MICROBIAL EXTRACTION OF HYDROGEN FROM LUNAR DUST

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If molecular hydrogen in lunar dust can be made available to the hydrogenases of bacteria, then several microbial pathways exist for the potential liberation of hydrogen, carbon dioxide, and methane using relatively simple apparatus. Intermediate products include microbial biomass and short chain organic acids such as acetate. The hydrogen could be harvested, and carbon dioxide, phosphate, nitrogen, and trade nutrients could be recycled. All reactions suggested in this paper, or similar ones, have been demonstrated on Earth, with the exception of the initial utilization of hydrogen from lunar fines by bacterial hydrogenases. However, it is possible to test these reactions on extant samples of lunar fines. If potentially toxic elements in lunar soils present problems for such processes, bacterial tolerance can be induced by plasmid transfer or by selection among cells subjected to increasing levels of these elements.

INTRODUCTION

A future permanent human colony on the Moon should be as independent from Earth as possible. Rocket fuel, for instance, should be produced from lunar resources to reduce the high costs of transportation from Earth (Duke *et al.*, 1985). Detailed analyses of samples brought back during the Apollo missions have shown that lunar regolith fines contain, mostly in their 20 μm size fraction, substantial amounts of extractable hydrogen (Bustin *et al.*, 1984). The H_2 concentrations varied from 50–220 ppm; average values given were 100–150 ppm, or 100–150 μg/g regolith fines. This hydrogen can be released by heat. The economic viability of the lunar base could be greatly improved if the hydrogen held in the lunar regolith fines could be harvested by bacteria, which would be needed anyway to aid in the recycling of human organic wastes.

Bacteria offer particularly versatile mechanisms of harvesting lunar hydrogen as they contain self-correcting features such as multiple homeostatic abilities to regulate their microenvironment. They show genetic versatility that allows them to compensate for a wide variety of conditions. Bacteria can be thought of as self-replicating catalysts with the power to adapt to their environment.

