A research-campaign whitepaper for consideration by the National Academies of Sciences, Engineering and Medicine (NASEM) in the development of NASA's Decadal Survey on Biological and Physical Sciences Research in Space 2023-2032

# What to Take? When to Make? How to Break Even?

## Avoid Mistakes in Microbial Biomanufacturing in Support of Human Near-to-Deep-Space Exploration

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**Executive Summary**

In this whitepaper, we call for the concept of biomanufacturing to be expanded and widely adopted as a support function of human Space-travel. To demonstrate the impact and value of this strategy, we identify the specific offworld scenarios where the concept is most applicable, as well as the vital inventories that can be made available thereby. This will serve to increase capabilities of human operations beyond Earth-orbit and allow for extended mission design through greater autonomy while minimizing risks through redundancy. To this end, we sketch the potential routes and systems to arrive at these goals, in the form of specialized microbial cell factories that can most meaningfully leverage the resources available along the journey. The strategic vision presented here relies heavily on Synthetic Biology as it integrates with major plans for *In Situ* Resource Utilization and highlights applications that engineered biology is uniquely suited to address. We finish by advocating for the research and development investments that need to be made in order to significantly increase readiness of these technologies over the coming decade. This dovetails with current efforts to return humans to the Moon with Mars on the horizon. Besides ensuring the feasibility and sustainability of crewed Space exploration and habitation, the advancement of these technologies may spawn a new scalable microgravity-based biotechnology industry that contributes to the creation of a circular economy on Earth.

RESEARCH CAMPAIGN
ESTIMATED COST: ~$100,000,000
PROJECT DURATION: 10 years

# Introduction

The downlink of imagery from the Perseverance Rover has captivated a world still gripped by quarantine and provided an opportunity for an imagination to sojourn across national borders—beyond Earth—to new worlds. With reinvigorated curiosity and eager for future Space missions of increasing technical complexity, we prepare for the return of human footprints on the Moon[5] and aspire onward to the next stage of Space exploration with transits[6] to and habitation[7] on Mars. This requires new technological paradigms to both enable such a grand vision and decrease the cost and risk of these missions[8]. Efforts to modernize mission architectures[9]—combinations of inter-linked system elements that together realize mission goals[10]—will need to leverage an array of enabling technologies including biotechnology.

Microbial biomanufacturing has the potential to provide integrated solutions for remote, austere, or compromised locations, especially in scenarios where supply chains for consumable and durable goods do not or cannot operate. Crewed deep-Space exploration missions are the ultimate synthesis of these constraints. When integrated effectively into mission architectures, sophisticated biotechnologies will

| Scenario | | Abiotic | Biological |
|---|---|---|---|
| LEO[1] | advantage | in certain cases manufacturing in microgravity may yield a premium product | bioprinting without structural stabilization and scaffold-free tissue engineering |
| | | re-use/-purposing of infrastructure on-orbit for strategic reduction of up-mass | more extensive recycling allows for tighter loop-closure |
| | drawback | no *in situ* resource utilization possible | no *in situ* resource utilization possible |
| | | in many cases ability to re-supply outweighs infrastructure-investment | in many cases ability to re-supply outweighs infrastructure-investment |
| | | microgravity makes certain processes more difficult | microgravity makes aqueous processes challenging |
| Lunar[2] | advantage | strategic outpost for infrastructure as stepping stone to the solar system | allows resources to be exploit that are not accessible otherwise |
| | | gravity, may allow certain processes to be adopted more readily | gravity, allows gas/liquid separation → operation of aqueous processes |
| | | may in some cases save mass/cost of re-supply | may in some cases expand mission capabilities |
| | drawback | limited portfolio and amount/density of available resources | resources are limited and processes are largely dependent on abiotic ISRU |
| | | ability to re-supply and delivery may often outweigh infrastructure investment | ability to re-supply and delivery may outweigh infrastructure investment |
| Interplanetary[3] | advantage | re-supply not feasible → recycling and loop-closure compulsory | increased redundancy through flexibility: can allow *ad hoc* solution of complex incidental problems |
| | | systems proven in LEO are readily transferable/adaptable | may allow more complete loop-closure and recycling |
| | drawback | no *in situ* resources available (only recycling/production from stock) | no *in situ* resources available (only recycling/production from stock) |
| | | microgravity makes certain processes more difficult | microgravity makes aqueous processes challenging |
| Martian[4] | advantage | no supply-chain, just-in-time response not feasible → ISRU, LC and ISM compulsory | especially suited to leverage the available *in situ* resources to expand capabilities |
| | | | allows resources to be exploit that are not accessible otherwise |
| | | gravity, may allow certain processes to be adopted more readily | gravity, allows gas/liquid separation → operation of aqueous processes |
| | drawback | high infrastructure investment | high maintenance |
| | | not as resilient | more susceptive to drift |

**Table 1:** Comparison of biotic vs. abiotic *in situ* (bio)manufacturing approaches across different Space-travel scenarios and destinations, including Low Earth Orbit (LEO), Luna, Mars, and Interplanetary Space (excluding cislunar, which is similar to LEO / Luna).

significantly de-risk crewed missions allowing for increased autonomy, modularity, and sustainability[11]. This ties in tightly with the concepts of *in situ* resource utilization (ISRU) and loop-closure (LC) – savings in up-mass can allow for more complex mission architecture with more extensive goals[12, 13]. Progressive advancement and wider implementation of *in situ* (bio)manufacturing (ISM / bio-ISM) as missions expand will lead to greater independence, at some point conceivably enabling human footholds across the solar system to become largely self-sufficient. The biotechnology paradigm is appropriate for that purpose, as fixed carbon, nitrogen and other resources (e.g., water, oxygen, phosphorus, sulfur) can be produced, recovered, and/or recycled in compact autonomous systems that are analogous to nature's biogeochemical cycles[14–17].

