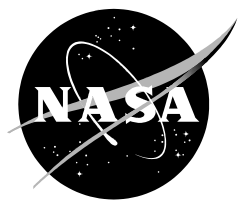


NASA/CP—2008—214564



Lunar Regolith Biomineralization Workshop Report

*Compiled and Edited by:
Bonnie P. Dalton, NASA (retired)
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Idaho Falls, Idaho*

Report of a workshop sponsored by and
held at NASA Ames Research Center, Moffett Field, CA
May 5-6, 2007

September 2008

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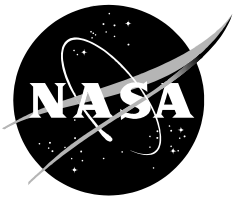
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Space Administration

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Executive Summary

On May 5 and 6 of 2007, NASA Ames Research Center hosted a workshop entitled Lunar Regolith Biomineralization. The workshop was co-organized and sponsored by NASA Ames Research Center and the Idaho National Laboratory, Idaho Falls, ID. In the NASA Vision for Space Exploration, in situ resource utilization (ISRU) will be a relevant issue in man's long-term presence in planetary exploration. The goal of the two-day interdisciplinary workshop was to address the feasibility of biologically based mining of the lunar regolith along with identification of views and concepts for moving this topic forward to NASA. NASA has the vision; Idaho National Laboratory has the experience in related Earth applications. Thus with Ames researchers' foundations in astrobiology and life support, the ongoing ISRU program at Johnson, and academic and geomicrobiology research interests, it was felt there could be a blend to make this an exciting discussion to determine pathways toward lunar regolith utilization.

The intent of the workshop was to gather researchers in government, industry, and academia from various backgrounds ranging from geomicrobiology, to extremophile microbiology, to heavy metal cycling in marine biology, along with biomineralization engineers to determine the feasibility of the lunar regolith biomineralization and next steps both in ground studies and space experiments. The first day sessions included presentations followed by breakout sessions, which reported their discussions and findings the next day. The final agenda is shown at the end of this report along with a white paper addressing regolith biomineralization which was circulated a week after the meeting per agreement by the attendees. The presentations and discussions indicated that, given the availability of water, microorganisms might be able to extract metals and other solid resources from lunar materials. However some workshop participants as well as several leading biochemists and microbiologists who were consulted after the workshop indicated that microorganisms cannot extract molecular oxygen directly from lunar silicates and metal oxides. Discussions identified the need to further explore a broad range of potentially useful metabolisms, as well as the potential for genetic bioengineering to assist in overcoming specific challenges presented by lunar regolith. With sustained manned presence, excess carbon and water would likely be available to overcome some of the primary difficulties identified during the discussions. It was agreed that additional characterization of the lunar regolith is necessary, and that ground-based simulant experiments should be strongly considered in order to understand how terrestrial microbes might interact with native mineral materials and the very different redox / mineral characteristics of lunar regolith. Additionally the impact of exposure to lunar gravity and radiation conditions must be taken into consideration, and therefore focussed satellite precursor missions to assess the impact of these factors on utilitarian microbial metabolisms (for life support and / or biomineralization ISRU potentials) are also recommended. Investigators must address the interplay of radiation, gravity and other key factors that will affect bioprocesses on the Moon and its regolith.

Lunar Regolith Biomining

Introduction

On May 5th and 6th, 2007, NASA Ames Research Center hosted a workshop entitled Lunar Regolith Biomining. The workshop was co-organized and sponsored by NASA Ames Research Center and the Idaho National Laboratory, Idaho Falls, ID. The goal of the two-day interdisciplinary workshop was to address the feasibility of biologically based mining of the lunar regolith along with identification of views and concepts for moving this topic forward to NASA. Ames Research Center was the focal point in defining the new discipline of Astrobiology which now has active research groups at NASA Field Centers, and throughout academia. Researchers in this area will be prime contributors in determining the potential for lunar regolith biomining, but information was also gleaned from researchers in other government agencies and industry.

Workshop presentations were selected to provide background in topics of interest that served as the foundation for discussion in the subsequent breakout sessions. The first topical area included the history, status, and issues with biomining on Earth to familiarize all attendees with current activities. These presentations related the primary considerations in existing biomining, e.g., microbes of choice, pH of reactions, time and temperature, specific mining applications and locations, and benefits and/or limitations of biomining. The second area reviewed existing research efforts addressing biomining of planetary surfaces (Mars, Moon), including microbial considerations, and chemical necessities in biomining and biofuel production. The last element pertained to other non-biological considerations and influences in biomining efforts on the lunar surface such as radiation fluxes and effects, and the application of small satellite experiments to learn more about the lunar and Martian surfaces.

Following the presentations, the workshop attendees divided into three breakout sessions to discuss areas of interest in greater detail and to define “next steps” in determining the feasibility of lunar regolith biomining. Topics for each of the three breakout sessions included:

- (A) Bio-communities of choice, target product(s), and suggested ground studies
- (B) Physical/environmental issues and ground studies
- (C) Development of reference experiments for the Astrobiology Small Payloads Workshop scheduled for June 2007. Payloads could be developed for small satellites and Exploration Systems Mission Directorate (ESMD) Constellation flights to low earth orbit (LEO), high earth orbit (HEO), lunar orbit, and the lunar surface.

The results of these three breakout sessions are summarized in the report. The report also includes a list of the participants.

Presentations

History, Current Status, and Issues with Biomining on Earth

Dr. Frank Roberto of Idaho National Laboratory (INL) opened the Workshop presentations with the **Biohydrometallurgical Approaches for Mining Lunar Regolith**. Dr. Roberto related that bioleaching is a well-known phenomenon as seen in Rio Tinto, Spain with evidence of cement manufacturing being practiced as far back as Phoenician times and vestiges found of Roman-era metal recovery throughout Europe. Bioleaching of minerals is a naturally occurring process with microbial ecology including acidophiles, mesophiles, moderate and extreme thermophiles along with pH ranges from 1-3 and temperatures ranging from 25°C to 100°C, as shown in Figure 1:

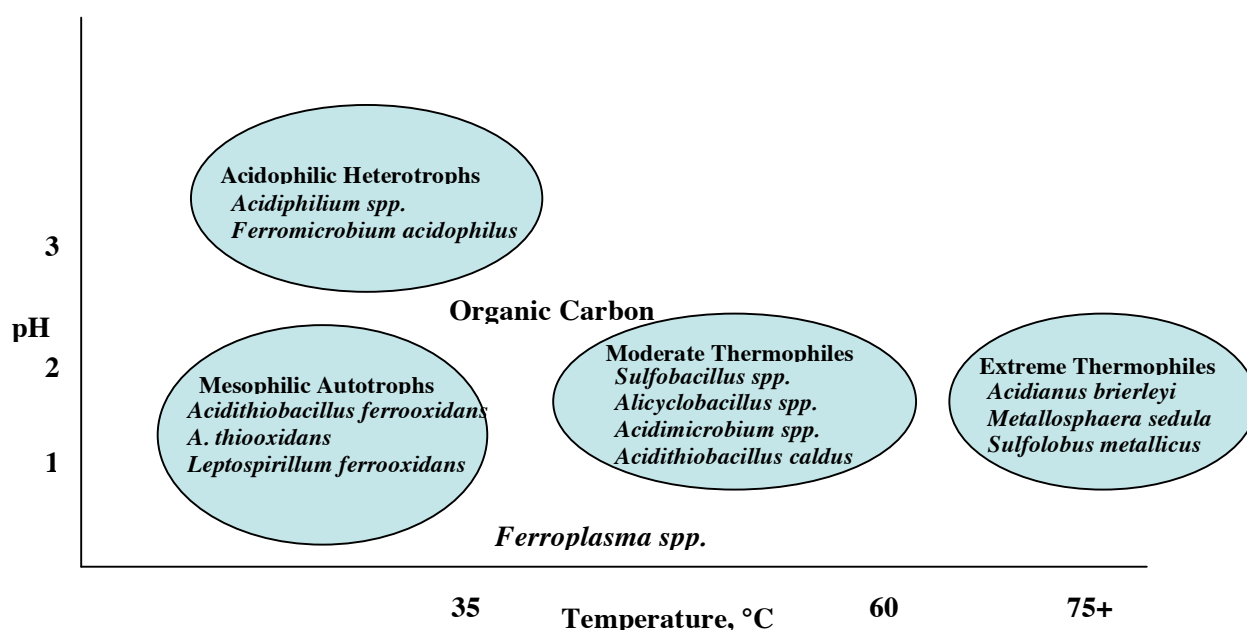


Figure 1: The microbial ecology of acidic, metal-rich environments- an expanding view through the application of metagenomics.

Bioleaching of sulfide minerals now represents the largest-scale commercial bioprocessing worldwide. Types of bioleaching in practice (by their industrial names) include dump leaching, heap leaching of copper and gold, BIOX[®] processing of gold concentrates, and BacTech moderate thermophile leaching of gold concentrate. INL is currently focusing on thermophile bioleaching with known and novel species, found primarily in Yellowstone National Park. Laboratory thermophilic leach studies yielded 65% copper recovery vs. 21% recovery where the control column was not inoculated with thermophilic microbes.

