methods include culturing the non-naturally occurring microbial organisms under conditions and for a sufficient period of time to produce 6-aminocaproic acid, 1,6-hexanediol, caprolactone, caprolactam, hexamethylenediamine.

[0007] In one aspect provided are non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding an aldehyde dehydrogenase enzyme that reacts with adipyl-CoA to form adipate-semialdehyde, wherein the aldehyde dehydrogenase has greater catalytic efficiency for adipyl-CoA as a substrate as compared to succinyl-CoA, acetyl-CoA, or both as substrates, and/or the aldehyde dehydrogenase has higher turnover number for adipyl-CoA substrate as compared to succinyl-CoA, acetyl-CoA, or both succinyl-CoA and acetyl-CoA substrates. In one example, provided are a non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding an aldehyde dehydrogenase enzyme that reacts with adipyl-CoA to form adipate-semialdehyde, wherein the aldehyde dehydrogenase has greater catalytic efficiency for adipyl-CoA substrate as compared to succinyl-CoA, acetyl-CoA, or both succinyl-CoA and acetyl-CoA substrates, and/or the aldehyde dehydrogenase has higher turnover number for adipyl-CoA substrate as compared to succinyl-CoA, acetyl-CoA, or both succinyl-CoA and acetyl-CoA substrates.

[0008] In one aspect, provided are methods of producing adipate-semialdehyde comprising culturing a non-naturally occurring microorganism of any one of the above aspects and examples for a sufficient time period and conditions for producing adipate-semialdehyde.

[0009] In one aspect, provided are methods of producing 6-aminocaproic acid (6ACA) comprising culturing a non-naturally occurring microbial organism of any one the above aspect and examples for a sufficient time period and conditions for producing 6ACA. In some examples, the methods further include recovering 6ACA from the microbial organism, fermentation broth, or both.

[0010] In one example provided are methods of producing hexamethylene diamine comprising culturing a non-naturally occurring microbial organism of any one of the above aspects and examples for a sufficient time period and conditions for producing hexamethylene diamine. In some examples, the methods further include recovering hexamethylene diamine from the microbial organism, fermentation broth, or both. In some examples, the non-naturally occurring microbial organism comprises two, three, four, five, six or seven exogenous nucleic acid sequences each encoding a hexamethylene diamine pathway enzyme.

[0011] In one example, provided are methods of producing 6-aminocaproic acid, 1,6-hexanediol, caprolactone, caprolactam, hexamethylenediamine comprising culturing a non-naturally occurring microbial organism of any one of the above examples and examples for a sufficient time period and conditions for producing 6-aminocaproic acid, 1,6-hexanediol, caprolactone, caprolactam, and hexamethylenediamine. In some examples, the methods further include recovering 6-aminocaproic acid, 1,6-hexanediol, caprolactone, caprolactam, and hexamethylenediamine.from the microbial organism, fermentation broth, or both. In some examples, the non-naturally occurring microbial organism comprises two, three, four, five, six or seven exogenous nucleic acid sequences each encoding 6-aminocaproic acid, 1,6-hexanediol, caprolactone, caprolactam, hexamethylenediamine pathway enzymes.

[0012] In one aspect, provided are bioderived 6-aminocaproic acid, hexamethylenediamine, or caprolactam synthesized using the disclosed methods.

[0013] In some examples, the aldehyde dehydrogenase enzyme of the non-naturally occurring microbial organism does not comprise the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

[0014] In some examples, the aldehyde dehydrogenase enzymeof the non-naturally occurring microbial organism comprises an amino acid sequence having at least about 60% amino acid sequence identity to at least 25, 50, 75, 100, 150, 200, 250, 300, or more contiguous amino acids of any of SEQ ID NOs: 4, 7, 11, 15, 17, 19, 24, 25, 27, 28, 31-33, 36, 38, 40-42, 44, 45, 47, 53, 58-60, 63, 65-67, 74, 75, 77, 80, 82, 84, 86-88, 90, 91, 94, 95, 97, 100, 101, 103, 107, 109, 111, 112, 117, 134, 135, 137, 145, 146, 148-150, 152, 157-159, 164-167, 176, 187, and 188.

[0015] In some examples, the aldehyde dehydrogenase enzyme of the non-naturally occurring microbial organism comprises an amino acid sequence having at least about 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% amino acid sequence identity to at least 25, 50, 75, 100, 150, 200, 250, 300, or more contiguous amino acids of any of SEQ ID NOs: 4, 7, 11, 15, 17, 19, 24, 25, 27, 28, 31-33, 36, 38, 40-42, 44, 45, 47, 53, 58-60, 63, 65-67, 74, 75, 77, 80, 82, 84, 86-88, 90, 91, 94, 95, 97, 100, 101, 103, 107, 109, 111, 112, 117, 134, 135, 137, 145, 146, 148-150, 152, 157-159, 164-167, 176, 187, and 188. In some examples, the aldehyde dehydrogenase enzyme uses NADH as a cofactor.

