New Lunar Economy: A Prospectus on Lunar Microbial Survival

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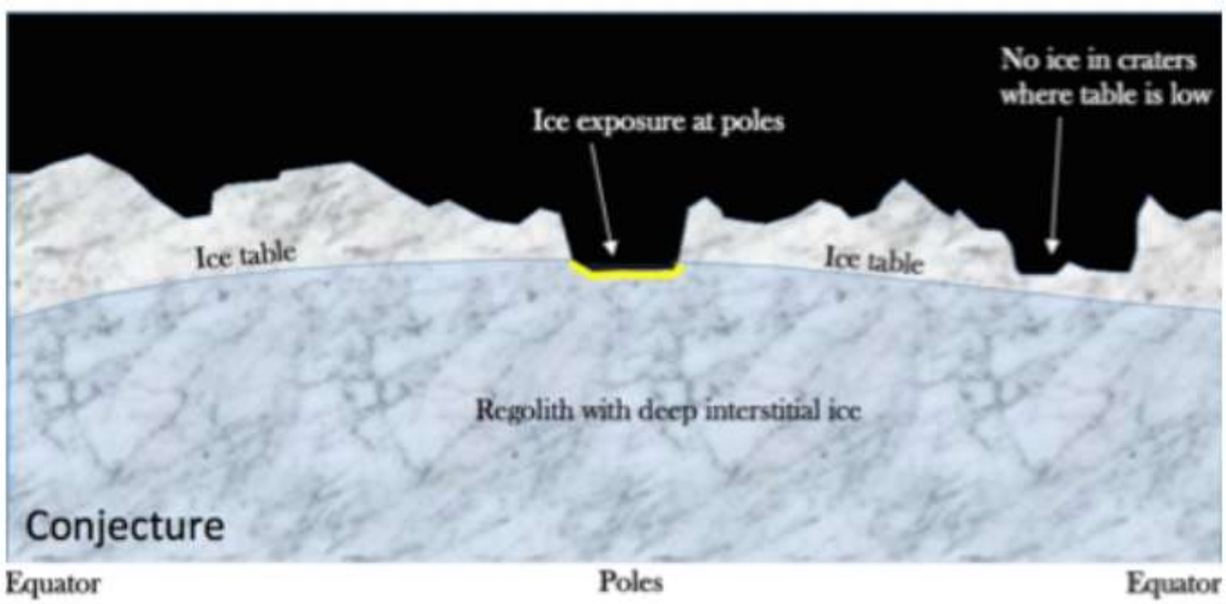


Illustration of a very deep and stable ice layer that has been in place since near the Moon's formation (e.g. Cannon, Deutsch, Head, & Britt, 2020)

Abstract
No definitive evidence of microbials have been found to exist in lunar regolith. Spacecraft-sourced microbes may present a microbial presence on the Moon. Microbial contamination cannot be ruled out. To capture the protective properties of spacecraft hardware, the Lunar Microbial Survival (LMS) model characterizes the bioburden residing on spacecraft and payloads. Auger-facilitated water extraction operations may uncover development of the bioburden expression. More intentionally, however, would be microbial-induced biomining for magnetic extraction of intracellular, soluble iron. This paper proposes further microbial studies related to environmental effects of lunar water harvesting and other lunar mining activities along with their respective remediation.
Keywords: Lunar Microbial Survival, Biomining, Space bioprocess engineering

Background
Lunar samples in sealed containers from the USSR's Luna missions have been examined, photographed from which hundreds of pictures were published in an atlas in 1979 without any notice of biological nature. Further study of the photographs was later undertaken by two biologists at the Russian Academy of Sciences, Stanislav I. Zhmur, Institute of the Lithosphere of Marginal Seas, and Lyudmila M. Gerasimenko, Institute of Biology who noticed that a few of the particles in the photographs were virtually identical to fossils of known biological species. Specifically, some spherical particles from the Luna 20 regolith plainly resembled fossils of modern coccoidal bacteria like *Siderococcus* or *Sulfolobus* in their scale, distribution, form, and the distortion of the spheres that occur during fossilization [O.D. Rode, A.V. Ivanov, M.A. Nazarov, A. Cimbalnikova, K. Jurek and V. Hejl. *Atlas of Photomicrographs of the Surface Structures of Lunar Regolith Particles*, Boston: D. Reidel Publishing Co., 1979].

