International Journal of Astrobiology 10 (4): 315–324 (2011)
doi:10.1017/S1473550411000164

Synthetic geomicrobiology: engineering microbe–mineral interactions for space exploration and settlement

Charles S. Cockell

Open University, Milton Keynes MK7 6AA, UK
e-mail: c.s.cockell@open.ac.uk

Abstract: Synthetic geomicrobiology is a potentially new branch of synthetic biology that seeks to achieve improvements in microbe–mineral interactions for practical applications. In this paper, laboratory and field data are provided on three geomicrobiology challenges in space: (1) soil formation from extraterrestrial regolith by biological rock weathering and/or the use of regolith as life support system feedstock, (2) biological extraction of economically important elements from rocks (biomining) and (3) biological solidification of surfaces and dust control on other planetary surfaces. The use of synthetic or engineered organisms in these three applications is discussed. These three examples are used to extract general common principles that might be applied to the design of organisms used in synthetic geomicrobiology.

Received 18 February 2011, accepted 14 April 2011, first published online 27 May 2011

Key words: synthetic geomicrobiology, biomining, soil formation, regolith, Mars, moon.

Introduction

Geomicrobiology deals with the study of the interactions of microbes with minerals (Konhauser 2007; Dong & Yu 2007; Erhlich & Newman 2009). On the Earth, geomicrobiology has a wide diversity of applications to practical problems that have both social and economic relevance. They include: (1) the use of microorganisms to accelerate the recovery of economically valuable elements from ores (called biomining) (Schippers *et al.* 2010), (2) the use of microorganisms in bioremediation of contaminated soils (Gadd 2010) and (3) the use of microorganisms to produce minerals of practical use (Templeton & Knowles 2009).

Geomicrobiology is a science that arose from the recognition that for microorganisms to exist in and on the Earth's crust for over 3.5 billion years, they have developed an exquisite set of capabilities for manipulating minerals (Benzerara & Menguy 2009), either dissolving them to access useful elements (Welch *et al.* 2002; Glowa *et al.* 2003), or creating new minerals, often as a result of their energy-yielding reactions (e.g. Kappler *et al.* 2004; Miot *et al.* 2009). These capabilities intersect with humanity's similar need to manipulate and exploit the Earth's crust. In this common need humans have learnt to exploit the many capabilities of microbial interactions with minerals.

Just as humans have used geomicrobiology on the Earth, it is inevitable that they will also use this science in space (Cockell 2010). There are a wide variety of potential applications of microbe–mineral interactions in space, including the use of microorganisms in fuel cells and the production of novel minerals (catalysts) required in space applications.

In this paper, previously published data are described from experiments in three specific areas of geomicrobiology applied to space settlement. These data are used to propose new concepts and possible approaches in synthetic biology. The data are also used to propose attributes that might be introduced into engineered microbes in each of these applications and to identify a set of 'core' attributes that might be introduced into any microorganisms used in space geomicrobiology.

Soil formation/microbial reactor feed from extraterrestrial regolith

Background

Soils are vital for a range of extraterrestrial uses, particularly in plant and crop growth (Ming & Henninger 1994). Although from a terminological perspective, some extraterrestrial regoliths might be classified as soils (Certini & Scalenghe 2010), planetary regolith is generally a poor substrate for agricultural use. Low organic carbon to sustain fungi and bacteria associated with the rhizosphere, poor water-holding potential because of low organic carbon, unweathered mineral constituents that result in low biological accessibility of their bioessential elemental constituents, are just some of the factors that make asteroidal, lunar or Martian regolith suboptimal substrates for plant growth without modification.

However, although most regoliths are suboptimal for growth, with some modification they are potentially useful. Volcanic soils (andisols), the weathering products of volcanic rocks such as basalt, are recognized to be some of the most productive soils on the Earth (Dahlgren *et al.* 1993). The abundance of basaltic and other volcanic rocks on the lunar (Greenhagen *et al.* 2010) and Martian surface (e.g. Ruzicka *et al.* 2001; Christensen *et al.* 2004) make these materials potentially useful for plant growth since they could provide

