

Toward Synthetic Biological Approaches to Resource Utilization on Space Missions

Supplementary Materials

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S1 Tabular Supporting Information

Table S1 summarizes the mechanisms of action and the outputs produced by organisms that take in carbon dioxide, as detailed by [1]. Table S2 provides a similar summary of organisms that utilize and produce various nitrogen compounds; these organisms also play a role in the microbial nitrogen cycle [2–6].

Table S3 details the methane-oxygen propellant production mechanisms and mass requirements for each of the Mars scenarios described in [7]. The associated mass costs for two different production options of a similar fuel mix for a lunar module ascent are also listed in Table S3.

Table S4 summarizes the Mars ISRU mass cost of strain *KN-15* of *Methanobacterium* and strain Marburg of *Methanobacterium thermoautotrophicum* when a flow rate constraint of 3,000 g of water/h is imposed by incorporating a 6 kg electrolyzer that was recently validated in [8]. Table S5 provides a similar summary of the Moon ISRU mass cost, based on complete carbon dioxide extraction from lunar regolith that has been excavated by a 108 kg excavator (scaled up from [9]).

Table S6 presents the unoptimized mass cost of nutrients and growth media, when extrapolated from the literature requirements, for Mars-based methane bioproduction over the anticipated manufacture period of 205 days.

Table S1: Summary of CO₂ Fixation in Autotrophs [1]

Product	Organisms	Mechanism	Energetic Cost
Glyceraldehyde 3-phosphate, which is useful for terpenoid biosynthesis [10].	Plants, algae, cyanobacteria, many aerobic or facultative aerobic proteobacteria belonging to the alpha, beta, and gamma subgroups, <i>Sulfobacillus</i> spp., iron and sulfur-oxidizing members of the firmicutes, some mycobacteria, green nonsulfur bacteria of the genus <i>Oscillochloris</i> (phylum <i>Chloroflexi</i>), and (anaerobic) photoheterotrophic growth of some purple bacteria (e.g., <i>Rhodobacter</i> , <i>Rhodospirillum</i> , and <i>Rhodospseudomonas</i>).	Reductive Pentose Phosphate Benson) Cycle, with key enzyme RubisCO.	Nine ATP equivalents and six NADPHs are required to synthesize one glyceraldehyde-3-phosphate molecule.
Acetyl-CoA, which must be converted to other intermediates of the carbon metabolism: pyruvate, phosphoenolpyruvate, oxaloacetate, and 2-oxoglutarate. It is a useful starting point for a number of high value chemicals and biofuels [11].	The green sulfur bacterium <i>Chlorobium limicola</i> (<i>Chlorobi</i>), anaerobic or microaerobic members of <i>Aquificae</i> , <i>Proteobacteria</i> (especially of the delta and epsilon subdivisions), and <i>Nitrospirae</i> (e.g., <i>Nitrospira</i> and <i>Leptospirillum</i>).	Reductive Citric Acid (Arnon-Buchanan) Cycle, which reverses the reactions of the oxidative citric acid cycle (Krebs cycle) and forms acetyl-CoA from two CO ₂ s.	In <i>Chlorobium</i> , two ATP equivalents are required to form pyruvate, and three additional ATPs are required to convert it to triose phosphates.
Acetyl-CoA, which can be used to generate acetate or methane in the process of energy conservation, and also used for the assimilation of a variety of C ₁ compounds like carbon monoxide, formaldehyde, methanol, methylamine, methylmercaptane, and methyl groups of aromatic O-methyl ethers/esters.	Prokaryotes that live close to the thermodynamic limit such as acetogenic bacteria and methanogenic archaea, anaerobic ammonia-oxidizing planctomycetes, sulfate-reducing bacteria (<i>Desulfobacterium</i> sp., <i>Deltaproteobacteria</i>), and autotrophic <i>Archaeoglobales</i> (<i>Euryarchaeota</i>) growing by means of anaerobic respiration.	Reductive Acetyl-CoA (Wood-Ljungdahl) Pathway, which requires strict anoxic conditions and has high energetic efficiency.	In methanogens, no additional ATP input is required, but in bacteria, an additional ATP equivalent is required.
Acetyl-CoA, which is used to synthesize pyruvate and then other central precursor molecules.	Mostly anaerobic autotrophic representatives of <i>Thermoproteales</i> and <i>Desulfurococcales</i> , and a facultative aerobe of the <i>Desulfurococcales</i> , <i>Pyrolobus fumarii</i> at very low O ₂ concentrations.	Dicarboxylate / 4-Hydroxybutyrate Cycle, which is anaerobic.	Five ATP equivalents are required to synthesize one pyruvate, and three additional ATP equivalents are required to synthesize one triose phosphate from pyruvate.
Acetyl-CoA, which is used to produce succinyl-CoA, which is then oxidatively converted to oxaloacetate, pyruvate and phosphoenolpyruvate.	(Micro)Aerobic <i>Sulfolobales</i> , and the mesophilic aerobic “marine group I” <i>Archaea</i> (“ <i>Thaumarchaeota</i> ”).	3-Hydroxypropionate / 4-Hydroxybutyrate Cycle, which is aerobic.	Nine ATP equivalents are required to generate three molecules of pyrophosphate, and three additional ATP equivalents are required to synthesize one triose phosphate from pyruvate.
Glyoxylate, which is converted to the cellular building blocks in a second cycle. The bi-cycle allows coassimilation of fermentation products such as acetate, propionate, and succinate, or 3-hydroxypropionate, an intermediate in the metabolism of the osmoprotectant dimethylsulfoniopropionate.	The green non-sulfur phototrophs of the <i>Chloroflexaceae</i> family, which grow preferentially under photoheterotrophic conditions. The only autotrophic representative of this family found so far is <i>Chloroflexus aurantiacus</i> .	3-Hydroxypropionate (Fuchs-Holo) Cycle, which has high efficiency carboxylases compared to RubisCO.	Seven ATP equivalents are required for pyruvate synthesis, and an additional three ATPs are required for triose phosphate.

