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Towards a Biomanufactory on Mars

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ABSTRACT

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 in the coming century.

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

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Introduction

Future space missions of increasing technical complexity – such as transits to[1] and habitation[2] on Mars – will require new technological paradigms[3]. Efforts to modernize mission architectures[4] – combinations of inter-linked system elements that together realize mission goals[5] – will need to leverage an array of enabling technologies including biotechnology[6–8]. Extended human stay in space or upon the surface of alien worlds like Mars introduces new mission elements that require innovation; among these are the biotechnological elements that support human health, reduce costs, and increase operational resilience. The potential for a Mars mission in the early 2030s[9] 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[5]. The replicative capacity of biology reduces mission launch cost by producing goods on-demand using *in situ* resources[10], recycling waste products[11], and interacting with other biological processes for stable ecosystem function[12]. 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 on deep space missions. Biological systems also provide robust utility via genetic engineering, which can provide solutions to unforeseen problems and lower inherent risk[6,13]. 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[14]. A biomanufactory for deep space missions[15] based on *in situ* resource utilization and composed of integrated subunits capable of producing food, pharmaceuticals, and biomaterials (Fig. 1) will greatly reduce launch and resupply cost, and is therefore critical to the future of human-based space exploration[6,8].

Feasibility, Needs, and Mission Architecture

Planning for a Mars mission began in the 1950s with a vision by Von Braun to send ten spacecrafts harboring seventy crew-members[16]. As times changed, technology and stakeholder goals[17,18] evolved, and now proposals include small crews supported by predeployments[9]. To expedite mission design, a Design Reference Architecture (DRA) can provide requirements and initial technology specifications[5]. The most recent National Aeronautics and Space Administration (NASA) DRA for Mars from 2009 offers no specifics for a biomanufacturing-driven mission[13] due to the novelty of space bioengineering. We propose that the DRA should be updated to account for innovations in this field. Here, we outline a foundation for a component focused on the biotechnological support of a long-term mission with six crew-members and duration of ∼500 sols (a sol is a Martian day and lasts slightly longer, by ∼40 min, than an Earth day) of surface operations flanked by two interplanetary transits of ∼210 days[19] for production of food, medicine and materials. We further assume predeployment cargo consisting primarily of *in situ* resource utilization (ISRU) hardware for Mars-ascent propellant production, which is to be launched from Earth to a target landing location. Additional supplies such as habitat assemblies[20,21], photovoltaics[22,23], experimental equipment, and other

Figure 1. Artist interpretation of Crewed Martian Biomanufactory (Artwork by Desiree Ho) powered by photovoltaics, fed via atmospheric ISRU, and capable of food & pharmaceutical synthesis (FPS), *in situ* manufacturing (ISM), and biological loop closure (LC).

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non-living consumables[24] will be included. We assume that crew will travel from Earth to low Earth orbit (LEO) then board an interplanetary craft for the journey to Martian orbit where the crew will descend to the surface in a separate craft, allowing the large transit vehicle to remain in orbit. Once on Mars, the crew will assemble their habitat from cargo. Following a surface campaign, the crew will leave Mars in a fueled ascent craft, board the interplanetary vehicle, and return to Earth orbit[9]. For this DRA, we envision that the Environmental Control and Life Support Systems (ECLSS) will rely on the development and integration of biotechnologies into standard methods for maintaining astronaut health and enabling mission success. While such integration between LSS and biotechnology has been proposed[11,12], we distinguish our proposed biomanufactory from prior ECLSS in that biotechnology serves as the primary driver for the ISRU, *in situ* manufacturing (ISM), food & pharmaceutical synthesis (FPS), and loop closure (LC) technologies (Fig. 2).

The requirements for sustaining a human population in terms of food, medicine, and gas exchange can impose important feasibility constraints[25–27] on the closed-loop life support system. The chief feasibility constraints are driven by the physiological profile of a crewmember (CM), 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[28,29]. 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[9]. As the length of a mission increases from ∼30 to ∼500 sols of surface operations, demands on the quantity and quality of consumables increase dramatically. Taking food systems as an example, consumable food mass scales nearly linearly with the increased demand. Storage of larger quantities of food necessitates additional refrigeration, including corresponding power and cooling systems. Furthermore, consumables must be maintained longer in harsher environments, increasing both financial and mass costs. But, caloric intake alone does not fully describe the consumption cost of astronaut

