frontiers in Astronomy and Space Sciences

PERSPECTIVE
published: 19 July 2021
doi: 10.3389/fspas.2021.711550

Towards a Biomanufactory on Mars

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A crewed mission to and from Mars may include an exciting array of enabling biotechnologies that leverage inherent mass, power, and volume advantages over traditional abiotic approaches. In this perspective, we articulate the scientific and engineering goals and constraints, along with example systems, that guide the design of a surface biomanufactory. Extending past arguments for exploiting stand-alone elements of biology, we argue for an integrated biomanufacturing plant replete with modules for microbial *in situ* resource utilization, production, and recycling of food, pharmaceuticals, and biomaterials required for sustaining future intrepid astronauts. We also discuss aspirational technology trends in each of these target areas in the context of human and robotic exploration missions.

Keywords: space systems bioengineering, human exploration, *in situ* resource utilization, life support systems, biomanufacturing

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Astrobiology, a section of the journal Frontiers in Astronomy and Space Sciences

Received: *18 May 2021*
Accepted: *01 July 2021*
Published: *19 July 2021*

Citation:
Berliner AJ, Hilzinger JM, Abel AJ, McNulty MJ, Makrygiorgos G, Averesch NJ H, Sen Gupta S, Benvenuti A, Caddell DF, Cestellos-Blanco S, Doloman A, Friedline S, Ho D, Gu W, Hill A, Kusuma P, Lipsky I, Mirkovic M, Luis Meraz J, Pane V, Sander KB, Shi F, Skerker JM, Styer A, Valgardson K, Wetmore K, Woo S-G, Xiong Y, Yates K, Zhang C, Zhen S, Bugbee B, Clark DS, Coleman-Derr D, Mesbah A, Nandi S, Waymouth RM, Yang P, Criddle CS, McDonald KA, Seefeldt LC, Menezes AA and Arkin AP (2021) Towards a Biomanufactory on Mars. Front. Astron. Space Sci. 8:711550. doi: 10.3389/fspas.2021.711550

1 INTRODUCTION

Extended human stay in space or upon the surface of alien worlds like Mars introduces new mission elements that require innovation (Musk, 2017); among these are the biotechnological elements (Menezes et al., 2015a; Menezes et al., 2015b; Nangle et al., 2020a) that support human health, reduce costs, and increase operational resilience. The potential for a Mars mission in the early 2030s (Drake et al., 2010) underscores the urgency of developing a roadmap for advantageous space biotechnologies.

A major limiting factor of space exploration is the cost of launching goods into space (Wertz and Larson, 1996). The replicative capacity of biology reduces mission launch cost by producing goods

FIGURE 1 | Artist's rendering of a crewed Martian biomanufactory powered by photovoltaics, fed *via* atmospheric ISRU, and capable of food and pharmaceutical synthesis (FPS), *in situ* manufacturing (ISM), and biological loop closure (LC). Artwork by Davian Ho.

on-demand using *in situ* resources (Rapp, 2013), recycling waste products (Hendrickx et al., 2006), and interacting with other biological processes for stable ecosystem function (Gòdia et al., 2002). This trait not only lowers initial launch costs, but also minimizes the quantity and frequency of resupply missions that would otherwise be required due to limited food and pharmaceutical shelf-life (Du et al., 2011) on deep space missions. Biological systems also provide robust utility *via* genetic engineering, which can provide solutions to unforeseen problems and lower inherent risk (Menezes et al., 2015a; Berliner et al., 2019). For example, organisms can be engineered on-site to produce a pharmaceutical to treat an unexpected medical condition when rapid supply from Earth would be infeasible (McNulty et al., 2021). A so-called "biomanufactory" for deep space missions (Menezes, 2018) based on *in situ* resource utilization and composed of integrated biologically-driven subunits capable of producing food, pharmaceuticals, and biomaterials (**Figure 1**) will greatly reduce launch and resupply cost, and is therefore critical to the future of human-based space exploration (Menezes et al., 2015a; Nangle et al., 2020a).

2 FEASIBILITY, NEEDS, AND MISSION ARCHITECTURE

The standard specifications for Mars exploration from 2009 (Drake et al., 2010) to 2019 (Linck et al., 2019) are not biomanufacturing-driven (Berliner et al., 2019) due to the novelty of space bioengineering. Here, we outline biotechnological support to produce food, medicine, and specialized construction materials on a long-term mission with six crew-members and surface operations for ~500 sols (a Martian sol is ~40 min longer than an Earth day) flanked by two interplanetary transits of ~210 days (Miele and Wang, 1999). We further assume predeployment cargo that includes *in situ* resource utilization (ISRU) hardware for Mars-ascent propellant production (Sanders, 2018), which is to be launched from Earth to a mission site. Additional supplies such as habitat assemblies (Hoffman and Kaplan, 1997; Cohen, 2015), photovoltaics (Landis, 2000; Landis et al., 2004), experimental equipment, and other non-living consumables (Benton, 2008) will be included.

The proposed biomanufactory would augment processes for air generation and water and waste recycling and purification—typically associated with Environmental Control and Life Support Systems (ECLSS) (Gòdia et al., 2002; Hendrickx et al., 2006)—since its needs overlap but are broader, and drive a wider development of an array of ISRU, *in situ* manufacturing (ISM), food and pharmaceutical synthesis (FPS), and loop closure (LC) technologies (**Figure 2**).

Food, medicine, and gas exchange to sustain humans imposes important ECLSS feasibility constraints (Yeh et al., 2005; Yeh et al., 2009; Weber and Schnaitmann, 2016). These arise from a crewmember (CM) physiological profile, with an upper-bound metabolic rate of ~11–13 MJ/CM-sol that can be satisfied through prepackaged meals and potable water intake of 2.5 kg/CM-sol (Liskowsky and Seitz, 2010; Anderson et al., 2018). Sustaining a CM also entails providing oxygen at 0.8 kg/CM-sol and recycling the 1.04 kg/CM-sol of CO_2, 0.11 kg of fecal and urine solid, and 3.6 kg of water waste within a habitat kept at ~294 K and ~70 kPa. Proposed short duration missions lean heavily on chemical processes for life support with consumables sent from Earth (Drake et al., 2010). As the length of a mission increases, demands on the quantity and quality of consumables increase dramatically. As missions become more complex with longer surface operations, biotechnology offers methods for consumable production in