Microbial metabolic pathways give rise to a multitude of compounds, many of the same that are available through petrochemistry (which is not available in Space), as well as a spectrum of bio-based alternatives. In addition, many cases exist where biology is the only option to obtain a critical compound, often at unrivaled purity and selectivity[18, 19].

The paradigm of implementing biomanufacturing meaningfully entails enhanced sustainability, contingency and increased capability of deep-Space exploration. While the advantages of biotic over abiotic approaches and vice versa as life-support functions for long-term Space exploration have been identified and discussed at length[13, 20–23], additional attention is required to formulate an actionable roadmap for deploying biomanufacturing-based systems within upcoming exploration campaigns. Further, it should be clarified that biomanufacturing is distinguished from purely remediative and extractive functions such as biomining[24] – rather offworld biomanufacturing corresponds to any deployable system that leverages biology as the primary driver in producing mission-critical items. This can include the product of one-off materials for downstream processing such as plastics or the biotransformation of waste materials to usable feedstocks for other processes. To focus and prioritize technology development, Table 1 highlights when ISM is most applicable to specific Space-travel scenarios. Table 1 demonstrates that the advantages and drawbacks of offworld biomanufacturing are driven by the mission environment and the resulting concept-of-operations (CONOPS). ISM, specifically through biological technologies, in support of Space exploration is most impactful the deeper humans venture into Space, where supply chains cannot reach in time. Feasibility is highest where fundamental resources exist that allow for ISRU, which is to some degree coincidental with increased distance from Earth (destinations Luna to Mars).

## Biomanufacturing CONOPS

As shown in Table 1, the primary factors predicted to govern the success of biomanufacturing are the availability of *in situ* resources and the availability of logistic resupply of cargo in the form of feedstocks. These factors can be used to qualitatively discriminate cases of mission CONOPS as shown in Figure 1A. While each such mission case will be equipped with specialized inventory elements, a generalization of available resources further aids in comparing mission profiles; as shown in Figure 1B, such elements may include solar cells, tools, reactors, control hardware, experimental scientific apparatuses, and habitat assemblies which will be transported as either pre-deployment cargo or with the crew. These elements will be used to assemble the larger integrated habitation and biomanufactory based agricultural or ISRU systems. The CONOPS of each cases will conform to specific inventory needs as they relate to astronaut requirements and the environmental context informs the specification of feedstocks used for the manufacturing of such inventory elements as shown in Figure 1C.

Case 1 considers a biomanufacturing system deployed on Luna in which the high availability of logistic resupply would take the form of an uninterrupted and stable communication and successful deployment of scheduled cargo and crew from Earth. Due to the dearth of *in situ* carbon and nitrogen resources on Luna[25], the scale of any biomanufacturing-driven product will be constrained by the supply-chain and the agency in recycling these elements at each phase in their life-cycle[1] as outlined by the purple lines in Figure 1C. In the area of food for example, extant technologies used to produce nutritionally complete foods for LEO will provide the supermajority of calories. However, the lack of reliance on *in situ* food production allows for testing and development of the systems required for cases 2-3. Systems that have achieved TRL 5 and beyond are well suited to be implemented and evaluated in case 1. Such technologies include: automated end-to-end lithoautotrophic and fungal fermentation of macronutrients and optimized photobioreactors for photoautotrophic fermentation of micronutrients. This is transferable for therapeutics and materials. While these systems currently exist in isolation or partially integrated in laboratory and industrial contexts, building automated end-to-end, compact systems will be a key requirement for case 1 food production. The raw materials will derive nearly entirely from cargo.