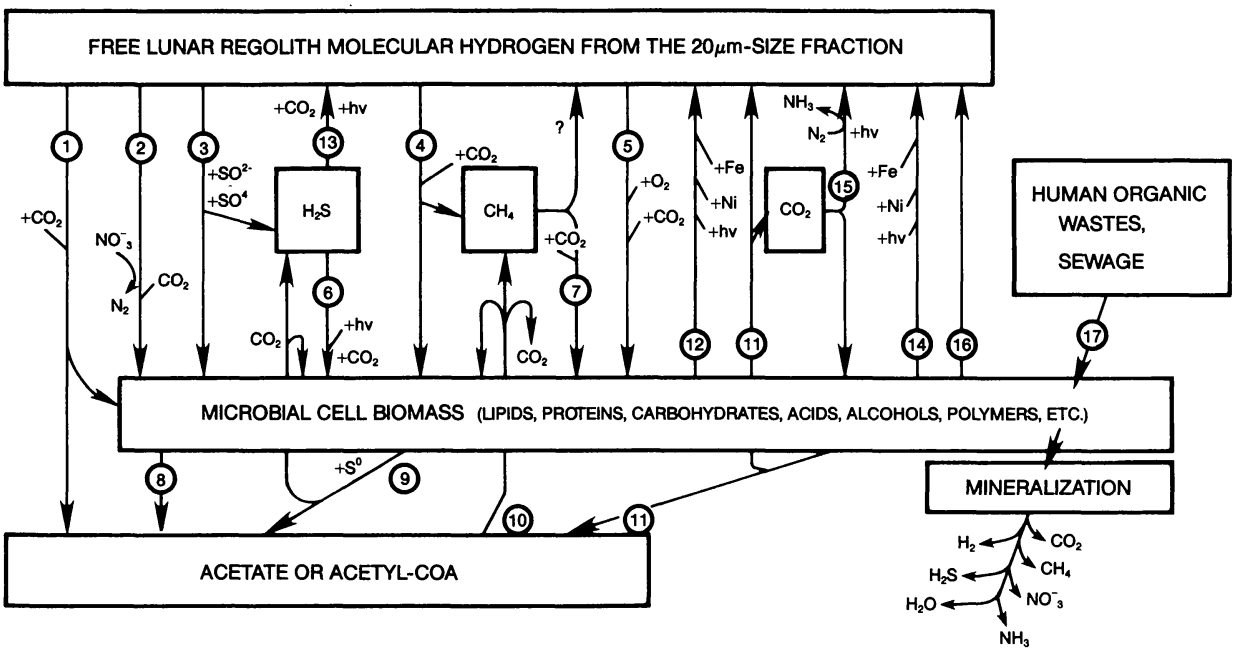


Figure 1. Possible reactions by which molecular hydrogen could be harvested from lunar dust with microbial activities. 1-Acetobacterium woodii, A. carbinolicum, Methanobacterium thermoautotrophicum, Clostridium thermoaceticum, Sporomusa sphaeroides. 2-Paracoccus denitrificans. 3-Desulfovibrio vulgaris; Campylobacter *sp.* 4-Methanobacterium *spp. 5-see Table 2.* 6-Chromatiaceae, chlorobacteria. 7-Rhodopseudomonas gelatinosa. 8-Acetobacterium carbinolicum, Ilyobacter polytrophus. 9-Thermococcus celer, Desulfuroccus mucosus. 10-Methanosarcina barkeri. 11-Acetomicrobium flavidum, Acidaminobacter hydrogenoformans. *12-Purple bacteria.* 13-Chromatiaceae. 14-Rhodospirillaceae. 15-Cyanobacteria. 16-Escherichia coli. *17-Anaerobic sewage fermentations; aerobic treatment.*

We propose that especially anaerobic bacteria could serve as a mechanism for harvesting the lunar hydrogen. The microbial biomass and organic compounds into which the hydrogen would be fixed initially could then be converted to molecular hydrogen by employing special bacterial strains or consortia and special conditions (Fig. 1). With the exception of the initial reaction whereby hydrogen is released from lunar dust, and whereby the bacteria are subjected to possibly toxic regolith mineral components, all subsequent steps are well established in microbial systems existing on Earth.

In a first step, anaerobic bacteria utilizing the hydrogen could create organic acids, CO_2, H_2S, or CH_4, as well as biomass. As in terrestrial sewage plants, anaerobic digesters could produce more methane from this biomass. Immobilized anaerobic and phototrophic bacteria in illuminated reaction chambers could generate CO_2 and H_2. After harvesting the hydrogen, the CO_2, phosphate, nitrogen, and trace nutrients could be recycled.

At a later stage, with oxygen available from iron-oxide-containing minerals, aerobic processes could generate water from the bacterial oxidation of hydrogen. Also, aerobic methane-oxidizing bacteria would convert CH_4 and CO_2 into water and biomass. The

water could be dissociated into oxygen and hydrogen. Aerobic hydrogen release by N_2-fixing cyanobacteria (blue-green algae) would be another possibility that could be explored.

ENVIRONMENTAL CONDITIONS NEEDED FOR BACTERIAL CULTIVATION

The conditions required are relatively simple to engineer. The necessary bacterial cultures can be brought from Earth in the form of either lyophilyzed or deeply-frozen strains. Lyophilization (freeze-drying) is performed by exposing well-grown cultures to very high vacuum in the cold. The dried cells are sealed in glass vials while attached to the vacuum line. Lyophilized cells are extremely resistant to noxia such as temperature changes, *etc.* As they do not grow in this dry state, they cannot express mutations occurring occasionally due to radiation. Transportation from Earth to the Moon in a spaceship would, therefore, be possible. No measurable losses of viability were noted with *Hydrogenomonas eutropha* Z1 and *Escherichia coli* K 12 when flown aboard the Russian satellite Cosmos 368 or aboard the automatic lunar station Zond 8 (Taylor, 1974).

The pressure would be optimized by engineering studies and would most likely not exceed several Earth atmospheres. Fermenters for all of these reactions could be opaque, but in the case of photodissociation of organics by purple or green bacteria, some protection from high light intensities and from short wavelength ultraviolet light would be needed. Nitrogen, sulfur, and possibly phosphorus, as well as most trace minerals necessary for the functioning of specific enzymes, would be available from lunar regolith fines. The relative concentrations of these might have to be altered.