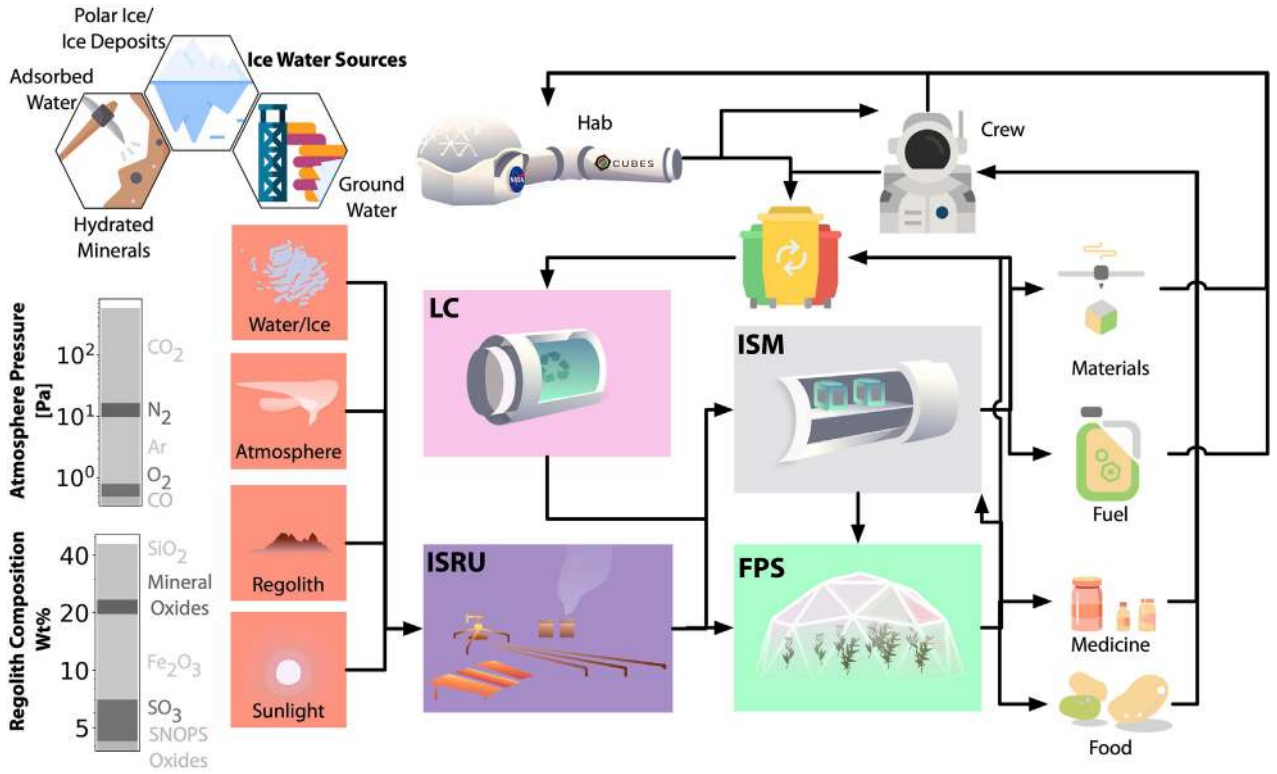

FIGURE 2 | Proposed surface operations are drawn from inventories of *in situ* resources (red) such as ice, atmosphere, regolith, and sunlight. Atmospheric feedstocks of carbon and nitrogen are biologically fixed *via* the ISRU (*in situ* resource utilization) biomanufactory components (including abiotic processes, purple), providing the source of biopolymer manufacturing *via* the ISM (*in situ* manufacturing) component (grey) and food *via* the FPS (food and pharmaceutical synthesis) component (green), which are used for astronaut consumption and utilization during mission operations. Waste from each of these elements is collected and fed into the LC (loop closure) element (pink) to maximize efficiency and reduce the cost of supply logistics from Earth.

the form of edible crops and waste recycling through microbial digestion (Hendrickx et al., 2006). Advancements in biomanufacturing for deep space exploration will ensure a transition from short-term missions such as those on the ISS that are reliant on single-use-single-supply resources to long-term missions that are sustainable.

2.1 Biomanufactory Systems Engineering

Efficiency gains in a biomanufactory come in part from the interconnection (**Figure 2**) and modularity of various unit operations (**Figures 3–6**) (Crowell et al., 2018). However, different mission stage requirements for assembly, operation, timing, and productivity can lead to different optimal biomanufactory system configurations. A challenge therefore exists for technology choice and process optimization to address the high flexibility, scalability, and infrastructure minimization needs of an integrated biomanufactory. Current frameworks for biomanufacturing optimization do not dwell on these aspects. A series of new innovations in modeling processes and developing performance metrics specific to ECLSS biotechnology is called for, innovations that can suitably capture risk, modularity, autonomy, and recyclability. Concomitant invention in engineering infrastructure will also be required.

3 FOOD AND PHARMACEUTICAL SYNTHESIS

An estimated ~ 10,000 kg of food mass is required for a crew of six on a ~ 900 days mission to Mars (Menezes et al., 2015a). Food production for longer missions reduces this mission overhead and increases food store flexibility, bolsters astronaut mental health, revitalizes air, and recycles wastewater through transpiration and condensation capture (Vergari et al., 2010; Kyriacou et al., 2017). Pharmaceutical life support must address challenges of accelerated instability [~ 75% of solid formulation pharmaceuticals are projected to expire mid-mission at 880 days (Menezes et al., 2015a)], the need for a wide range of pharmaceuticals to mitigate a myriad of low probability medical risks, and the mismatch between the long re-supply times to Mars and often short therapeutic time windows for pharmaceutical treatment. Pharmaceutical production for longer missions can mitigate the impact of this anticipated instability and accelerate response time to unanticipated medical threats. In early missions, FPS may boost crew morale and supplement labile nutrients (Khodadad et al., 2020). As mission scale increases, FPS may meet important food and pharmaceutical needs (Cannon and Britt, 2019). A biomanufactory that focuses on oxygenic photoautotrophs, namely plants, algae and cyanobacteria, enhances simplicity, versatility, and synergy with intersecting life

support systems (Gòdia et al., 2002; Wheeler, 2017) and a Martian atmosphere has been shown to support such biological systems (Verseux et al., 2021). While plant-based food has been the main staple considered for extended missions (Drake et al., 2010; Anderson et al., 2018; Cannon and Britt, 2019), the advent of cultured and 3D printed meat-like products from animal, plant and fungal cells may ultimately provide a scalable and efficient alternative to cropping systems (Cain, 2005; Pandurangan and Kim, 2015; Hindupur et al., 2019).