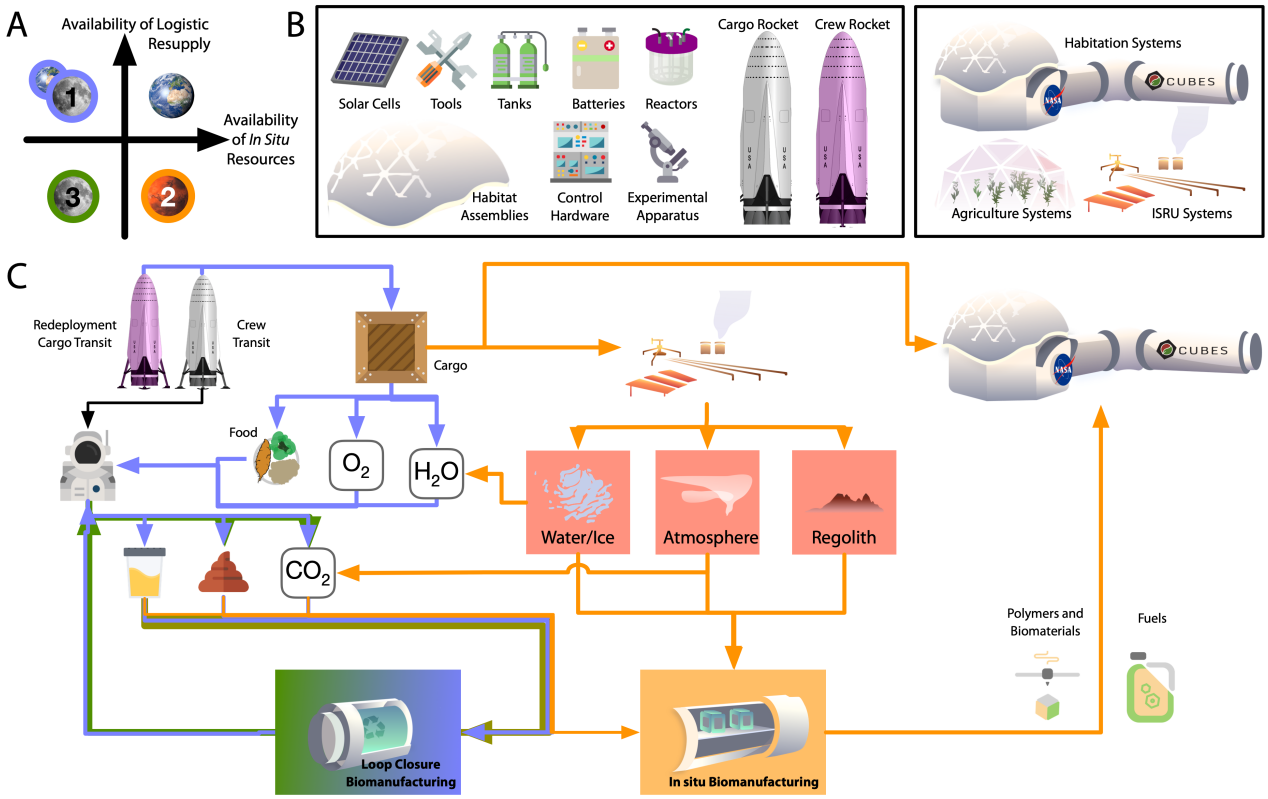


**Figure 1:** **(A)** Categorization of context-specific biomanufacturing mission cases mapped on a qualitative spectrum for the availability of *in situ* resources and the availability of logistic resupply. Cases are colored in correspondence with CONOPS outline. **(B)** Left box corresponds to a generalization of cargo elements common across mission profiles. Right box corresponds to a generalization of assembled habitation and biomanufacturing systems. **(C)** Outline of biomanufacturing CONOPS with arc colors corresponding to mission cases from (A). Cases either rely on LC-driven biomanufacturing (case 1 circuit colored purple; case 3 circuit colored green) or ISRU-driven biomanufacturing (case 2 circuit colored orange). *In situ* resources are highlighted in red.

Case 2 considers a biomanufacturing system deployed on Mars in which poor logistic resupply is governed by an increased interplanetary distance but with much higher availability of *in situ* resources. Meaningful ISM in this context will require significantly scaled up versions of the systems brought to TRL 8-9 in case 1. Here, while a portion of the food, therapeutics or materials requirements will be brought in cargo as in case 1, *in situ* utilization of water, atmosphere, and regolith must be implemented to increase mission flexibility and resilience. Equipment automation becomes the backdrop of an increasingly diverse set of inventory items. For food, nutritional completeness, palatability together with customization of texture, flavor, and format will be of central importance. Such technologies include: scaled up and modular fermentation and bioprocesses, optimized genetically engineered microbial strains to efficiently produce intermediates (e.g., ingredients), and formulation systems to assemble the final products (e.g., the meals).

Case 3 considers a biomanufacturing system deployed on Luna where logistic resupply has been interrupted in the course of planned mission operations. Here, sustained operation must rely on LC-driven biomanufacturing. In this scenario, food production is used as a stopgap measure to extend the food supply until the next resupply. Given the resource paucity of Luna, the raw materials will be sourced from organic waste, derivatization of cargo materials, and textitin situ water. Technologies include: semi-integrated scaled up lithoautotrophic fermentation for caloric and nutritionally complete but simple food formats and optimization of microbial cultivation in minimal media based on white, grey and black water.

# Biomanufacturing Approaches

## Rational Coupling of Biological Systems and Resources

The range of microbial taxa being proposed and investigated for Space-applications is still narrow and often limited to the few model organisms (e.g., *E. coli*, *S. cerevisiae*) whose rise to popularity in Earth-based applications is mostly rooted in legacy. Past, present and future microbial cell factories (incl. synthetic analogs like protocells and cell-free systems) should be identified and future targets recommended, basing their selection on application- (feedstock/product pairing, scale, continuity, and responsiveness of

the respective process) and scenario-specific criteria (environmental parameters) as has recently been put forward[26]. Likewise, there needs to be a shift in paradigm regarding the feedstocks - sugars or other purified multi-carbon compounds (e.g., oils and fatty acids) will likely not be the prime substrates of biotechnology in Space, but rather the products/intermediates (e.g., $CO_2$ and derivable single-carbon compounds, crude or waste biomass) in a manufacturing chain or loop that serves life-support (LC elements such as regeneration of oxygen and waste reclamation). Critical consideration for recycling of resources at the elemental level (i.e., a stochastic accounting of chemicals) should guide selection and utilization of the applicable feedstocks. Any dead-end, non-recyclable resource will eventually require a resupply from Earth. For auxiliary functions (e.g., materials for additive manufacturing), production volume is more decisive than continuity and response time (as is critical in case of food and therapeutics), therefore requiring the adaptation of widely available resources, either directly (where available), or through (physico)chemical means (e.g., as secondary beneficiary of propellant production from *in situ* resources). Figure 2 is a breakdown of possible production routes/flow of resources in case of bio-ISM for inventory items outgoing from scenario-dependent *in situ* resources.