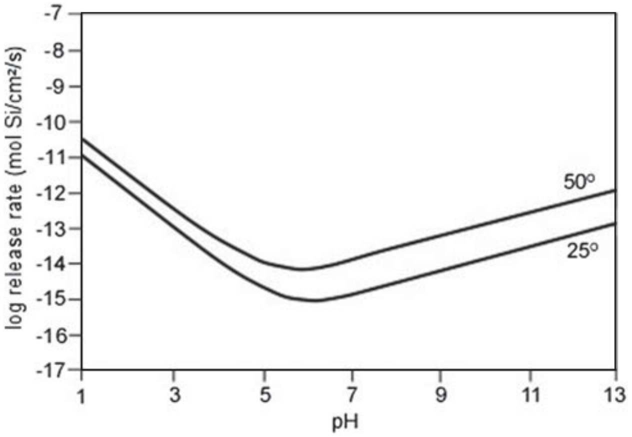

Fig. 1. Typical pH dependence of bulk basalt glass dissolution at two temperatures expressed as the silica release rate (adapted from Gislason & Oelkers, 2003).

many bioessential cations, such as Mg, Ca, Fe and K, reducing mass and energy costs in the transport of these nutrients from elsewhere. On Mars specifically, some of these volcanic rocks have already been weathered into more biologically available alteration phases such as smectites (Christensen *et al.* 2004), although these materials are still likely to suffer from low carbon content.

One way of improving planetary regolith is to weather it using microorganisms, thus improving the carbon content (by the introduction of growing organisms), releasing bioessential cations and improving the water-holding potential of the material by elevating its organic carbon content.

Material altered in this way might be used as nutrient feedstock for bioreactors in life-support systems that have microbial components (Hendrickx & Mergeay 2007; Lehto *et al.* 2007). Feedstock provision in this way will reduce mass and energy costs in the transport of nutrients from Earth or other locations to the location of the life-support system.

Rocks can be broken down (weathered) by a number of mechanisms. However, one effective means is to change the pH (Gislason & Oelkers 2003; Gudbrandsson *et al.* 2008), which alters the rate of exchange reactions at the surface of silicate materials and thus alters the rate of the Si release and the matrix-modifying cations (Fig. 1). Weathering can generally be accelerated by reducing or increasing the pH.

A reduction of pH can be effected by introducing into the material heterotrophic bacteria with an appropriate organic source that causes the production of acid. An example is glucose, which when converted to gluconic acid, causes a reduction in pH and an increase in the weathering rates of basaltic rocks (Wu *et al.* 2007). One disadvantage of this approach is the need to provide an organic source for the bacteria to weather the rocks, which may have a large mass cost.

Another way of increasing the weathering rate is to increase the pH. Although some minerals, such as olivines and pyroxenes, display a reduced weathering rate at increasing pH values (Wogelius & Walthier 1991; Knauss *et al.* 1993; Oelkers & Schott 2001), many minerals such as plagioclase and glasses show the type of relationship illustrated in Fig. 1, such that bulk rocks, including crystalline basalts, will tend to be weathered faster (and will be broken down more effectively) at higher pH values since some of their constituent minerals will be broken down more quickly. One way in which an increase in pH can be accomplished is with photosynthetic organisms that elevate the pH (Budel *et al.* 2004) by causing a net production of hydroxyl ions after consuming CO_2 from bicarbonate. This might be achieved using algae or cyanobacteria. On Mars, this CO_2 supply could come from pressurized Martian atmosphere. On the Moon and asteroids, it would have to be supplied by CO_2 manufacturing.

Experimental: Methods and results

A variety of cyanobacteria of potential use in space applications were tested for their ability to accelerate the weathering of rocks found in extraterrestrial environments as reported by Olsson-Francis & Cockell (2010). Rocks used by these authors included basalt (found on the moon and Mars), anorthosite (found on the moon) and rhyolite (a silica-rich material used to gain generic insights into the effects of silica content on weathering rates applied to silica-rich rocks on any planetary bodies).

The organisms tested were: *Gloeocapsa* strain OU_20, isolated from a rock-dwelling epilithic community obtained in the south of the UK and exposed to Low Earth Orbit (Olsson-Francis *et al.* 2010a); *Leptolyngbya* strain OU_13 and *Phormidium* strain OU_10, both isolated from the same rock-dwelling community exposed to Mars simulated conditions; *Chroococcidiopsis* 029, an extremophilic microorganism naturally associated with rocks in many environments, including extreme desert environments (Billi *et al.* 1996a, 2000; Cockell *et al.* 2005); *Arthrospira platensis*, which can be used as a food supplement (Janssen *et al.* 2010); *Synechococcus elongatus*, a rapidly growing cyanobacterium that has previously received attention in the synthetic biological production of biofuels (Deng & Coleman 1999) and *Anabaena cylindrica*, which forms akinetes (resting-state cells) (Adams & Carr 1981).