Examining the mineralogy of the regolith, basalt will be the major source of materials. Any process proposed would need to identify what we want to recover. As part of INL's initial proposal to NASA, there are several assumptions:

1. The Lunar regolith is a poor resource compared to terrestrial minerals, so recoveries will not be very efficient at first.

2. There will be need for environmental control to sustain any microorganisms, and a determination of the amount of resources needed and available for use (water, oxygen etc.) for bioleaching as compared to the need for these same resources for other purposes.

Other elements to consider for lunar regolith biomining include:

- Some hydrogen and oxygen need to be available initially
- Environmental control of temperature to maintain liquid water
- Shielding/using radioresistant microorganisms
- Power available (possibly from solar or nuclear source)
- Scaling for final design
- Materials to be recovered could include: iron, aluminum, titanium, chromium, and elemental oxygen.
- Desirable microbial characteristics including radioresistance and cold/heat resistance (mesophiles 4-45°C, psychrophiles < -15°C).

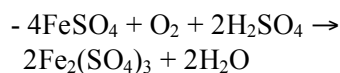
Dr. Roberto pointed out that terrestrial analogs are available including the effects from low pH environment, high temperature environment, high radiation (natural or engineered sites), cold environments, and deep subsurface.

Several questions followed the presentation, which provided additional information:

1. Particle size affects leaching efficiency and duration. Fine particles will leach faster, but on Earth this requires much energy since physically decreasing particle size (comminution) prior to the leaching process is expensive.
2. Currently there is no commercial bioleaching of aluminum. It can be done at the bench level, but industry is not interested.
3. The rate of leaching is temperature dependent. There are orders of magnitude difference between 4°C and 100°C. A paper has been published showing leaching is even possible at -20°C, but it is at a very slow rate. With thermophilic organisms, grams of metals have been liberated per day in the laboratory by only milligrams of microorganisms.

The second presentation relating current status of biomining on Earth was by Dr. James Brierley. His presentation addressed **Development of Terrestrial Bioleaching and Mineral Biooxidation Processes**. Common characteristics of biohydrometallurgy, the industrial application of biomining, are that it is water based, it is an aerobic microbial oxidation of iron and sulfur with ferric iron as the prime oxidant of minerals, and generally involves sulfide minerals. Dr. Brierley reiterated that common applications include bio-oxidation pretreatment of refractory precious metal ores and concentrates, bioleaching of copper, and bioleaching of cobalt. He defined bio-oxidation as **exposing** the metal value, which is a pre-treatment; bioleaching is **extracting** the mineral value. The micro-chemical processes are illustrated in Figure 2.

•Role of microbes



•Ferric oxidation of sulfide minerals

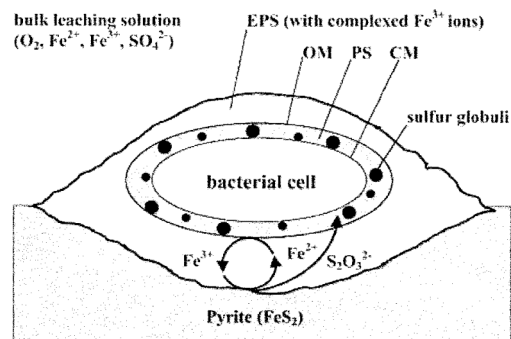
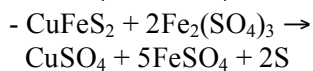
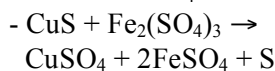
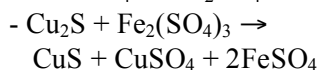
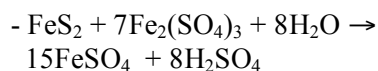


Figure 2: The bioleach process.

A microenvironment is produced by the bacteria that carry all of the compounds needed for the bioleaching of minerals. In the sequence of oxidation of the mineral, the iron arsenate oxidizes first and does so very rapidly. After 46 days, the iron sulfide (FeS_2) pyrite starts to oxidize beginning in areas with cracks, where the mineral is accessible. The redox potential affects how quickly a mineral region in the rock is oxidized. Many genera and species of bacteria are involved in oxidation; in industry, mesophiles are used. These include: *Acidithiobacillus ferrooxidans*, *A. thiooxidans* + *Leptospirillum ferrooxidans* + *Sulphobacillus* (these form spores and bioleach at 50°C), + thermophilic archaea (these bioleach at 78°C). If pyrite is euhedral (crystalline), it is very resistant to bioleaching (needs to be porous). Aeration is critical for the bioleaching process to help the growth of the microbes.

Discovery of the role of the acidophilic iron oxidizing bacteria was first reported in 1947. The first documentation of commercial applications was in the 1957 Kennecott Copper dump bioleach for copper recovery. The biggest applications of biomining are currently in mining of precious metals in South Africa, Brazil, Chile, Peru, Australia, and China. Industrial methods now include heap bioreactors, heap and dump reactors, and run-of-mine heap reactors. In operations such as the Escondida mine in Chile, as much as 180,000 to 245,000 tons of copper are recovered per annum, and 1.56 billion tons of copper reserves remain. The Escondida operators estimate that in 25 years they will be recovering 64 million tons per annum. The pad size at a site such as this is 2000 meters wide, 4900 meters long and 126 meters high.

Advances in Commercialization of Heap Bioreactors

Current commercial processes are depicted in the pictures at site locations shown in figures 3 and 4.



Figure 3: Active ventilation by forced air ~ 1995, The Quebrada Blanca Cu Leach.



Figure 4: Copper bioleaching; heap and dump reactors in the Atacama Desert of northern Chile.

At all these sites, appropriate aeration for microbial growth has been essential whether in a heap process or a tank process with inoculum. The Newmont Mining Corporation using a trade name BIOPRO (bio-oxidation pretreatment) has recovered 12,172 kg of gold over a five-year period from 2000-2005. Dr. Brierley appropriately posed the question, “Where next for these microbe miners?”

Research Efforts

Both Drs. Roberto and Brierley spoke briefly of ongoing research in their laboratories. Dr. Roberto's work focused primarily on the extremophiles obtained from Yellowstone National Park. Dr. Brierly introduced an anaerobic archaean—*Acidianus brierleyi*, which he first reported on in 1982. This microbe is involved in oxidation of sulfur and reduction of molybdate (MoO_4) with a resultant “molybdenum blues” coloration.

Dr. Paul Todd of Space Hardware Optimization Technology (SHOT) related his company's research experiences in his presentation, **Terrestrial Extremophiles for Extraterrestrial Environments**.

Dr. Todd's graphic, Figure 5, depicts the elemental composition (by percent) of lunar soil.

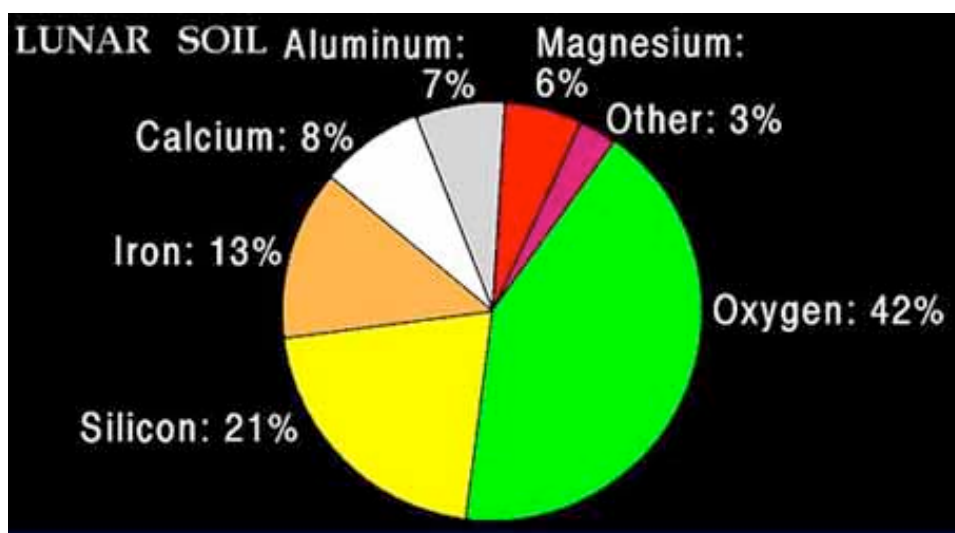


Figure 5: Elemental composition (by percent) of lunar soil.

In addition to the seven major elements, there are twelve minor elements including phosphorous, implanted wind hydrogen and helium at 50 ppm and traces of carbon and nitrogen. Minerals present include:

- Whitlockite & Apatite $\text{Ca}(\text{PO}_4)_2$ & $\text{Ca}_5(\text{PO}_4)_3(\text{OH}, \text{F}, \text{Cl})$
- Plagioclase, $\text{Ca}(\text{Na})\text{Al}_2\text{Si}_2\text{O}_8$
- Pyroxene, $(\text{Mg}, \text{Fe}, \text{Ca})\text{SiO}_3$
- Olivine, $(\text{Mg}, \text{Fe})_2\text{SiO}_4$
- Ilmenite, FeTiO_3

In the discussion that followed this presentation Dr. David McKay related that there is carbon within the first few hundred angstroms at the surface of regolith particles, very accessible by microorganisms. In addition 1% of the carbon in the soil is present as carbides (M_xC_y).