## Specific Areas of Research and Development

Food—because of its significant payload requirement and lack of robust abiotic modes of production—is the most immediate and essential application of biotechnology in Space. Current gaps in end-to-end biomanufacturing, particularly in the areas of automation, nutritional completeness, and palatability, mean that early near- and deep-Space missions will rely on pre-packaged calorically dense food similar to what is currently program for LEO[22]. Plants, likely grown in artificially-illuminated hydro-, aeroponics, vertical farms, lose many of their advantages (e.g., low technical barrier to entry and high TRL across different modes of production, processing, and formulation) when translated to more resource-constrained environments such as Luna or Mars. Therefore, extensive focus needs to be given to developing microbially-derived foods. Fermentation technologies have shorter boot-up and production timelines (days to weeks), smaller footprint, are more resource efficient, have higher proportion of edible biomass, higher diversity of flavor, texture, and nutrition than plants / crops. Further, there are substantial and meaningful knock-on effects to incentivising innovation of bio-ISM. The area of food production would likely see the most near-term advantages as an increasing number of food tech companies seek to produce food more efficiently, sustainably, and deliciously than current legacy methods. Improving cell culture, bioprocess, and food formulation technologies, would support the growth and proliferation of emerging commercial efforts to make food people want to eat in a scalable and sustainable way.

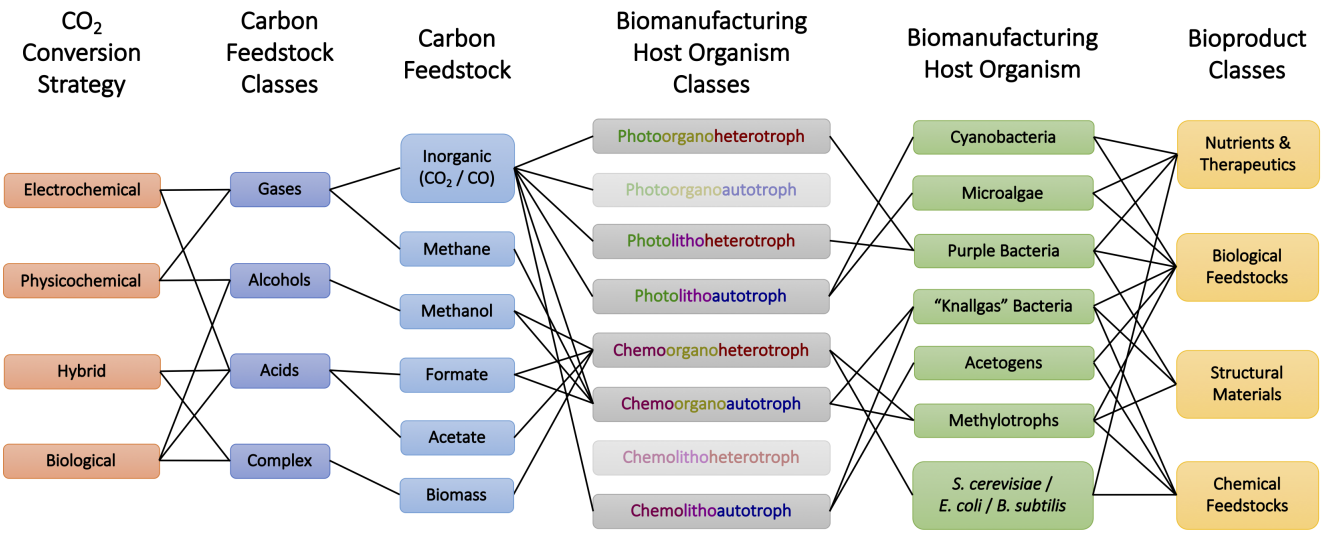


**Figure 2:** Breakdown of different routes for bioproduction of inventory elements from carbon dioxide, either as *in situ* or recovered resource. Connecting arcs represent possible paths for carbon compound conversion of intermediates to products through different means of conversion. Usability of different feedstocks is tied to nutritional mode of the microbial host organism (more than one nutritional mode is possible for certain organisms). Classes of products were assigned to the respective microbes in respect to the nutritional mode and other characteristics of the chassis (e.g., aerobic/anaerobic, prokaryotic/eukaryotic, rate and robustness), rather than ability to (naturally) derive the respective compounds (technically metabolic engineering allows any compound to be produced in any organism, with very few exceptions, e.g., in case of glycosylated proteins). Products (biological feedstocks) may or may not comprise some of the initial feedstocks, hence a staged bioprocess, i.e., two consecutive rounds of microbial conversion to upcycle carbon is conceivable.

Safeguarding the physical and mental health of the crew demands a plan for long-term therapeutic self-sufficiency, with *in situ* production playing an increasing role as mission scenarios advance. Biomanufacturing is a promising candidate for a subset of medicines with short shelf-lives and inconsistent demand, in particular those that prevent or counteract osteopenia, antimicrobials and biologics for radioprotection[22]. An implication of establishing point-source on-demand manufacturing in Space in all three areas (food, pharmaceuticals, materials), but most significant in case of pharmaceuticals production, is that it can be translated back to Earth for use in remote, resource-limited areas.

