon frequency of each amino acid in a gene encoding a native *Pseudomonas denitrificans* protein; and

using the codon frequency of the native protein to replace the 10 codons at the 5'-terminus of the first gene with an optimized 10 codons, wherein the codon for each amino acid of the optimized 10 codons is present at the same frequency as the codon frequency of the native protein,

wherein the gene encoding a native *Pseudomonas denitrificans* protein is mmsA.

2. The nucleic acid of claim 2, wherein the nucleic acid comprises a sequence that is 95% identical to a sequence selected from the group consisting of SEQ ID Nos: 29, 30, and 31 (Opt1-3).