Dr. Todd pointed out that the challenge for microbial survival may be ultraviolet (UV) radiation between 200-300 nm along with temperature variations from $+120^\circ\text{C}$ to -170°C . The research conducted at SHOT was a result of a meeting in 2004 by a group of scientists to discuss and identify a community of organisms to be utilized in early experiments addressing planetary surfaces. This early meeting was focused on Mars, but is applicable to the Moon. During this meeting, the *ecopoiesis* concept was discussed and defined as the emergence of living, eventually self-sustaining ecosystems. Then followed scaling

rules for test beds, e.g., gas concentrations, heat capacities, heat transfer, light and radiation intensities, biomass, partial gravity, and mechanical properties. As a result of the meeting, an Ecopoiesis Test Bed chamber was developed. Candidate microorganisms were suggested with valuable characteristic tolerances and included:

- Radiation (*Deinococcus radiodurans*)
- Hyperbaric/anaerobic (*Bacillus infernos*)
- Vacuum (*Streptococcus mitis*)
- High saline (*Haloferax volcanii*)
- Sulfurous environment (*Thiobacillus* species)
- Spore dormancy (*Bacillus subtilis*)
- Low temperature (*Anabaena*, other cyanobacteria)
- Cyanobacteria are mesotrophic (mesophilic, or autotrophic, or possibly both), can fix nitrogen, can act endolithotrophically, are resistant to high carbon dioxide (CO₂).

It was deemed that autotrophy is essential. Dr. Todd suggested that the community consider using pioneer autotrophic organisms first, and then use the products as a food source for bioleaching chemolithotrophs. Cyanobacteria and halobacteria (halophilic bacteria) meet many of the pioneer characteristics for environment compatibility, but no halobacterium that is an autotroph has been found. Dr. Todd related that since these organisms have many of the genes to tolerate many different environmental challenges, such as radiation damage, they might be used as a genetic source for bioengineering a candidate autotroph that can withstand environmental challenges. As an example of the organisms' capabilities, the cyanobacterium, *Synechococcus*, can rebound from a temporary complete depletion of CO₂. In addition, SHOT's research showed that cyanobacteria in their Mars simulant chamber did not lose any esterase activity between light and dark cycles – 5 weeks/100mbar CO₂ exposure.

It was reported that in three simulation experiments with lithotrophs, described as a desert varnish community, DV8, at the Mars Desert Research Habitat site near Hanksville, Utah, there was significant survivorship in each experiment. Although the number of individual strains within the community diminished, two very significant organisms emerged: a cyanobacterium and a manganese oxidizing bacterium, which grow robustly in culture. These organisms prefer to be maintained in mixed culture. The cyanobacteria are very prolific and the manganese oxidizer is still capable of manganese utilization after exposure. Using 2-(p-iodophenyl)-3-p- (nitrophenyl)-5 phenyltetrazolium chloride and 4', 6'-diamidino-2-phenylindole assays shortly after retrieval, relatively high numbers of metabolically active cells (~20-32%) were shown. The conclusion is that organisms are maintaining some level of activity in the chamber, as confirmed by rapid growth in culture immediately post exposure.

To address lunar regolith biomineralization, Dr. Todd proposed selected single organism experiments where atmospheric composition is varied, followed by repeating the experiment with regolith simulant JSC-1 and identifying minimum atmosphere requirements and introducing bio-communities. Though a small amount of CO₂ is present, it would be necessary to add supplemental CO₂ to attain a 200-mbar atmosphere. The audience also suggested using radiation heat that is not lethal. In response to the question of heat at night, Dr. Todd suggested, "Let everything freeze and then restart during the next light period." Others suggested using photovoltaic power during the night. It was also noted that regolith is a good insulator, and, depending on how deep the microorganisms would be placed, temperature could be kept constant. The other alternative suggested was to work at the poles where there is constant light of varying intensity.

Dr. David McKay's (NASA Johnson Space Center) presentation, **Considerations on Lunar Bio-Leaching**, tied in current JSC efforts addressing in situ resource utilization (ISRU) for life support and propellants. Dr. McKay illustrated the necessity for oxygen production in any Moon exploration or

stationary site with quantitative data. To be operationally and/or economically feasible, 10 metric tons (MT)/year of oxygen would have to be produced using no more than 2 MT of hardware and material inputs. Thus far his team has demonstrated the capability to produce at least 1 MT per year. The JSC team has focused primarily on physiochemical extraction. Following 30 years of research, with 20 different processes emerging, the NASA Constellation Program selected three approaches for development. These included 1) hydrogen reduction of lunar soil, 2) electro-winning (magna electrolysis, molten salt electrolysis, or molten oxide electrolysis, and 3) carbo-thermal reduction of lunar soil.

Biological/microbial processing is a relatively new idea, but would eventually fit with the anaerobic processing of crew waste and trash, which will be essential for long term presence. Hydrogen reduction of lunar soil is dependent on extremely high temperatures, e.g., 1050° C. The carbothermal reduction also requires high temperatures (>1800° C). By design, the JSC team developed their hydrogen and carbo-thermal reduction processes to share a significant amount of hardware. The research team is also assessing the possibilities of a solar concentrator vs. electrical power for the reduction reaction heat source. Clearly, there are challenges in reduction, but there may also be alternative biotechnology using a mutated strain of the mold *Aspergillus niger*. The lithotrophic cyanobacteria may also prove beneficial in this application. Based on their work thus far, Dr. McKay's team believes that cyanobacteria may have the ability to break oxygen-iron bonds to get iron, thus liberating oxygen as a byproduct. The challenge will be to uncover the fate of the oxygen after liberation. Based on their work thus far, Dr. McKay's team believes that cyanobacteria may have the ability to break oxygen-iron bonds to get iron, potentially liberating oxygen as a byproduct. They postulate this based on the observation of pitting of mineral surfaces or dissolution of mineral substrate in liquid culture with cyanobacteria. They recommend further study based on these preliminary results to elucidate the mechanism of the mineral dissolution, the fate of the mineral bound oxygen, and overall expected yield rates.

Pursuing the role of microbials, Dr. McKay's group is developing:

- Biotechnology approaches to extract iron, titanium, aluminum, and oxygen
- A cyanobacterial consortium for efficient methane production
- Cyanobacterial strains with increased productivity of organic and amino acids
- Advanced photobioreactors for extraterrestrial cultivation of cyanobacteria
- Optimal genomes and species selection.

The JSC team's work is at a technical readiness level (TRL) 2 with the development of a membrane photobioreactor. The short-term goal in their microbial processing is to reach a TRL 3-4, which they believe can be achieved, if they:

- Select and characterize 2-3 cyanobacteria species with the best ability to etch lunar soil
- Document the release of different elements and oxygen from lunar simulants of different origin/composition induced by cyanobacterial (CB) etching
- Evaluate the best conditions for etching CB using lunar simulants as feedstock
- Produce preliminary bioreactor systems requirements
- Design and construct a small prototype reactor

The group's long-term goals of biomining for Mars exploration and lunar colonization include supplementing physical-chemical ISRU by:

- Oxygen production for propellants
- Providing metals for electroplating and powder metallurgy applications (advanced manufacturing)
- Supplying high-protein food supplements for crews
- Supplying greenhouses with nutrients to enhance food production
- Supplying organic components to methanogenic reactors for continuous methane production

- Biogenic air regeneration: removal of CO₂, decomposition to oxygen and carbon, return of oxygen to breathing atmosphere.

The ultimate plan would be to recycle all biodegradable waste to recover the carbon, nitrogen, water, and phosphorus.

It should be noted that while some believe that there may be sources of water ice in some permanently shadowed craters at the lunar poles and that these sources, if they exist, could potentially offer a source for the extraction of oxygen, several attendees asserted that biomining O₂ from other lunar materials was “infeasible in the foreseeable future.”

Dr. Patrick Fu, of the University of Hawaii presented the concept of **Metabolic Engineering for the Biofuel Production**. Dr. Fu’s research was of interest to this session since it has been suggested that a cyanobacterial mutant could be created which would reduce iron in lunar regolith in a manner suited to lunar resources, e.g., little or no water. Dr. Fu has created a cyanobacterial mutant to produce ethanol.

Dr. Fu related that yeast use a glucose pathway to make ethanol, but the amount of ethanol produced per unit of biomass is low, so people have introduced the xylose pathway. Co-fermentation of the two sugars will result in a greater amount of ethanol. Ethanol production from agricultural crops is a negative gain for cost. Agricultural crops take months to grow, and input energy associated with biomass production and processing significantly reduce the economic yields from traditional fermentation processes. Cyanobacteria engineered to produce ethanol yield a much simpler process, which only takes five days versus several months. Only carbon dioxide (CO₂) and solar energy are needed. There is a positive cost gain. The cyanobacteria convert CO₂ directly to ethanol. Because carbon goes directly to the production of ethanol, growth is less at the expense of ethanol production than might otherwise be the case. High lighting intensity is needed, and that is the limiting resource. High cell density is also a limiting factor, so a larger surface area is beneficial to the process. Dr. Fu compared the symbiotic relationship of coral algae, where the algae use light to produce energy and as a result produce food for the coral. He posed the questions: “Can this type of synergy be exploited for extraterrestrial habitation? Could this thus be applied to biomining with a tap into biodiversity?”