Biomanufacturing has the potential to generate consumable and durable goods made of plastics, metals, and ceramics as mission objects, with uses and sizing ranging from small replacement parts and functional tools to physical components of the life-supporting habitat. Although, in case of ground operations, manufacturing with soil and rock as components of buildings and structures will likely be large by volume, regolith is not very suited for most other applications, given its limited flexibility and plasticity. In combination with additive manufacturing, bioplastics will make up the majority of high-turnover items with regular demand, while also accounting for contingencies. Such polymeric constructs can be derived from basic feedstocks in a more compact and integrated way than chemical synthesis, in as short as a one-step bioprocess[13, 22]. Further, especially high-performance polymers have a range of applications in Space Technology, including for example ballistic protection. Analogous polymers or the chemical feedstocks thereof can be obtained through biomanufacturing[27, 28]. Technologies for production of biomaterials, in particular bioplastics, from *in situ* resources like carbon dioxide are immediately relevant to providing solutions for the most pressing challenges on Earth, which are mitigation of greenhouse gas emissions through carbon capture and neutrality, as well as reduction of environmental pollution with non-biodegradable materials. Biomaterials production from inorganic carbon is therefore an enabling technology for the evolution of a circular economy and sustainable (bio)chemical industry on Earth.

### Technologies Needed in Support of Specific Research Areas

Much of the technology development described above relies heavily on Synthetic Biology, which will require an iterative approach to optimize microbial cell factories for a desired function (design-build-test-learn cycle). Therefore, the time-intensive and high-cost of genetic engineering and 'omics studies, as well as their vetting in a real offworld environment must be taken into account for timelines. Further, to conduct the studies that would advance the technological readiness of biomanufacturing, it is essential that the next Decadal Survey supports hardware development. Specifically, standardized, versatile, and scalable bioreactor systems capable of providing the environmental conditions for handling and cultivation of microbes in different offworld scenarios are required, combined with autonomous data acquisition for process and hardware performance characterization.

## Campaign Roadmap

To verify, open, and update campaign specifications and evolve the technological readiness of areas described above requires scientists and engineers from various areas spanning biology, chemistry, and physics to work together to advance microbial cell factories and build cross-compatible and scalable processing systems within the confines and stressors of Space. Biomolecular, bioprocess, and biosystems engineering must be integrated with pre-processing as well as downstream processing of resources and products, respectively, and tied in with mission-support infrastructure and logistics. Coordination mission specialists are critical to deploy tests into Space under different constraints (scenarios) and build long-term partnerships and understanding between the public and private sector. We argue that such groundwork requires long-lived multidisciplinary centers that are secure from volatility of markets and swings of political agendas to perform the large-scale, long-term science necessary to succeed. Thus, our proposed campaign roadmap will be based on expanding the mandate and resources of NASA's Space Technology Research Institute (STRI) Program and the installment of a dedicated Innovation Hub for the coming decade. A congruent argument was made in the associated whitepaper "Space Bioprocess Engineering on the Horizon" and funding considerations are analogous. Such a center would conduct fundamental technology development and advance TRLs in partnership with industry. We further propose such a center to be attached to a dedicated Space-based R&D Hub (the "Field Station" for the Innovation Hub). Service providers would dedicate and manage resources both on ISS (near term) and next-generation Space station(s) (second half of decade). This would be the pipeline to ensure testing, prototyping, and maturation of technologies in Space with assigned, predictable launches, hardware and support.

## Appendix - Supporting Information on Specific Research Needs

The study of Space biomanufacturing is still limited to small-scale microgravity experiments[3, 29, 30] (e.g., BioRock[31] or Rhodium Inflight Biomanufacturing[32]). To found these technologies as viable manufacturing platforms in Space, advancement of biological technologies, particularly in respect to scaling and adaptation of Bioprocess Engineering and Synthetic Biology to the relevant (offworld) environments (e.g., LEO, Mars, Luna, etc.) will require extensive R&D[13]. To this end, there is a need for advancement of eLife/digital life modeling and simulation to better predict manufacturing outcomes (e.g., performance drift of microbial models). Computer-aided modeling, albeit an essential tool to investigate potential without risk (e.g., algae-based biofuel production on Mars[33], autonomous biomining on the Moon[34] using mass balances and growth kinetics, fluid dynamics of bioreactor operations in different planetary gravities[35]), will, however, never be able to fully de-risk a technology before deployment. Further, most modeling studies and analog experiments fail or are unable to consider in an integrated way impacts of non-Terran environmental constraints, such as the effects of combined (micro- or partial-) gravity and cosmic radiation (both the high-energy but low-flux background of cosmic galactic rays, as well as the greater concern presented by very high-fluence bursts of solar energetic particles accelerated by a coronal mass ejection). Space-analog R&D efforts should therefore account for combined impacts when considering mechanisms of microbial biomanufacturing, in dry-, as well as wet-lab. This serves to establish a profound and reliable understanding of cell/culture stability, viability, and identity in Space, answering the question how (engineered) microbes behave (different) in the Space environment. To this end improved automated real-time microbial 'omics and product monitoring with smart operational and product quality control decision making and implementation (e.g., data-backed targeted cell sorting, genetic and epigenetic editing, environmental conditions adjustments, etc.) is applicable[36].