FPS organisms for Mars use must be optimized for growth and yields of biomass, nutrient, and pharmaceutical accumulation. Providing adequate and appropriate lighting will be a challenge of photoautotrophic-centric FPS on Mars (Massa et al., 2007; Kusuma et al., 2020). Developing plants and algae with reduced chloroplast light-harvesting antenna size has the potential to improve whole-organism quantum yield by increasing light penetration deeper into the canopy, which will reduce the fraction of light that is wastefully dissipated as heat and allow higher planting density (Friedland et al., 2019). Developing FPS organisms for pharmaceutical production is especially complicated, given the breadth of production modalities and pharmaceutical need (e.g., the time window of intervention response, and molecule class) (McNulty et al., 2021). Limited-resource pharmaceutical purification is also a critically important consideration that has not been rigorously addressed. Promising biologically-derived purification technologies (Werner et al., 2006; Mahmoodi et al., 2019) should be considered for processing drugs that require very high purity (e.g., injectables).

Developing FPS growth systems for Mars requires synergistic biotic and abiotic optimization, as indicated by lighting systems and plant microbiomes. For lighting, consider that recent advancements in LED efficiency now make LEDs optimal for crop growth in extraterrestrial systems (Hardy et al., 2020). The ideal spectra from tunable LEDs will likely be one with a high fraction of red photons for maximum production efficiency, but increasing the fraction of shorter wavelength blue photons could increase crop quality (Johkan et al., 2010; Kusuma et al., 2021). Similarly, higher photon intensities increase production rates but decrease production efficiency. Understanding the associated volume and power/cooling requirement tradeoffs will be paramount to increasing overall system efficiency.

For microbiomes, consider that ISS open-air plant cultivation results in rapid and widespread colonization by atypicaly low-diversity bacterial and fungal microbiomes that often lead to plant disease and decreased plant productivity (Khodadad et al., 2020). Synthetic microbial communities (SynComs, **Figure 3A**) may provide stability and resilience to the plant microbiome and simultaneously improve the phenotype of host plants *via* the genes carried by community members. A subset of naturally occurring microbes are well known to promote growth of their plant hosts (Hassani et al., 2018), accelerate wastewater remediation and nutrient recycling (Nielsen, 2017), and shield plant hosts from both abiotic and biotic stresses (Caddell et al., 2019), including opportunistic pathogens (Bishop et al., 1997; Ryba-White et al., 2001; Leach et al., 2007). While SynCom design is challenging, the inclusion of SynComs in life support systems represents a critical risk-mitigation strategy to protect vital food and pharma resources. The application of SynComs to Mars-based agriculture motivates additional discussions in tradeoffs between customized hydroponics versus regolith-based farming, both of which will require distinct technology platforms and applied SynComs.

3.1 FPS Integration Into the Biomanufactory

Our biomanufactory FPS module has three submodules: crops, pharmaceuticals, and functional foods (**Figure 3**). The inputs to all three submodules (**Figure 3**) are nearly identical in needing H_2O as an electron donor, CO_2 as a carbon source, and light as an energy source, with the required nitrogen source being organism-dependent (e.g., *Arthrospira platensis* requires nitrate). H_2O, CO_2, and light are directly available from the Martian environment. Fixed nitrogen comes from the biomanufactory ISRU module. The submodules

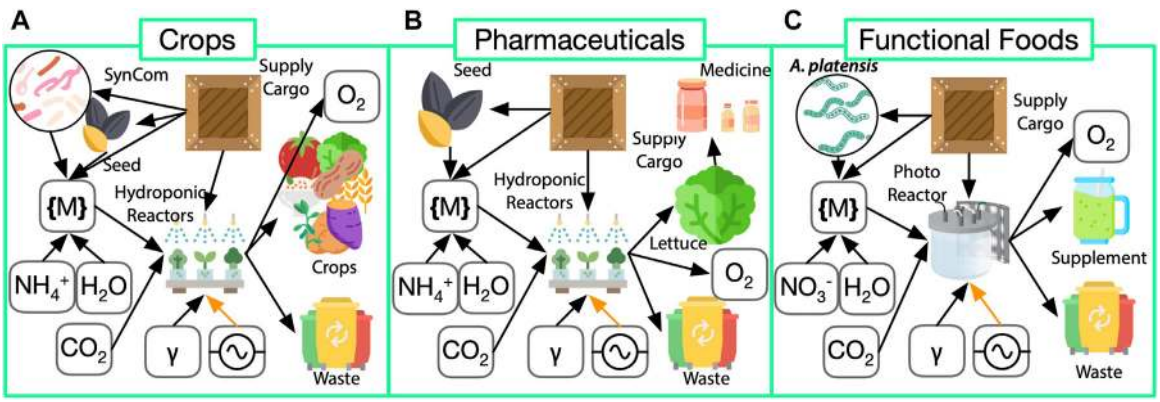

FIGURE 3 | FPS (green in **Figure 2**) system breakdown for biomanufactory elements of **(A)** crops, **(B)** a biopharmaceutical, and **(C)** functional food production. In all cases, growth reactors require power (electrical current symbol ⏦) and light (γ). **(A)** Crop biomass and oxygen gas (O_2) are produced from hydroponically grown plants using seeds and the set of media elements ({M}) derived from supply cargo. The reactor is also supplied with an ammonium (NH_4^+) nitrogen source and CO_2 carbon source from ISRU processes. **(B)** In a similar fashion, medicine can be produced from genetically modified crops such as lettuce **(C)** Functional foods such as nutritional supplements are produced *via* autotrophic growth of *Arthrospira platensis*. In all cases, biomass is produced, collected, and inedible biomass is distributed to the LC module for recycling. Orange lines indicate additional power supply to the system.

output O_2, biomass, and waste products. However, the crop submodule (**Figure 3A**) chiefly outputs edible biomass for bulk food consumption, the pharmaceutical submodule (**Figure 3B**) synthesizes medicines, and the functional foods submodule (**Figure 3C**) augments the nutritional requirements of the crop submodule with microbially-produced vitamins (e.g., vitamin B_{12}). These outputs will be consumed directly by crew-members, with waste products entering the LC module for recycling.

All submodules will have increased risk, modularity, and recyclability relative to traditional technological approaches. Increased risk is associated with biomass loss due to lower-than-expected yields, contamination, and possible growth system failure. Increased modularity over shipping known pharmaceuticals to Mars derives from the programmability of biology, and the rapid response time of molecular pharming in crops for as-needed production of biologics. Increased recyclability stems from the lack of packaging required for shipping food and pharmaceuticals from Earth, as well as the ability to recycle plant waste using anaerobic digestion.

At a systems integration level, FPS organism care will increase the crew time requirements for setup, maintenance, and harvesting compared to advance food and pharmaceutical shipments. However, overall cost impacts require careful scrutiny: crop growth likely saves on shipping costs, whereas pharmaceutical or functional food production on Mars may increase costs relative to shipping drugs and vitamins from Earth.