During the questions that followed his presentation, Dr. Fu noted:

- Other phenotypic changes in the cyanobacteria making ethanol included color change to a green/yellow.
- Other atmospheric products that could be used included carbon monoxide (CO), and methane (CH₄). A New Zealand company has reportedly used CO.
- The bioengineered gene is maintained in the bacteria in the presence of a wild-type community by integrating the gene into the chromosome.
- As for genetic drift on the Moon due to issues such as unknown selective pressures, the cyanobacteria were put in excess ethanol and high heat, and the cyanobacteria turned white. The bacteria were still alive and more active than the green bacteria. When put into fresh medium the bacteria returned to their normal morphology and color. Ethanol production went down.

Dr. Ron Oremland of the United States Geological Survey (USGS) presented another aspect to consider in regolith biomining during his presentation **The Ecology of Arsenic**. There are microbes that oxidize Arsenic: As(III) to As(V). Arsenate respiration is needed for growth. As(III) is the electron donor in this process. Bacteria that reduce selenium could do the same to arsenic. Arsenic is common in meteorites and is a byproduct of mineral mining--arsenic trioxide. It is not a great oxidant, but is good enough. The oxidation process releases water. There are arsenate resistance genes to protect the bacteria. In studies at Searles Lake, CA it was found that at low depths below the brine level interface, the As(V) and As(III) levels go up and evidence of metabolic products are seen. *Halarsenobacterium silvermanii*

was found in the sediment, which is not an archaeon. This organism reduces arsenate to arsenite. The organism has not been found in Mono Lake, even though As(V) reduction has been observed in Mono Lake. It is a member of a known group of arsenate respirers.

Based on an analysis of As respirers, archaea do not use the same mechanism as *Halarsenobacterium silvermanii*. This may indicate that *H. silvermanii* is related to the Archaea and were present at a geological time when As was very abundant. Searles Lake has a high concentration of borate. Though all processes are not known, the organism may have implications for bioleaching of regolith, specifically As(III) production under anaerobic conditions.

During the questions that followed it was related that arsenate production was also tested with hydrogen and sulfate growth support. The reduced presence of arsenate on Earth is believed to be a result of arsenate migrating into the core of the Earth and recycling to the surface as a result of volcanism.

Non-biological Considerations and Influences

Dr. Tore Straume from Ames Research Center and the lead in radiation studies at Ames, presented **Ionizing Radiation on the Surface of the Moon**. Dr. Straume shared data from Wilson (2006) of proton energy spectra for six large solar particle events (SPEs) gathered since 1956. In response to a later question, the Wilson data was collected using satellites. There are measurements from L1, which are completely outside of the magnetosphere.

Galactic cosmic rays (GCR) consist principally of hydrogen, helium, carbon, and iron nuclei with energies in the GeV range. It is felt that some of the galactic cosmic radiation particles can penetrate fairly deep into the regolith. Solar particle events consist of protons with energy from 1-1000 MeV. In solar radiation, the vast majority are protons with very low energy (20-150 MeV). We know that it takes 20 MeV to penetrate the space suit, and 80 MeV to penetrate to the bone marrow. This level was seen for an SPE in August of 1972 on the Moon.

The calculated dose-rate during 1972 SPE (lunar EVA in 0.3 g/cm² spacesuit). For a 0.3 g/cm² dense space suit, the dose rises steeply at over 1000 rads/hr, which exceeds any dose limit on the skin. For the marrow, the level only goes up 20 rads/hr with 1000 rads/hr for 2-3 hours for skin exposure, 10 rads/hr for 10 hours for bone marrow. Data from the Apollo flights are very relevant for any human presence on the Moon.

In all activities, shielding depths depend on materials used. At depths of 1 cm, sterilizing doses will not kill microorganisms. As for radiosensitivity of microorganisms—a large solar event would kill all microorganisms at the surface, but most would live at 1cm depth. High-speed protons take approximately an hour to get to the Moon. High mass ejections take days with many different particles. The particles are at the interfaces of the shockwave. Those with energies greater than 10 MeV arrive before those greater than 100 MeV. Dr. Straume expressed the belief that bacteria will survive a major SPE event if the microbial leaching is conducted at 1 cm depth because the regolith will reduce the energy to a few thousand rads. This is in contrast to human cells sensitivity in the hundreds of rads level.

During the question and answer session following this presentation, several other items of interest were addressed. *Deinococcus radiodurans* evolved a mechanism of DNA repair associated with radiation resistance where protein scaffolding keeps the DNA together very tightly so that the broken ends remain close together. This helps facilitate higher fidelity repair. Desiccation drove the evolution of this mechanism because desiccation causes DNA breaks.

In response to whether the radiation vector/depth is proven or just a model, and what is the uncertainty in the depth measurement and what is the nominal, Dr. Straume responded that there is a need to validate the calculations and models, and the best approach would be to put a densitometer on the Moon. The question was then posed if the modeling had to be on the Moon. The response was that the secondary production of radiation occurs on the Moon via interactions between GCR, SPE and lunar materials, and you wouldn't see the "free flyers" (if the experiments were performed on Earth). Neutrons are more damaging than the protons, so neutrons will have more biologically damaging effects. This needs to be validated on the Moon. Dr. Straume also reminded the audience that we cannot think of radiation alone, but must address the interplay of radiation and gravity affecting bioprocesses on the Moon and its regolith.

Though Dr. Chris McKay's presentation was not the last on the agenda, it is being presented last in this report since it was an expanded view—looking also at the plans for Mars with the urging that much could be learned from precursor missions to the Moon. The title was **Following the Dirt Road to the Moon and Mars**. Dr. McKay proposed a science-driven mission to the Moon as first priority to investigate the lunar dust. His priorities on near term low-cost missions to the Moon and Mars are: 1a) science, 1b) hazard and engineering, 2) plant growth media, 3) radiation shielding, and 4) biomining.

McKay proposed use of the Ames Genesat for Lunar and Mars microsattellites. He suggested 1) microarray analysis of lunar dust and use of electrostatic forces to collect nanodust (vs. a mechanical collector), 2) testing of magnetic and electrostatic materials that repel dust, and 3) microfluidic reactions with lunar dust including water and other solvents. Though his primary emphasis and planning has been toward revealing life on Mars, he urged preliminary missions to the Moon to eliminate greater costs and uncertainty in Mars missions.

Addressing biomining, McKay suggested using microbial consortia that are demonstrated to bioleach lunar simulants as a radiation and soil hazard assay. In conclusion, he urged that we focus toward determining how to piggyback different research experiments together to take advantage of limited resources.

Responding to a comment about a linkage between biomining and hazard reduction, i.e., bacteria release compounds that break down fine particles, Dr. McKay said, "Maybe biomining can mobilize factors in the regolith that can be more readily detected—again linkage between one application to one of the near term mission driver research applications."

At the conclusion of the presentations, the group divided into the three breakout sessions and spent the remainder of the day in their discussions. The next morning, the group leaders presented the results of those discussions.

Breakout Sessions

Session A: Bio-communit(ies) of choice, target product(s), and suggested ground studies

Oxygen was deemed to be the most important potential product that could be recovered from lunar regolith, providing essential materials for life support and propellants. This was displayed as follows:

Exogenous resource	Endogenous resource	Product output
EARTH	MOON	-oxygen for
H ₂ O, CO ₂	C(~200g/ton)	life support
(initially)	N,P	-oxidizer, fuel

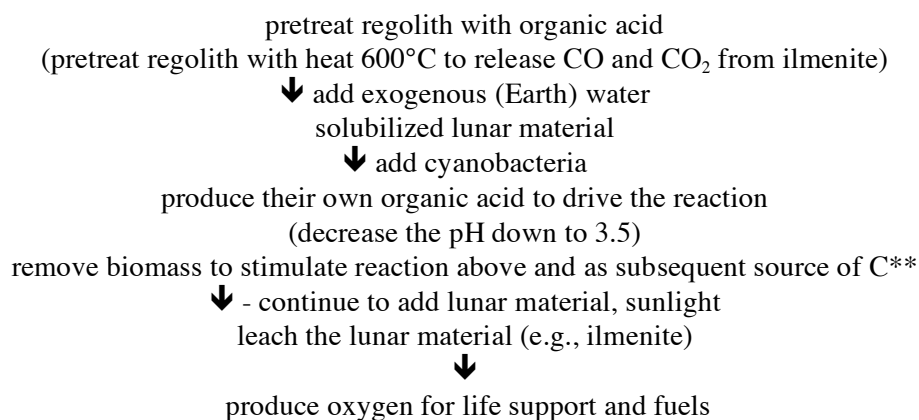
Environmental Control: >200mb atmosphere; liquid water

The question was posed: Is the endogenous carbon (C) sufficient and available? JSC has a plan to use the C from regolith, using the Sabatier reactor, which produces carbon dioxide (CO₂) and carbon monoxide (CO). They are using the oxygen (O₂) from water electrolysis. The requirements include: preliminary H₂O electrolysis, 300°C, and aluminum oxide (AL₂O₃) as a catalyst.