Table S2: Selections from the Microbial N₂ Cycle [2–6]

Product	Organisms	Mechanism
Ammonia	<i>Rhizobium</i> , <i>Frankia</i> , <i>Azotobacter</i> , <i>Beijerinckia</i> , <i>Azospirillum</i> , <i>Acetobacter</i> , cyanobacteria, <i>Anabaena</i> , <i>Clostridium</i>	Biological Nitrogen Fixation
Nitrite	Ammonia-Oxidizing Bacteria (AOB), e.g., <i>Nitrosomonas</i> , <i>Nitrosococcus</i> , and Ammonia-Oxidizing Archaea (AOA)	Aerobic Ammonia Oxidation, with CO ₂ as carbon source.
Nitrate	Nitrite-Oxidizing Bacteria (NOB), e.g., <i>Nitrobacter</i> , <i>Nitrospira</i>	Aerobic Nitrite Oxidation, with CO ₂ as carbon source
Nitrite, Nitric oxide, Nitrous oxide, Nitrogen	<i>Pseudomonas</i> , <i>Bacillus</i> , <i>Thiobacillus</i> , <i>Propionibacterium</i> , glycogen-accumulating organisms	Denitrification in low-oxygen or anaerobic conditions

Table S3: Mass Cost of Current Mars and Moon Ascent Propellant Production Approaches

Name	Production Option [7, 12]	Production Mechanism [7, 13]	Mass Delivered [T] [7, 13]
Mars Bring Methane	24,891 kg of O ₂ , 133 kg of N ₂ /Ar, bring 399 kg of H ₂ , bring 6,567 kg of CH ₄	Reverse Water Gas Shift 2CO ₂ + 2H ₂ → 2CO + 2H ₂ O followed by water electrolysis 2H ₂ O → 2H ₂ + O ₂	7.5
Mars Bring Hydrogen	24,891 kg of O ₂ , 133 kg of N ₂ /Ar, 6,567 kg of CH ₄ , bring 2,069 kg of H ₂	Sabatier CO ₂ + 4H ₂ → CH ₄ + 2H ₂ O and Reverse Water Gas Shift 2CO ₂ + 2H ₂ → 2CO + 2H ₂ O with water electrolysis 2H ₂ O → 2H ₂ + O ₂	3.3
Mars Find Water	24,891 kg of O ₂ , 133 kg of N ₂ /Ar, 6,567 kg of CH ₄ , excavate 16,788 kg of H ₂ O	Sabatier CO ₂ + 4H ₂ → CH ₄ + 2H ₂ O with water electrolysis 2H ₂ O → 2H ₂ + O ₂	2.7 with 3wt% H ₂ O in soil, 2.0 with 8wt% H ₂ O in soil
Moon Bring Hydrogen	7,600 kg of O ₂ , 2,160 kg of CH ₄ , bring 1,501 kg of H ₂ , bring 1,617 kg of C in plastic and packaging trash	Hydrogen reduction of FeO in a 10T capacity O ₂ plant followed by water electrolysis, pyrolysis of plastic and packaging trash with the excess H ₂ for CH ₄	4.1 with 5wt% FeO in regolith (1.501 of H ₂ + 1.617 C trash + 0.942 O ₂ plant), 3.7 with 14wt% FeO in regolith (1.501 of H ₂ + 1.617 C trash + 0.563 O ₂ plant)
Moon Find Water	7,600 kg of O ₂ , 2,160 kg of CH ₄ , bring 1,617 kg of C in plastic and packaging trash, excavate 8,588 kg of H ₂ O	Water electrolysis in a 10T capacity O ₂ plant, pyrolysis of plastic and packaging trash with the obtained H ₂ for CH ₄	1.8 (1.617 C trash + 0.190 O ₂ plant)