Introduction

During the Apollo/Viking era of the 1960s and 1970s, prelaunch microbial surveys of robotic and crewed vehicles documented a wide diversity of microorganisms present on spacecraft surfaces [1]. Bioburdens on Moon-bound vehicles were estimated by using traditional culture-based assays yielding between $1x10^4$ and $2 x10^8$ viable mesophilic bacterial species. Recovered microorganisms were composed of 80% nonspore-forming bacteria, 10% spore-forming bacteria, and 10% eukaryotic species vehicle [2]. Based on this literature, several general conclusions include

- 80–95% of all culturable microorganisms recovered from spacecraft were mesophilic heterotrophic species considered indigenous to human, soil, or airborne ecologies.
- Microbial contaminants of spacecraft were generally composed of bacterial and fungal species that closely reflected the environments within which spacecraft are assembled.
- Microbial species recovered from spacecraft [3] were nearly identical to species observed in airborne samples in clean rooms.
- Unusual extremophiles from diverse terrestrial ecosystems, including nonculturable species, recovered from spacecraft [4], but precise estimates of the percentage of nonculturable species present on spacecraft surfaces were lacking.
- During the Apollo era, relaxed sanitation protocols correlated with increased microbial diversity [5].
- Many of the microbial species recovered from spacecraft surfaces were aerobic mesophilic species, although facultative anaerobes were recovered from some vehicles [6].
- The abundance of spore-forming bacteria (predominantly Bacillus spp.) ranged between 1% and 36% of the total culturable heterotrophic species recovered from spacecraft.
- And, the overall average of 12 assayed spacecraft was reported to be 10% of the total diversity for spore-forming Bacillus spp. [7].

Since the Luna 2 spacecraft impacted the Moon on September 14, 1959, 54 missions have either landed or crashed 77 vehicles or major components on the lunar surface [8]. Previous models of microbial survival on the Moon do not consider the permanently shadowed regions (PSRs). These regions shield their interiors from many of the biocidal factors encountered in space flight, such as UV irradiation and high temperatures, and this shielding reduces the rate at which microbial spores become nonviable. The Lunar Microbial Survival Model [9] to the environment found inside two PSRs craters, Shackleton and Faustini reduction rates of of -0.0815 and -0.0683 logs per lunation, respectively, which implies that it would take 30.0 years for Shackleton and 30.8 years for Faustini to accumulate a single Sterility Assurance Level of -12 logs of reduction. The lunar PSRs are therefore one of the least biocidal environments in the solar system and would preserve viable terrestrial microbial contamination for decades [10].

Problem

A recent study reported the possible existence of niches in the South Pole that microbes can survive [11]. Many profound scientific questions depend on the availability of pristine lunar samples: Did organics survive on the Moon and to what extent can they survive today? Is there a pre-biotic inventory sequestered in the ice? If biotic and/or prebiotic molecules survive on the Moon, will these bacteria prove to be harmful to astronauts, human, plant, and animal species or does this imply that life can survive on the Moon? Lunar polar regions may provide an inventory of volatiles and possibly pre-biotic organic materials carrying evidence for the formation/ evolution of life. However, keeping that evidence relatively uncontaminated with future lunar landings will be crucial for any measurable inventory [12].

Purpose

This paper proposes further microbial studies related to environmental effects of lunar water harvesting and other lunar mining activities along with their respective remediation.