In the first stage of an optimal system, the lunar fines would be added to anaerobic fermenters and the hydrogen harvested in the form of bacterial biomass and fermentation products. The second stage would utilize reactors in which phototrophic anaerobes (purple or green bacteria) could be immobilized on special surfaces and generate H_2 in the light. The reactor fluid and other gases containing CO_2, as well as phosphate, sulfur, nitrogen, trace minerals, vitamins, and water would be recycled, partly by microbial processes ("mineralization"), partly chemically.

All organic wastes and sewage generated by the lunar population would be collected and fermented much as in anaerobic sewage treatment facilities on Earth, to provide additional products for the photoreactors.

In the third stage, with oxygen available from iron-oxide-rich lunar minerals, aerobic autotrophic bacteria or unicellular green algae would be employed as well. Especially selected or engineered aerobic strains with high hydrogenase activity would be able to tolerate stress conditions of the lunar environment. These aerobic organisms could extract H_2 from the regolith in explosion-proof fermenters; their Knallgas reaction would form water and biomass.

Some recently discovered archaebacteria (*Sulfolobus ambivalens*) grow either anaerobically with H_2, S^0, and CO_2 to form H_2S and cell biomass or aerobically with

S^0, O_2, and CO_2 to form sulfate (Zillig *et al.*, 1985). To work with bacterial strains of great metabolic versatility would certainly be advantageous.

HYDROGEN UTILIZATION

The initial reaction, which is critical to the whole operation, requires the release of hydrogen from the lunar soil. Although pretreatments may be required, the essential interaction is between the enzyme hydrogenase in the bacteria and the lunar hydrogen. Well-known hydrogenases for the initial anaerobic step occur in two candidate microbes: the sulfate-reducing and the methanogenic bacteria. Sulfate-reducing bacteria form biomass and H_2S from SO_4^{2-}, H_2, and CO_2 (plus trace minerals and possibly some vitamins). The Michaelis-Menten constant K_m, for growth with hydrogen-limiting conditions for a number of SO_4 reducers, is $2 - 4 \times 10^{-6}$ Molar (Robinson and Tiedje, 1984). (The K_m is the concentration of H_2 at which growth is at the half maximal rate.) Methanogenic bacteria that form CH_4 and biomass from H_2 and CO_2 have K_m values of $6 - 7 \times 10^{-6}$ Molar hydrogen. Sulfate reducers thus can outcompete the methanogens for hydrogen. The H_2S generated from sulfate-reducing bacteria may pose corrosion problems in the reactors, as it does on Earth, but, of the organisms that have been well studied, these have the highest known affinity for hydrogen.

Table 1. Anaerobic Bacterial Hydrogen Consumption Yielding Various Reduced Compounds and Cell Biomass (CBM)

Organisms	Reactions	References
Paracoccus denitrificans	$5H_2 + 2H^+ + 2NO_3^- \longrightarrow N_2 + 6H_2O + CBM$	Schlegel and Schneider (1978)
Campylobacter sp.	$H_2 + S^0$ (or $S_2O_3^{2-}$, or SO_3^-) $\longrightarrow HS^- + H + CBM$	Laanbroek *et al.* 1978
Desulfovibrio vulgaris (strain Marburg)	$4H_2 + H^+ + SO_4^{2-} \longrightarrow HS^- + 4H_2O + CBM$	Schlegel and Schneider (1978)
Methanobacterium sp.	$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O + CBM$	Mah and Smith (1981)
Methanosphaera stadtmaniae	$H_2 + CH_3OH \longrightarrow CH_4 + H_2O + CBM$	Miller and Wolin (1985)
Vibrio succinogenes	$H_2 + fumarate^{2-} + \longrightarrow succinate^{2-} + CBM$	Schlegel and Schneider (1978)
Acetobacterium carbinolicum	$4H_2 + 2HCO_3^- + H^+ \longrightarrow acetate + 4H_2O + CBM$	Eichler and Schink (1984)
Acetobacterium woodii	$H_2 + CO_2 \longrightarrow acetate + CBM$	Ragsdale and Ljungdahl (1984)
Methanobacterium thermoautotrophicum	$8H^+ + 2CO_2 \longrightarrow$ acetyl-CoA (acetate) + CBM	Stupperich and Fuchs (1984)
Sporomusa sphaeroides	$28H^+ + 7CO_2 \longrightarrow acetate + CBM$	Möller *et al.* (1984)
Clostridium thermoaceticum	$H_2 + CO_2 + CoASH + methyl\text{-}THF \longrightarrow$ acetyl-CoA + THF + CBM	Pezachka and Wood (1984)