Addressing the question of bioleaching of the lunar material, cyanobacteria (as claimed in one presentation) are proposed agents for break down of ilmenite (FeTiO₃) to release iron (Fe), Oxygen (O) and Titanium (Ti). The oxygen is very reactive, but few questions remain on whether it comes from mineral dissolution or splitting water and where it goes. The O₂ is generally recognized as coming from H₂O. Mechanisms may need to be established to prevent this O from reacting, specifically by keeping pollutants out of the water.

Carbon for the bioreactor (short term) will come from Earth substances, recycling the cyanobacterial biomass and carbon present in the lunar regolith, while long term sources will include human organic waste and respired CO₂ that are expected to be in great excess once continuous manned presence is established.

The JSC workshop attendees were members of Session A and provided the following flow chart for oxygen production as part of their current ground program plans:



Session A recommended the following goals to promote biomining:

- 1) Prove the validity of biomining over physical chemical processes
 - a. Need to get funding and proof of concept within ~2 years
 - b. Use lunar simulants/curated lunar material

- 2) Consider hybrid solutions that might include physical-chemical approaches
- 3) Other regolith products are dependent on the success of these goals.

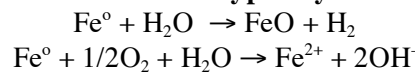
The group also posed other issues which need to be answered as ground studies go forward. These included:

- 1) Alternative strategies (there is little time to do massive screening; anaerobic process is desirable); use regolith simulants for screening.
- 2) A bioreactor needs to be designed to handle solids, methods of solids collection, and introduction into the bioreactor.
- 3) Need for additional environmental controls (radiation, shielding, insulation)
- 4) Storage of product.

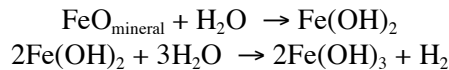
Session B: Physical/environmental issues and ground studies

Addressing physical/environmental issues, session members started by identifying useful products at the Moon for and from biomining. These included oxygen/oxidants, radiation shielding, water, materials including structural materials, e.g., iron and aluminum, building materials for structure and insulation, and a solar energy infrastructure. They also identified biogenic elements, carbon, nitrogen, oxygen, phosphorous, and sulfur along with trace metals. Physical factors identified included radiation, low pressure/containment, low gravity, temperature excursions, light, toxicity, dryness/lack of moisture, accessibility of target, and the regolith properties including grain size and permeability. Chemical factors identified included the fact that lunar materials are highly chemically reduced, which is inhibitory to organisms since they need reductants and oxidants for energy transduction and biosynthesis. In addition, there is toxicity to microbes involved in biomining (and to humans). Other chemical factors included: corrosive properties, water availability, carbon, nitrogen, hydrogen, and phosphorous availability. Carbon, nitrogen and hydrogen are available from solar wind with nitrogen better than carbon. Last in chemical factors is the low abundance and/or accessibility of target elements in the regolith and rocks. The prime chemical is the need for oxidants. The term “Oxidant Desert” was applied to the Moon. Lunar surface materials have astonishingly low oxygen fugacities; even water would be an important oxidant. The following chemical equations indicate ongoing reactions and issues:

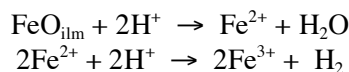
Lunar surface materials typically have >1 % Fe⁰



Iron mobilization/oxidation from minerals at neutral pH:



Iron mobilization/oxidation from minerals at low pH:



**But need an oxidant to produce H⁺ from lunar H reservoirs
To produce any O₂ biologically, need even more O (from H₂O or CO₂)**

This issue is addressed further in a white paper assembled by Dr. DesMarais and leading researchers in the areas of geomicrobiology and exobiology. The paper is presented in Appendix A and lists all contributors, some of whom were unable to attend this workshop.

Session B provided a listing of suggested studies enabled by lunar precursor missions. These included:

- Radiation flux measurements sensor on Moon, secondary radiation, matrix effects

- Microbial growth at low P, e.g., 25° C, 100 mbar
- Microbial growth: Toxicity, growth and performance—testing requires real lunar regolith
- Regolith/water interactions: Physical + Chemical effects, e.g., critical for ‘heap’ design
- Mapping real distribution of components at poles
- Lunar dust reactivity mission - (smallsat mission)

As part of their assignment, they also identified ground-based studies as follows:

- 1) Radiation experiment with lunar simulants and Apollo cores, radiogenic elements
- 2) Radiation effects on microbial biominers’ (microbes) survival
 - a. Survival of metabolic capabilities which make it a good biominer
- 3) “Heap” design with respect to physical, chemical environment, effect of pressure (P), gravity, scale, stability, transport
- 4) Harvest strategies
 - a. Wetting and solubilization, extraction, refinement
 - b. Post processing of harvested resource

A second set of ground based studies should be performed to determine future directions. Many of these will require actual regolith, e.g., electrostatic properties, toxicity, affinities in water. The coproduction of life support resources and biomining products (as needed by ISRU) must be targeted along with analysis of the synergy of biomining and hazard studies—how does the dust behave. Next, there will be a need to compare biomining vs. traditional ISRU trade studies. The last thing the group identified was the need for a very high fidelity regolith simulant and in great quantities.

The big question from the discussion of this presentation was, *“Can we do something with the regolith without bringing a lot of resources from the Earth to utilize in situ resources?”*

For simplicity, **Session C** changed their title to **Microbial Studies on Satellites**. They expressed the need to perform simulations of lunar and Martian environments addressing gravity, radiation, and regolith. These three elements will have effects on multiple levels, including the individual microbe (gene expression), population of microbes (mutation rates, shifts in gene frequencies), and the community (multiple bio systems and chemical interactions). They suggested using the existing GeneSat system with its multi-well growth chambers. This would allow for bioengineered clonal cultures, wild-type species, and a multi-species microbial community. Experiments should commence in low earth orbit (LEO) to assess gravity effects and to use a simulated regolith. The next step would be high earth orbit (HEO) where cosmic ray environment could be experienced and assessed. Last would be microbial activity on the lunar surface—the real thing. An added note is that these will have to be long duration studies. It was brought out in the session that some analytical capabilities in any satellite experiments should include: imaging, optical density, gas sensors, fluorescence IR detection, GC mass spectrometry, microarrays, temperature sensors, radiation dosimeters, accelerometers, microfluidics, data storage downlink, and commanding capability. Some of these capabilities do already exist in the GeneSat equipment. The group also emphasized that such experiments are a necessary part of the total exploration initiative and can benefit the ISRU activities, Environmental Controlled Life Support Studies (ECLSS), human health and also affect the planetary protection aspects of the astrobiology program.

The primary intent of this group, as indicated in the introduction to the report, was to develop reference experiments for the Astrobiology Small Payloads Workshop which was conducted at Ames, June 18-20, 2007.

Conclusion

Although the question of the feasibility of regolith biomineralization is not fully answered, the presentations and discussions indicated that, given a source of water, the extraction of metals and other solid resources might be feasible. However the assessment by one of the discussion subgroups and by other leading biochemists and microbiologists (see Appendix A) is that microorganisms cannot extract molecular oxygen directly from lunar silicates and metal oxides. With sustained manned presence, excess carbon and water would likely be available to overcome some of the primary difficulties identified during the discussions. Simultaneously, the JSC Team is actively pursuing an ISRU program for a lunar landing with a recognition that far in the future regolith biomineralization may be possible. Participating Ames researchers indicated that microorganisms also can play key roles in human life support systems. In order to move forward on the subject of this workshop, we must learn more about the lunar regolith and the physical/chemical interactions that could exist. The consensus was that a *good* lunar simulant of sizeable quantity is sorely needed. There was also consensus that work done toward understanding the lunar surface and chemical or biological interactions on the lunar surface is an essential step to further defining potential roles for biological systems on Mars. The editors of this report wish to thank all the participants for their contributions, and Dr. Pete Worden, NASA/ARC director, for his enthusiastic support of this activity.

Appendix A

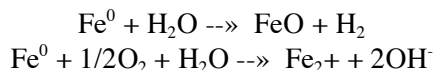
Biomining Lunar Oxygen: Realities and their Consequences

David Des Marais, May 10, 2007

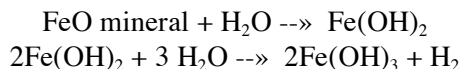
This statement by Des Marais and additional commentaries by Hoehler, Blankenship and Pierson in the pages that follow evaluate a proposal to extract molecular oxygen from lunar materials. That proposal was presented at the Biomining Workshop held at Ames Research Center on May 5 and 6, 2007. The commentary below draws from researchers who are recognized internationally for their expertise in redox chemistry, photosynthesis and biogeochemistry. These experts can discuss this topic further with the reader; their contact information is at the end of this white paper.