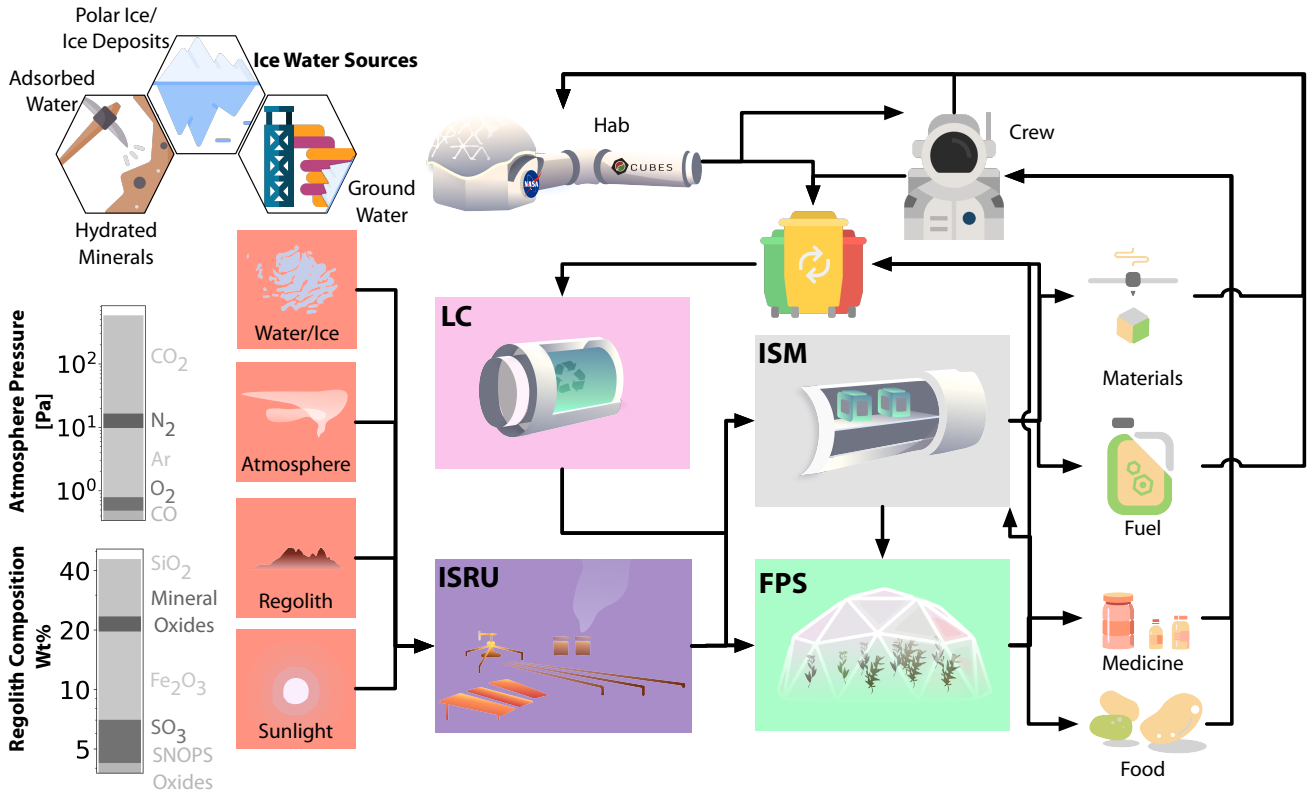

Figure 2. Proposed surface operations are drawn from inventories of *in situ* resources (red) such as ice, atmosphere, regolith, and sunlight. Atmospheric feedstocks of carbon and nitrogen are biologically fixed via the ISRU (*in situ* resource utilization) biomanufactory components (including abiotic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

sustainability: pharmaceutical needs must also be met in order to ensure crewmember health. The risk of having compromised consumables necessitates additional reserves to match astronaut needs in terms of important elemental components, such as carbon, nitrogen, and phosphorus[30]. While some physico-chemical means exist for recycling a subset of these elements, they are usually mass and energy intensive[31], and generally need additional downstream processing[32]. As missions become more complex with longer surface operations, biotechnology offers methods for consumable production in the form of edible crops and waste recycling through microbial digestion[11]. Advocacy and advancement in biomanufacturing for deep space exploration will ensure a transition from short-term missions reliant on single-use-single-supply resources to long-term missions that are sustainable.

Biomanufactory Systems Engineering

We propose a main the benefit of a biomanufactory is the efficiency gained through interconnection (Fig. 2) and modularity of the various unit operations (Figs. 3-6)[33]. However, at every mission stage where different choices of the active operations is needed, the requirements for assembly and initiation of operations – as well as the requirements for timing and amount of productivity – lead to different optimal configurations of the system. There is a challenge in creating a ECLSS framework for technology choice and process optimization that specifically addresses the high degree of flexibility, scalability, and infrastructure minimization needed for an integrated biomanufactory.

The uncertainty in process parameters on a new planet are likely to be very high and amplify the risks associated with each element, thus these metrics have outsized importance compared to classical chemical engineering systems on earth. The requirements for exceptionally high efficiency, low and reusable waste streams, and provable containability to prevent environmental contamination, place higher value on 'sustainability' performance parameters and stronger requirements on integrated co-design than in earth bound systems. To recognize the advantages of a plant that can scale on demand and can flexibly change processes as unexpected new biosynthesis processes are needed, a high premium on modularity and compatible design comes to the fore. Finally, to meet the demand that biomanufacturing processes should start before crew arrival and should minimize the need for crew intervention thereafter, automation becomes a premium. Current frameworks for advanced manufacturing optimization don't focus as heavily on these aspects and creating a robust approach for space requires a series of new innovations in modeling processes and the development of performance metrics specific to the ECLSS environment such as risk, modularity, autonomy, and recyclability. Concomitant invention in the engineering infrastructure will also be required.

Food and Pharmaceutical Synthesis

An estimated ∼10,000 kg of food mass is required for a crew of six on a ∼900 day mission to Mars[6]. 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. Pharmaceutical life support must overcome accelerated instability (∼75% of solid formulation pharmaceuticals are projected to expire mid-mission at 880 days[6]) and long re-supply times. Pharmaceutical production for longer missions can be expected to mitigate the impact of this anticipated instability and accelerate response time to unanticipated medical threats. In earlier missions, it is likely that the FPS will be used in a capacity to boost crew morale and supplement labile nutrients[34]. As the mission scale increases, FPS to meet food and pharmaceutical needs becomes more important[35]. We propose a biomanufactory focus on oxygenic photoautotrophs, namely plants, algae and cyanobacteria, in FPS production systems to enhance simplicity, versatility, and synergy with intersecting life support systems[12,36]. While plant-based food has been the main staple considered for extended missions[9,28,35], the advent of cultured and 3D printed meats and meat-like products from animal, plant and fungal cells may ultimately provide a scalable and efficient alternative to cropping systems[37–39].

Mission duration and environmental parameters such as CO_2 levels will have a significant influence on the FPS platform tradeoff and selection, such as calorie-rich agronomic crops[40] versus nutrient-rich horticultural crops[41]. FPS may also include select edible species of cyanobacteria and microalgae, such as *Arthrospira platensis* and *Chlamydomonas reinhardtii*, which provide higher growth rates, generally higher protein contents, and require lower photon intensities than food crops for optimal growth, but at the cost of increased reactor complexity[42–44].

Development of specific FPS organisms for use on Mars is needed to optimize growth and maximize yields of biomass, nutrient, and pharmaceutical accumulation. These optimized organisms may be developed either through the selection of pre-existing strains and/or genetic engineering and breeding. For example, providing adequate and appropriate lighting will be a primary challenge of photoautotrophic-centric FPS on Mars[45,46]. 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[47].

The selection and development of FPS organisms for pharmaceutical production is an especially complicated endeavour, given the breadth of production modalities and pharmaceutical need (e.g. time window of intervention response, molecule class), as has been recently reviewed in detail[14]. For example, transgenic cyanobacterial production may provide an advantage

for orally bioavailable small molecule targets (e.g. acetaminophen), while transient plant-based production is well-suited for rapid response and complex molecule countermeasures (e.g. cytokine therapy for acute radiation sickness). Furthermore, limited resource pharmaceutical purification is a critically important consideration that has not been rigorously addressed in literature to date. Given promising biologically-derived purification technologies[48,49] , this should also be considered for processing drugs that require very high purity (e.g. injectables).

Development of FPS growth systems for Mars require biotic and abiotic optimization. Two key considerations for technological development are the lighting systems and plant microbiome. The recent advancements in LED efficiency have implicated this technology as the optimal lighting system for crop growth in extraterrestrial systems[50]. For maximum production efficiency the ideal spectra from tunable LEDs will likely be one with a high fraction of red photons, but increasing the fraction of shorter wavelength blue photons could increase crop quality. Higher photon intensities increase production rates but decrease production efficiency. Understanding this tradeoff, in the context of volume and power/cooling requirements, will be paramount to increasing overall system efficiency.