Since the reaction of the lunar hydrogen with these bacteria is the untested portion of our harvesting scheme, we propose that a large number of different bacterial types be tested. Suggested anaerobic bacteria are listed in Table 1. Products of hydrogen fixation are water, sulfide, methane, succinate or acetate, and cell biomass.

It is also possible to create microbial biomass and water from hydrogen aerobically, once oxygen becomes available either as an import or as a locally produced commodity. The process requires digesters with vigorous aeration, the engineering of which is well known. A selected list of aerobic bacterial candidates is given in Table 2. The list contains representatives of very different physiological groups. Some aerobic H_2 bacteria excrete large amounts (up to 5g/l) of organic compounds, especially when NH_4^+ and oxygen are limiting (Vollbrecht *et al.*, 1978).

Table 2. Aerobic Bacteria with the Ability for Hydrogen Consumption and the Formation of Water and Cell Biomass (CBM)

Organisms	References	Organisms	References
Pseudomonas saccharophilia	Schlegel and Schneider (1978)	*Corynebacterium* sp.	Jochens and Hirsch, (personal communication (1985)
Alcaligenes eutropha	Schlegel and Schneider (1978)	*Azospirillum lipoferum*	Malik and Schlegel (1981)
Seliberia sp.	Schlegel and Schneider (1978)	*Derxia gummosa*	Malik and Schlegel (1981)
Comamonas sp.	Schlegel and Schneider (1978)	*Rhizobium japonicum*	Malik and Schlegel (1981)
Hydrogenobacter sp.	Schlegel and Schneider (1978)	*Microcyclus aquaticus*	Malik and Schlegel (1981)
Xanthomonas autotrophicus	Schlegel and Schneider (1978)	*Microcyclus eburneus*	Malik and Schlegel (1981)
Rhizobium leguminosarum	Schlegel and Schneider (1978)	*Renobacter vacuolatum*	Malik and Schlegel (1981)
Mycobacterium phlei	Hirsch (1961)	*Rhodopseudomonas capsulata* (dark)	Gogotov (1984)
Nocardia opaca	Hirsch (1961)	*Rhodospirillum rubrum* (dark-grown)	Gogotov (1984)
Nocardia hydrocarbonoxides	Hirsch (1961)	*Rhodopseudomonas acidophila* (dark)	Schlegel and Schneider (1978)
N. petroleophila	Hirsch (1961)	*Gloeobacter* sp. 7421	Howarth and Codd (1985)
N. autotrophica	Hirsch (1961)	*Synechococcus* sp. 6307	Howarth and Codd (1985)
Nocardia sp. from Antarctic rocks	Hirsch (personal communication, 1985)		

METHANE UTILIZATION

Organic wastes from human activities and the combined cellular biomasses produced anaerobically or aerobically would be fermented in anaerobic digesters resulting in the production of methane, carbon dioxide, and possibly some short chain organic acids, using technology already well studied on Earth.

Numerous species of aerobic bacteria are known to oxidize methane (Whittenbury *et al.*, 1970): *Methylomonas, Methylycoccus, Methylosinus* spp. From the anaerobic fermenters they could harvest methane, a particularly attractive product as it is easily handled. These bacteria form water and cell biomass in the presence of oxygen. They can also fix nitrogen if the level of oxygen is low. The water produced would be harvested and electrochemically dissociated into hydrogen and oxygen. It could also be dissociated biologically by using immobilized cyanobacteria (Kayano, *et al.*, 1981).