[0016] In some examples, the aldehyde dehydrogenase enzyme of the non-naturally occurring microbial organism comprises an amino acid sequence having at least about 60% amino acid sequence identity to at least 25, 50, 75, 100, 150, 200, 250, 300, or more contiguous amino acids of any of SEQ ID NOs: 7, 28, 60, and 107. In some examples, the aldehyde dehydrogenase comprises an amino acid sequence having at least about 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% amino acid sequence identity to at least 50, 75, 100, 150, 200, 250, 300, or more contiguous amino acids of any of SEQ ID NOs: 7, 28, 60, and 188. In some examples, the aldehyde dehydrogenase enzyme uses NADH as a cofactor.

[0017] In some examples, the aldehyde dehydrogenase enzyme of the non-naturally occurring microbial organism comprises an amino acid sequence having at least about 60% amino acid sequence identity to at least 25, 50, 75, 100, 150, 200, 250, 300, or more contiguous amino acids of any of SEQ ID NOs: 53, 77, 82, 94, and 152. In some examples, the aldehyde dehydrogenase enzyme uses NADH, NADPH, or both as a cofactor.

[0018] In some examples, the aldehyde dehydrogenase enzyme of the non-naturally occurring microbial organism has higher catalytic efficiency for adipyl-CoA substrate compared to succinyl-CoA substrate. In some examples, the catalytic efficiency of the aldehyde dehydrogenase enzyme for adipyl-CoA substrate is at least twice as high as the catalytic efficiency for succinyl-CoA substrate.

[0019] In some examples, the aldehyde dehydrogenase enzyme of the non-naturally occurring microbial organism has greater catalytic efficiency for adipyl-CoA substrate compared to acetyl-CoA substrate. In some examples, the catalytic efficiency of the aldehyde dehydrogenase enzyme for adipyl-CoA substrate is at least five times as high as the catalytic efficiency for acetyl-CoA substrate. In some examples, the

aldehyde dehydrogenase enzyme has higher turnover number for adipyl-CoA substrate as compared to acetyl-CoA substrate.

[0020] In some examples, the aldehyde dehydrogenase enzyme of the non-naturally occurring microbial organism further reacts with 6-aminocaproyl-CoA to form 6-aminocaproate semialdehyde.

[0021] In some examples, the non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding an aldehyde dehydrogenase enzyme converts more adipyl-CoA to adipate semialdehyde than a control microbial organism substantially identical to the non-naturally occurring microbial organism, with the exception that the control microbial organism does not comprise the exogenous nucleic acid encoding an aldehyde dehydrogenase enzyme.

[0022] In some examples, at least one exogenous nucleic acid encoding an aldehyde dehydrogenase enzyme that reacts with adipyl-CoA to form adipate-semialdehyde is heterologous to the microbial organism.

[0023] In some examples, the non-naturally occurring microbial organism comprises a 6-aminocaproic acid pathway. In some examples, the 6-aminocaproic acid pathway comprises: (i) transaminase, (ii) 6-aminocaproate dehydrogenase, or both (iii) transaminase and 6-aminocaproate dehydrogenase enzymes. In some examples, the non-naturally occurring microbial organism further comprises one or more additional exogenous nucleic acids encoding one or more of the 6-aminocaproic acid pathway enzymes. In some examples, the exogenous nucleic acids encoding one or more of the 6-aminocaproic acid pathway enzymes is heterologous to the microbial organism.

[0024] In some examples, the non-naturally occurring microbial organism comprises a hexamethylenediamine pathway. In some examples, the hexamethylenediamine pathway comprises (i) 6-aminoacaproyl CoA transferase, (ii) 6-amino caproyl CoA synthase, (iii) 6-amino caproyl CoA reductase, (iv) hexamethylenediamine transaminase, (v) hexamethylenediamine dehydrogenase, (v) or a combination of one or more of the enzymes (i)-(v). In some examples, the microbial organism further comprises one or more additional exogenous nucleic acids encoding one or more of the hexamethylenediamine pathway enzymes such as carboxylic acid reductase (CAR) that converts 6-aminocaproate to 6-aminocaproate semialdehyde. The 6-aminocaproate semialdehyde can subsequently be converted to hexamethylene diamine. In some examples, the exogenous nucleic acids encoding one or more of the hexamethylenediamine pathway enzymes is heterologous to the microbial organism.

[0025] In some examples, the non-naturally occurring microbial organism comprises a caprolactam pathway. In some examples, the caprolactam pathway comprises aminohydrolase enzyme. In some examples, the microbial organism further comprises one or more additional exogenous nucleic acids encoding aminohydrolase enzyme. In some examples, the exogenous nucleic acids encoding aminohydrolase enzyme is heterologous to the microbial organism.