To study cyanobacterial growth, media were prepared using volcanic rock (crushed to powder) and sterilized in ddH_2O. The media either had no amendments or was supplemented with 10 mM $(NH_4)_2SO_4$, 10 mM $NaNO_2$. The media were supplemented with 10 mM $(NH_4)_2SO_4$ because the cyanobacterial growth benefited from low concentration of sulphates. Some media was supplemented with 10 mM $NaNO_2$ because the non-nitrogen fixers, *A. platensis, Chroococcidiopsis* 029 and *S. elongatus* required nitrogen. Preliminary experiments showed that the optimal rock/ddH_2O ratio was 1 g of rock (< 100 μm in diameter)/5 ml of ddH_2O. For the reported experiments 25 ml of liquid media and 5 g of rock were used. Cultures were grown in polymethylpentene flasks soaked overnight in 1% HNO_3. Details of the experimental procedure are reported in Olsson-Francis & Cockell (2010).

Experiments showed that in the absence of supplements, the nitrogen-fixing organisms (all organisms other than *A. platensis,*

Table 1. *Biomass achieved on extraterrestrial analogue material and final concentration of Ca with organisms compared to a control (data taken from Olsson-Francis & Cockell 2010). Data after 45 days of experimental duration*

Organism	Biomass (g biomass/kg basalt)[a]	Biomass (g biomass/kg basalt)[b]	Final [Ca] Rhyolite (μM)[c]	Final [Ca] Basalt (μM)[c]	Final [Ca] Anorthosite (μM)[c]
Anabaena cylindrica	0.49 ± 0.02	0.49 ± 0.09	11.1	43.5	43.5
Chroococcidiopsis sp. 029	0.33 ± 0.11	N.D.	6.51	37.1	26.0
Arthrospira platensis	0.10 ± 0.02	N.D.	0.218	35.2	32.6
Synechococcus elongatus	0.69 ± 0.03	N.D.	7.63	36.8	36.6
Phormidium strain OU_10	0.11 ± 0.03	N.D.	14.5	55.2	32.4
Leptolyngbya strain OU_13	0.46 ± 0.01	0.22 ± 0.07	13.2	49.5	26.5
Gloeocapsa strain OU_20	0.02 ± 0.01	0.01 ± 0.03	8.51	51.2	19.9
CONTROL	N/A	N/A	0.251	29.1	8.2

[a] Experiment supplemented with 10 mM $(NH_4)_2SO_4$ and 10 mM $NaNO_2$. When no supplements were added only *A. cylindrica* (0.49 ± 0.09 g/kg rock), *Leptolyngbya* OU_13 (0.22 ± 0.07 g/kg rock) and *Gloeocapsa* OU_20 (0.01 ± 0.03 g/kg rock) grew to yield measurable biomass.

[b] No supplements (organisms grown in ddH_2O). N.D., not determined (no growth recorded).

[c] The concentration of Ca is shown here, but other metals were examined by these authors. These values are with supplemental N and S (see point 1). The concentrations of Ca in each of the rock types are also different (rhyolite ~1.2%, basalt ~11%, anorthosite 15% expressed as CaO equivalent).

Chroococcidiopsis 029 and *S. elongatus, Phormidium* OU_13), were capable of growing on all three rock types (Table 1). In all three rock types the weathering rate (measured as the release of bioessential elements) was about twice as great as non-biological control experiments. The fastest weathering rates were generally achieved by *A. cylindrica*. Weathering rates were correlated to growth rates (measured as increase in protein concentration in the flasks) of the organisms. The highest biomass without additives was achieved with *A. cylindrica*. With additional sulphate and nitrogen, *S. elongatus* achieved the highest growth.

One observation that comes to light is that the fastest-growing organisms (e.g. *A. cylindrica*), are not the most extremophilic. Extremophilic organisms (e.g. *Chroococcidiopsis* spp.) are generally slow growing. Using existing (non-engineered) organisms generally results in a trade-off between rapid growth rate and ability to tolerate extremes, a trade-off which is likely to be encountered in space applications.