BIOLOGICAL RELEASE OF HYDROGEN

The terminal stage of the process involves the production of H_2. Numerous phototrophic bacteria can be grown under conditions where hydrogen is released in the light. We have summarized some of these reactions in Table 3; they are examples of a large number

Table 3. Microbial Hydrogen Evolution from Various Substrates

Organisms	Reactions	References
Rhodopseudomonas sp., *Thiocapsa* sp., *Chromatium* sp., *Thiocystic* sp.	lactate, acetate, ethanol, S^0, or $S^{2-} \xrightarrow[\text{anaerobic}]{\text{light}} H_2$ + oxidized substrate + CBM	Gogotov (1984); Matheron and Baulaigne (1983)
Rhodopseudomonas sp. (strain MPBE 2271)	malate $\xrightarrow[\text{anaerobic}]{\text{light}} H_2 + CO_2$ + CBM	Mitsui *et al.* (1983)
Escherichia coli	sugars, pyruvate, or formate $\longrightarrow X + 2H^+ + CBM + \longrightarrow H_2$	Adams *et al.* (1981)
Acetomicrobium flavidum	hexose + $2H_2O \xrightarrow{58°C} 4H_2 + 2CO_2$ + 2 acetate + CBM	Soutschek *et al.*(1984)
Clostridium sp.	pyruvate or molasses $\xrightarrow{\text{ferredoxin}} H_2$ + CBM	Schlegel and Schneider (1978); Suzuki *et al.*(1983)
Rhodopseudomonas gelatinosa	$CH_4 + CO_2 \xrightarrow[\text{anaerobic}]{\text{light}}$ CBM + H_2?	Wertlieb and Vishniac (1967)
Nostoc sp. *Gloeobacter* sp. 7421	$N_2 + CO_2 \xrightarrow[\text{air}]{\text{light}} H_2 + H_2O + O_2 + NH_3$ + CBM	Kerfin and Böger (1982), Howarth and Codd (1985)
Synechococcus sp.	$N_2 + Co_2 \xrightarrow[\text{air}]{\text{light}} H_2 + H_2O + O_2 + NH_3$ + CBM	Mitsui *et al.*(1983)
Acetaminobacter hydrogenoformans	glutamate $\xrightarrow[\text{anaerobic}]{} H_2$ + acetate + CO_2 + propionate or formate	Stams and Hansen (1984)

of similar reactions known to occur in bacteria and even in green algae. In the latter case, strains of *Chlorella pyrenoidosa, C. vulgaris,* or *Selenastrum gracile* actively produce hydrogenase approximately four hours after onset of anaerobiosis (Kessler and Maifahrth, 1960). The hydrogen production of immobilized cells of *Clostridium butyricum* has been studied quantitatively by Suzuki *et al.* (1983). Three kilograms of wet cells produced, from molasses, 400–800 ml/min^{-1} H_2 continuously over a 2-month period. An engineering analysis of photosynthetic hydrogen production systems was carried out by Herlevich *et al.* (1983), who calculated the cost of production.

Possibly a reisolation of the *Rhodopseudomonas* observed by Wertlieb and Vishniac (1967) would be attempted. This bacterium oxidized CH_4 with CO_2 in light, anaerobically, with the formation of cell biomass. Under nitrogen-limiting conditions, it might be possible to induce the organism to release hydrogen in the light. Of course, it is also possible to develop an abiotic process to photochemically release hydrogen from methane.

The metabolic versatility of non-sulfur purple bacteria (*Rhodospirillaceae*) could be advantageous. These organisms readily utilize hydrogen as an electron donor for photoautotrophic growth (Stanier *et al.*, 1976). The mechanisms used by these anaerobes resemble those of aerobic hydrogen bacteria (Schneider and Schlegel, 1977), but aerobic hydrogen consumption requires the absence of light. In the presence of light, under anaerobic conditions, nitrogen limitation induces N_2-fixation, and their nitrogenase releases H_2.