Table S4: Plant Mass Cost and Savings of Proposed Mars Ascent Methane Propellant Production Approaches

Name	Production Option	Production Mechanism	Mass and Time Summary
Mars Bring Hydrogen High Vol. Prod. Low Gas Conc.	24,891 kg of O ₂ , 133 kg of N ₂ /Ar, 6,567 kg of CH ₄ , bring 1,651 kg of H ₂	<i>Methanobacterium</i> strain <i>KN-15</i> 65 L volume in 100 L bioreactor 32 kg of biosynthesized CH ₄ /day 2,998 g of flowing H ₂ O/h 64 kg of electrolyzed O ₂ /day and Reverse Water Gas Shift 11,792 kg additional total O ₂ after separate electrolysis	Current Plant: 356 kg [7] Proposed Plant: 217 kg (193 kg bioreactor estimate + 6 kg electrolysis unit × 2 + 12 kg RWGS unit [14]) Plant Reduction: 39% CH ₄ Completion: 205 days O ₂ Completion: 205 days (390 days without RWGS) Current H ₂ : 2,069 kg [7] Proposed H ₂ : 1,651 kg H₂ Reduction: 20%
Mars Bring Hydrogen Low Vol. Prod. High Gas Conc.	24,891 kg of O ₂ , 133 kg of N ₂ /Ar, 6,567 kg of CH ₄ , bring 1,651 kg of H ₂	<i>M. thermoautotrophicum</i> 157 L volume in 200 L bioreactor 32 kg of biosynthesized CH ₄ /day 2,998 g of flowing H ₂ O/h 64 kg of electrolyzed O ₂ /day and Reverse Water Gas Shift 11,792 kg additional total O ₂ after separate electrolysis	Current Plant: 356 kg [7] Proposed Plant: 263 kg (239 kg bioreactor estimate + 6 kg electrolysis unit × 2 + 12 kg RWGS unit [14]) Plant Reduction: 26% CH ₄ Completion: 205 days O ₂ Completion: 205 days (390 days without RWGS) Current H ₂ : 2,069 kg [7] Proposed H ₂ : 1,651 kg H₂ Reduction: 20%
Mars Find 3wt% H ₂ O High Vol. Prod. Low Gas Conc.	24,891 kg of O ₂ , 133 kg of N ₂ /Ar, 6,567 kg of CH ₄ , excavate 14,849 kg of H ₂ O (includes bioreactor working volume and solvent for an hour of nutrient flow, assuming recycling)	<i>Methanobacterium</i> strain <i>KN-15</i> 65 L volume in 100 L bioreactor 32 kg of biosynthesized CH ₄ /day 2,998 g of flowing H ₂ O/h 64 kg of electrolyzed O ₂ /day Separate electrolysis of the excavated H ₂ O for H ₂ also yields 13,108 kg O ₂	Current Plant: 545 kg [7] (527 kg if 8wt% H ₂ O [7]) Proposed Plant: 205 kg (193 kg bioreactor est. + 2×6 kg electrolysis units) Plant Reduction: 62% (61% for 8wt% H₂O) CH ₄ Completion: 205 days O ₂ Completion: 205 days Current H ₂ O: 16,788 kg [7] Proposed H ₂ O: 14,760 kg H₂O Reduction: 12%
Mars Find 3wt% H ₂ O Low Vol. Prod. High Gas Conc.	24,891 kg of O ₂ , 133 kg of N ₂ /Ar, 6,567 kg of CH ₄ , excavate 14,938 kg of H ₂ O (includes bioreactor working volume and solvent for an hour of nutrient flow, assuming recycling)	<i>M. thermoautotrophicum</i> 157 L volume in 200 L bioreactor 32 kg of biosynthesized CH ₄ /day 2,998 g of flowing H ₂ O/h 64 kg of electrolyzed O ₂ /day Separate electrolysis of the excavated H ₂ O for H ₂ also yields 13,108 kg O ₂	Current Plant: 545 kg [7] (527 kg if 8wt% H ₂ O [7]) Proposed Plant: 251 kg (239 kg bioreactor est. + 2×6 kg electrolysis units) Plant Reduction: 56% (55% for 8wt% H₂O) CH ₄ Completion: 205 days O ₂ Completion: 205 days Current H ₂ O: 16,788 kg [7] Proposed H ₂ O: 14,760 kg H₂O Reduction: 12%