This review finds that the oxygen biomining strategy proposed at the Ames workshop is impossible to execute due to several fundamental factors. These factors arise from the chemical nature of lunar materials as well as the machinery of living organisms. Lunar regolith and rocks are chemically highly reduced; the lunar surface is an “oxidant desert.” While oxygen atoms are indeed abundant in lunar materials, this oxygen is in a chemically reduced state and therefore occurs as a mild reductant, not as an oxidant. Oxygen atoms bonded directly to the abundant iron atoms in lunar minerals are indeed readily chemically accessible, but this iron occurs only as ferrous and metallic iron. In order to extract oxygen from lunar materials, ferrous iron necessarily must be reduced to its metallic state.

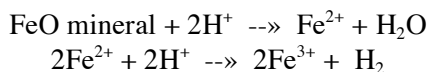
Water (H₂O) must be added to lunar materials in order to establish habitable conditions that would sustain any microbial “biominers.” However lunar materials are so chemically reduced that they will consume considerable amounts of H₂O before aqueous conditions can persist and sustain life. Metallic iron (Fe⁰) is present both in lunar regolith and in basalts at >1 wt. % (bulk regolith has 12 wt. % total Fe). This Fe⁰ rapidly consumes H₂O, for example,



Thus a considerable amount of oxygen is consumed even before microorganisms can become metabolically active. Lunar materials are so reduced that H₂O acts as an oxidant! An ongoing supply of oxidant (from Earth?) must be added the lunar material process stream, both initially to “prime the pump” by stabilizing an H₂O inventory and establishing aqueous conditions, and also continuously thereafter, because Fe⁰ in the lunar material would otherwise quickly consume the H₂O in the bioreactor. Even after aqueous conditions are established and Fe⁰ is totally consumed in a batch of lunar material, the reactions that can mobilize iron from its host minerals and oxidize it do not release oxygen and can actually consume even more oxidant. Oxygen that was formerly associated with the iron will end up in a reduced state in a stable chemical product. For example, at neutral to slightly alkaline pH:



Microorganisms have not developed the biochemical machinery to extract O₂ from these insoluble products, even if these products are solubilized. Iron ions that are extracted from minerals and oxidized at low pH are more soluble, for example:



Note that H₂O is produced when FeO is reacted. However an ongoing supply of H⁺ is required to facilitate these reactions, and H⁺ is an oxidant. Because hydrogen in lunar regolith is chemically reduced; an oxidant is required to produce H⁺. This discussion illustrates the unavoidable chemical consequences of the highly reduced state of lunar materials: they are formidable sinks of oxidants, not only when water is added but also when iron is mobilized. In summary, even if microorganisms could extract O₂ directly from lunar materials (which they cannot, see below), substantial quantities of oxidants must be consumed before microbes produce the first O₂ molecule.

Microbial sources of molecular oxygen. This section and those that follow explain in greater detail why microorganisms cannot execute a net extraction O₂ from lunar materials. Please read the attached subsequent essays by Dr. Robert Blankenship, a leading authority on the biochemistry of microbial photosynthesis, as well as the comments by Dr. Tori Hoehler, a recognized authority on the energetics of microbial biogeochemistry.

Oxygen generation is highly unfavorable thermodynamically from virtually all sources, even more so from reduced lunar materials. No known biological process converts Fe²⁺ and/or Fe³⁺ to FeO. Entirely novel biochemical processes and microorganisms must be devised to do so. Non-O₂-producing phototrophic bacteria indeed can oxidize H₂ to H₂O, Fe₂⁺ to Fe₃⁺, or H₂S to SO₄ but they require CO₂, a mild oxidant, which they reduce to organic carbon. These microorganisms cannot produce O or O₂ as a byproduct of these reactions. Cyanobacteria produce O₂ as a byproduct of the extraction of H from H₂O to synthesize organic biochemicals from CO₂. Oxygen-producing photosynthetic machinery cannot be adapted to produce O₂ from FeO instead of from H₂O. In fact FeO cannot even reach the reaction center in cyanobacteria where O₂ is produced. The reaction center in cyanobacteria where O₂ is generated cannot be modified by bioengineering to utilize FeO. Bioengineering can indeed create novel combinations of genetic and biochemical functions in microorganisms. Such functions must already exist somewhere in living systems, and this is not the case with some of the key steps in the proposed biomining scheme. The proposed extraction of O from FeO by cyanobacteria is far beyond the current reach of bioengineering technology.

Risk. The high risk associated with the proposal to biomine oxygen from lunar materials with cyanobacteria goes beyond the fact that it will not work. The continuing advocacy of this proposal would diminish the creditability of its advocates, and it might also diminish the perceived credibility of truly meritorious applications of biomining on the Moon.

Promising applications of biomining. The use of microbial ecosystems to recover and recycle oxidants and other key nutrients in functioning human life support systems is highly promising and does not require the innovation of unknown and potentially impossible biological capabilities. Biomining methods to extract metals on Earth are also well established and some of these might be adapted for the Moon. Such alternative applications rest on firm foundations of known biological capabilities and demonstrated principles of biotechnology.

Resources for additional information. Individuals who contributed to this set of reports and/or have relevant expertise are available to discuss this subject further.

Photosynthesis:

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Biological O₂ production from lunar regolith material:

Tori Hoehler, May 10, 2007

Biological O₂ production from lunar regolith material

Tori Hoehler, May 10, 2007

Overview: The problem of extracting O₂ from its native state in the lunar regolith (as illustrated here by ilmenite, FeTiO₃, the regolith mineral most commonly considered in abiotic O₂-producing schemes) boils down to trying to wrench electrons from the second most electronegative element on the periodic table. Chemically, this is (theoretically) possible in two ways: (1) Ilmenite could be reacted with a chemical oxidizing agent having a higher reduction potential than O₂. The supply of oxidant would have to be stoichiometrically greater than the yield of reductant, since the ferrous iron in ilmenite would be oxidized before any oxygen might conceivably be produced. All viable oxidants that meet the needed criteria (of having a higher reduction potential than O₂) are of higher molecular weight than O₂. Thus, the cost associated with supplying oxidant for this process (in terms of payload weight) would substantially outweigh the savings associated with harvesting O₂ from the regolith (likewise, any scheme to recycle such oxidant in situ using solar energy could be more productively and cost-effectively employed directly to oxygen production). (2) Ilmenite oxidation could be coupled – in an energetically unfavorable and therefore energy-requiring reaction – to reduction of a chemical oxidant with a lower reduction potential than O₂ (e.g., protons, CO₂, etc.). As in (1), this solution would require net import of oxidant to the Moon, with associated cost problems. It would almost certainly also require a biological (or, at very least, enzymatic) agent for coupling the process to the needed energy input. (3) Barring provision of an external agent to accept electrons from O₂, the electrons would have to be accepted within the ilmenite molecule itself, in the reaction $2\text{FeTiO}_3 \rightarrow 2\text{Fe} + \text{O}_2 + 2\text{TiO}_2$. Thermodynamically, this reaction is *extremely* unfavorable. The O₂ fugacity maintained by this reaction at equilibrium is 10⁻⁹³ atmospheres (corresponding to vastly less than one molecule of O₂ for the entire volume of the moon). Energy – almost certainly light energy – would be required to drive the process forward for production of viable amounts of O₂. The energy required is significantly greater than that transduced into liberation of O₂ during oxygenic photosynthesis.

1. **Known O₂-producing metabolisms.** There is one known metabolism among all terrestrial organisms that produces O₂ – oxygenic photosynthesis.
2. **Thermodynamics of O₂ production.** Thermodynamically, O₂ production is unfavorable from all but a few oxygen-bearing compounds. The compounds that do readily produce O₂ (that is, the ones that will yield O₂ without input of energy) – e.g., hydrogen peroxide, ozone, etc., are typically highly reactive and often, for that reason, find use as disinfectants (agents that tend to destroy living things). None of these compounds is present in significant abundance in the lunar regolith. To provide them would worsen, not solve, the cost problem associated with lifting O₂ to the moon, since, pound-for-pound, the mass of oxidant required to be lifted to the moon would be greater than the yield of O₂ obtained there. For all other compounds, O₂ production is not energy yielding and does not, therefore, represent a thermodynamically viable product of metabolism (where metabolism is the process of energy extraction from the environment). If O₂ is to be produced biologically, it will have to be as an unavoidable by-product of metabolism (a product of a reaction that an organism is obligated to perform for reasons other than energy harvesting). It will also require significant investment of energy to bring this about. Organisms would need (a) a good reason to divert significant energy into this process, (b) a biochemical means of carrying out the reaction, and (c) a mechanism for transducing the required amount of energy into the oxygen-producing reaction.
3. **Oxygen production by water photolysis.** The known metabolism of O₂ production by photosynthesis (as carried out by cyanobacteria and plants) exemplifies this point. The reaction $2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$ is extremely unfavorable in the forward direction. When written relative to production of H₂, $2\text{H}_2\text{O} \rightarrow \text{O}_2 + 2\text{H}_2$, the free energy change under physiological conditions is about +470 kJ/(mol O₂). Photosynthetic organisms obtain this energy by harvesting two photons of light, using antenna pigments that absorb at specific wavelengths, and invest it by *combining* these photon energies through an intricate and finely tuned electron transport scheme.