Advances in cell-based biorefinery monitoring should complement and may be developed from process analytics tools and instrumentation for Earth-based (semi)automated continuous at-line and in-process production quality control. Such quality-by-design and process-validation approaches may enlist various scalable line production (e.g., microfluidic devices, magnetic traps, centrifuges, etc.), sensor (e.g., precision

| | Food | Pharmaceuticals | Materials |
|---|---|---|---|
| TRL 8-9 | pre-packaged food and supplements | pre-packaged pharmaceuticals and medication | pre-assembled structures, durable goods and consumables |
| TRL 6-7 | hydro-, aeroponics, vertical farming; fungal fermentation for protein, engineered heterotrophs and autotrophs for fats, carbs, protein, flavors, fiber and micronutrients | scale continuous production to meet on-demand and/or stockpiled biomedical resource needs | on-orbit additive manufacturing of small replacement parts from cargo-materials |
| TRL 4-5 | integrated and automated bioprocesses, gas and organic carbon fermentation scale up with mission constraints; nutritionally complete microbe-based food production and formulation | integrated interoperable process analysis tools and instrumentation for formulation design and production; validation of end-to-end automated identity, purity, and formulation screenings | integrated and automated bioprocesses, gas and organic carbon fermentation scale up with mission constraints; additive manufacturing demonstration with natural biopolyesters |
| TRL 1-3 | engineered microbes to produce several ingredients simultaneously, autotroph-heterotroph co-culture, end-to-end cell-to-food production system, *in situ* resource purification technologies | engineered microbes for desired biomedical products (e.g., therapeutic proteins) for quality-by-design and process-validation manufacturing; end-point biomedical products conform to completed TRL9 for pharmaceutical design to market; production system development and low-resolution quality control | engineered microbes to precision produce novel, high-performance materials and feedstocks not otherwise accessible from regular feedstocks (abiotic or biological) |

**Table 2:** Current technology readiness levels (TRLs) of in-Space food, pharmaceuticals and materials production technologies. Separate sets of TRL can be established for design, study, approval (safety and efficacy), marketing and production technology/process research, development, and implementation. Here, the latter are presented, which is what most biomanufacturing work considers and is consistent with the whitepaper theme.

spectroscopy, molecular probe, electrochemical detection, etc.), and computational (e.g., AI/ML-powered meta-learning inferential virtual screening, analytical modeling and testing, etc.) technologies to help optimize end-to-end identity, purity, formulation, and contamination screenings for small- and macro-molecules. This serves to answer the question of what environmental conditions need to be controlled to optimize bio-processes in Space and what technologies are needed to monitor the efficiency of these.

Biomanufacturing advantages and disadvantages for prioritized manufacturing technologies (e.g., microfluidic bioreactors, chip-based bioreactors, etc.), protocols, stock (food, pharmaceuticals, structural materials, etc.), and production outcomes (e.g., controlled quality, scalable yield, titer, rate, etc.) need to be validated across the different Space/extraterrestrial environments. To increase amenability of these fields and provide appropriate conditions for technology maturation, research support should be meaningfully distributed between ground studies (basic research and analogs), simulation platforms (parabolic flights, suborbital flights), lower-Earth orbit (dedicated satellites, ISS, private space stations) and cislunar Space (Gateway, dedicated satellites), as well as full investigations on the Lunar (Artemis missions) and Martian surface (robotical). More biomanufacturing prototyping with current and emerging biomanufacturing systems and different microbial models can and should be accomplished with Earth-based simulated Space/extraterrestrial environments to accelerate R&D at improved time and cost effectiveness. Not only will that bridge knowledge gaps and provide reduction to practice for Space/extraterrestrial applications, but it may render significant outcomes to improve manufacturing paradigms for Earth production needs.

The effectiveness of in-Space biomanufacturing systems needs to be assessed through systems analysis to prioritize technologies by means of significant criteria. Traditionally, metrics have been developed as a means to quantify specific attributes of a system. One of the more widely used metrics developed for life-support systems, ESM (Equivalent Systems Mass), converts mass, volume, power, cooling, and crew times as a single metric equivalent to the predicted upmass required[37, 38]. While it has become a standard metric for comparing biomanufacturing systems[39, 40], it does not include aspects such as cost, risk, sustainability, recyclability, complexity, modularity, reliability, robustness, resilience, readiness, safety, etc. Other metrics have since been developed to assess these areas, but prioritization of the unique requirements of each design warrant a more holistic systems analysis rather than dependence on any single one. bio-ISM systems will need to be developed to have similar, if not more favorable aspects over traditional systems and resupply in a systems analysis.

The design, study, approval (safety and efficacy), for pharmaceuticals in Space applications is to be assessed separate from their production. The problem is that label and off-label Space application of therapeutics approved for use on Earth may have diminished with possible hazardous therapeutic consequences for Astronauts, regardless of production quality or post-production degradation. This situation results from changes in human physiology and drug action in Space environments. Therefore, clinical trials for already approved drugs may need to be conducted in non-Terran environments. Regardless, current TRLs for pharmaceutical classes (and specific drugs) approved for Earth use are tenuous for Space use without full knowledge for safety and efficacy in Space environments, with major impacts on pharma design, prescription, and production strategies. Even off-label use drugs are studied on Earth to ensure wanted patient outcomes and considerable impact on demand and production quality and quantity targets.

For offworld ISM of materials and structures a multitude of different approaches exists, many of them are still inhibited by the extent of initially required critical infrastructure[41–43]. Nevertheless, autonomous 3D-printing of infrastructure relying on composites with regolith has been proposed and prototyped by companies like Made in Space, however, this still requires significant up-mass of auxiliary equipment to allow for e.g., stripping and processing of topsoil, as well as the raw material for the binding resin. If, however, the binding material (polymeric resins and thermosets such as aramids and arylates) could also be derived or produced on-site from *in situ* resources, an additive manufacturing method may become immediately more feasible. Ultimately Engineered Living Materials"[44] may provide the game-changing solution for the most pressing issues of deep-Space habitation – these would allow the tailored construction of structural and supporting components, possibly in combination with 3D-bioprinting abolishing the need for extensive downstream processing of bioproducts into purified materials.

