

Topical: Biologically Facilitated Processes Towards Sustainable Space Exploration

A topical white paper 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.

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Abstract

Finding sustainable approaches to achieve independence from terrestrial resources will be pivotal to the success of space exploration in the next decade (2023-2032). The use of microorganisms could help achieve this aim, by performing biological *in situ* resource utilization (bio-ISRU). Here, we propose a series of processes of interest for the acquisition of structural and essential materials, waste recycling, soil formation and energy production, highlighting potential outcomes and recommendations to improve their feasibility in the space environment.

Background

Microorganisms can facilitate a myriad of processes in support of sustainable, long-term human exploration of space. In addition to or complementing mechano/physico/chemical approaches to *in-situ* resource utilization (ISRU), life support systems and similar processes, biologically-facilitated methods must be considered for the next decade of NASA's Biological and Physical Sciences (BPS) Division research and development portfolio (Montague et al., 2012; Nangle et al., 2020; Castelein et al., 2021; Keller et al., 2021). Here, we present a series of potential outcomes from these approaches, which include biomining, bioremediation, biological waste reclamation, soil formation for crops and food production, concrete production and myco-structures, and energy production. The approaches ("potential outcomes") presented here are based on processes and technologies currently implemented on Earth at different readiness levels, while taking into account difficulties and specificities of the space environment. Selecting the most suitable microorganisms and processes for any given space application is non-trivial, as terrestrial technologies may not be directly adaptable to the harsh space environment, and extensive investigations are pivotal to their success (Averesch, 2021). For this reason, we include recommendations (in bold) and guiding questions for consideration by the National Academies of Sciences, Engineering and Medicine (NASEM) Steering Committee in their development of NASA's Decadal Survey on BPS Research in Space 2023-2032.

Crewed missions on the Artemis Program plan to visit Lunar locations south of 79°S and near Permanently Shadowed Regions (PSRs), which have been designated as Category II-L, and allow living organisms to be included on payloads and studied, as per NASA's recent Interim Directive (NID) 8715.128 (nodis3.gsfc.nasa.gov/OPD_docs/NID_8715_128.pdf). This white paper does not address potential negative outcomes of the use of organisms in inhabited areas, such as possible pathogenesis and or biodegradation of materials, as these topics are addressed in other white papers. However, potential mitigation of the above concerns could be achieved by selecting microorganisms with appropriate biosafety levels and devising suitable bioengineering techniques, as well as implementing appropriate containment procedures, when relevant.

The potential outcomes from the biologically facilitated processes described herein can increase the sustainability of human exploration of space, namely the plans to return-to-stay to the Moon under the Artemis Program. Towards the end of the 2023-2032 decade and beyond, these processes may support missions to Mars and may be translatable to Earth, improving biomining-related approaches and bioremediation, for instance. In longer term, the bio-ISRU and BPS processes proposed here could support microbial-based space biotechnologies, in order to enhance the sustainability of extraterrestrial settlements, and in turn benefit terrestrial applications by

leveraging these cutting-edge space advancements, according to the United Nations 2030 agenda for Sustainable Development Goals (SDGs, sdgs.un.org).

Potential Outcomes

The potential outcomes from biologically-supported ISRU (bio-ISRU) and biologically facilitated processes (BFP) include:

1. Biomining of metals, metalloids and minerals. The plan for developing infrastructure on the Moon and in cis-lunar space would require cost-effective supplies (e.g., structural metals, O₂, mineral nutrients, volatiles) obtainable by harnessing *in-situ* natural resources available on celestial bodies (Menezes et al., 2015b, 2015a; Santomartino et al., in review). Biomining, the use of microorganisms to sustainably extract valuable metals from minerals and mine waste (Schippers et al., 2013), could be adapted for space applications (Volger et al., 2020) to recover metals from regolith or waste. Chemolithoautotrophs (e.g., iron and/or sulfur oxidising microorganisms) could be suitable for biomining of sulfide minerals, occurring in a variety of settings on Mars (Franz et al., 2019). Microorganisms of other nutritional preferences (e.g., organotrophs), consortia or bioengineering techniques could be used elsewhere: the Lunar surface is mainly composed of silica-saturated rocks, with a general lower sulfur composition (Anand et al., 2012; Brounce et al., 2020, Cockell and Santomartino, in press). Since the science regarding the ability to perform biomining under Lunar and Martian conditions is scarce (Castelein et al., 2021), **efforts toward advancing our knowledge on microbial growth and bioleaching capacity from extraterrestrial regolith under space conditions, and toward the development of bioengineering and synthetic biology approaches, is compulsory.**