Table S5: Plant Mass Cost and Savings of Proposed Moon Ascent Methane Propellant Production Approaches

Name	Production Option	Production Mechanism	Mass and Time Summary
Moon Bring Hydrogen High Vol. Prod. Low Gas Conc.	7,600 kg of O ₂ , 2,160 kg of CH ₄ , bring 958 kg of H ₂ , excavate 48,000 kg of regolith/day	<i>Methanobacterium</i> strain <i>KN-15</i> 43 L volume in 50 L bioreactor 21 kg of biosynthesized CH ₄ /day 1,965 g of flowing H ₂ O/h 42 kg of electrolyzed O ₂ /day (equivalent to a plant producing 15T of O ₂ annually)	Current: 2,559 kg or 2,180 kg at 5wt% FeO or 14wt% FeO in regolith, respectively [13] (1,617 kg of C trash + 942 kg or 563 kg, respectively, for 10T O ₂ plant) Proposed: 331 kg (165 kg bioreactor est. + 6 kg electrolysis unit + 80 kg excavator × 2) Reduction: 87% (85% for 14wt% FeO case) CH ₄ Completion: 103 days O ₂ Completion: 181 days (less if excavated water also electrolyzed) Current H ₂ : 1,501 kg [13] Proposed H ₂ : 958 kg H₂ Reduction: 36%
Moon Bring Hydrogen Low Vol. Prod. High Gas Conc.	7,600 kg of O ₂ , 2,160 kg of CH ₄ , bring 958 kg of H ₂ , excavate 48,000 kg of regolith/day	<i>M. thermoautotrophicum</i> 103 L volume in 200 L bioreactor 21 kg of biosynthesized CH ₄ /day 1,965 g of flowing H ₂ O/h 42 kg of electrolyzed O ₂ /day (equivalent to a plant producing 15T of O ₂ annually)	Current: 2,559 kg or 2,180 kg at 5wt% FeO or 14wt% FeO in regolith, respectively [13] (1,617 kg of C trash + 942 kg or 563 kg, respectively, for 10T O ₂ plant) Proposed: 405 kg (239 kg bioreactor est. + 6 kg electrolysis unit + 80 kg excavator × 2) Reduction: 84% (81% for 14wt% FeO case) CH ₄ Completion: 103 days O ₂ Completion: 181 days (less if excavated water also electrolyzed) Current H ₂ : 1,501 kg [13] Proposed H ₂ : 958 kg H₂ Reduction: 36%
Moon Find Water High Vol. Prod. Low Gas Conc.	7,600 kg of O ₂ , 2,160 kg of CH ₄ , excavate 4,911 kg of H ₂ O from 32,400 kg of regolith/day (includes bioreactor working volume and solvent for an hour of nutrient flow, assuming recycling)	<i>Methanobacterium</i> strain <i>KN-15</i> 29 L volume in 50 L bioreactor 14 kg of biosynthesized CH ₄ /day 1,326 g of flowing H ₂ O/h 28 kg of electrolyzed O ₂ /day Separate electrolysis of the excavated H ₂ O in the regolith yields the remaining 3,275 kg O ₂	Current: 1,807 kg [13] (1,617 kg of C trash + 190 kg for 10T O ₂ plant) Proposed: 285 kg (165 kg bioreactor est. + 2×6 kg electrolysis units + 108 kg excavator) Reduction: 84% CH ₄ Completion: 153 days O ₂ Completion: <153 days Current H ₂ O: 8,588 kg [13] Proposed H ₂ O: 4,851 kg H₂O Reduction: 44%
Moon Find Water Low Vol. Prod. High Gas Conc.	7,600 kg of O ₂ , 2,160 kg of CH ₄ , excavate 4,911 kg of H ₂ O from 32,400 kg of regolith/day (includes bioreactor working volume and solvent for an hour of nutrient flow, assuming recycling)	<i>M. thermoautotrophicum</i> 70 L volume in 100 L bioreactor 14 kg of biosynthesized CH ₄ /day 1,326 g of flowing H ₂ O/h 28 kg of electrolyzed O ₂ /day Separate electrolysis of the excavated H ₂ O in the regolith yields the remaining 3,275 kg O ₂	Current: 1,807 kg [13] (1,617 kg of C trash + 190 kg for 10T O ₂ plant) Proposed: 313 kg (193 kg bioreactor est. + 2×6 kg electrolysis units + 108 kg excavator) Reduction: 83% CH ₄ Completion: 153 days O ₂ Completion: <153 days Current H ₂ O: 8,588 kg [13] Proposed H ₂ O: 4,851 kg H₂O Reduction: 44%