As has been shown on the ISS, it is not feasible to cultivate plants in aseptic conditions, and unanticipated changes in temperature and humidity cause shifts in plant microbiome composition[34]. Applying synthetic microbial communities (SynComs) to plants (Fig. 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[51], accelerate wastewater remediation and nutrient recycling[52], and shield plant hosts from both abiotic and biotic stresses[53], including opportunistic pathogens[54–56]. 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. While SynCom design is challenging, inclusion of SynComs in life support systems represents a critical risk-mitigation strategy to protect vital food and pharma resources.

FPS Integration into the Biomanufactory

In order to supply astronauts with proper nutrition and pharmaceuticals on a voyage to Mars, the FPS module of our biomanufactory is broken into three submodules: crops, pharmaceuticals, and functional foods (Fig. 3). Here, we discuss each submodule, the integration of these components in the scope of the biomanufactory as a whole, and provide justifications for use of these modules over technological alternatives.

As the FPS is based solely on oxygenic photoautotrophs, the inputs to all three submodules (Fig. 3) are nearly identical in needing water as an electron donor, carbon dioxide as a carbon source, and light as an energy source, with the required nitrogen source being organism-dependent (e.g. *A. platensis* requires nitrate). While CO_2, H_2O, and light will be supplied from

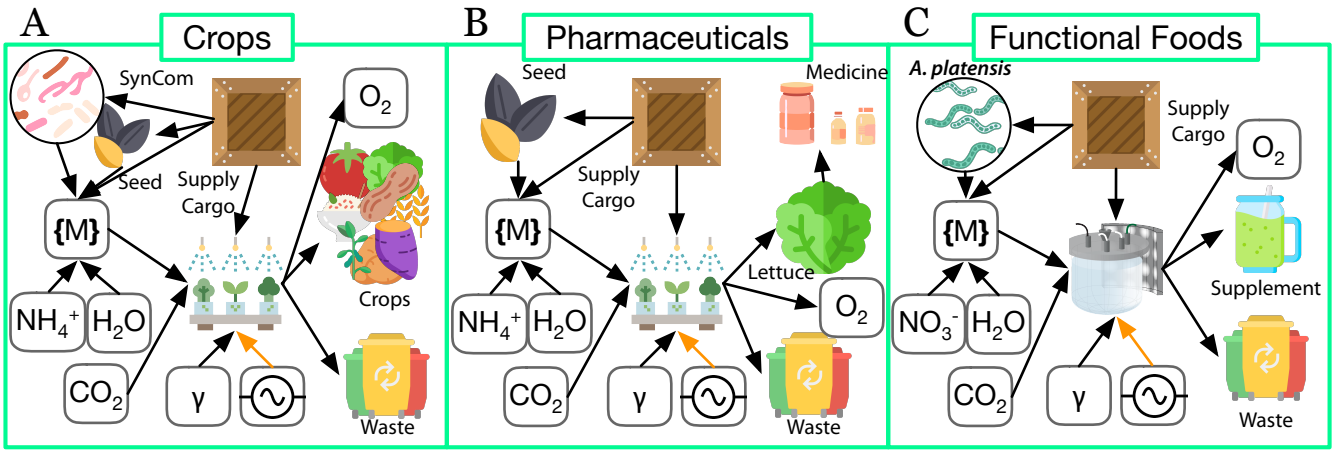

Figure 3. FPS (green) system breakdown for biomanufactory elements of **(A)** crops, **(B)** a biopharmaceutical, and **(C)** functional food production. In all cases, growth reactors require power (electrical current symbol) and light (γ). **(A)** Crop biomass and oxygen gas (O_2) are produced from hydroponically grown plants using seeds and media ({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 *A. platensis*. In all cases, biomass is produced, collected, and inedible biomass is distributed to the LC for recycling.

the Martian environment without biological alteration, fixed nitrogen will be supplied from the ISRU module, and will help set nitrogen-fixation requirements for the system. Outputs from each module are similar in that they produce O_2, biomass, and waste products. However, the crop submodule (Fig. 3A) is specifically tailored to output edible biomass for bulk food consumption, the pharmaceutical submodule (Fig. 3B) is designed for the output of medicines, and the purpose of the functional foods submodule (Fig. 3C) is to output nutritional requirements not met by the crop submodule, such as microbially-produced vitamins (e.g. vitamin B_{12}). These outputs, excluding waste products, will be consumed directly by crew-members, with waste products entering the LC module for recycling.

Across the FPS, we expect that all submodules will have increased risk, modularity, and recyclability, while having decreased autonomy, relative to traditional technological approaches. The FPS module increases mission risk in food and pharmaceutical availability associated with biomass loss due to lower-than-expected yields, contamination, and possible growth system failure. The programmability of biology, such as the rapid response time of molecular pharming in crops for as-needed production of biologics, supports this increased modularity over shipping a known set of pharmaceuticals to Mars. 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. Growth of FPS organisms increases crew time requirements for setup, maintenance, and harvesting as compared to simply shipping food and pharmaceuticals ahead of time. Cost may fluctuate between the FPS submodules as 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.

In situ Manufacturing

Maintaining physical FPS systems such as hydroponic and/or aeroponic reactors requires cultivation chambers, chamber and plant support structures, tubing connections, spray nozzles, and tools. Such physical objects represent elements of an inventory that for the short missions will likely be a combination of predeployment cargo and supplies from the crewed transit vehicle[9]. As mission duration increases, so does the quantity, diversity of composition, and complexity of construction for elements in the inventory. While the extent to which ISM would be used on initial exploration missions is not yet specified in the current DRA[9], recent developments[57–59] imply that ISM will be critical for the generation of commodities and consumables made of plastics[60], metals[61], composite-ceramics[62], and electronics[63] as mission objects, with uses ranging from functional tools[64] to physical components of the life-supporting habitat[58,65].

While a precise manifest for surface operations of ∼500 sols is yet to be published, the composition of consumable constructs is likely to be largely plastic and with a size profile on the order small parts to bench-top equipment. Biotechnology in combination with additive manufacturing can produce such polymeric constructs from versatile feedstocks, and because compact microbial bioreactors[66], operating at ambient conditions, allow for simpler production than those common to terrestrial chemical processes. The versatility of microbial metabolisms allows us to tap into *in situ* resources such as CO_2 from the atmosphere, methane (CH_4) from abiotic Sabatier processes[67], and biologically synthesized C2 compounds, such as acetate as well as waste biomass.