[0026] In some examples, the non-naturally occurring microbial organism comprises a 1, 6-hexanediol pathway. In some examples, the 1, 6-hexanediol pathway comprises the following enzymes: a 6-aminocaproyl-CoA transferase or synthetase catalyzing conversion of 6ACA to 6-aminocaproyl-CoA; a 6-aminocaproyl-CoA reductase catalyzing conversion of 6-aminocaproyl-CoA to 6-aminocaproate

semialdehyde; a 6-aminocaproate semialdehyde reductase catalyzing conversion of 6-aminocaproate semialdehyde to 6-aminohexanol; a 6-aminocaproate reductase catalyzing conversion of 6ACA to 6-aminocaproate semialdehyde; an adipyl-CoA reductase adipyl-CoA to adipate semialdehyde; an adipate semialdehyde reductase catalyzing conversion of adipate semialdehyde to 6-hydroxyhexanoate; a 6-hydroxyhexanoyl-CoA transferase or synthetase catalyzing conversion of 6-hydroxyhexanoate to 6-hydroxyhexanoyl-CoA; a 6-hydroxyhexanoyl-CoA reductase catalyzing conversion of 6-hydroxyhexanoyl-CoA to 6-hydroxyhexanal; a 6-hydroxyhexanal reductase catalyzing conversion of 6-hydroxyhexanal to HDO; a 6-aminohexanol aminotransferase or oxidoreductases catalyzing conversion of 6-aminohexanol to 6-hydroxyhexanal; a 6-hydroxyhexanoate reductase catalyzing conversion of 6-hydroxyhexanoate to 6-hydroxyhexanal; an adipate reductase catalyzing conversion of ADA to adipate semialdehyde; and an adipyl-CoA transferase, hydrolase or synthase catalyzing conversion of adipyl-CoA to ADA.

[0027] In some examples, the non-naturally occurring microbial organism comprises pathways from adipate or adipyl-CoA to caprolactone. In some examples, the pathways from adipate or adipyl-CoA to caprolactone comprises the following enzymes: adipyl-CoA reductase, adipate semialdehyde reductase, 6-hydroxyhexanoyl-CoA transferase or synthetase, 6-hydroxyhexanoyl-CoA cyclase or spontaneous cyclization, adipate reductase, adipyl-CoA transferase, synthetase or hydrolase, 6-hydroxyhexanoate cyclase, 6-hydroxyhexanoate kinase, 6-hydroxyhexanoyl phosphate cyclase or spontaneous cyclization, phosphotrans-6-hydroxyhexanoylase.

[0028] In some examples, the aldehyde dehydrogenase of the non-naturally occurring microbial organism is derived from a prokaryotic species. In some examples, the aldehyde dehydrogenase enzyme is derived from *Acidaminococcus*, *Collinsella*, *Peptostreptococcaceae*, or *Romboustsia*.

[0029] In some examples, the non-naturally occurring microbial organism comprises a species of *Acinetobacter, Actinobacillus, Anaerobiospirillum, Aspergillus, Bacillus, Clostridium, Corynebacterium, Escherichia, Gluconobacter, Klebsiella, Kluyveromyces, Lactococcus, Lactobacillus, Mannheimia, Pichia, Pseudomonas, Rhizobium, Rhizopus, Saccharomyces, Schizosaccharomyces, Streptomyces, and Zymomonas*. In some examples, the non-naturally occurring microbial organism is a strain of *Escherichia. coli*.

[0030] In some examples, the culturing is performed in a fermentation broth comprising a sugar.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] FIG. 1 shows exemplary pathways from succinyl-CoA and acetyl-CoA to 6-aminocaproate, hexamethylenediamine (HMDA), caprolactam. The enzymes are designated as follows: A) 3-oxoadipyl-CoA thiolase, B) 3-oxoadipyl-CoA reductase, C) 3-hydroxyadipyl-CoA dehydratase, D) 5-carboxy-2-pentenoyl-CoA reductase, E) 3-oxoadipyl-CoA/acyl-CoA transferase, F) 3-oxoadipyl-CoA synthase, G) 3-oxoadipyl-CoA hydrolase, H) 3-oxoadipate reductase, I) 3-hydroxyadipate dehydratase, J) 5-carboxy-2-pentenoate reductase, K) adipyl-CoA/acyl-CoA transferase, L) adipyl-CoA synthase, M) adipyl-CoA hydrolase, N) adipyl-CoA reductase (aldehyde forming), O) 6-aminocaproate transaminase, P) 6-aminocaproate dehydrogenase, Q) 6-aminocaproyl-CoA/acyl-CoA transferase, R) 6-aminocaproyl-CoA synthase, S) amidohydrolase, T) spontaneous cyclization, U) 6-aminocaproyl-CoA reductase (aldehyde forming), V) HMDA transaminase, W) HMDA dehydrogenase, X) adipate reductase, Y) adipate kinase, Z) adipylphosphate reductase.