Table S6: Unoptimized Nutrient Mass Requirements for 205 Days of Mars Methane Propellant Production

Name	Production Mechanism	Nutrient Mass Summary
Mars Bring Hydrogen High Vol. Prod. Low Gas Conc.	<i>Methanobacterium</i> strain <i>KN-15</i> 65 L volume in 100 L bioreactor	18.66–21.66 g/L nutrient weight concentration (since NaHCO ₃ is between 5–8 g/L) [15, 16] ⇒ 1.21–1.41 kg of initial media in 63.6–63.8 kg transported H ₂ O 0.3–0.4/h dilution rate [16] ⇒ 19.5–26 L/h media flow rate ⇒ 1,791–2,862 kg of flowing media (includes 19.14–25.44 kg recycled flowing H ₂ O, 65 kg start media)
Mars Bring Hydrogen Low Vol. Prod. High Gas Conc.	<i>M. thermoautotrophicum</i> 157 L volume in 200 L bioreactor	15.17 g/L nutrient weight concentration [17] ⇒ 2.38 kg of initial media in 154.6 kg transported H ₂ O 0.15/h dilution rate [18] ⇒ 23.55 L/h media flow rate ⇒ 1,938 kg of flowing media (includes 23.19 kg recycled flowing H ₂ O, 157 kg start media)
Mars Find 3wt% H ₂ O High Vol. Prod. Low Gas Conc.	<i>Methanobacterium</i> strain <i>KN-15</i> 65 L volume in 100 L bioreactor	18.66–21.66 g/L nutrient weight concentration (since NaHCO ₃ is between 5–8 g/L) [15, 16] ⇒ 1.21–1.41 kg of initial media in 63.6–63.8 kg excavated H ₂ O 0.3–0.4/h dilution rate [16] ⇒ 19.5–26 L/h media flow rate ⇒ 1,708–2,773 kg of flowing media (excludes 19.14–25.44 kg recycled flowing H ₂ O, start H ₂ O)
Mars Find 3wt% H ₂ O Low Vol. Prod. High Gas Conc.	<i>M. thermoautotrophicum</i> 157 L volume in 200 L bioreactor	15.17 g/L nutrient weight concentration [17] ⇒ 2.38 kg of initial media in 154.6 kg excavated H ₂ O 0.15/h dilution rate [18] ⇒ 23.55 L/h media flow rate ⇒ 1,760 kg of flowing media (excludes 23.19 kg recycled flowing H ₂ O, start H ₂ O)

S2 Alternative Propellant and Generation Cost

There exists another rocket fuel candidate with specific impulse and density-specific impulse properties that are comparable to a methane-oxygen combination: the currently uncommon blended monopropellant, nitrous oxide-hydrocarbon, where the hydrocarbon is propane, ethane, ethylene, acetylene, etc. Nitrous oxide (N₂O) [19] has been analyzed for spacecraft propulsion as a monopropellant [20, 21] and as a blended monopropellant, for instance with propane (abbreviated as NOP) [22, 23], ammonia [24], and paraffin when mixed with oxygen (known as Nytrox) [25]. Nitrous oxide’s appeal lies in its specific impulse properties (which are particularly high as a blended monopropellant), its lack of toxicity, and its relative ease of storage and handling. NASA has also studied nitrous oxide monopropellant rockets and their potential applications [26, 27], and is interested in proprietary blends of nitrous oxide and hydrocarbons for potential use as a Mars ascent vehicle propellant. These blends are collectively known as Nitrous Oxide Fuel Blends X (NOFBX) [28–31], where each fuel blend has X replaced by a two-digit number that designates the hydrocarbon used and its mixture ratio. These hydrocarbons include ethane, ethylene and acetylene [32].