The cyanobacteria make this investment (and produce oxygen) specifically as a means of *obtaining electrons* for biosynthesis (= reduction of CO₂). Note that the oxygen yield of this process depends directly on provision of CO₂, the ultimate sink for electrons from water, in stoichiometric proportion.

4. **Oxygen production from ilmenite by biological metabolism?** Can we envision that cyanobacteria or any other organism will produce O₂ from something other than water? More specifically, can we envision that such production is possible by microbes utilizing ilmenite (2FeTiO₃ → 2Fe + O₂ + 2TiO₂)? Kinetic issues will certainly come into play, since we are considering a solid mineral: the organism would need to find a way to dissolve or chelate the mineral to get it inside the cell, and this would represent an additional and significant energetic cost to the organism. But let's assume that such a mechanism exists. The free energy change for the production of O₂ from ilmenite, as written above, is about +526 kJ/(mol O₂) – that is, it requires substantially more energy to get the oxygen out of ilmenite than to get it from water. This quantity of energy is so large that it likely rules out all but phototrophic metabolisms (Chemotrophic organisms are certainly capable of investing energy into chemical reactions, but must do so by expending ATP. To liberate O₂ from ilmenite would require that the energy of about 10 ATP be invested into a single reaction step. Enzymes that coordinate the reaction of this many molecules in a single step are unknown and extremely unlikely on biochemical/chemical grounds.) As with cyanobacteria, any phototrophic organism that might carry out the production of O₂ from ilmenite would have to have (a) a good reason for doing so, because of the extremely high energetic cost, (b) a way of channeling the needed energy into the reaction, and (c) a biochemical mechanism for doing so.
5. **Is an iron-reducing, oxygen-producing metabolism out there, undiscovered?** Bearing in mind that no organism is currently known to produce O₂ from ilmenite (or, more generally, from FeO, or from any other compound besides water), two possibilities remain: (1) There may be an as-yet unknown metabolism that carries out the desired reaction or (2) The desired metabolism might be “genetically engineered”. With reference to (1), let us again consider that an organism would need a good reason to have evolved a capability to do this process, since it will otherwise represent a (very large) waste of energy. To do so as a means of obtaining electrons (as the cyanobacteria do with water) would be redundant with respect to extant and energetically more favorable mechanisms. Any organism that might conceivably metabolize ilmenite for the purposes of obtaining electrons would be consistently presented with two alternative sources. First, in ilmenite itself, ferrous iron is a far more accessible (and energetically much less costly) source of electrons. Organisms are known that are capable of phototrophically oxidizing ferrous iron for the purpose of electron harvesting*. Second, water must be present for the organism to function. Use of water as an electron donor to photosynthesis is well known. Thus, it is not clear why O₂ production from ilmenite as a means of harnessing electrons would ever have had reason to evolve. It is not clear that any other reason (beyond electron-harvesting) exists for biology having developed a means of producing O₂ from ilmenite.
6. **Genetic engineering?** As it currently exists, genetic engineering allows us to take desired bits of metabolic capability (as a specific excerpt of the genome) from one organism and have them expressed in another organism. Because there is not currently a known metabolic capability for O₂ production from ilmenite (and because, I would argue, one probably doesn't exist) this is not a viable option. Protein engineering allows us to take an existing protein and, by modifying the sequence of amino acids that comprise it, alter its structure and (to a limited extent) its function. This is the only conceivable route to a biological system that makes O₂ from ilmenite. But in this case, we'd be talking not just about “tweaking” a single protein through a simple modification. The water-lysing enzyme itself would first have to be altered at a fundamental level – that is, both the binding site (which is conformationally and electrostatically adapted to water) and the remaining three dimensional structure (e.g., the channel through which substrate water passes into the binding site) would have to be comprehensively altered. In essence, a completely new enzyme would have to be designed and created. The ability to engineer new enzymes for specific catalysis, from scratch, lies well beyond the current grasp of biotechnology. Importantly, the

water-lysing step is one component in a closely connected sequence of events that also involves light harvesting (through antenna pigments that are tuned to specific wavelength ranges, which may be unsuitable for capturing and delivering the photon energies required for ilmenite lysis) and a series of electron transport steps. The water-lysing enzyme is an integral component in this chain and substantial alteration to accommodate the new function of ilmenite lysing would require that the new enzyme be “shoe-horned” into the existing (or likewise modified) photosynthetic apparatus). This amounts to recreating, almost from scratch, the apparatus that took more than one billion years to arise on Earth. I consider it extremely unlikely that this could be accomplished with currently or conceivably available biotechnology on a timescale relevant to lunar exploration.

Bottom line for research on biological production of O₂ on the moon: We know that O₂ production by microorganisms is possible – specifically, by cyanobacteria, using water as a substrate. No other means of O₂ production is known and I would argue on evolutionary and biochemical grounds that such a metabolism is unlikely to exist. I believe that genetic or protein engineering to create such a capacity is not feasible with current capabilities (or those conceivably available through advance of technology over the relevant time scale) because of the extensive alteration required. If microbes are to be considered in reference to O₂ biomining, the focus should be on cyanobacteria using locally available water. In this case, a carbon source would have to be provided in comparable stoichiometric abundance to the O₂ produced. Since electrolysis of water using electricity harvested from photovoltaic panels could accomplish the same thing, the relative costs, robustness, and engineering considerations of the abiotic and biotic systems should be considered.

* A note on iron cycling by microorganisms: Oxidation of ferrous to ferric iron by both anoxygenic phototrophic and oxygen-respiring bacteria is well known. Note, however, that the handling of ferrous iron by such organisms, even if conceived in terms of a hypothetical reaction in which FeO is converted to Fe³⁺ and “O”, does not solve the problem of O₂ production. The issue in creating O₂ is not to break the Fe-O bond – which will cleave heterolytically to yield “Fe²⁺” and “O²⁻” – it is to wrench electrons from one of the most electronegative elements on the periodic table. This will be highly energy demanding in all but a few situations, none of which represent cost-viable solutions to the problem at hand. Lacking the participation of a chemical species more oxidized than O₂, breakage of the Fe-O bond will simply result in O “keeping” the electrons (as “O²⁻”) and reacting with local water or protons to form hydroxide or water.

Cyanobacteria and Fe(II)

Beverly Pierson May 15, 2007

My lab has been studying the interactions of cyanobacterial-dominated microbial mats in high iron environments. Our earlier observations have been published and our most recent studies in collaboration with George Luther's lab are in a manuscript currently in review for possible publication in *Geochimica Cosmochimica Acta*. In this recent work we determined that rapid light-dependent oxidation of soluble Fe(II) in natural hot spring waters was mediated by oxygen produced by photosynthetic cyanobacteria. We studied the kinetics using microelectrodes.

All of our work has focused on the oxidation of soluble Fe(II). We have never studied interactions of cyanobacteria with metallic iron. Our earlier published studies showed that some cyanobacterial mats were stimulated by Fe(II) while others were inhibited by it.

In our current work, we have been studying the physiology of the entire photosynthetic community by measuring CO₂ fixation activity in suspensions of microbial mat in the absence of Fe(II) and in the presence of various concentrations of Fe(II). This work has not yet been submitted for publication but has been presented at meetings. In this work we have consistently seen a stimulation of CO₂ fixation in the presence of Fe(II) (200-600 μM) in some cyanobacterial populations. Inhibition is often seen at higher levels. Our data have been obtained from natural populations, not pure cultures, and can vary with the microbial mat source. These studies are complex to interpret but suggest the possible presence of direct photosynthetic oxidation of Fe(II) by some cyanobacteria. This "photoferrotrophic" activity by cyanobacteria has also been suggested by others but has not been proven--certainly not in our lab.

I would like to review some of the biological problems associated with iron-dependent photosynthesis that must be considered.

(1) If a functional photosystem II (PSII) is present that oxidizes water, it will do so. Other reductants pose serious difficulties.

(2) It could be possible to engineer cyanobacteria without a functional PSII reaction center. However, cyanobacteria are autotrophs and in order to fix CO₂ into organic carbon using PSI alone, a reductant must be supplied.

(3) Sulfide works as such a reductant and some cyanobacteria use it on Earth today, not because they have no PS(II) but because sulfide inhibits it and can be used by PSI.

(4) We and others suspect that in some cyanobacteria, soluble Fe(II) may function similarly, inhibiting PS(II) while supplying electrons to PSI to sustain CO₂ fixation.

(5) However, oxidation of Fe(II) poses some unique biological problems. Oxidation of Fe(II) produces Fe(III) which is not soluble at prevailing intracellular pH and will rapidly precipitate inside the cells. Consequently the membrane-bound reaction centers that would photo-oxidize the Fe(II) must be located in the cytoplasmic membrane where they can extract electrons from the Fe(II) at the surface of the cell leaving the insoluble ferric products in the periplasm or outside the cell. The efficient gathering of weak light energy by numerous internal thylakoids would be useless.

