However, these methods reduce the available arable land for food production and are expensive.

Given the high demand for methionine and the relative high cost and unreliability of fermentable carbohydrates, there is a need in the art for alternative and improved methods for biologically producing methionine in a cost-effective manner. The present disclosure meets such needs, and further provides other related advantages.

## SUMMARY

In an aspect, there is provided a non-natural methanogenic archaea, wherein the non-natural methanogenic archaea metabolizes a $CO_x$ substrate, optionally in the presence of $H_2$, to produce methionine at a higher level than a parent methanogenic archaea and wherein the non-natural methanogenic archaea expresses at least one polypeptide chosen from:

(a) a polypeptide having an amino acid sequence as set forth in at least one of SEQ ID NOS.:4, 8, or 32;

(b) a MMP1359 protein (SEQ ID NO: 3) that is mutated, wherein the protein has a mutation at residue D439, wherein the residue numbering corresponds to residue positions of MMP1359 protein from *Methanococcus maripaludis* S2 DSM14266; or

(c) an ortholog of SEQ ID NO.:3 that is mutated, wherein the ortholog has a mutation at residue D439, wherein the residue numbering corresponds to residue positions of MMP1359 protein from *Methanococcus maripaludis* S2 DSM14266; or

(d) a MMP1358 protein (SEQ ID NO: 7) that is mutated, wherein the protein has a mutation at residue G114, wherein the residue numbering corresponds to residue positions of MMP1358 protein from *Methanococcus maripaludis* S2 DSM14266; or

(e) an ortholog of SEQ ID NO.:7 that is mutated, wherein the ortholog has a mutation at residue G114, wherein the residue numbering corresponds to residue positions of MMP1358 protein from *Methanococcus maripaludis* S2 DSM14266; or

(f) a C2A1821 protein (SEQ ID NO: 30) that is mutated, wherein the protein has a mutation at residue S489, wherein the residue numbering corresponds to residue positions of C2A1821 protein from *Methanosarcina acetivorans* C2A; or

(g) an ortholog of SEQ ID NO.:30 that is mutated, wherein the ortholog has a mutation at residue S489, wherein the residue numbering corresponds to residue positions of C2A1821 protein from *Methanosarcina acetivorans* C2A.

In another aspect, there is provided a method for producing methionine, comprising culturing a non-natural methanogenic archaea as defined herein in the presence of a substrate comprising $H_2$ and $CO_2$, CO, or CO and $CO_2$ under conditions for a time sufficient to produce methionine, wherein the non-natural methanogenic archaea: (a) expresses one or more sulfur assimilation polypeptides having increased activity as compared to a parent methanogenic archaea; (b) overexpresses one or more sulfur assimilation polypeptides; or (c) comprises altered regulation of one or more sulfur assimilation polypeptides, wherein the non-natural methanogenic archaea produces methionine at a higher level than a parent methanogenic archaea.

In another aspect, there is provided a system for producing methionine, comprising:

(a) a source of gas comprising a $CO_x$ substrate, optionally in the presence of $H_2$;

(b) a bioreactor comprising a non-natural methanogenic archaea as defined herein comprising an exogenous nucleic acid molecule encoding a sulfur assimilation polypeptide; and

(c) a connector disposed between the gas source and the bioreactor to allow flow of the gas into the bioreactor;

wherein the non-natural methanogenic archaea metabolizes the $CO_x$ substrate, optionally in the presence of $H_2$, to overproduce methionine as compared to a parent methanogenic archaea.

## DETAILED DESCRIPTION

The present disclosure provides compositions and methods for metabolic engineering (e.g., altering genes, gene expression, gene expression regulation) of

hydrogenotrophic microorganisms to produce higher levels of methionine as compared to a wild-type or parent organism.

By way of background, many microorganisms, including those classified as Archaea and hydrogenotrophic microorganisms, produce methionine via biosynthetic pathways that share several enzymes that are involved in the production of other amino acids, such as the aspartate pathway amino acids. One or more of these amino acid biosynthesis enzymes are subject to feedback regulation, repression of gene expression, or both. For example, aspartokinase, the first committed enzyme involved in directing carbon flux into the biosynthesis of industrially important amino acids (e.g., methionine), is allosterically inhibited from phosphorylating aspartate by threonine and lysine in Coryne bacterium glutamicum (Sano and Shiio, J. Gen. Appl. Microbiol. 16:373, 1970; Yoshida et al, J. Mol. Biol. 368:521, 2007). Another enzyme, homoserine O-succinyltransferase is subject to feedback regulation by methionine and ^-adenosylmethionine (Born and Blanchard, Biochem. 35: 14416, 1999). Homoserine dehydrogenase is the first committed enzyme in the methionine/ threonine biosynthetic pathways, but it has to compete for aspartyl semialdehyde with the first enzyme committed to the lysine biosynthetic pathway, dihydrodipicolinate synthase. Hence, whether carbon flux goes toward methionine or lysine will depend on which enzyme obtains the substrate.