What is claimed is:

1. A non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding an aldehyde dehydrogenase enzyme that reacts with adipyl-CoA to form adipate-semialdehyde, wherein the aldehyde dehydrogenase has greater catalytic efficiency for adipyl-CoA as a substrate as compared to succinyl-CoA, acetyl-CoA, or both as substrates, and/or the aldehyde dehydrogenase has higher turnover number for adipyl-CoA substrate as compared to succinyl-CoA, acetyl-CoA, or both succinyl-CoA and acetyl-CoA substrates.

2. The non-naturally occurring microbial organism ofclaim 1, wherein the aldehyde dehydrogenase comprises an amino acid sequence having at least about 60% amino acid sequence identity to at least 25 contiguous amino acids of any one of SEQ ID NOs: 4, 7, 11, 15, 17, 19, 24, 25, 27, 28, 31-33, 36, 38, 40-42, 44, 45, 47, 53, 58-60, 63, 65-67, 74, 75, 77, 80, 82, 84, 86-88, 90, 91, 94, 95, 97, 100, 101, 103, 107, 109, 111, 112, 117, 134, 135, 137, 145, 146, 148-150, 152, 157-159, 164-167, 176, 187, and 188.

3. The non-naturally occurring microbial organism of any one of claims 1-2, wherein the aldehyde dehydrogenase comprises an amino acid sequence having at least about 60% amino acid sequence identity to at least 25 contiguous amino acids of any one of SEQ ID NOs: 7, 28, 60, or 107.

4. The non-naturally occurring microbial organism of any one of claims 1-3, wherein the non-naturally occurring microbial organism comprises a 6-aminocaproic acid pathway, a hexamethylenediamine pathway, and a caprolactam pathway.

5. The non-naturally occurring microbial organism any one of claims 1-4, wherein the 6-aminocaproic acid pathway comprises: (i) transaminase, (ii) 6-aminocaproate dehydrogenase, or both (iii) transaminase and 6-aminocaproate dehydrogenase enzymes.

6. The non-naturally occurring microbial organism of any one of claims 1-4, wherein the hexamethylenediamine pathway comprises (i) 6-aminoacaproyl CoA transferase, (ii) 6-amino caproyl CoA synthase, (iii) 6-amino caproyl CoA reductase, (iv) hexamethylenediamine transaminase, (v) hexamethylenediamine dehydrogenase, (v) or a combination of one or more of the enzymes (i)-(v).

7. The non-naturally occurring microbial organism of any one of claims 1-4, wherein the microbial organism further comprises one or more additional exogenous nucleic acids encoding aminohydrolase enzyme.

8. The non-naturally occurring microorganism of any of claims 1-7, wherein the aldehyde dehydrogenase enzyme is derived from *Acidaminococcus*, *Collinsella*, *Peptostreptococcaceae*, or *Romboustsia*.

9. The non-naturally occurring microbial organism of any of claims 1-8, wherein the non-naturally occurring microbial organism comprises a species of *Acinetobacter, Actinobacillus, Anaerobiospirillum, Aspergillus, Bacillus, Clostridium, Corynebacterium, Escherichia, Gluconobacter, Klebsiella, Kluyveromyces, Lactococcus, Lactobacillus, Mannheimia, Pichia, Pseudomonas, Rhizobium, Rhizopus, Saccharomyces, Schizosaccharomyces, Streptomyces, and Zymomonas*.

10. A method of producing adipate-semialdehyde comprising culturing a non-naturally occurring microorganism of any one of claims 1-9 for a sufficient time period and conditions for producing adipate-semialdehyde.

11. A method of producing 6-aminocaproic acid (6ACA) comprising culturing a non-naturally occurring microbial organism of any one of claims 1-10 for a sufficient time period and conditions for producing 6ACA.

12. The method of claim 11, further comprising recovering 6-aminocaproic acid (6ACA), hexamethylenediamine, caprolactone, 1,6-hexanediol, caprolactam from the non-naturally occurring microbial organism, fermentation broth, or both.

13. The method of claim any one of claims 10-12, wherein the non-naturally occurring microbial organism comprises two, three, four, five, six, or seven exogenous nucleic acids each encoding a caprolactam pathway enzyme.

14. The method of any one of claims 10-13, wherein the culturing is performed in a fermentation broth comprising a sugar.

15. A bioderived 6-aminocaproic acid, hexamethylenediamine, 1,6-hexanediol, caprolctone, or caprolactam synthesized from the method of any one of claims 10-14.