The targeted materials are polyhydroxyalkanoates (PHAs), biological polyesters that are produced by a variety of organisms[68]. While the dominant natural PHA is poly(3-hydroxybutyrate) (PHB), microbial production of various co-polymers has been demonstrated, expanding the range of physical properties that can be achieved. This is commonly accomplished through co-feeding with fatty acids or hydroxyalkanoates. PHAs such as poly-lactic acid (PLA) polymers have been produced by e.g. *E. coli*[69], albeit to much lower weight % than is observed of PHA in organisms producing them naturally. PHA composition has been modulated in various other organisms; even in methanotrophs production of highly crystaline 3-hydroxybutyrate-containing copolymers with fractions of hydroxypropionate, valerate, and hydroxyhexanoate[70–72] has been demonstrated[73–76]. These co-substrates could be sourced from additional process inputs or generated *in situ* by metabolic engineering. The rapid development of synthetic biology tools for non-model organisms opens the opportunity to modulate PHA production in high PHB producers to tune polymer properties and derive a range of high-performance materials with enhanced, application-specific properties. The intracellularly accumulating bioplastics need to be sufficiently purified to allow downstream processing. The required degree of purity here determines the approach, and required secondary resources, as discussed further below. Fused filament fabrication (FFF) 3D printing is one method that has been applied for PLA processing, which may be extendable to other bio-polyesters: Microbially-produced PHAs are thermally melted, extruded into filaments and subsequently fed into an FFF printer, which constructs the part by layer-by-layer deposition. The primary use of this 3D printing will be for high turnover equipment such as hydroponic farming reactors as well as replacement parts and tools to solve unpredicted problems or ones that can otherwise not be accounted for[77]. FFF has been shown to works well in microgravity[77,78]. Once printing is operational, optimally it will be integrated in-line with the production of bioplastics and filament extrusion; early integrated testing of this ISM capability may prove difficult on robotic missions due to the lengthy supply chain and difficulty to operate autonomously and could instead be broken up into the modules microbial production, recovery/purification, filament extrusion, and 3D printing processes.

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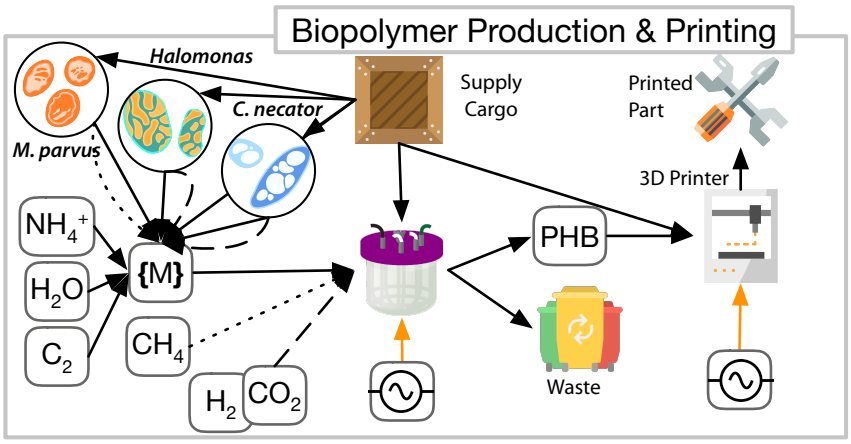

Figure 4. ISM (grey) systems breakdown for biomanufactory elements of biopolymer production & 3D printing. 3D printed parts are fabricated from bioproduced plastics. Biopolyesters such as PHB, along with the corresponding waste products are formed in cargo-supplied reactors with the aid of microorganisms. A variety of available carbon feedstocks may serve as substrates for auto-, hetero-, or mixotrophic microorganisms such as *C. necator*, *Methylocystis parvus* and various halophile species like *Halomonas*. All three microbes are capable of using C_2 feedstocks (like acetate), 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).

The greatest challenges in order to advance the described ISM technologies pertain the optimization of the production process (bioreactors) as well as downstream processability of the biomaterials: Out of the three candidates (*Cupriavidus*, *Methylocystis*, *Halomonas*) that can suffice the requirements for bioplastics production, each requires a different set of parameters to optimize their use, strongly affecting reactor design and operation. As shown in Figure 4, 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 would allow versatility in the microbe selection, but may be less efficient and autonomous than engineering a single organism like *C. necator* to use CO_2 directly from the atmospheric inventory. Alternatively, in the event that CH_4 is produced abiotically for ascent propellant[4], a marginal fraction of total CH_4 would be sufficient for production of enough plastic and without additional hardware costs associated with C_2 production via ISRU. Lastly, relying on *Halomonas* in combination with acetate as substrate may allow much more rapid production of the required quantities of material, but with availability constraints of the substrate when compared to CH_4 or $CO4_2/H_2$. Further, the bioplastic recovery and purification process presents a major challenge: to circumvent the use of halogenated organic solvents (and associated increase in up-mass for recycling equipment), an osmolysis process[79] may be employed with the halophile[80,81], which, however, still requires significant amounts of water. An alternative applicable to all three proposed organisms may be the utilization of acetate or methanol as solvents[82,83], which may be available through other modules of the biomanufactory. The high crystallinity of certain PHAs causes them to have a narrow melting range, placing operational constraints on the temperature-control crucial for 3D printing. Further, PHB is brittle, warps during extrusion and 3D printing, hampering its application in order to make a functional tool[84]. Work-arounds may be additives and co-polymerization, which ultimately depends on what biology can provide[85]. This underlines the need to develop bio-platforms for more diverse PHAs through synthetic biology.

ISM Integration into the Biomanufactory

ISM of biomaterials can reduce the mission cost, increase modularity, and improve system recyclability compared to abiotic approaches, which only offer the benefits of a more streamlined process (Fig 4). In an abiotic approach, plastics will be included in the payload thereby causing high energy demands at launch. As with elements of FPS and ISRU, Biomanufacturing overcomes the constraints of the abiotic route by allowing for plastics with tuned properties and controlled production volume on the Martian surface therefor reducing mission risk. The high modularity of independent plastic production, filament production, and 3D printing allows for a versatile process, but with these added steps, more resources must be directed to systems operations. This effort overall maximizes resource use and recyclability, by utilizing mission waste streams and even recycling printed parts.

Major complications from ISM are introduced during integration; the complexity of converting waste resources to usable plastic products introduces many complex processing steps with few simple solutions[86–88]. With the system drawing primarily from the waste streams of an already established Martian base, plastic production will be limited to surface operations and so

requirements for prototyping will not require on-orbit testing. The major proving ground for this technology will be on the Lunar surface where it must be setup and operated with only astronaut intervention.