Methods and Results

A literary survey of three proposed harvesting/ mining activities on the Moon described in (1) ProSPA-enabled regolith sampling yields of water; (2) microbial-catalyzed intracellular biomass accumulated iron; and (3) activity of auger-facilitated water extraction from excavated icy regolith, were reviewed.

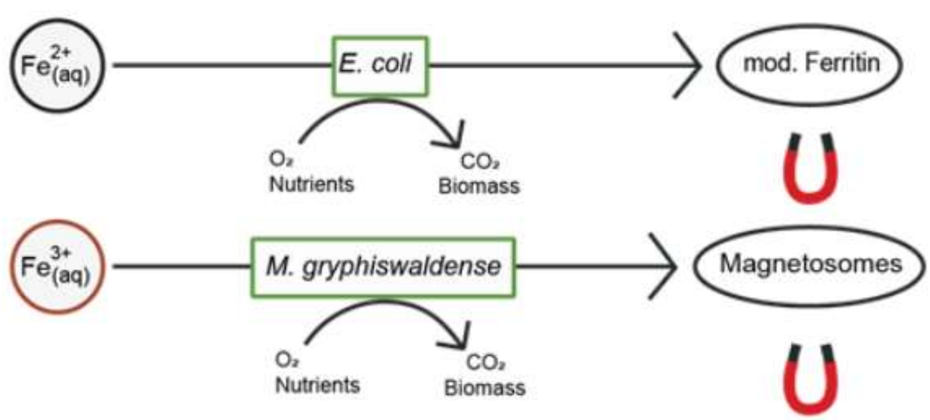
ProSPA-enabled regolith sampling yields of water

Hydrogen reduction of iron oxide-bearing minerals in the lunar regolith, such as ilmenite, has long suggested a potential method for producing water on the Moon to support exploration. Reduction of lunar regolith tested in gas-flowing systems utilized pumps to re-circulate gases. Alternatively, a static system negated the need for a more complex system where gases were continuously pumped away. For example, static PROSPECT Sample Processing and Analysis (ProSPA) instrument measured volatiles in the lunar regolith and optimized a procedure to extract water from ilmenite [13]. The PROSPECT package will be flown on the Luna-27 lander, currently scheduled for launch in 2025, and consists of the PROSPECT Sample Excavation and Extraction Drill (ProSEED), and the PROSPECT Sample Processing and Analysis (ProSPA) suite [14]. It aims to extract samples, determine their volatile inventory, and assess resource potential of the Moon through ISRU experiments. Therefore, ProSPA operations will enable regolith sample yields of water to be measured at any landing site location [15]. Spacecraft-sourced microbial contamination cannot be ruled out. To capture the protective properties of spacecraft hardware, the Lunar Microbial Survival (LMS) model divides the bioburden residing on spacecraft and payloads into three categories: (1) spores residing on the exterior surfaces of spacecraft that are subject to the greatest variations in temperature, solar UV, solar wind particles (SWPs), and all other effects without shielding; (2) spores residing on the shallow interior of spacecraft but that are in thermal contact with the spacecraft exterior surfaces; and (3) spores residing deep within the spacecraft and thermally insulated from the spacecraft exterior surfaces heated directly by solar irradiation. Thus, spores in the second category are considered shielded from all deleterious effects except for vacuum and heat, whereas those in the third category are subject only to the long-term effects of cis-lunar vacuum [16].

Microbial-catalyzed intracellular biomass accumulated iron

Microorganisms may be used in production processes of self-reproducing modifiable nanofactories, catalyzing a wide range of chemical conversions. Some branches of microbial life on earth have developed a metabolism around the use of metal oxides as electron donors or acceptors [17]. A subsection of these organisms utilize solid metal oxides as substrate, converting them to more soluble forms, which makes them interesting for use in mining operations [18]. Such use of microorganisms on earth is widespread and is actively used in the biomining of copper, cobalt, gold, uranium and other metals [19]. In the case of copper, biomining accounts for more than 20% of the yearly worldwide production [20]. These facts give a promising outlook on the application of biomining in space exploration [21]. Lab-scale experiments on the interaction between bacteria and lunar regolith simulant confirm this expectation [22]. However, so far, no design for full-scale biomining operations in space has been presented [23]. It can be hypothesized that the construction and maintenance of an extra-terrestrial base will also rely on iron. Considering the abundance of iron in both Lunar and Martian regolith at 5-22 wt% and 17.9 wt%, respectively, this element is likely to be useful in construction-oriented ISRU [23a]. The general process of using bacteria for mining applications in space, is the dissolution and accumulation of specific resources from Lunar regolith:

(1) Accumulation of dissolved iron in concentrated form, allowing for magnetic extraction.



(2) Leaching of iron from mineral ores, bringing it in a dissolved state, allowing for precipitation of magnetic particles and ores.

The constructed E. coli overexpressing a modified ferritin complex, has a dysfunctional iron export mechanism and an improved iron import mechanism [24]. The combination leads to a high intracellular iron concentration. The resulting capability to swim along magnetic field lines is commonly known as magnetotactic behavior [25]. In the accumulation processes by E. coli and M. gryphiswaldense, a significant uptake of extracellular dissolved iron is expected. The accumulation process in E. coli, takes slightly over 50 hours to consume the initially provided lactate (7.2 g/L). The predicted growth profile, initially exponential, changes into a linear profile after 15 hours. The decreasing dissolved oxygen concentration suggests growth limited by the oxygen transfer rate. However, the extracellular iron concentration does not decrease notably. The overexpression of the encapsulated ferritin provides an estimated 20-50 encapsulated ferritin complexes per cell, or a biomass iron concentration of only 8-20 /g, too low a value to significantly affect extracellular concentration. The model for M. gryphiswaldense predicts batch growth for 39 hours to fully consume the initial lactate and ammonium. The decreasing level of dissolved oxygen indicates its consumption. Again, the concentration of extracellular iron does not decrease notably. M. gryphiswaldense reportedly accumulates iron to a concentration of 4.4 mg/g_x [26], three orders of magnitude more than the proposed E. coli strain. Still, this is not enough to have a substantial impact on the extracellular iron concentration. For the bioaccumulation of iron to be a feasible approach to ISRU activities, the amount of iron accumulated in one gram of biomass should outweigh the amount of transported nutrients required to generate that one gram of biomass. In the case of M. gryphiswaldense growing on lactate, it means that one gram of lean biomass should contain 2.8 grams of iron, i.e. 74% of total biomass should be iron. For E. coli growing on lactate, these values are 7.73 gram and 88.5% [27].

Activity of auger-facilitated water extraction from excavated icy regolith.

No studies have determined microbial colonies indigenous to Lunar regolith. However, the following ISRU icy regolith mining operations may determine otherwise. The results of recent studies conducted by the Lunar Reconnaissance Orbiter [3] made it possible to assume that there is "permafrost" near the lunar poles with a relatively high content of water ice (up to 5% by weight). Here, water ice is supposed to provide the life support system for a future lunar station and act as a source of hydrogen–oxygen fuel for flights into deep space [28]. In order to minimize power, only excavation and water extraction are performed in the PSR; a water tanker is included to deliver the extracted water to a processing plant located on the crater's ridge, where the water is purified, electrolyzed into oxygen and hydrogen, liquefied, and stored. ISRU processing components on the ridge outside the PSR are also defined and sized, but they were not packaged on a lander. The distance between the ridge plant and the PSR system is assumed to be 3.5 km. Power inside the PSR is assumed to be provided by a previously delivered nuclear fission power plant. While the components of the ISRU plant safely operate about 60 m from the power plant. The lander is assumed to land 1 km away to avoid any damage to the power plant from blast ejecta during landing. A cable cart is included that will unroll a cable connecting the ISRU components to the power supply. The processing components located on the ridge were assumed to be powered by a solar array system. Minimum success criteria for the Pilot Plant were defined as one 'year operation', so no energy storage system was initially included in the conceptual design. The top-level objective was to produce 1000 kg of oxygen from 1125 kg of water extracted from the icy regolith in a permanently shadowed region. The regolith was assumed to contain 5 percent water by mass underneath a 20-cm thick desiccated layer of regolith [29]. The components included in the ISRU PSR plant are the excavator, regolith hopper, water extraction reactor, fluid and electrical umbilical for tanker connection, command and data handling, communications and tracking, power management and distribution, thermal control, and structures and mechanisms. For the pilot plant, the excavator gathers about 40 kg of icy regolith in each of the two bucket drums and drives 100 m to the lander, then backs up the ramp onto the payload deck and empties one bucket drum into the hopper. After emptying the first drum, the excavator waits until that regolith has been processed, then spins around and empties the second. The excavator drives down the ramp, gathers the processed regolith that has been deposited on the ground, and drives 100 m to a dump site. The excavator then drives another 100 m to the excavation site and the cycle is repeated. Table 1 lists the time and energy associated with each step in the excavation cycle based on measured power and time in terrestrial tests at a velocity of 0.44 m/s.