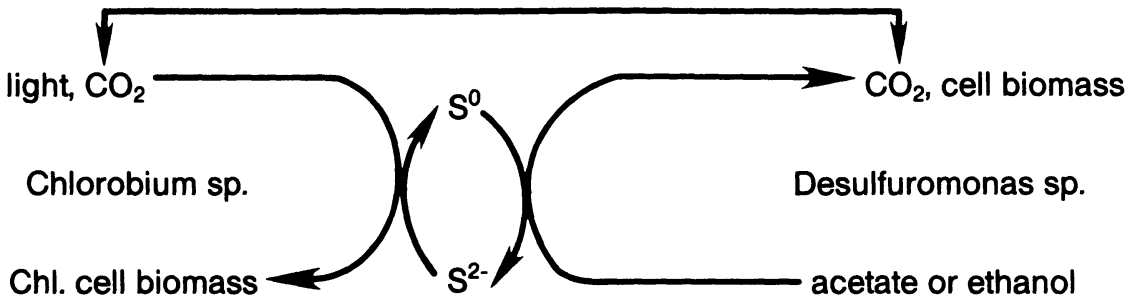
INTERMEDIATE REACTIONS YIELDING ACETATE

Conversion of various organic excretion products to acetate may become necessary. Acetate is a possible precursor for methane (Mah *et al.*, 1978) or photosynthetic hydrogen evolution (Table 3). *Ilyobacter polytrophus* ferments 3-hydroxybutyrate, crotonate, pyruvate, citrate, glucose, fructose, malate, or fumarate, to form acetate as well as some formate, propionate, and butyrate (Stieb and Schink, 1984). Another acetogenic bacterium is *Acetobacterium carbinolicum*, which ferments formate, methanol, aliphatic alcohols, 1,2-diols, 2,3-butanediol, acetoin, glycerol, lactate, pyruvate, or hexoses (Eichler and Schink, 1984). Glutamate is converted to acetate, CO_2, formate, propionate, and H_2 by *Acidaminobacter hydrogenoformans* (Stams and Hansen, 1984). Such versatile bacteria would be preferred to fermentation specialists that could utilize only few compounds.

FORMATION OF REDUCED SULFUR COMPOUNDS

Hydrogen fixation by sulfate-reducing bacteria has already been mentioned (Table 1). Several newly discovered anaerobic bacteria utilize organic compounds as hydrogen donors and S^0 as the hydrogen acceptor. *Desulfurococcus mucosus* and *D. mobilis* grow with yeast extract or casein as carbon and hydrogen source and need S^0 as the acceptor, producing sulfide and CO_2 (Zillig *et al.*, 1982). These organisms are extremely thermophilic, requiring 85°C for optimal growth. Similar reactions with peptides are carried out by *Thermococcus celer*, a moderately halophilic thermophile that requires 88°C and 40 g/l of NaCl for growth (Zillig *et al.*, 1983). Finally, sulfide production from S^0 and acetate

has been described for *Desulfuromonas acetoxidans*, an anaerobic bacterium that lives syntrophically with green phototrophic sulfur bacteria of the genus *Chlorobium* (Pfennig and Biebl, 1976):



The sulfide-hydrogen that accumulates in such reactions would be transferred into purple bacterial biomass (*Chromatiaceae*) through their anaerobic photosynthesis. Under aerobic conditions, the sulfide-reducing equivalents could be harvested by sulfide-oxidizing bacteria such as *Beggiatoa* spp., *Thiothrix* spp., *Thiobacillus* spp., *etc.*

POSSIBLE TOXICITY OF LUNAR REGOLITH COMPONENTS

In the first reactions proposed here, the H_2-utilizing bacteria and their enzymes will be in direct contact with the lunar regolith fines. Analyses of Apollo soil samples taken from mare, highland, or basin ejecta show relatively high concentrations of some potentially toxic elements (Table 4). Although some of the elements occur in higher concentrations

Table 4. Concentration Ranges of Selected Lunar Soil Elements with Potential Toxicity to Microorganisms

Element	Concentration Range in Lunar Soil (μg/g soil)
As	0.01–0.41
Ba	85.7–767.5
Be	1.2–5.5
Cd	0.03–0.8
Cr	700.0–3600.0
Cu	6.4–31.0
F	37.0–278.0
Mn	500.0–1900.0
Ni	131.0–345.0
Pb	0.8–10.0
Se	0.03–0.39
Sr	104.2–234.0
U	0.26–3.48
Zn	6.3–49.0

From Freitas and Gilbreath (1982)

on Earth, their combined presence could be detrimental to normal terrestrial bacteria. Lunar samples containing high chromium and manganese may have to be tested for such toxic effects.