In situ Resource Utilization

ISRU reduces launch mass required for mission operations by enabling the conversion of native materials from the atmosphere and regolith into useful consumables or feedstocks for downstream processes[10]. Given the primary abundance of CO_2, along with smaller concentrations of N_2 (Fig. 2), in the Martian atmosphere, past research priority has been their abiotic conversion to CH_4 and NH_3 via the Sabatier and Haber-Bosch processes[67,89,90]. Both of these processes also require a source of hydrogen gas (H_2), which can come from the electrolysis of water-ice[91,92]. With high temperature and pressure requirements, these reactions – generally intended for the production of large quantities of ascent bipropellant with combinations of hydrocarbon cryogenic liquid CH_4[4,93] or hydrazine with an oxidizer such as liquid O_2 – are energy intensive and thus limited by power availability[28].

Power generation and initial resource selection and management for ISRU technology platforms represent prima facie shaping of the biomanufactory. Both, nuclear reactors[94] and solar cells[22,23], can supply sufficient energy for biomanufactory processes[28]. However, transit and/or mining of fissile material represents a significant hazard to crewmembers and the Martian environment, so solar cells coupled to power-storing devices such as batteries or fuel cells represents a more practical option for initial missions. CO_2 and N_2 supplied to bioreactors as carbon and nitrogen sources for autotrophic and diazotrophic microbial growth will require compression and fractionation since the atmospheric concentrations ($\sim$0.57 kPa and $\sim$0.016 kPa, respectively) are significantly lower than the partial pressure ($\sim$40 kPa) typically required for effective biological fixation[95–97].

Although photosynthetic organisms such as cyanobacteria are attractive for pharmaceutical or functional food production, higher demand for carbon-rich feedstocks and other chemicals necessitates a more rapid and efficient CO_2-fixation strategy. Physicochemical conversion is similarly inefficient due to its high temperature and pressure requirements. An emerging strategy, termed electromicrobial production (EMP), in which reducing power is transferred from abiotic electrodes to microbes can offer rapid and efficient CO_2-fixation at ambient temperature and pressure. This transfer of reducing power is achieved either by direct attachment of microbes to the electrode surface, where electrons are transferred directly to the organism as an energy source, or with the aid of a mediator molecule such as molecular hydrogen (H_2) or formate ($HCOO^-$) that is produced at a cathode and subsequently consumed by microbes for CO_2 fixation[98–100]. This strategy has been used to produce a wide variety of platform chemicals including acetate[101], isobutanol[102], poly-hydroxybutyrate (PHB)[103], and sucrose[104], and therefore represents a flexible and highly promising ISRU platform technology. While mediated EMP is more suitable in the near-term, direct electron transfer mechanisms may obviate metal electrodes entirely, so this strategy may be better suited for fully recyclable processing.

Following a similar analysis, 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 limits throughput[105]. 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 significant cellulosic biomass waste from FPS processes, can facilitate recycling organic matter into fertilizer that further supports crop growth. However, the use of regolith in such scenarios is hampered by widespread perchlorate detection[106–108], indicating that decontamination is necessary prior to enrichment or use. Such dechlorination can be achieved via biological perchlorate reduction using one of many dissimilatory perchlorate reducing organisms[109–112].

ISRU Integration into the Biomanufactory

While short-term forays rely on such abiotic production primarily for basic astronaut consumables like O_2[113] and fuel, longer-term missions necessitate placing a higher priority on the efficiency and scalability of ISRU systems along with a significantly expanded product spectrum. This prompts a biomanufactory 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 for plants and other organisms, 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 (Fig. 5A), CO_2 is supplied as the carbon source, and electrons are supplied as H_2 or directly via a cathode. The purposed outputs of this submodule are fixed carbon products (e.g. acetate or sucrose), which are then used as inputs for the other ISRU submodules (Fig. 5B,C) in addition to the ISM module (Fig. 2). The inputs to the nitrogen fixation submodule (Fig. 5B) include fixed carbon feedstocks, N_2, and light. 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

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regolith enrichment submodule (Fig. 5C) include regolith, fixed carbon feedstocks, and N_2. Regolith enrichment outputs include soil for the FPS module (in the event that soil-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.

Biological processes for CO_2- and N_2-fixation and regolith processing have the potential to minimize energy requirements and reduce reactor operation hazards by operating at near-ambient temperatures and pressures; effectively decreasing cost and risk metrics. Additionally, flexibility in production, enabling just-in-time production, can be achieved by on-demand genetic engineering or pre-deployment generation of a library of readily-stored production strains without any changes in the supporting infrastructure. Alternately, 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. Finally, 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[114,115].

Loop Closure and Recycling

Waste stream processing to recycle essential elements will reduce material requirements in the biomanufactory. Inedible crop mass, human excreta, and other mission wastes are rich in carbon and nitrogen. Thus, converting wastes to value-added compounds for the biomanufactory is useful. While the traditional focus of waste management for space missions has been on water recovery and efficient waste storage through warm air drying and lyophilization[25,28], the generation of methane, a propellant fuel, has gained gradual attention. Incineration of waste, followed by a Sabatier reaction[116] or waste pyrolysis[117] yields methane. In the first instance[116], incineration oxidizes waste to gases such as carbon dioxide, carbon monoxide and steam, following which, carbon dioxide and carbon monoxide yield methane on reaction with hydrogen (derived from the steam) in the Sabatier reactor. On the other hand, pyrolysis, a process at inert atmosphere, can be conducted in series of stages at increasingly higher temperature[117]. At relatively lower temperature (~400°C), tar is the primary product along with syngas and char as byproducts. At higher temperature, the hydrocarbon liquids in tar can be cracked to yield more gaseous products, mainly H_2, CO, H_2O, CO_2 and CH_4. Thus, methane yield is less than that obtained by incineration, followed by Sabatier reaction. The literature[118] also presents a thermal degradation reactor that can operate under varying conditions promoting pyrolysis, gasification or incineration; alkanes, alkenes, aldehydes and ketones were also detected as minor products in tar alongside the major gaseous products.

Rather than relying solely on these abiotic technologies, microbial treatment of mission wastes to recover resources is also an option. Aerobic composting produces CO_2 and a nutrient-rich extract for plant and microbial growth[119,120]. However, this process requires oxygen, which will likely be a limiting resource. Hence, anaerobic digestion, a multi-step microbial process that can produce a suite of end-products, is the most promising waste treatment technique for a Mars biomanufactory[121,122].