4 *IN SITU* MATERIALS MANUFACTURING

Maintaining FPS systems requires cultivation vessels/chambers, support structures, plumbing, and tools. Such physical objects represent elements of an inventory that, for short missions, will likely be a combination of predeployment cargo and supplies from the crewed transit vehicle (Drake et al., 2010). As mission duration increases, so does the quantity, composition diversity, and construction complexity of these objects. The extent of ISM for initial exploration missions is not currently specified (Drake et al., 2010). Nevertheless, recent developments (Owens et al., 2015; Moses and Bushnell, 2016; Owens and De Weck, 2016) imply that ISM will be critical for the generation of commodities and consumables made of plastics (Carranza et al., 2006), metals (Everton et al., 2016), composite-ceramics (Karl et al., 2020), and electronics (Werkheiser, 2015) as mission objects, with uses ranging from functional tools (Grenouilleau et al., 2000) to physical components of the life-supporting habitat (Owens et al., 2015).

Plastics will make up the majority of high-turnover items with sizes on the order of small parts to bench-top equipment, and will also account for contingencies (Prater et al., 2016). Biotechnology—specifically synthetic biology—in combination with additive manufacturing (Rothschild, 2016) has been proposed an a critical element towards the establishment of offworld manufacturing (Snyder et al., 2019) and can produce such polymeric constructs from basic feedstocks in a more compact and integrated way than chemical synthesis, because microbial bioreactors operate much closer to ambient conditions than chemical processes (Malik et al., 2015). The versatility of microbial metabolisms allows direct use of CO_2 from Mars' atmosphere, methane (CH_4) from abiotic Sabatier processes (Hintze et al., 2018), and/or biologically synthesized C_2 compounds such as acetate, as well as waste biomass.

A class of bioplastics that can be directly obtained from microorganisms (Naik et al., 2008) are polyhydroxyalkanoates (PHAs). While the dominant natural PHA is poly (3-hydroxybutyrate) (PHB), microbes can produce various co-polymers with an expansive range of physical properties (Myung et al., 2017). This is commonly accomplished through co-feeding with fatty acids or hydroxyalkanoates, which get incorporated in the polyester. These co-substrates can be sourced from additional process inputs or generated *in situ*. For example the PHA poly-lactic acid (PLA) can be produced by engineered *Escherichia coli* (Jung et al., 2010), albeit to much lower weight percent than is observed in organisms producing PHAs naturally. PHA composition can be modulated in other organisms (Rehm, 2010). The rapid development of synthetic biology tools for non-model organisms opens an opportunity to tune PHA production in high PHB producers and derive a range of high-performance materials.

Before downstream processing (melting, extrusion/molding), the intracellularly accumulating bioplastics need to be purified. The required degree of purity determines the approach and required secondary resources. Fused filament fabrication 3D-printing, which works well in microgravity (Prater et al., 2016; Prater et al., 2018), has been applied for PLA processing and may be extendable to other bio-polyesters. Ideally, additive manufacturing will be integrated in-line with bioplastics production and filament extrusion.

4.1 ISM Integration Into the Biomanufactory

Figure 4 depicts the use of three organism candidates from genera *Cupriavidus*, *Methylocystis*, and *Halomonas* that can meet bioplastic production. This requires a different set of parameters to optimize their deployment, which strongly affects reactor design and operation. These microbes are capable of using a variety of carbon sources for bioplastic production, each with a trade-off. For example, leveraging C_2 feedstocks as the primary source will allow versatility in the microbe selection, but may be less efficient and autonomous than engineering a single organism like *Cupriavidus necator* to use CO_2 directly from the atmosphere. Alternatively, in the event that CH_4 is produced abiotically for ascent propellant (Musk, 2017), a marginal fraction of total CH_4 will be sufficient for producing enough plastic without additional hardware costs associated with ISRU C_2 production. Relying on *Halomonas* spp. in combination with acetate as substrate may allow very rapid production of the required bioplastic, but substrate availability constraints are higher than for CH_4 or CO_2/H_2. A terminal electron acceptor is required in all cases, which will almost certainly be O_2. Supplying O_2 safely without risking explosive gas mixtures, or wasting the precious resource, is again a question of reactor design and operation. Certain purple non-sulfur alphaproteobacteria (e.g., *Rhodospirillum rubrum* (Brandl et al., 1989; Heinrich et al., 2016) and

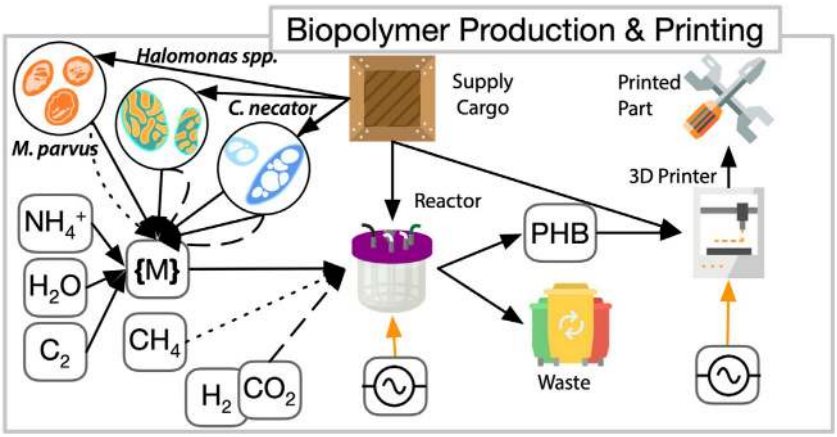

FIGURE 4 | ISM (grey in **Figure 2**) systems breakdown for biomanufactory elements of biopolymer production and 3D-printing. 3D printed parts are fabricated from bioproduced plastics. Biopolyesters such as PHB, along with corresponding waste products, are formed in cargo-supplied reactors with the aid of microorganisms. A variety of available carbon feedstocks can serve as substrates for aerobic auto-, hetero-, or mixotrophic microorganisms such as *Cupriavidus necator*, *Methylocystis parvus* and *Halomonas* spp. All three microbes are capable of using C_2 feedstocks (like acetate, indicated by solid line), while *C. necator* and *Methylocystis* can also use C_1 feedstocks. The former utilizes a combination of CO_2 and H_2 (large dotted line), while only *M. parvus* can leverage CH_4 (small dotted line).

Rhodopseudomonas palustris (Doud et al., 2017; Touloupakis et al., 2021)) also feature remarkable substrate flexibility and can produce PHAs (Averesch, 2021).