Activity	Power		Digging		Disposal	
	Terrestrial[a] (W)	Lunar[b] (W)	Time (min)	Energy (W-hr)	Time (min)	Energy (W-hr)
Digging/Receive Slag	215	215	2.5	9.0	2	7.2
Drive Empty	200	65			8.5	9.2
Drive Full	210	66	8.5	9.4	8.5	9.4
Dump	155	155	5[c]	2.6	< 1	2.6
Totals			**16**	**21.0**	**20**	**28.4**

[a]Tests run at 0.44 m/s; hotel load 53 W
[b]Assume velocity = 0.2 m/s; hotel load 53 W
[c]1.0 minute for depositing in hopper; remaining time is low-energy positioning

Table 1 Excavator specifications per delivery/disposal cycle

The hopper is set on top of the lander payload deck to provide easy access for the excavator. Inside the hopper a horizontal auger transports the regolith to one end where a vertical tube connects it to the water extraction reactor. Inside the water extraction reactor, another auger is used to facilitate the water extraction from the excavated icy regolith. This auger-dryer method is based on terrestrial soil dryers where the objective is to dry the soil. However, in the case of polar water processing, the evolved water is the valuable product [31]. Avionics provide command, control, and health management to the subsystems, and enable autonomous control of ISRU components. The avionics subsystem is based on commercially available military or space grade components from proven vendors, all of which have a strong flight heritage. The communication subsystem provides local proximity communications operations up to 3 km, as well as a data link to the Gateway in lunar orbit at a minimum of 300 kbps. The antennas are placed near the corners of the hardware deck to avoid interference from the lander's solar panel (remains stowed), and to maintain a clear path for the excavator. Power during nominal operations is provided through the external cable connection to the mobile power cart. Thermal management for the components on the PSR lander is minimal and is focused on heating to maintain minimum operating temperatures in the 50 K environment. The electronics enclosure that houses the electronics is wrapped with 25 layers of multi-layer insulation and contains heaters for maintaining temperature. The ice tanker is the component that connects the PSR plant and the Ridge plant. There are two tankers such that one tanker is always connected to the PSR plant collecting the extracted water. The tanker was initially designed as a liquid water tanker, where the incoming water vapor would be condensed and transported as a liquid [32]. The power solution selected for this conceptual design was to assume a nuclear reactor had been landed in the PSR on a previous demonstration mission. That lander also carries a cable cart that deploys a power cable between the nuclear reactor and the ISRU PSR plant. To determine the distance between the two landers, several factors were considered. While the PSR components only need to be 60 m away from the reactor to protect the electrical system, plume impingement analysis recommends landing at least 1 km from any existing asset to protect it from sand-blasting damage. Assuming a 50-m landing accuracy for the CLPS lander delivering the ISRU PSR Plant, total distance between the nuclear reactor and the PSR Plant will be up to 1.1 km. Assuming a 15 percent pathfinding margin, the cable needs to be 1.25 km long [33].