Finally, the concept of a "Space Biofoundry" (i.e., automated infrastructure for engineering and analytics of biological systems[45]), which may allow microbial cell factories to be constructed at destination as the need arises, based on design-plans transmitted from Earth, should be assessed for feasibility to be accommodated in mission designs.

## References


[1] Marc Giulianotti et al. "Opportunities for Biomanufacturing in Low Earth Orbit: Current Status and Future Directions". In: *Preprints* (2021).

[2] Gary C Smith. "Some Biological Considerations for a Permanent, Manned Lunar Base". In: *The American Biology Teacher* 57.2 (1995), pp. 92–98. ISSN: 0002-7685. DOI: 10.2307/4449930. URL: https://doi.org/10.2307/4449930.

[3] Paul Westgate et al. "Bioprocessing in space". In: *Enzyme and microbial technology* 14.1 (1992), pp. 76–79. ISSN: 0141-0229.

[4] A E Drysdale, M K Ewert, and A J Hanford. "Life support approaches for Mars missions". In: *Advances in Space Research* 31.1 (2003), pp. 51–61. ISSN: 0273-1177.

[5] Marshall Smith et al. "The Artemis Program: An Overview of NASA's Activities to Return Humans to the Moon". In: *2020 IEEE Aerospace Conference*. IEEE, 2020, pp. 1–10. ISBN: 1728127343.

[6] Humphrey W Price et al. "Human missions to Mars orbit, Phobos, and Mars surface using 100-kWe-class solar electric propulsion". In: *AIAA Space 2014 Conference and Exposition*. 2014, p. 4436.

[7] Robert P Mueller et al. *Nasa Centennial Challenge: Three Dimensional (3d) Printed Habitat, Phase 3*. Tech. rep. 2019. URL: https://ntrs.nasa.gov/citations/20190032475.

[8] Christopher L Moore. "Technology development for human exploration of Mars". In: *Acta Astronautica* 67.9-10 (2010), pp. 1170–1175. ISSN: 0094-5765.

[9] Elon Musk. "Making Humans a Multi-Planetary Species". In: *New Space* 5.2 (2017), pp. 46–61. ISSN: 2168-0256.

[10] James Richard Wertz and Wiley J Larson. *Reducing space mission cost*. Microcosm Press Torrance, CA, 1996. ISBN: 1881883051.

[11] Aaron J. Berliner et al. "Space Bioprocess Engineering on the Horizon". In: *In Preperation* (2021).

[12] Robert W Moses and Dennis M Bushnell. *Frontier in-situ resource utilization for enabling sustained human presence on mars*. 2016. URL: https://ntrs.nasa.gov/citations/20160005963.

[13] Aaron J Berliner et al. "Towards a Biomanufactory on Mars". In: *Frontiers in Astronomy and Space Sciences* 8 (2021), p. 120. ISSN: 2296-987X. DOI: 10.3389/fspas.2021.711550. URL: https://www.frontiersin.org/article/10.3389/fspas.2021.711550.

[14] Harald Helisch et al. "Close the gap–Potential of microalgal biomass for closed ECLSS and future in-situ resource utilization in space". In: 49th International Conference on Environmental Systems, 2019.

[15] Ernst Messerschmid and Reinhold Bertrand. "Environmental control and life support system". In: *Space Stations*. Springer, 1999, pp. 109–145.

[16] Alan R Johnson. "Biodiversity requirements for self-sustaining space colonies". In: *Futures* 110 (2019), pp. 24–27. ISSN: 0016-3287.

[17] Christine Escobar and James Nabity. "Past, present, and future of closed human life support ecosystems-a review". In: 47th International Conference on Environmental Systems, 2017.

[18] Sang Yup Lee et al. "A comprehensive metabolic map for production of bio-based chemicals". In: *Nature Catalysis* 2.1 (2019), pp. 18–33. ISSN: 2520-1158. DOI: 10.1038/s41929-018-0212-4. URL: https://doi.org/10.1038/s41929-018-0212-4.

[19] *Agile Biofoundry Metabolic Map*. URL: https://agilebiofoundry.org/metabolic-map-3/.

[20] Amor A Menezes et al. *Towards synthetic biological approaches to resource utilization on space missions*. Vol. 12. 102. The Royal Society, 2015, p. 20140715.

[21] Amor A. Menezes et al. "Grand challenges in space synthetic biology". In: *Journal of The Royal Society Interface* 12.113 (2015), p. 20150803. ISSN: 1742-5689. DOI: 10.1098/rsif.2015.0803. URL: http://rsif.royalsocietypublishing.org/lookup/doi/10.1098/rsif.2015.0803.

[22] Shannon N Nangle et al. "The case for biotech on Mars". In: *Nature Biotechnology* 38.4 (2020), pp. 401–407. ISSN: 1546-1696.

[23] Cyprien Verseux et al. “Sustainable life support on Mars–the potential roles of cyanobacteria”. In: *International Journal of Astrobiology* 15.1 (2016), pp. 65–92. ISSN: 1473-5504.

[24] Yosephine Gumulya, Luis Zea, and Anna H Kaksonen. “In situ resource utilisation: The potential for space biomining”. In: *Minerals Engineering* 176 (2022), p. 107288. ISSN: 0892-6875.

[25] Ian A Crawford. “Lunar resources: A review”. In: *Progress in Physical Geography* 39.2 (2015), pp. 137–167. ISSN: 0309-1333.