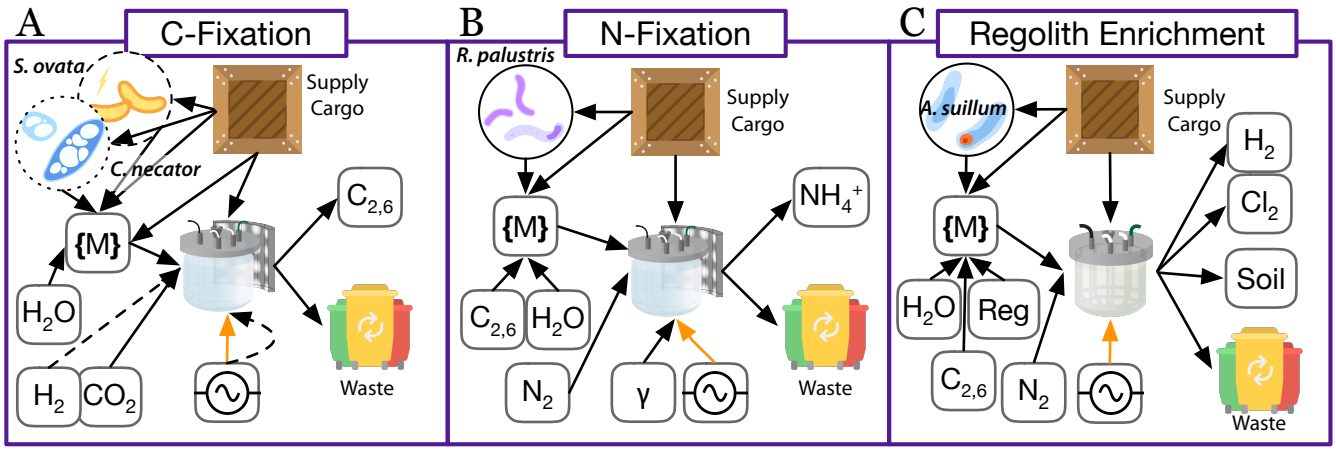

Figure 5. ISRU (purple in Fig. 2) system breakdown of biomanufactory elements. **(A)** Carbon fixation with the autotrophic bacteria *Sporomusa ovata* or *Cupriavidus necator* through electrosynthesis or lithoautotrophic fixation of C1-carbon (electricity or H_2 as the electron donor). **(B)** Microbial 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.

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Digestion products methane and volatile fatty acids (VFA, such as acetic acid) are substrates for polymer-producing microbes, and digestate, with fixed nitrogen, phosphorus and potassium, can be ideal for plant and microbial growth (Fig. 6). Even further, CH_4 and CO_2 as products are also useful as energy sources in the form of biogas and H_2, as a byproduct, can also be a source of renewable energy[123,124]. While abiotic strategies can yield multiple end-products which can be useful for specific scenario, anaerobic digestion products are perfect fit as recycled streams for the ISM and FPS processes and so, we mostly focus on the latter. Any waste treatment technique warrants shipment of additional infrastructure and utilities, thereby contributing to the launch mass of the mission. Accordingly, the extent of loop closure that is obtainable from a specific waste treatment route must be analyzed to balance yield with infrastructure and logistics costs incurred.

Anaerobic digestion performance is a function of the composition and pretreatment of input waste streams, 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, and pH[121–123,125,126]. Many of these process parameters exhibit trade-offs between product yield and necessary resources. For instance, higher loading of waste reduces water demand, albeit at the cost of process efficiency. While certain conditions such as increased temperature and pH produce increasing amount of ammonia, a corrosive and potentially toxic gas, it can be removed by various conventional and novel technologies[127].

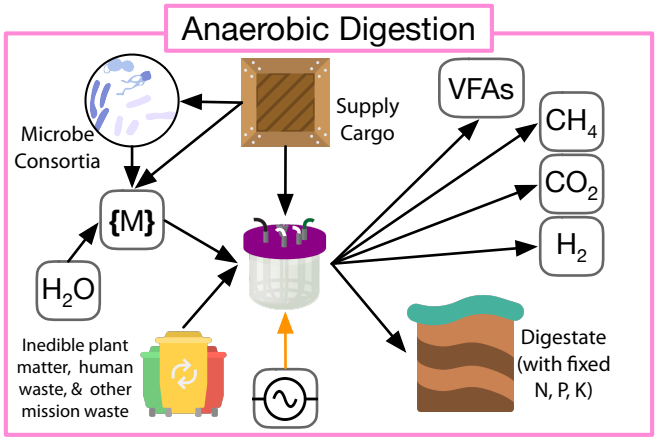

Figure 6. LC-based (pink) anaerobic digestion of mission waste such as inedible plant matter, microbial biomass, human, and other wastes produce methane, volatile fatty acids (VFAs) along with digestate rich with key elemental nutrients (N, P, K), thereby supplementing ISRU operation.

LC Integration into the Biomanufactory

The waste from FPS and ISM sections and human waste are inputs for anaerobic digester, while the recycled products supplement ISRU section. As noted above, depending on the configuration of the waste streams from the biomanufactory and other mission elements, the organic loading rates, solid retention times, operating temperatures and pH of the AD process can be varied to alter the efficiency and output profile. All these diverse options and concomitant impacts call for optimization of waste processing design and operation, and identification of the optimal end-product distribution based on a loop closure metric[128] against mission production profiles, mission horizon, biomanufacturing feedstock needs, and possible use of left over products by other mission elements beyond the biomanufactory.

One can also qualitatively compare anaerobic digestion with abiotic strategies of waste treatment (incineration and pyrolysis) on the basis of various critical attributes. For instance, power demand for anaerobic digestion is lower since the operating temperature (∼35-55°C) is reduced compared to abiotic processes (∼500-600°C). This strategy also mitigates risk and increases modularity significantly, as it is capable of producing a variety of products with diverse end-uses. Process autonomy typically relies on the use of process models that are a function of microbial kinetics. Despite numerous modeling studies[129,130], satisfactory bioprocess operation under the uncertainties of space missions such as altered gravity and exposure to galactic cosmic radiation is still an open question and warrants more detailed studies.

Discussion and Roadmap

Each of the elements and areas for the biomanufactory described above require further development and study if they are to be deployed in a mission context. This needs to be done in concert with the planned NASA missions that provide critical opportunities to test subsystems and models necessary to evaluate the efficacy and technology readiness levels level (TRL)[131] of

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a biomanufactory of the sort proposed. Figure 7 is our attempt to place what critical elements of a biomanufactory roadmap into this context. We label a number of the critical mission stages using the labels RMA-S and RMA-L which refer to Mars surface missions with short (∼30 sols) and long (>500 sols) durations respectively. Through integrating upcoming missions (en route, planned, and proposed) of the ISS (color: gold), Luna (color: blue), and Mars (color: red) with Earth-based developments (color: black), we aim for a first biomanufactory deployment during a proposed RMA-L1 mission at ∼2040 and staged testing of components in the missions preceding.