Bioplastic recovery and purification is a major challenge. To release the intracellular compound, an osmolysis process (Rathi et al., 2013) may be employed with the halophile (Tan et al., 2011; Chen et al., 2017). However, the transfer of cells into purified water and separation of the polyesters from the cell debris, potentially through several washing steps, may require substantial amounts of water. An alternative and/or complement to the common process for extraction of PHAs with halogenated organic solvents, is to use acetate or methanol as solvents (Anbukarasu et al., 2015; Aramvash et al., 2018). This is applicable independent of the organism and the inputs can be provided from other biomanufactory modules.

The high crystallinity of pure PHB makes it brittle and causes it to have a narrow melting range, resulting in warp during extrusion and 3D-printing. Such behavior places operational constraints on processing and hampers applications to precision manufacturing (Marchessault and Yu, 2005). Workarounds may be through additives, biocomposite synthesis, and copolymerization. However, this ultimately depends on what biology can provide (Müller and Seebach, 1993). There is a need to advance space bio-platforms to produce more diverse PHAs through synthetic biology.

ISM of biomaterials can reduce the mission cost, increase modularity, and improve system recyclability compared to abiotic approaches. In an abiotic approach, plastics will be included in the payload, thereby penalizing up-mass at launch. As with elements of FPS and ISRU, ISM increases flexibility and can create contingencies during surface operations, therefore reducing mission risk. The high modularity of independent plastic production, filament formation, and 3D-printing allows for a versatile process, at the cost of greater resources required for systems operations. Overall, this maximizes resource use and recyclability, by utilizing mission waste streams and byproducts for circular resource management.

5 *IN SITU* RESOURCE UTILIZATION

Biomanufacturing on Mars can be supported by flexible biocatalysts that extract resources from the environment and transform them into the complex products needed to sustain human life. The Martian atmosphere contains CO_2 and N_2 (Menezes et al., 2015a). Water and electrolytically produced O_2 and H_2 are critical to mission elements for any Mars mission. It is very likely that the expensive and energy-intensive Sabatier plants (Clark and Clark, 1997; Meier et al., 2017; Hintze et al., 2018) for CH_4 production will be available per Design Reference Architecture (DRA 5.0) (Drake et al., 2010). While a Haber-Bosch plant could be set up for ammonia production, this is neither part of the current DRA (Drake et al., 2010) nor exceptionally efficient. Thus, for a biomanufactory, we must have carbon fixation reactors to fix CO_2 into feedstocks for non-methanotrophs, and have nitrogen fixation reactors to fix N_2 to fulfill nitrogen requirements for non-diazotrophs. Trace elements and small-usage compounds can be transported from Earth, or in some cases extracted from the Martian regolith. In the case where power is provided from photocollection or photovoltaics, light energy will vary with location and season, and may be critical to power our bioreactors.

Although photosynthetic organisms are attractive for FPS, a higher demand for carbon-rich feedstocks and other chemicals necessitates a more rapid and efficient CO_2 fixation strategy. Physicochemical conversion is inefficient due to high temperature and pressure requirements. Microbial electrosynthesis (MES), whereby reducing power is passed

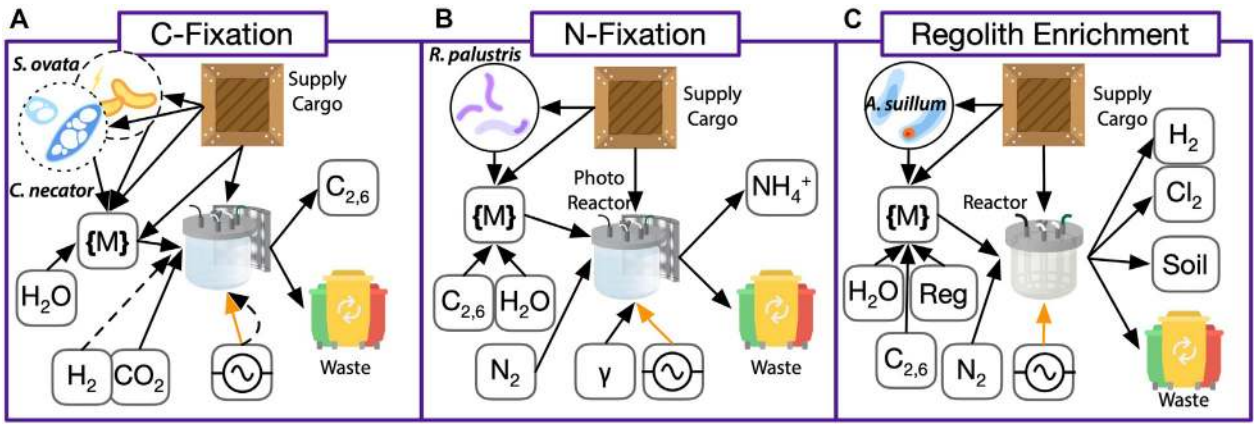

FIGURE 5 | ISRU (purple in **Figure 2**) system breakdown of biomanufactory elements. **(A)** Carbon fixation with the autotrophic bacteria *Sporomusa ovata* or *Cupriavidus necator* through electrosynthesis or lithoautotrophic fixation of C_1-carbon (cathodes or H_2 as the electron donor). **(B)** Microbial nitrogen fixation with diazotrophic bacteria like *Rhodopseudomonas palustris* growing photoheterotrophically **(C)** Regolith (Reg) enrichment using the perchlorate-reducing microbe *Azospira suillum*. Black lines represent material and energy flows related to biological consumption and production. Orange lines indicate additional power supply to the system.

from abiotic electrodes to microbes to power CO_2 reduction, can offer rapid and efficient CO_2 fixation at ambient temperature and pressure (Abel and Clark, 2020). MES can produce a variety of chemicals including acetate (Liu et al., 2015), isobutanol (Li et al., 2012), PHB (Liu et al., 2016), and sucrose (Nangle et al., 2020b), and therefore represents a flexible and highly promising ISRU platform technology (Abel et al., 2020).

Biological N_2-fixation offers power- and resource-efficient ammonium production. Although photoautotrophic N_2 fixation with, for example, purple non-sulfur bacteria, is possible, slow growth rates due to the high energetic demand of nitrogenase limit throughput (Doloman and Seefeldt, 2020). Therefore, heterotrophic production with similar bacteria using acetate or sucrose as a feedstock sourced from electromicrobial CO_2-fixation represents the most promising production scheme, and additionally benefits from a high degree of process redundancy with heterotrophic bioplastic production.