	Mass			Power		
	Basic (kg)	Growth[a] (kg)	Total (kg)	Basic (W)	Growth (30%) (W)	Total (W)
PSR Plant	**275**	**50**	**325**	**1809**	**544**	**2353**
Bus	114	24	138	45	14	59
Command & Data Handling	10.3	3.1	13.4	24.3	7.3	31.6
Communications &Tracking	13.5	1.4	14.9	3.8	1.2	5.0
Electrical Power Subsystem	43.0	12.3	55.3	11.5	3.5	15.0
Thermal Control	3.8	0.7	4.5	5.4	1.6	7.0
Water Capture and Transfer	15.7	1.0	16.6			
Structures and Mechanisms	28.1	5.1	33.1			
ISRU System	161	26	187	1764	530	2294
Water extractor and hopper	95	19	114	1475	443	1918
Water tanker hotel/survival				115	35	150
Excavator	66	7	73	174[b]	52	226

[a]Mass growth based on AIAA Standard
[b]Only needed for 6.5 hrs every 12.5 days

Table 2 Mass and power summary for PSR plant

One key remaining challenge is supplying power to the water extraction plant in the PSR. While the separate mission dedicated to landing a nuclear power plant in the PSR is a reasonable assumption for a full-scale production plant that will require a significant, dedicated power source, requiring three lander missions for a pilot plant is not an attractive approach. Additional analysis is in work evaluating other options to provide power to the PSR plant [34].

Discussion

Space bioprocess engineering (SBE) is an emerging multi-disciplinary field to design, realize, and manage biologically driven technologies specifically with the goal of supporting life on long term space missions. A coherent strategy for the long-term development of this field is lacking. There is almost no formal definition of the scope, performance needs and metrics, and technology development cycle for these systems. Payload volume, mass, and power requirements are made as small as possible and are limited in envelope by their carrier system. One of the most compelling aspects of biotechnology is the ability of such systems to adapt to these constraints relative to certain industrial alternatives [35].

Closed ecological systems (CESs) are ecosystems without any matter exchange with outside environment [36]. CESs are necessary for long-term manned space missions, which aims at minimizing support from Earth. They are composed of several specific compartments that together reproduce the main functionalities of an ecological system in continuous mode of operation and under controlled conditions. CESs are autonomous systems integrating various generation, recycling, and consumption subsystems with the storing capability to solve potential imbalance of key elements in the loop.

As mine influenced water (MIW) loads are sequestered or remediated, the net water products biochemistry change. Depending on MIW loads, influences such as iron, aluminum and manganese are either directly or indirectly remediated by the self-organized biofilms. Direct bioremediation (DBR) is when a microbe uses the influence in its metabolic triplet to respire, grow and reproduce [37]. The production of in-situ or laboratory-cultured biomass functioning as biological oxygen demand (BOD) when reduced environments are desired, is an example of indirect bioremediation (IDB). The biomass may not be a direct carbon source for DBR of influences, but it can use up all of the dissolved O_2 in a load, indirectly leading to the redox ladder and the selective pressure that allows the next most energetic couplet to outcompete the one below it on the redox ladder [38].

Conclusion

No definitive evidence of microbials have been found to exist in lunar regolith. Spacecraft-sourced microbes may present a microbial presence on the Moon. Microbial contamination cannot be ruled out. To capture the protective properties of spacecraft hardware, the Lunar Microbial Survival (LMS) model characterizes the bioburden residing on spacecraft and payloads. Auger-facilitated water extraction operations may uncover development of the bioburden expression. More intentionally, however, would be microbial-induced biomining for magnetic extraction of intracellular, soluble iron. This paper proposes further microbial studies related to environmental effects of lunar water harvesting and other lunar mining activities along with their respective remediation.

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