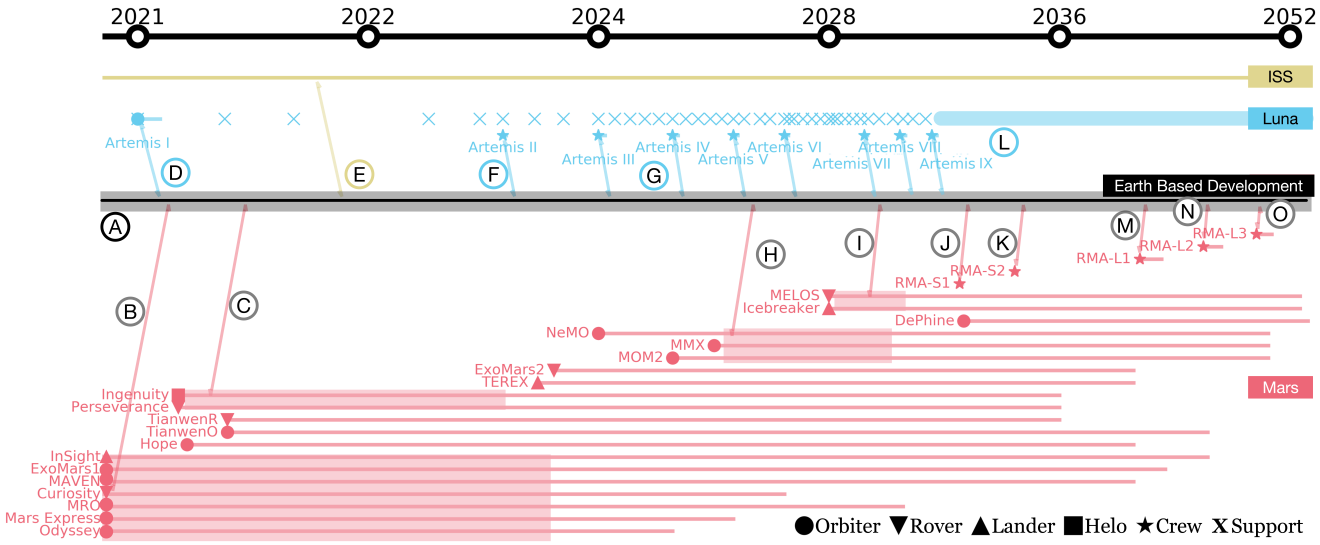

Figure 7. Proposed roadmap from 2021 to 2052 in $\log_2$-scale time of Earth-based developments (black) and their relationships to ISS (gold), Lunar (blue), and Martian (red) missions. Missions noted range in status from currently operational to enroute, to planned to proposed. RMA-S corresponds to a 30-sol mission and RMA-L correspond to missions with more than 500 sols of surface operations. RMA-L1 corresponds to 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.

There are many ethical issues associated with any mission to Mars, let alone a crewed mission and one bringing along other organisms that could conceivably contaminate the environment. In tandem with the technical evaluation of the roadmap, it is necessary to ensure we address theses issues, especially those that obtain to the biotechnological support of it(Fig. 7Ⓐ). International recognition of space as the "common heritage of humankind" should require that space exploration is carried out to benefit all peoples[132–134]. Ideally then, a fully rendered Design Reference Mission would culminate in an agreement as to whether the venture's benefits outweigh the risks and a memorandum of understanding as to upholding planetary protection guidelines[135], meeting financial constraints, and mitigating foreseeable societal disparities. A human expedition to Mars is projected to cost between $150 billion[136,137] and nearly $1 trillion[138]. Since this massive resource deployment could alternately be used to address political, economic, and sustainability challenges on Earth, minimizing the financial cost of the mission and maximizing societal benefit is necessary. We argue that biotechnology may offer a path to reduce the financial cost and augment the scientific benefits of long-duration missions. The technology demonstrated on such missions can limit harm to and extraction from extraterrestrial locations through its strong focus on sustainability and containment, and additionally can provide an alternative less environmentally detrimental method for manufacturing on Earth. However, this alternate system could simply be used to further exacerbate the inequities between communities that have the resources necessary to develop such a system and those that do not, both on Earth and via increased access to extraterrestrial resources[139]. Further, reliance on biotechnology can increase the risk of forward biological contamination[140]. Planetary protection policies that disrupt the settler colonialist framework of space exploration are necessary, then, to provide answers or frameworks to address extant ethical questions surrounding deep-space exploration, especially on Mars[134,141]. These policies should be developed in large part by centering the perspectives of those who have experienced the worst harms from previous colonization events, in particular Indigenous peoples[142], and must explicitly consider how to equitably deploy on Earth the biotechnology developed for crewed extraterrestrial missions[143]. Critically, scientists and engineers developing these technologies cannot be separate or immune to such policy development.

Autonomous Martian Surface Missions

Figure 7Ⓑ denotes the interconnection between current Martian mission objects such as the InSight[144] lander (symbol: △), Curiosity[145] rover (symbol: ▽), and ExoMars1[146], MAVEN[147], MRO[148], Mars Express[149], Odyssey[150] orbiters (symbol: ◯) to the Earth-based development of process elements for a biomanufactory (Figs.3-6). While diverse in objectives, these Martian missions have rendered invaluable data aiding in the design specification of the biomanufactory, mainly in the presence of regolith and atmospheric resources which scope the biotechnology platform in terms of ISRU. Together with the en route autonomous surface missions of Ingenuity[151] and Perseverance[152] (Fig. 7Ⓒ), these missions provide a roadmap for continued mission development in terms of landing location based on biosignatures[153,154]. The breakdown of biomanufactory elements (Figs.3-6) show that biotechnology will require ample water in media (symbol: $\{M\}$), atmospheric gas feedstocks, and power which can be bounded by measurements from autonomous missions. Unlike previous surface payloads that were limited to on-planet analysis, the upcoming rover missions offer an opportunity for data analysis and sample return, promising to shape the design scope of ISRU processes such as regolith decontamination from perchlorate and nitrogen enrichment for crop growth. In conjunction to these active and en route missions, additional orbiters (NeMO[155]) and lander/rover pairs (ExoMars2) (Fig. 7Ⓗ) have been planned and will aid in the selection of a landing site for short term martian exploration missions (Fig. 7Ⓙ,Ⓚ). Such locations will be determined based on water/ice mining/availability with planned mission operations like Icebreaker/MELOS[156] in (Fig. 7Ⓘ). Upcoming rover missions offer additional opportunities beyond measurements of climate and geology; more importantly, these missions can be deployed with specific payloads for experimental validation of biomanufactory elements. Low TRL biotechnologies can be flown as experimental packages in upcoming rovers and landers, offering the possibility for TRL advancement of specific biology-driven subsystems. Planning for such specific experimental packages are likely to require coordination with and continuation from ISS and CubeSat payloads (Fig. 7Ⓔ). For example, biologically-driven ISRU processes (Fig. 5) for C-and-N-fixation will need to be evaluated for specific efficiency values from which downstream technology can be scaled in terms of reactor size and quantity. These Mars-based experiments can be compared against their ISS and Earth-based analogues for understanding the impact of Martian gravity on efficiency.