Regolith provides a significant inventory for trace elements (Fe, K, P, S, etc.) and, when mixed with the substantial cellulosic biomass waste from FPS processes, can facilitate recycling organic matter into fertilizer to support crop growth. However, regolith use is hampered by widespread perchlorate (Catling et al., 2010; Cull et al., 2010; Navarro-González et al., 2010), indicating that decontamination is necessary prior to enrichment or use. Dechlorination can be achieved *via* biological perchlorate reduction using one of many dissimilatory perchlorate reducing organisms (Byrne-Bailey and Coates, 2012; Davila et al., 2013; Wetmore et al., 2015; Bywaters and Quinn, 2016). Efforts to reduce perchlorate biologically have been explored independently and in combination with a more wholistic biological platform (Llorente et al., 2018). Such efforts to integrate synthetic biology into human exploration missions suggest that a number of approaches should be considered within a surface biomanufactory.

5.1 ISRU Integration Into the Biomanufactory

A biomanufactory must be able to produce and utilize feedstocks along three axes as depicted in **Figure 5**: CO_2-fixation to supply a carbon and energy source for downstream heterotrophic organisms or to generate commodity chemicals directly, N_2-fixation to provide ammonium and nitrate for plants and non-diazotrophic microbes, and regolith decontamination and enrichment for soil-based agriculture and trace nutrient provision. ISRU inputs are submodule and organism dependent, with all submodules requiring water and power. For the carbon fixation submodule (**Figure 5A**), CO_2 is supplied as the carbon source, and electrons are supplied as H_2 or directly *via* a cathode. Our proposed biocatalysts are the lithoautotrophic *Cupriavidus necator* for longer-chain carbon production [e.g., sucrose (Nangle et al., 2020b)] and the acetogen *Sporomusa ovata* for acetate production. *C. necator* is a promising chassis for metabolic engineering and scale-up (Nangle et al., 2020b), with *S. ovata* having one of the highest current consumptions for acetogens characterized to date (Logan et al., 2019). The fixed-carbon outputs of this submodule are then used as inputs for the other ISRU submodules (**Figures 5B,C**) in addition to the ISM module (**Figure 2**). The inputs to the nitrogen fixation submodule (**Figure 5B**) include fixed carbon feedstocks, N_2, and light. The diazotrophic purple-non sulfur bacterium *Rhodopseudomonas palustris* is the proposed biocatalyst, as this bacterium is capable of anaerobic, light-driven N_2 fixation utilizing acetate as the carbon source, and has a robust genetic system allowing for rapid manipulation (Doloman and Seefeldt, 2020; Abel et al., 2020). The output product is fixed nitrogen in

the form of ammonium, which is used as a feedstock for the carbon-fixation submodule of ISRU along with the FPS and ISM modules. The inputs for the regolith enrichment submodule (**Figure 5C**) include regolith, fixed carbon feedstocks, and N_2. *Azospira suillum* is a possible biocatalyst of choice due to its dual use in perchlorate reduction and nitrogen fixation (Bywaters and Quinn, 2016). Regolith enrichment outputs include soil for the FPS module (in the event that solid support-based agriculture is selected instead of hydroponics), H_2 that can be fed back into the carbon fixation submodule and the ISM module, chlorine gas from perchlorate reduction, and waste products.

Replicate ISRU bioreactors operating continuously in parallel with back-up operations lines can ensure a constant supply of the chemical feedstocks, commodity chemicals, and biomass for downstream processing in ISM and FPS operations. Integration of ISRU technologies with other biomanufactory elements, especially anaerobic digestion reactors, may enable (near-)complete recyclability of raw materials, minimizing resource consumption and impact on the Martian environment (MacElroy and Wang, 1989; Pogue et al., 2002).

6 LOOP CLOSURE AND RECYCLING

Waste stream processing to recycle essential elements will reduce material requirements in the biomanufactory. Typical feedstocks include inedible crop mass, human excreta, and other mission wastes. Space mission waste management traditionally focuses on water recovery and efficient waste storage through warm air drying and lyophilization (Yeh et al., 2005; Anderson et al., 2018). Mission trash can be incinerated to produce CO_2, CO, and H_2O (Hintze et al., 2013). Pyrolysis, another abiotic technique, yields CO and H_2 alongside CH_4 (Serio et al., 2008). The Sabatier process converts CO_2 and CO to CH_4 by reacting with H_2. An alternate thermal degradation reactor (Caraccio et al., 2013), operating under varying conditions that promote pyrolysis, gasification, or incineration, yields various liquid and gaseous products. The fact remains however, that abiotic carbon recycling is inefficient with respect to desired product CH_4, and is highly energy-intensive.

Microbes that recover resources from mission wastes are a viable option to facilitate loop closure. Aerobic composting produces CO_2 and a nutrient-rich extract for plant and microbial growth (Ramirez-Perez et al., 2007; Ramirez-Perez et al., 2008). However, this process requires O_2, which will likely be a limited resource. Hence, anaerobic digestion, a multi-step microbial process that can produce a suite of end-products at lower temperature than abiotic techniques ($\sim$35–55°C compared to $\sim$500–600°C, an order of magnitude difference), is the most promising approach for a Mars biomanufactory (Meegoda et al., 2018; Strazzera et al., 2018) to recycle streams for the ISM and FPS processes. Digestion products CH_4 and volatile fatty acids (VFA, such as acetic acid) can be substrates for polymer-producing microbes (Myung et al., 2015; Chen et al., 2018). Digestate, with nutrients of N, P, and K, can be ideal for plant and microbial growth (Möller and Müller, 2012), as shown in **Figure 6**. Additionally, a CH_4 and CO_2 mixture serves as a biogas energy source, and byproduct H_2 is also an energy source (Schievano et al., 2012; Khan et al., 2018).

Because additional infrastructure and utilities are necessary for waste processing, the extent of loop closure that is obtainable from a treatment route must be analyzed to balance yield with its infrastructure and logistic costs. Anaerobic digestion performance is a function of the composition and pretreatment of input waste streams (crop residuals, feces, urine, end-of-life bioproducts), as well as reaction strategies like batch or continuous, number of stages, and operation conditions such as organic loading rate, solids retention time, operating temperature, pH, toxic levels of inhibitors (H_2S, NH_3, salt) and trace metal requirements (Rittmann and McCarty, 2001; Schievano et al., 2012; Aramrueang et al., 2016; Liu et al., 2018; Meegoda et al., 2018; Strazzera et al., 2018). Many of these process parameters exhibit trade-offs between product yield and necessary resources. For example, a higher waste loading reduces water demand, albeit at the cost of process efficiency. There is also a potential for multiple co-benefits of anaerobic digestion within the biomanufactory. Anaerobic biodegradation of nitrogen-rich protein feedstocks, for example, releases free NH_3 by ammonification. While NH_3 is toxic to anaerobic digestion and must thus be managed (Rittmann and McCarty, 2001), it reacts with carbonic acid to produce bicarbonate buffer and ammonium, decreasing CO_2 levels in the biogas and buffering against low pH. The resulting digestate ammonium can serve as a fertilizer for crops and nutrient for microbial cultures.