The mass of a nitrous oxide-hydrocarbon blended monopropellant that is required to lift a Mars ascent vehicle to orbit can be determined as follows. Since the amount of thrust that is required for ascent is $\Delta V = 5.625$ km/s [7], and the specific impulse of the methane-oxygen propellant is $I_{sp} = 371$ s (at a nozzle expansion ratio of 200 and a chamber pressure of 68 atm) [33] with a fuel mass $m_f = 31,458$ kg (Table S3), the ideal rocket equation,

$$\Delta V = I_{sp} g \ln \frac{(m_1 + m_f)}{m_1},$$

where $g = 9.80665$ m/s² suggests that the mass of the Mars ascent vehicle, m_1 , is about 8,518 kg. Using the ideal rocket equation again, along with a specific impulse of $I_{sp} = 325$ s taken from that of NOFBX [31] (which has a theoretical maximum I_{sp} of 345 s; NOP to vacuum has a comparable I_{sp} of 312 s [23]), the mass of a nitrous oxide-hydrocarbon blended monopropellant that is required for ascent computes to about 41,237 kg. It is anticipated that this entire required mass will be launched from Earth if this blended monopropellant is selected for Mars ascent, although the “potential for *in situ* top-off” has also been identified [30]. It is possible to convert carbon dioxide to ethylene with the electrochemical approach in [34].

S3 Biological Nitrous Oxide-Hydrocarbon Generation Methods and Costs

There is a significant potential fuel benefit to producing nitrous oxide biologically on Mars, perhaps in combination with a hydrocarbon to achieve a mixture that has a high specific impulse. This benefit results from reducing the launch mass cost of complete nitrous oxide-hydrocarbon propellant delivery. As stated in Section 2.4, we seek a manufacture process that utilizes a feedstock of biologically-produced acetate, which can be made with hydrogen electrolyzed from water that is extracted from the Martian soil. Such a process exists: the biological manufacture of ethane and propane from acetate can proceed with the organisms and conditions described in [35]; alternatively, ethane can be electrolyzed from acetate; and lastly, the so-called Glycogen Accumulating Organisms (GAOs) [36] take up acetate anaerobically to produce nitrous oxide via denitrification at an efficiency of either 90% with a nitrate nitrogen (NO₃-N) load, or 95% with a nitrite nitrogen (NO₂-N) load. The latter efficiency is the highest efficiency known [37]. Other biological nitrous oxide production mechanisms are also available [38], and the conditions for producing nitrous oxide are well-studied [39–47]. There is evidence that the idea of combining biologically-produced nitrous oxide with a rocket engine is actively being researched, for instance to power a wastewater treatment plant [48].

At an 8.0:10.0 mixture ratio of nitrous oxide to ethane [32], about 18,328 kg of the blend is nitrous oxide, and the remainder 22,909 kg is ethane. This ratio is selected from among the possibilities in [32] because it maximizes the percentage content of nitrous oxide, which has a greater acetate conversion efficiency than ethane. That is, the Kolbe electrolysis stoichiometry of



requires 2 mol of acetate for every 1 mol of ethane produced, while GAOs require 17.20 mol of acetate for every 14.78 mol of N₂O produced with an NO₂-N load [36], and are therefore more efficient at acetate conversion. A total of 100,589 kg of acetate is required to produce the necessary ethane and nitrous oxide for the blended monopropellant. However, even with two 2,000 L bioreactors (that, together with a soil processing plant, weigh about as much as the shipped mass for the “Bring Hydrogen” case in Table S3), only 29.6 kg of acetate can be produced per day at the experimental autotrophic production rate of 7.4 g/L/day; hence, 3,398 days are required to biomanufacture the requisite acetate. Because additional reactor(s) are also needed to accomplish nitrification upstream of GAO nitrous oxide production, the overall mass and time costs render nitrous oxide-ethane biomanufacture impractical for now.

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