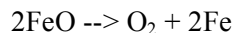
(6) Engineering iron utilizing cells requires more than engineering a reaction center redox function (difficult if not impossible in itself), but also requires major biological alterations to avoid any internal generation of Fe(III).

(7) Maintaining physiologically suitable pH in a bioreactor poses several challenges. I will cite 3 obvious ones below that we have had to consider and that have caused numerous frustrations in trying to obtain and sustain iron-oxidizing cultures of cyanobacteria. Buffering can be costly and must be considered because most cells do not function well over a very wide range of pH. Large fluctuations in either direction could not only affect the inorganic chemistry but also could be severely detrimental to the biological entities. The following 3 factors must be considered when growing autotrophic cyanobacteria in the presence of high levels of reduced iron: (a) The presence of any oxygen at all will rapidly oxidize Fe(II) to Fe(III) over a wide pH range. Even at pH 5.3 (the lowest we have used) the oxidation is rapid. (b) The oxidation of Fe(II) to Fe(III) is an acidification reaction. (c) CO₂ fixation rapidly raises the pH.

Photosynthetic Oxygen Production and Biomining

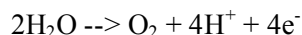
Robert Blankenship, May 9, 2007

The core proposal for lunar biomining is to have cyanobacteria carry out the following transformation:

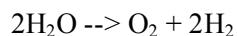


The iron in FeO would be reduced to metallic Fe and the O is being oxidized to O₂. I am not aware of any biological system that is able to produce metallic Fe. Certainly, cyanobacteria are not capable of this

transformation and I can't imagine that they could be engineered to do this. They do carry out the transformation:

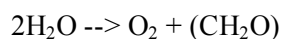


Here the water is oxidized to form O_2 --all of the O_2 that cyanobacteria produce comes from water through the action of Photosystem 2. The electrons are normally used to reduce CO_2 to organic matter. Some people are working to have them produce H_2 , so that the overall reaction would be:

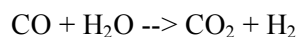


This is roughly equivalent thermodynamically to what the organisms are able to do normally. However, there have been major problems making that work. The enzymes that make H_2 are very sensitive to O_2 , so the products poison the catalysts. A lot of people are working extremely hard to accomplish this, but so far without much luck. Here they are leaving alone the part of the system that produces O_2 , which is the Mn center of Photosystem 2. No one has succeeded in doing genetic engineering on this system to alter the substrate selectivity. It has an active site buried well inside the membrane protein complex. Water diffuses down a long tunnel to get to the site of oxidation. It is inconceivable that a bulk phase mineral could go down that same tunnel. Mobilized Fe^{2+} in aqueous solution might be imagined to be able to be oxidized to Fe^{3+} , as is done by some purple photosynthetic bacteria, but that doesn't really help as the goal is to reduce the Fe, not oxidize it.

What the cyanobacteria could do is produce O_2 according to the following reaction:

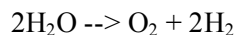


where CH_2O is organic matter. This requires that an ample source of H_2O and CO_2 be provided. You could make do with CO , as some other bacteria can convert it to CO_2 and H_2 according to the following reaction:



But that also requires a substantial source of H_2O , which I understand is difficult.

The bottom line is that I don't see any way to biologically transform FeO into Fe. It is probably worth comparing the thermodynamics of the processes as well. The reaction



has a standard state free energy change of about $+450 \text{ kJ mol}^{-1}$. The energy to drive it comes from light. I could not find thermodynamic data on FeO . I imagine that it is even more positive. But the real issue is with the enzymes that would be expected to do the transformations. I know of no enzymes in biology that produce metallic Fe. Without question, no known photosynthetic organism can do anything remotely like that and I think it would be extremely difficult to make it work using genetic engineering. You would be creating whole new metabolisms without anything to work from. Genetic engineering does pretty well when you have an enzyme that already does something similar and you want to alter its properties or maybe expand the range of possible substrates a bit. But starting completely from scratch, especially using minerals as substrates would be like shooting in the dark. Some enzymes do interact with minerals, but they are not at all well understood as a group.

Appendix B

Agenda

Day 1, Saturday May 5, 2007

8:00 - 8:15 am	Coffee
8:15 - 8:30 am	Pete Worden , Center Management Welcome

Presentations

8:30 - 9:00 am	Frank Roberto , INL – Proposal, History, and Current Status on Bio-mining and Regolith Applications
9:00 - 9:45 am	Paul Todd , SHOT – Terrestrial Extremeophiles for Extraterrestrial Environments
9:45 - 10:30 am	David McKay , NASA JSC – Considerations on Lunar Bio-leaching
10:30 - 10:45 am	Break
10:45 - 11:30 am	Ron Oremland , USGS – The Microbial Arsenic Cycle in Extreme Environments
11:30 - 12:15 pm	Chris McKay , NASA ARC – Lunar and Mars Studies
12:15 - 1:00 pm	Lunch with workshop discussions
1:00 - 1:45 pm	James Brierley , Brierley Consultancy LLC – Advances and Issues for Application of Bio-mining by Industry
1:45 - 2:30 pm	Patrick Fu , U. Hawaii at Manoa – Metabolic Engineering for the Biofuel Production
2:30 - 3:15 pm	Tore Straume , NASA ARC – A brief overview of the ionizing radiation doses on the surface of the Moon.
3:15 - 3:30 pm	Break
3:30 - 5:30 pm	Breakout Sessions as noted below

- a. Bio-community of choice for regolith interactions and suggested ground studies. Facilitator: Frank Robertob
- b. Physical/environmental issues and suggested ground studies. Facilitator: David Des Marais
- c. Develop reference experiments for the Astrobiology Small Payloads Workshop in June. Payloads could be developed for small satellites and ESMD Constellation test flights to LEO, HEO, lunar orbit, and the lunar surface. Facilitator: Orlando Santos

Day 2, Sunday May 6, 2007

9:00 - 10:30 am	Break Out Sessions Summary Presentations (30 Minutes Each)
10:30 - 12:00 pm	Next Steps Discussion

Appendix C

WORKSHOP ATTENDEES

NAME		ORGANIZATION	LOCATION	
David	Bayless	Ohio University	Athens	OH
Sharmila	Bhattacharya	NASA Ames Research Center	Moffett Field	CA
Paul	Blum	University of Nebraska	Lincoln	NE
James	Brierley	Brierly Consultancy, LLC	Highlands Ranch	CO
Igor	Brown	NASA Johnson Space Center	Houston	TX
Bin	Chen	NASA Ames Research Center	Moffett Field	CA
Jacob	Cohen	USRA, NASA Headquarters	Washington	DC
Bonnie P	Dalton	NASA Ames Research Center, Ret.	Moffett Field,	CA
Wanda L	Davis	SETI Inst./NASA Ames	Moffett Field	CA
Dave	Des Marais	NASA Ames Research Center	Moffett Field	CA
Lauren	Fletcher	Stanford University/ NASA ARC	Moffett Field	CA
Pengcheng	Fu	University of Hawaii at Manoa	Honolulu	HI
Beverly	Girten	NASA Ames Research Center	Moffett Field	CA
Edward	Goolish	NASA Ames Research Center	Moffett Field	CA
Esther	Hill	Lockheed Martin, Ames Research Center	Moffett Field	CA
John	Hogan	NASA Ames Research Center	Moffett Field	CA
Steve	Howe	Idaho National Laboratory	Idaho Falls	ID
Linda	Jahnke	NASA Ames Research Center	Moffett Field	CA
Jeff	Jones	NASA Johnson Space Center	Houston	TX
John	Karcz	SETI Inst./NASA Ames Research Center	Moffett Field	CA
Bishun	Khare	SETI Inst./NASA Ames Research Center	Moffett Field	CA
Melissa	Kirven-Brooks	NASA Ames Research Center	Moffett Field	CA
Timothy	Lee	NASA Ames Research Center	Moffett Field	CA
Darlene	Lim	NASA Ames Research Center	Moffett Field	CA
Oana	Marcu	NASA Ames Research Center	Moffett Field	CA
Andrew	Mattioda	SETI Inst./NASA Ames Research Center	Moffett Field	CA
David	McKay	NASA Johnson Space Center	Houston	TX
Lee	Morin	NASA Johnson Space Center	Friendswood	TX
Ronald	Oremland	US Geological Survey	Menlo Park	CA
Lee	Prufert-Bebout	NASA Ames Research Center	Moffett Field	CA
Frank	Roberto	Idaho National Laboratory	Idaho Falls	ID
Farid	Salama	NASA Ames Research Center	Moffett Field	CA
Chad	Saltikov	University of CA, Santa Cruz	Santa Cruz	CA
Orlando	Santos	NASA Ames Research Center	Moffett Field	CA
Kevin	Sato	Lockheed Martin, Ames Research Center	Mtn. View	CA
Marianne	Steele	Lockheed Martin, Ames Research Center	Moffett Field	CA
Carol	Stoker	NASA Ames Research Center	Moffett Field	CA
Paul	Todd	SHOT, Inc	Greenville	IN
Pete	Worden	NASA Ames Research Center	Moffett Field	CA