6.1 LC Integration Into the Biomanufactory

FPS and ISM waste as well as human waste are inputs for an anaerobic digester, with output recycled products supplementing the ISRU unit. Depending on the configuration of the waste streams from the biomanufactory and other mission elements, the operating conditions of the process can be varied to alter the efficiency and output profile. Open problems include the design and optimization of waste processing configurations and operations, and the identification

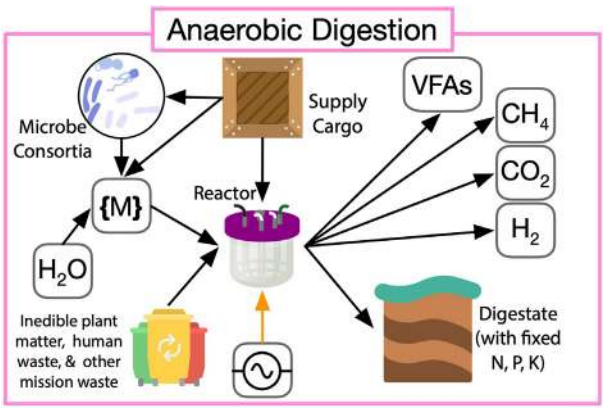

FIGURE 6 | LC-based (pink in **Figure 2**) anaerobic digestion of mission waste such as inedible plant matter, microbial biomass, human, and other wastes produce methane, volatile fatty acids (VFAs), and digestate rich with key elemental nutrients (N, P, K), thereby supplementing ISRU operation.

of optimal end-product distributions based on a loop closure metric (Benvenuti et al., 2020) against mission production profiles, mission horizon, biomanufacturing feedstock needs, and the possible use of leftover products by other mission elements beyond the biomanufactory. A comparison with abiotic waste treatment strategies (incineration and pyrolysis) is also needed, checking power demand, risk, autonomy, and modularity benefits.

7 DISCUSSION AND ROADMAP

Biomanufactory development must be done in concert with planned NASA missions that can provide critical opportunities to test subsystems and models necessary to evaluate efficacy and technology readiness levels (TRLs) (Mankins, 1995). **Figure 7** is our attempt to place critical elements of a biomanufactory roadmap into this context. We label critical mission stages using Reference Mission Architecture (RMA)-S and RMA-L, which refer to Mars surface missions with short (~ 30 sols) and long (> 500 sols) durations, respectively.

Reliance on biotechnology can increase the risk of forward biological contamination (Piaseczny et al., 2019). Beyond contamination, there are ethical issues that concern both the act of colonizing a new land and justifying the cost and benefits of a mission given needs of the many here on earth. Our roadmap begins with the call for an extensive and ongoing discussion of ethics (**Figure 7** Ⓐ). Planetary protection policies can provide answers or frameworks to address extant ethical questions surrounding deep-space exploration, especially on Mars (Rivkin et al., 2020; Tavares et al., 2020). Critically, scientists and engineers developing these technologies cannot be separate or immune to such policy development.

7.1 Autonomous Martian Surface Missions

Figure 7 Ⓑ denotes the interconnection between current Martian mission objects (Mars InSight Landing Pres, 2018; Mars Science Laboratory L, 2012; Baldwin et al., 2016; MAVEN Press Kit, 2013; Mars Reconnaissance Orbit, 2006; Mars Express: A Decade of Observing the Red Planet, 2013; Mars Odyssey Arrival Pres, 2001) and Earth-based process development elements for a biomanufactory (**Figures 3–6**). Together with *en route* autonomous surface missions (Mars Helicopter/Ingenuity, 2020; Mars 2020 Perseverance La, 2020) (**Figure 7** Ⓒ), these missions provide a roadmap for continued mission development based on landing location biosignatures (Bussey and Hoffman, 20162016; Vago et al., 2017). The biomanufactory (**Figures 3–6**) will require ample water in media, atmospheric gas feedstocks, and power that can be bounded by measurements from autonomous missions. Upcoming sample return missions offer an opportunity to shape the design of ISRU processes such as regolith decontamination from perchlorate and nitrogen enrichment for crop growth. Additional orbiters (Jedrey et al., 2016) and lander/rover pairs (**Figure 7** Ⓗ) have been planned and will aid in the selection of a landing site for short term Martian exploration missions (**Figure 7** Ⓙ, Ⓚ). Such locations will be determined based on water/ice mining/availability (McKay et al., 2013) as depicted in **Figure 7** Ⓘ. These missions can be deployed with specific payloads to experimentally validate biomanufactory elements. Low TRL biotechnologies can be flown as experimental packages on upcoming rovers and landers, offering the possibility for TRL advancement of biology-driven subsystems. Planning for such testing will require coordination with, and validation on, ISS and satellite payloads (**Figure 7** Ⓔ), for instance, to understand the impact of Martian gravity, to contrast levels of radiation exposure, and so on.

7.2 Artemis Operations

The upcoming lunar exploration missions, Artemis (Smith et al., 2020) and Gateway (Crusan et al., 2019), provide additional opportunities for integration with Earth-based biomanufactory development. Early support missions (**Figure 7** Ⓓ, Ⓕ) will provide valuable experience in cargo predeployment for crewed operations, and is likely to help shape logistics development for short-term (**Figure 7** Ⓙ, Ⓚ) as well as long-term Mars exploration missions (**Figure 7** Ⓜ) when a biomanufactory can be deployed. Here we present a subset of Artemis efforts as they relate to mission elements with opportunities for testing and maturing biomanufacturing technology. Although ISRU technologies for the Moon and Mars will be sufficiently distinct due to different resource availabilities, crewed Artemis missions (**Figure 7** Ⓕ, Ⓖ) provide a testing ground for crewed Mars bioprocess infrastructure. Later Artemis missions (**Figure 7** Ⓛ) also provide a suitable environment to test modular, interlocked, scalable reactor design, as well as the design of compact molecular biology labs for DNA synthesis and transformation. Since these technologies are unlikely to be mission critical during Artemis, their TRL can be increased and their risk factors studied through in-space evaluation.

The Artemis missions also provide a testbed to evaluate the space-based evolution of microbes and alterations of seedstocks as a risk inherent to the biological component of the biomanufactory. This risk can be mitigated by incorporating backup seed and microbial freezer stocks to reset the system. However, ensuring that native and/or engineered traits remain robust over time is critical to avoid the resource penalties that are inherent to such a reset. Consequently, while optimal organisms and traits can be identified and engineered prior to a mission, testing their long-term performance on future NASA missions prior to inclusion in life support systems will help to assess whether engineered traits are robust to off-planet growth, whether microbial communities are stable across crop generations, and whether the *in situ* challenges that astronauts will face when attempting to reset the biomanufacturing system are surmountable. Quantifying these uncertainties during autonomous and crewed Artemis missions will inform tradeoff and optimization studies during the design of an enhanced life support system for Martian surface bio-operations.