[26] Nils Jonathan Helmuth Averesch. “Choice of Microbial System for In-Situ Resource Utilization on Mars”. In: *Frontiers in Astronomy and Space Sciences* 8 (2021), p. 116. ISSN: 2296-987X. DOI: 10.3389/fspas.2021.700370. URL: https://www.frontiersin.org/article/10.3389/fspas.2021.700370.

[27] Nils J H Averesch et al. “Biocatalytic Formation of Novel Polyesters with para-Hydroxyphenyl groups in the Backbone {\textendash} Engineering Cupriavidus necator for production of high-performance materials from CO2 and electricity”. In: *bioRxiv* (2021). DOI: 10.1101/2021.12.12.472320. URL: https://www.biorxiv.org/content/early/2021/12/13/2021.12.12.472320.

[28] Nils J H Averesch and Lynn J Rothschild. “Metabolic engineering of Bacillus subtilis for production of para-aminobenzoic acid – unexpected importance of carbon source is an advantage for space application”. In: *Microbial Biotechnology* 12.4 (2019), pp. 703–714. DOI: https://doi.org/10.1111/1751-7915.13403. URL: https://sfamjournals.onlinelibrary.wiley.com/doi/abs/10.1111/1751-7915.13403.

[29] Paul Todd. “Space Bioprocessing”. In: *Bio/Technology* 3.9 (1985), pp. 786–790. ISSN: 1546-1696. DOI: 10.1038/nbt0985-786. URL: https://doi.org/10.1038/nbt0985-786.

[30] Gerda Horneck, David M Klaus, and Rocco L Mancinelli. “Space Microbiology”. In: *Microbiology and Molecular Biology Reviews* 74.1 (2010), pp. 121–156. DOI: 10.1128/MMBR.00016-09.

[31] Charles S Cockell et al. “Space station biomining experiment demonstrates rare earth element extraction in microgravity and Mars gravity”. In: *Nature communications* 11.1 (2020), pp. 1–11. ISSN: 2041-1723.

[32] *Establishing Biomanufacturing Processes for Human Systems in Remote Environments*. URL: https://www.nasa.gov/mission_pages/station/research/experiments/explorer/Investigation.html?#id=8306.

[33] Nicholas S Kruyer et al. “Designing the bioproduction of Martian rocket propellant via a biotechnology-enabled in situ resource utilization strategy”. In: *Nature Communications* 12.1 (2021), p. 6166. ISSN: 2041-1723. DOI: 10.1038/s41467-021-26393-7. URL: https://doi.org/10.1038/s41467-021-26393-7.

[34] B A E Lehner et al. “End-to-end mission design for microbial ISRU activities as preparation for a moon village”. In: *Acta Astronautica* 162 (2019), pp. 216–226. ISSN: 0094-5765. DOI: https://doi.org/10.1016/j.actaastro.2019.06.001. URL: https://www.sciencedirect.com/science/article/pii/S0094576519300694.

[35] R Volger et al. “Theoretical bioreactor design to perform microbial mining activities on mars”. In: *Acta Astronautica* 170 (2020), pp. 354–364. ISSN: 0094-5765. DOI: https://doi.org/10.1016/j.actaastro.2020.01.036. URL: https://www.sciencedirect.com/science/article/pii/S0094576520300564.

[36] Allison J Lopatkin and James J Collins. “Predictive biology: modelling, understanding and harnessing microbial complexity”. In: *Nature Reviews Microbiology* 18.9 (2020), pp. 507–520. ISSN: 1740-1534. DOI: 10.1038/s41579-020-0372-5. URL: https://doi.org/10.1038/s41579-020-0372-5.

[37] Julie A Levri, David A Vaccari, and Alan E Drysdale. *Theory and application of the equivalent system mass metric*. Tech. rep. 2000.

[38] Julie Levri et al. *Advanced life support equivalent system mass guidelines document*. Tech. rep. 2003. URL: https://ntrs.nasa.gov/citations/20040021355.

[39] Saige Drecksler et al. “Effects of Space Biomanufacturing on Fuel Production Alternatives for Mars Exploration”. In: 2020 International Conference on Environmental Systems, 2020.

[40] Alexander J Benvenuti et al. "Design of Anaerobic Digestion Systems for Closed Loop Space Biomanufacturing". In: *50th International Conference on Environmental Systems (ICES)*. 2020.

[41] Timothy Cichan et al. "Mars base camp: An architecture for sending humans to Mars by 2028". In: *2017 IEEE Aerospace Conference*. IEEE, 2017, pp. 1–18. ISBN: 1509016139.

[42] Timothy Cichan et al. "Mars base camp: An architecture for sending humans to Mars by 2028". In: *2017 IEEE Aerospace Conference*. IEEE, 2017, pp. 1–18. ISBN: 1509016139.

[43] J Gruenwald. "Human outposts on Mars: engineering and scientific lessons learned from history". In: *CEAS Space Journal* 6.2 (2014), pp. 73–77. ISSN: 1868-2510.

[44] Peter Q Nguyen et al. "Engineered Living Materials: Prospects and Challenges for Using Biological Systems to Direct the Assembly of Smart Materials." eng. In: *Advanced materials (Deerfield Beach, Fla.)* 30.19 (May 2018), e1704847. ISSN: 1521-4095 (Electronic). DOI: 10.1002/adma.201704847.

[45] Maciej B Holowko et al. "Building a biofoundry". In: *Synthetic biology (Oxford, England)* 6.1 (2021), ysaa026. ISSN: 2397-7000. DOI: 10.1093/synbio/ysaa026. URL: https://europepmc.org/articles/PMC7998708.