Crewed Artemis and Gateway Operations

The upcoming Lunar exploration missions, Artemis[157] and Gateway[158], provide additional opportunities for integration with Earth-based development of a biomanufactory. The unmanned support (symbol: X) Artemis I mission (Fig. 7Ⓓ) of ∼25 days in 2021 will serve as the beginning of support development, performance testing, and communication systems setup for crewed (symbol: ⋆) missions to Luna beginning with the ∼10 day flight test of Artemis II in 2023 and the ∼30 day return to the lunar surface in Artemis III in 2024 (Fig. 7Ⓕ)[157,159,160]. The early support lunar exploration missions will provide valuable experience in predeployment of cargo for downstream crewed operations and is likely to help shape logistics development of both the short term Martian exploration missions (Fig. 7Ⓙ,Ⓚ) and the longer term missions (Fig. 7Ⓜ) where we propose to deploy the martian surface biomanufactory. Both the initial deployment of these support packages and downstream transit of crew in the early Artemis program will also provide opportunities for development of the space launch systems, crew capsule, and next-generation spacesuits, feeding design constraints and mission-context testing opportunities prior to the first crewed Martian mission. These initial Artemis missions will also provide the foundational Power & Propulsion Element (PPE)[161] and Habitation & Logistics Outpost (HALO) modules for the establishment of the Gateway system for downstream crewed transportation infrastructure to Mars and continued in Artemis IV-IX through 2030. The specifics of these elements will shape the biomanufactory in terms of habitat design and construction, and propellant selection. Since RMA-L1 ECLSS will be integrated with the biomanufactory, the envelope of these elements will provide insight into the demands that must be met with biotechnology. As of now, no planned lunar surface missions after 2030 have been made public by NASA, but are likely to follow in concurrent operation to Martian missions (Fig. 7Ⓛ). Since Luna has a different environmental inventory compared to Mars, the ISRU technologies will be sufficiently distinct. However, since both mission sets will be crewed, the Artemis missions provide a testing ground for bioprocess infrastructure and operations testing. Factors such as microgravity on the Artemis mission can be treated as an opportunity to evaluate biotechnology for transit from Earth to Mars, its construction and operation, and the recycling and use of some of the common waste streams predicted to be the same on the Moon and Mars. The use of later Artemis missions also provide a mission-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 and without sufficient increase in a loss of mission or crew.

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, but ensuring that native and/or engineered traits remain robust over time is critical to avoiding the resource penalties 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

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their 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 the *in situ* challenges astronauts will face when attempting to reset them. Quantifying these uncertainties during crewed and autonomous Artemis missions will help to inform the optimization of tradeoffs when designing a bio-enhanced life support system for Martian surface operations.

Initial Human Exploration of Mars

Crewed surface operations of ∼30 sols by four to six astronauts are projected by the current DRA[9] to begin in 2031 (Fig. 7Ⓙ) with an additional mission similar in profile in 2033 (Fig. 7Ⓚ). Given the short duration, a mission-critical biomanufactory as described is unlikely to be deployed given both the the inherent risk of the low TRL technology components and the lack of value that can be offered by biology in a short timeframe at high cost of reactor hardware. However, the short-term, crewed missions RMA-S1 and RMA-S2 provide an opportunities for increasing the TRL of biomanufactory elements to the requisite levels required for the following ∼500 sol surface missions RMA-L1 (Fig. 7Ⓜ) in 2040 and RMA-L2 (Fig. 7Ⓝ) in 2044. Such opportunities lie with elements being testing during RMA-S1 and RMA-S2 operations and additional collection of data for further Earth-based development and scoping of technologies. Building on the abiotic ISRU from early Artemis missions and the payload packages from NeMO and ExoMars2, we propose that RMA-S1 carry experimental systems for C-and-N-fixation processes such that a realized biomanufactory element can be properly scaled (Fig. 5). Since RMA-S1 and RMA-S2 will be crewed, regolith process testing becomes more feasible to be tested onsite on the surface of Mars, than during the complex sample return missions. 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 would be the growth of crops under various conditions (Fig. 3A) to validate that a plant microbiome can provide a prolonged benefit in enclosed systems, and to determine the resiliency in the event of pathogen invasion/loss of microbiome function due to evolution. Additionally, TRL for crop systems would need to be evaluated in the case of knowledge gaps from microgravity. For example, plant hydraulics may be affected by gravity but have not yet been studied extensively due to the to-date difficulties in generating an altered gravity field (e.g. like that of a Martian environment)[162].

While conducting these experiments, the RMA-S1 and RMA-S2 crews will be exposed for the first time to surface conditions after interplanetary travel, allowing for initial assessment of the resulting health effects and comparing them to the operation on the lunar surface (Fig. 7Ⓕ), identifying the realistic design constraints for pharmaceutical and functional food needs and the corresponding biomanufactory element requirements (Fig. 3B,C). The RMA-S1 and RMA-S2 mission ISRU and FPS experiments will also provide insight into the inventory requirements for downstream scaling of the biomanufactory inventory in RMA-L1 and RMA-L2. With a projected inventory for these missions, ISM technologies such as bioplastic synthesis and additive manufacture (Fig. 4) can be evaluated for sufficient TRL. Furthermore, performance of loop closure strategy, yielding a range of desired products can also be tested. This will be quite useful in properly estimating the impact of changes in waste stream characteristics owing to the different prevailing conditions on recycling. Even though production of biogas through anaerobic treatment of waste, has a TRL of 7-8[163], the TRL for the process with multiple products, in Martian gravity is still an open question.

On Moving Forward

In this perspective, we have outlined the design and deployment of a biomanufactory for surface operations during a 500 day human exploration mission on Mars. Our extension of previous cases for exploiting stand-alone elements of biology in an integrated biomanufacturing system demonstrate the importance of bringing together the systems of resource utilization, production, and recycling, of food, pharmaceuticals, and biomaterials to sustain the astronauts of the future. Additionally, we outlined the envelope of future design, testing, and deployment of the biomanufactory in the context of a roadmap that spans Earth-based system development, testing on the ISS, integration with Lunar missions, and initial construction during shorter-term human exploration of Mars. As expected, the path towards this biomanufactory will be replete with challenges in technology development, scientific scope, sociopolticial impact, and ethical considerations. But that is part of the excitement, part-and-parcel, of the journey to Mars.

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Competing Interests

The authors declare that there is no conflict of interest.