2. Extraction of Rare Earth Elements (REEs), vanadium, silicon and other elements of interest for manufacturing processes. The use of biomining technologies to extract elements of manufacturing interest is particularly promising. For instance, Rare Earth Elements (REEs), essential for electronic device production, tend to occur at low abundances in most areas of the Moon and Mars but some important exceptions have been highlighted (e.g., McLeod and Krekeler, 2017). The Oceanus Procellarum region of the Moon's near-side is particularly enriched in REEs, although higher-resolution mapping is needed. Hydrothermally altered Martian regolith breccia represented by one meteorite is also relatively REE-rich, hinting at the potential for impact craters to host REE resources (Liu et al., 2016; McLeod and Krekeler, 2017). Vanadium, a widespread trace element in Lunar and Martian rocks with several practical applications in metallurgy, catalysis, and batteries, could enhance material resistance under space stressors. The microbial extraction of these elements from basalt on the International Space Station (ISS) has been demonstrated (Santomartino et al., 2020, Cockell et al. 2020, 2021), but **further investigations are required to scale up the system, improve the process, and adapt the technology for other elements of interest (e.g., silicon) for manufacturing processes with biologically-mediated and -based methods.**

3. Waste (crew, crops, consumables and electronics) recovery. A closed-loop, or recycling and reuse of materials, is key for sustainable human exploration of space (Gòdia et al., 2002, Keller et al., 2021, Berliner et al., 2021, Nangle et al., 2021). Microbiologically supported processes can provide viable and efficient methods for recycling human and consumable (e.g., plastics) wastes, to reclaim water, fixed carbon and nitrogen, and inorganic byproducts (e.g., minerals, volatiles

etc.). Raw materials can be recovered from metallic structures and electronic devices (Urbina et al., 2019). Waste from (bio-)manufacturing compartments can be recovered and reused as feedstocks for further applications. All the principles and applications mentioned above could be included in regenerative life support systems (LSS). To this aim, **it is suggested that the development of biologically-based waste recycling methodologies implementable in space LSS should be a key priority.**

4. Bioremediation of habitat air, water and regolith. For future missions to be successful, a focus on water and waste recycling and purification related to Environmental Control and LSS (Berliner et al., 2021) and bioregenerative life support systems (BLSS; Häder, 2020) is necessary. Microbial bioremediation can support the removal of CO₂ from the atmosphere in habitats and supplement the generation of O₂. In addition, the captured CO₂ may subsequently be used also for bio-concrete production, as described later. Heavy metals and toxic compounds could be removed from Lunar and Martian regolith for further applications, e.g., as soil (Gadd, 2010; Brune and Bayer, 2012, Billi et al., 2021). Bioremediation may be supported with Proteobacteria, e.g., *Sphingomonas*, fungi such as *Penicillium* spp. (Baraniecki et al., 2002; Matsumura et al., 2015; Nilgiriwala et al., 2008; Ojuederie et al., 2017, Janssen et al., 2010, Leitão 2009) and bioengineering of other organisms. Current knowledge on the applicability of these processes in space is limited, therefore **better understanding the mechanisms of bioremediation, microbial behavior in the Lunar environment, and the optimization of process conditions is pivotal. The maturation of BLSS and bioremediation technologies is recommended as a top priority.**

5. Soil formation to help enable the cultivation of crops. For successful human settlements on the Moon or Mars, crews will have to grow their own food. Agricultural methods may include soil-based farming, hydroponics, cellular agriculture, insect farming, etc. By creating a sealed environment and closed-loop greenhouse-type infrastructure such as the ISS' Advanced Plant Habitat (APH) model, we can ensure sufficient light, water supply, soil-nutrients, and other necessary parameters for crop growth. As described above, microorganisms can bind and mobilize specific elements from the Lunar and Martian regolith (Lehner et al., 2018; Volger et al., 2020). This, together with the removal of toxic elements and compounds (bioremediation, see outcome 4), in particular for plants, can improve the potential of regolith as a plant growth substrate, which drastically reduces the amount of materials needed for the resupply of plant-based LSS. This circular approach, ultimately, combines key elements of the potential outcomes 1 to 4 while producing the food needed for a human presence and minimizing the amount of material transported from Earth. **Next to the maturation of the BLSS and biomining, we recommend having the combination of those as a core aspect of the future roadmap.**

6. Concrete bioproduction. Considering the highly corrosive and harsh Lunar environment (e.g., high doses of radiation, Lunar dust, extreme temperatures; de Rooij 2010), construction of resistant shielding structures and the material will be necessary. Microbiologically Induced Calcite Precipitation (MICP) is a biogeochemical process whereby microorganisms precipitate calcium carbonate (CaCO₃), a compound mainly found in limestone, which can increase soil strength and stiffness (Mujah et al., 2017). Some microorganisms, such as resistant *Bacillus* spp. (Seifan et al., 2016), can use carbon dioxide available in the atmosphere to form the carbonate, which can serve to produce self-healing or “living” structures partly made up of regolith. In a similar vein, self-healing concrete on Earth can be fed with organic waste materials (Vermeer et al., 2021). Using appropriate extremophiles for MICP may help in the production and maintenance of bioconcrete,

as well as space conditions-resistant structures on Lunar and planetary surfaces. On Earth, MICP is currently leveraged for the production of biocomposite, and biocementation is used as a novel geotechnical engineering approach. **We recommend that MICP and other biogeochemical processes be studied as a potential approach for the bioproduction of concrete for (habitable) structures, roads, launch pads, and other key assets on the Lunar surface.**