7.3 Human Exploration of Mars

Crewed surface operations of ~ 30 sols by four to six astronauts are projected (Drake et al., 2010) to begin in 2031 (**Figure 7** Ⓙ),

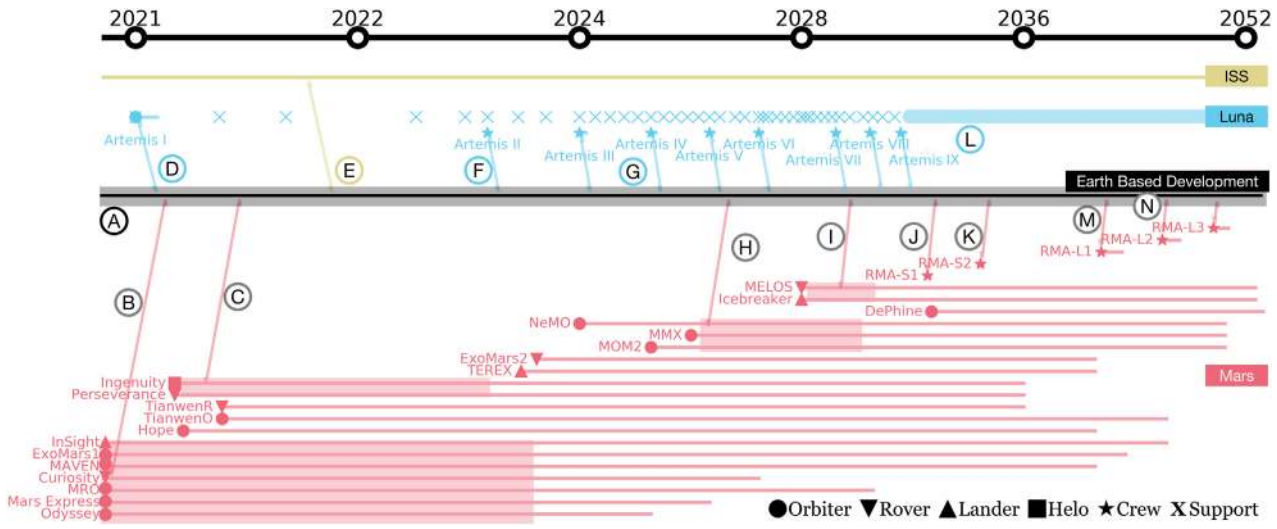

FIGURE 7 | Proposed roadmap from 2021 to 2052 in $\log_2$-scale time of Earth-based developments (black) and their relationships to ISS (gold), lunar (blue), and Martian (red) missions. Missions range in status from currently operational, to enroute, planned, and proposed. Reference Mission Architecture (RMA)-S is a 30-sol mission, and RMA-L are missions with more than 500 sols of surface operations. RMA-L1 is the mission target for deployment of a biomanufactory. An arrival at target location is denoted with a symbol to indicate its type as orbiter, rover, lander, helicopter, support, or crewed operations. Circled letters are colored by location and correspond to specific milestones or opportunities for biomanufactory development.

with an additional mission similar in profile in 2033 (**Figure 7** Ⓚ). Given the short duration, a mission-critical biomanufactory as described herein is unlikely to be deployed. However, these short-term, crewed missions RMA-S1, S2 provide opportunities to increase the TRL of biomanufactory elements for ~ 500 sol surface missions RMA-L1 (**Figure 7** Ⓜ) in ~ 2040 and RMA-L2 (**Figure 7** Ⓝ) in ~ 2044. Building on the abiotic ISRU from early Artemis missions, we propose that RMA-S1 carry experimental systems for C-and-N-fixation processes such that a realized biomanufactory element can be properly scaled (**Figure 5**). Since RMA-S1, S2 will be crewed, regolith process testing becomes more feasible to be tested onsite on the surface of Mars, than during a complex sample return mission. Additionally, while relying on prepacked food for consumption, astronauts in RMA-S1 will be able to advance the TRL of platform combinations of agriculture hardware, crop cultivars, and operational procedures. An example is growing crops under various conditions (**Figure 3A**) to validate that a plant microbiome can provide a prolonged benefit in enclosed systems, and to determine resiliency in the event of pathogen invasion or a loss of microbiome function due to evolution. Additionally, the TRL for crop systems can be re-evaluated on account of partial gravity and/or microgravity.

The RMA-S1 and RMA-S2 crews will be exposed for the first time to surface conditions after interplanetary travel, allowing for an initial assessment of health effects that can be contrasted to operations on the lunar surface (**Figure 7** Ⓕ), and that may be alleviated by potential biomanufactory pharmaceutical and functional food outputs (**Figures 3B,C**). The RMA-S1 and RMA-S2 mission ISRU and FPS experiments will also provide insight into the input requirements for downstream biomanufactory processes. ISM technologies such as bioplastic synthesis and additive manufacture (**Figure 4**) can be evaluated for sufficient TRL. Further, loop closure performance for several desired products can also be tested. This will help estimate the impact of waste stream characteristics changes on recycling (Brémond et al., 2018).

7.4 Moving Forward

We have outlined the design and future deployment of a biomanufactory to support human surface operations during a 500 days manned Mars mission. We extended previous stand-alone biological elements with space use potential into an integrated biomanufacturing system by bringing together the important systems of ISRU, synthesis, and recycling, to yield food, pharmaceuticals, and biomaterials. We also provided an envelope of future design, testing, and biomanufactory element deployment in a roadmap that spans Earth-based system development, testing on the ISS, integration with lunar missions, and initial construction during shorter-term initial human forays on Mars. The innovations necessary to meet the challenges of low-cost, energy and mass efficient, closed-loop, and regenerable biomanufacturing for space will undoubtedly yield important contributions to forwarding sustainable biomanufacturing on Earth. We anticipate that the path towards instantiating a biomanufactory will be replete with science, engineering, and ethical challenges. But that is the excitement—part-and-parcel—of the journey to Mars.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

AJB, JMH, AJA, and APA conceived the concept based on the Center for the Utilization of Biological Engineering in Space (CUBES) that was established by AJB, BB, DSC, DCD, AM, SN, RMW, PY, CSC, KAM, LCS, AAM, and APA. All authors wrote and edited the manuscript with grouped efforts led by JMH, AJA, AJB, MJM, NJHA, SSG, and GM.

FUNDING

This material is based upon work supported by NASA under grant or cooperative agreement award number NNX17AJ31G.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.