## CLAIMS

What is claimed is:

1. A non-natural methanogenic archaea, wherein the non-natural methanogenic archaea metabolizes a $CO_x$ substrate, optionally in the presence of $H_2$, to produce methionine at a higher level than a parent methanogenic archaea and wherein the non-natural methanogenic archaea expresses at least one polypeptide chosen from:

(a) a polypeptide having an amino acid sequence as set forth in at least one of SEQ ID NOS.:4, 8, or 32;

(b) a MMP1359 protein (SEQ ID NO: 3) that is mutated, wherein the protein has a mutation at residue D439, wherein the residue numbering corresponds to residue positions of MMP1359 protein from *Methanococcus maripaludis* S2 DSM14266; or

(c) an ortholog of SEQ ID NO.:3 that is mutated, wherein the ortholog has a mutation at residue D439, wherein the residue numbering corresponds to residue positions of MMP1359 protein from *Methanococcus maripaludis* S2 DSM14266; or

(d) a MMP1358 protein (SEQ ID NO: 7) that is mutated, wherein the protein has a mutation at residue G114, wherein the residue numbering corresponds to residue positions of MMP1358 protein from *Methanococcus maripaludis* S2 DSM14266; or

(e) an ortholog of SEQ ID NO.:7 that is mutated, wherein the ortholog has a mutation at residue G114, wherein the residue numbering corresponds to residue positions of MMP1358 protein from *Methanococcus maripaludis* S2 DSM14266; or

(f) a C2A1821 protein (SEQ ID NO: 30) that is mutated, wherein the protein has a mutation at residue S489, wherein the residue numbering corresponds to residue positions of C2A1821 protein from *Methanosarcina acetivorans* C2A; or

(g) an ortholog of SEQ ID NO.:30 that is mutated, wherein the ortholog has a mutation at residue S489, wherein the residue numbering corresponds to residue positions of C2A1821 protein from *Methanosarcina acetivorans* C2A.

2. The non-natural methanogenic archaea according to claim 1(b) or claim 1(c), wherein the mutation at D439 is a D439N substitution and the residue numbering corresponds to residue positions of MMP1359 from *Methanococcus maripaludis* S2 DSM14266.

3. The non-natural methanogenic archaea according to claim 1(d) or claim 1(e), wherein the mutation at G114 is a G114E substitution and the residue numbering corresponds to residue positions of MMP1358 from *Methanococcus maripaludis* S2 DSM14266.

4. The non-natural methanogenic archaea according to claim 1(f) or claim 1(g), wherein the mutation at S489 is a S489N substitution and the residue numbering corresponds to residue positions of C2A1821 from *Methanosarcina acetivorans* C2A.

5. The non-natural methanogenic archaea according to any one of claims 1-4, wherein the non-natural methanogenic archaea further comprises a deregulated aspartokinase activity, a methionine synthase, or both.

6. The non-natural methanogenic archaea according to claim 5, wherein the deregulated aspartokinase activity is an exogenous aspartokinase and is encoded by

(a) a mutant *lysC* gene comprising a mutation at a threonine binding site, optionally wherein the threonine binding site mutation is at residue

I272, D274, G277, E278, A279, D294, Q298, N372, N374, I375, or any combination thereof, wherein the residue numbering corresponds to residue positions encoded by *lysC* of *Corynebacterium glutamicum* ATCC 13032;

(b) a mutant *lysC* gene comprising a mutation at a lysine binding site, optionally wherein the lysine binding site mutation is at residue I291, I293, D294, T361, S381, E382, or any combination thereof, wherein the residue numbering corresponds to residue positions encoded by *lysC* of *Corynebacterium glutamicum* ATCC 13032;

(c) a mutant *lysC* gene comprising a mutation at a lysine and threonine binding site, optionally wherein the residue numbering corresponds to residue positions encoded by *lysC* of *Corynebacterium glutamicum* ATCC 13032; and/or

(d) a mutant *lysC* gene comprising a mutation at a site other than a lysine or threonine binding site, optionally wherein the mutation at a site other than a lysine and threonine binding site is at residue F283, N299, S301, S302, T308, T311, T336, G359, F364, M365, T380, R384, S386, or any combination thereof, wherein the residue numbering corresponds to residue positions encoded by *lysC* of *Corynebacterium glutamicum* ATCC 13032.

7. The non-natural methanogenic archaea according to any one of claims 1-6, wherein the non-natural methanogenic archaea further comprises an exogenous nucleic acid molecule encoding one or more polypeptides from a methionine biosynthetic pathway selected from aspartokinase, aspartyl semialdehyde dehydrogenase, homoserine dehydrogenase, homoserine *O* -acetyltransferase, homoserine *O*-transsuccinyltransferase, *O* -succinylhomoserine lyase, cystathionine γ-synthase, cystathionine β-lyase, *O* -acetylhomoserine sulfhydrylase, homocysteine *S*-methyltransferase, methionine synthase (cobalamin dependent or independent), or any combination thereof; and optionally wherein:

(a) (i) the exogenous nucleic acid molecule encodes a homoserine dehydrogenase, a serine acetyltransferase, or both, and optionally the homoserine dehydrogenase, serine acetyltransferase, or both are overexpressed and/or the homoserine dehydrogenase, serine acetyltransferase, or both are deregulated; or (ii) the exogenous nucleic acid molecule encodes a homoserine *O*-acetyltransferase, an *O*-acetylhomoserine sulfhydrylase, or both and optionally the homoserine *O*-acetyltransferase, *O*-acetylhomoserine sulfhydrylase, or both are overexpressed

and/or the homoserine *O*-acetyltransferase, *O*-acetylhomoserine sulfhydrylase or both are deregulated;

(b) the exogenous nucleic acid molecule encodes a methionine synthase, and optionally wherein the methionine synthase is overexpressed as compared to a parent methanogenic archaea lacking the exogenous nucleic acid molecule encoding methionine synthase.

8. The non-natural methanogenic archaea according to claim 7, wherein

(a) one or more nucleic acid molecules encoding polypeptides from a lysine biosynthetic pathway are knocked out or have reduced activity, and/or

(b) one or more nucleic acid molecules encoding polypeptides from a threonine biosynthetic pathway are knocked out or have reduced activity; and/or

(c) optionally wherein a nucleic acid molecule that encodes a dihydrodipicolinate synthase, a homoserine kinase, a threonine dehydratase, a threonine aldolase, a serine hydroxymethyl transferase, or any combination thereof are knocked out or encode a reduced activity dihydrodipicolinate synthase mutant, a homoserine kinase mutant, threonine dehydratase mutant, threonine aldolase mutant, serine hydroxymethyl transferase mutant, or any combination thereof.

9. The non-natural methanogenic archaea according to any one of claims 6-8, wherein the exogenous nucleic acid molecule is (a) integrated in the genome of the non-natural methanogenic archaea, (b) in a self-replicating vector in the non-natural methanogenic archaea.

10. The non-natural methanogenic archaea according to any one of claims 1-9, wherein the non-natural methanogenic archaea (a) is a lysine auxotroph, threonine auxotroph, glycine auxotroph, or any combination thereof, (b) has reduced phosphoenolpyruvate synthase activity, increased pyruvate kinase activity, or both, or (c) has increased pyruvate carboxylase activity, increased 5-methyltetrahydrofolate corrinoid/iron sulfur protein methyltransferase activity, increased pyruvate synthase, increased acetyl-CoA synthase, increased aspartate aminotransferase activity, or any combination thereof.

11. The non-natural methanogenic archaea according to any one of the preceding claims, wherein the $CO_x$ substrate is a $H_2/CO_x$ substrate

comprised of $H_2$, CO, and $CO_2$, and the $H_2/CO_x$ substrate is optionally comprised of syngas or water-gas shifted syngas.

12. The non-natural methanogenic archaea according to claim 11, wherein (a) the ratio of $CO_2$ to $H_2$ ranges from 1:50 to 10:1, respectively, (b) the ratio of $CO_2$ to $H_2$ ranges from 1:2 to 1:4, respectively; and optionally wherein the total amount of CO is no more than 1%.

13. The non-natural methanogenic archaea according to claim 1, wherein the methanogenic archaea does not produce cytochromes.

14. The non-natural methanogenic archaea according claim 1, wherein the methanogenic archaea produces cytochromes.

15. The non-natural methanogenic archaea according to any one of the preceding claims, wherein the non-natural methanogenic archaea (a) expresses or overexpresses an exporter of methionine and/or (b) further comprises an exogenous nucleic acid molecule that encodes an exporter of methionine.

16. A method for producing methionine, comprising culturing a non-natural methanogenic archaea according to any one of claims 1-15 in the presence of a substrate comprising $H_2$ and $CO_2$, CO, or CO and $CO_2$ under conditions for a time sufficient to produce methionine, wherein the non-natural methanogenic archaea: (a) expresses one or more sulfur assimilation polypeptides having increased activity as compared to a parent methanogenic archaea; (b) overexpresses one or more sulfur assimilation polypeptides; or (c) comprises altered regulation of one or more sulfur assimilation polypeptides, wherein the non-natural methanogenic archaea produces methionine at a higher level than a parent methanogenic archaea.

17. A system for producing methionine, comprising:

(a) a source of gas comprising a $CO_x$ substrate, optionally in the presence of $H_2$;

(b) a bioreactor comprising a non-natural methanogenic archaea according to any one of claims 1-15 comprising an exogenous nucleic acid molecule encoding a sulfur assimilation polypeptide; and

(c) a connector disposed between the gas source and the bioreactor to allow flow of the gas into the bioreactor;

wherein the non-natural methanogenic archaea metabolizes the $CO_x$ substrate, optionally in the presence of $H_2$, to overproduce methionine as compared to a parent methanogenic archaea.

18. The system according to claim 17, wherein the bioreactor is a liquid phase, bubble column, or trickle bed bioreactor.

19. The system according to claim 17, wherein the $CO_x$ substrate is a $H_2/CO_x$ substrate comprised of syngas or water-gas shifted syngas.