Microoganisms can develop extreme tolerances to heavy metals and some rare elements (Tyler, 1981). The detoxification can be accomplished by several different methods. Cell walls of bacilli and filamentous fungi can sequester heavy metals. Chitin or chitosan of *Rhizopus arrhizus* can sequester uranium or thorium at amounts of up to 20% of the organism's dry weight (Volesky *et al.*, 1983). Cadmium resistance in *Staphylococcus aureus* is, for example, plasmid-bound, due to an increased Cd-efflux system [2 Cd^{2+}/ $2H^{+}$ antiporter (see Foster, 1983)]. Arsenate resistance is also plasmid-bound and linked to arsenite and antimony resistance (Foster, 1983). In *Pseudomonas putida*, cadmium resistance is due to synthesis of three novel proteins rich in cysteine [MW 4000–7000 (see Higham *et al.*, 1985)].

Information on the physiological effects of most of these potentially toxic regolith components on microorganisms is scanty. Although some of the elements listed in Table 4 are probably needed as "trace elements," the concentration ranges offered to the bacteria are extremely critical. Therefore, experiments will have to be conducted in which a large number of bacterial test strains are exposed to various lunar soil samples under simulated lunar reactor conditions.

SOME FINAL THOUGHTS

At the present time, it does not seem to be possible to send experimental cultures of bacteria to the Moon to study their longevity and activities under lunar conditions. However, the "Biostack" experiments of the Apollo 16 and 17 missions have shown that living microorganisms can indeed be carried safely to the lunar surface and back (Taylor, 1974). *Bacillus subtilis* spores embedded in polyvinyl alcohol sheets were subjected to high-energy, multicharged ("HZE") particles, as indicated by tracks registered in special dosimeters. After this treatment, the *Bacillus subtilis* spores germinated, grew, and elongated just as well as the untreated ground controls did.

The quantitative aspects of the hydrogen-harvesting processes are difficult to assess. We assume an average bacterial cell composition as described in Table 5. We can further assume for the sake of calculation that respiration or other processes would not result

Table 5. Composition of an Average, Growing Bacterium

Water	80 %	
Dry matter	20 %	
carbon		45–55 %
oxygen		20 %
nitrogen		10–15 %
hydrogen		10 %
phosphorus		2–6 %
other elements		5 %

From Schlegel, 1984.

in loss of cell substance during the synthesis of 1 g of microbial biomass. Living bacterial substance should then be composed of water (800 mg/g) and dry matter (200 mg/g). This dry biomass would then consist of approximately 50% (or 100 mg) carbon, 20% (or 40 mg) oxygen, 10% (or 20 mg) nitrogen, 10% (or 20 mg) hydrogen, 5% (or 10 mg) phosphorus, and 5% (or 10 mg) of other elements.

To extract 20 mg hydrogen from an average lunar soil (size fraction $< 20\ \mu m$, containing 100 ppm hydrogen), we would need 200 g regolith fines. Since energy is indeed required to construct a living organism, one might have to spend perhaps 1–2 kg of lunar dust for the production of 1 g of living bacteria. With respect to carbon, and assuming that 5–10 g of soil contain 1 mg C, the construction of 1 g living bacteria would require at least 0.5–1.0 kg of soil. The availability of carbon for the bacteria has not been considered in the calculation, but elemental carbon has not been known to be utilized by bacteria. For the nitrogen, 200 g of soil would probably be sufficient. In total, 3–5 kg of lunar soil would allow the construction of 1 g living bacteria—always assuming that the elements can be offered in a "palatable" form.

The availability of versatile bacteria with many different metabolic possibilities for the utilization of lunar regolith hydrogen has been demonstrated. Next should be an assessment of actual yields of these reactions under (simulated) lunar conditions. Combinations of different organisms to form active consortia or syntrophic mixtures would have to be tested for optimal metabolic activity and longevity. Genetic engineering might be necessary to "train" the selected bacteria for tolerances to lunar stress conditions. Thus, artificial microcosms (ecosystems) will have to be created, and their homeostasis (ability to return to original activity after a perturbation) will have to be optimized, possibly by combining a large number of different microorganisms to perform a given reaction. This latter activity represents a challenge to present-day microbiologists who plan future lunar colonies.

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