7. Myco-architecture. Linked to outcome 6, myco-architecture refers to the production of resistant structural components and surfaces using fungi. NASA is already studying the process for space applications (<https://tinyurl.com/myco-architecture>), based on growing fungal mycelium with insulating, fire retarding, high compression and flexural strength characteristics, in a controlled fashion to form furniture and habitat shells. **Research aiming to use fungal mycelia and other microorganisms for shielding and structure formation, particularly under space conditions, should be supported to develop novel approaches for the production of structures and compartments on the Lunar surface.**

8. Energy Production. Microbial Fuels Cells (MFCs) utilize microbes to generate electricity. A new class of anaerobic bacteria ('electricigens', e.g., *Desulfuromonas*, *Geobacter* etc.) can reduce organic wastes to produce electricity. MFCs could be coupled with *in-situ* flow-through waste remediation systems to harness the reducing power of organic waste to generate electricity, and/or fuel. H₂ can be microbially produced by converting carbohydrates, or through nanoparticle production from Lunar regolith (Barton et al., 2014; Fang et al., 2019; Patel et al., 2021). Certain *Acinetobacter* species have been shown to produce nanoparticles from silicon dioxide molecules (Singh et al., 2008). Biofuel from bio-ISRU (e.g., using cyanobacteria) could also be a possibility for energy production (Keller et al., 2021). **Studies using electricigens, hydrogen and biofuel producing microorganisms from *in situ* resources should be supported to allow for *in situ* production of electricity with low energy input, and more generally for energy production.**

Hardware

To facilitate and optimize the studies that would enable these potential outcomes, **it is essential that the next Decadal Survey supports the development of (i) bioreactors capable of providing the environmental conditions needed for each of these processes, and (ii) live data acquisition systems for process and hardware performance characterization.** Of particular interest from a biological perspective would be the development of automated and live monitoring systems, which would allow the control of biotechnological process parameters (e.g., growth & production rate, titer and yield, pH, pO₂, as well as inputs and outputs) to characterize system performance.

Research Platforms and Funding Considerations

To provide appropriate conditions for technology maturation and open the field to a wider community, **preliminary studies should be supported in laboratories on Earth, simulation platforms (drop towers, clinostats, parabolic flights, suborbital flights, etc.) and in lower-Earth orbit (sortie flights, dedicated satellites, CubeSats, ISS / private space stations), and full investigations in cis-lunar space (Gateway, dedicated satellites) and on the Lunar surface (uncrewed CLPS landers and Artemis crewed missions).** Implementing the space facilities to enlarge the size of space experiments, in terms of sample replicates and tested experimental

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conditions, **should be also considered to improve the impact of the preliminary studies.** Given that these studies will likely heavily rely on synthetic biology, which will require an iterative approach to optimizing organisms to a desired function, **funding should take into account the high cost of genetic engineering and synthetic biology.**

Guiding Questions

Taking an approach based on feasibility, efficiency, and sustainability, the following guiding questions are suggested to be considered for inclusion into NASA's next Decadal Survey on Biological and Physical Research in Space:

Overarching Questions

- How can microbial biotechnology enhance the sustainability of long-term deep-space exploration missions and settlements?
- How can biomining and bio-ISRU mature to sustain BLSS, and *vice versa*?
- How can bio-ISRU and BFPs be conducted while adhering to Planetary Protection guidelines?
- Which bio-ISRU and BFPs technologies will, in the future, benefit Earth and Martian exploration?

Microbial Research

- What microorganisms and cultivation parameters optimize bio-ISRU processes in space?
- What are the detailed mechanisms of microbial bioremediation?
- How do microbes behave in the Lunar, Martian, and more broadly space environments?
- Which synthetic biology/bioengineering strategies should be developed for bio-ISRU?
- Which microorganisms can be easily engineered for bio-ISRU tasks?
- How can we engineer microorganisms to be able to operate under extreme radiation and altered gravity conditions in space?

Technology Development

- What are the infrastructure requirements to implement bio-ISRU processes (in space)?
- What technologies are needed to quantify and assess bio-ISRU processes efficiency?
- How do we collect, extract, or refine the products obtained by bio-ISRU to utilize them?
- Which bio-ISRU and BFP methodologies would be self-sustainable?

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