Discussion and application of synthetic geomicrobiology

The data obtained in these experiments show that without supplements and using only water, cyanobacteria can be used to accelerate weathering of rocks associated with extraterrestrial regolith. High silica content retards the release of bioessential cations as expected from chemical weathering investigations (Wolff-Boenisch *et al.* 2006). However, silica-rich rocks would not be a preferred substrate for plant growth. The rocks that can be most easily weathered by microorganisms are also the rocks that are the most productive for plant growth (e.g. basalts).

Organisms not only accelerate rock weathering, but also use some of the released cations to propagate, thus contributing carbon to the substrate. As the phototrophs used here are autotrophic, they contribute fixed carbon in biomass from CO_2 gas. This gives them an advantage on Mars, where pressurized atmospheric gas (95.32% CO_2) might be used as the source of carbon to generate fixed carbon. The fixation of nitrogen by some organisms can be used to obviate the requirement for abiotic nitrogen supplied as a mineral supplement (although atmospheric N_2 would be required; again, on Mars this can be supplied from the native atmosphere).

Although organisms derived from the natural environment can be used to carry out weathering as shown in these experiments, synthetic geomicrobiology could be used to develop 'superweathering microorganisms' (SWeMs) capable of carrying out rapid mineral dissolution. Some of the characteristics of a SWeM that these experiments bring to light are:

(1) Ability to rapidly change the pH of the solution to cause rock dissolution.

(2) As acidity or alkalinity might be detrimental to the subsequent use of the weathered material by other organisms, they might be engineered to reverse the pH at the end of the processing stage by the production of neutralizing metabolites.

(3) Tendency not to form biofilms that retard mineral dissolution from rock surfaces (i.e. disabled biofilm-formation genes).

(4) Cells might be engineered to produce elemental sequestration compounds such as siderophores (which bind iron) to improve non-pH-driven dissolution of rocks.

(5) Tendency to break down into labile carbon compounds. The organisms should break down into their constituent biomolecules as easily as possible to provide carbon to bacteria and fungi that might be used to enhance plant growth in the resulting material.

Biomining

Background

Biomining encompasses the use of microorganisms to assist in the release of economically important elements or compounds from rocks. On Earth, both copper and gold, for example, are now harvested using acidophilic iron-oxidizing bacteria in 'biomining' operations (Norris *et al.* 2000; Stott *et al.* 2003). The range of conditions in which biomining is accomplished on a commercial scale is still quite narrow. Generally, metals within pyritic ores are the current focus of commercial operations because the iron within them can be easily oxidized by iron-oxidizing bacteria such as *Acidithiobacillus* species that thrive within the acidic conditions produced from sulphide oxidation.

The process of biomining has a simple overall principle. In sulphide ores (pyritic ores), iron-oxidizing bacteria provide a source of Fe^{3+} that causes oxidation of the sulphidic minerals (Clark & Norris 1996; McGuire *et al.* 2001), producing sulphuric acid that sustains further rock dissolution, breaking apart the rock matrix to facilitate acquisition of useful minerals (e.g. Pronk & Johnson 1992; Schroeter & Sand 1993; Solisio *et al.* 2002; Rawlings 2005) such as nickel, copper and gold, among other metals. Sulphur-oxidizing microorganisms can also contribute to rock dissolution in biomining. The principle is based on the ability of microorganisms to accelerate rock weathering. Unlike the weathering of rocks caused by pH changes, in the case of most biomining applications, the breakdown of the rock relies on the direct alteration of the iron and sulphur valence state, which is being used as a source of energy by the microorganisms. The advantages of biomining over purely chemical methods are: (1) reduced need for chemicals to enhance rock dissolution or elemental release (this might be offset by the requirements to provide microbial growth nutrients, but if the rock contains bioessential elements these additional requirements can be minimized), (2) reduction or removal of the use of poisonous compounds (such as cyanides, traditionally used to leach rocks) and (3) reduction in energy requirements to effect rock dissolution.

In principle, there is no reason as to why biomining must be limited to these pyrite-specific reactions. Any microorganism that is capable of accelerating the weathering and dissolution of a rock that contains a commercially important element would be useful in biomining, even if the rate of weathering was increased only by a few-fold.

Most materials of interest in space do not have high concentrations of pyrites. Two important examples are most asteroidal material and lunar and Martian basalts.

Asteroidal material is known to harbour platinum group elements, volatiles and a variety of useful resources for human space settlements and terrestrial industries (Kryzanowski & Mardon 1990; Sonter 1997; Busch 2004). Microorganisms might be used to facilitate access to this material by enhancing the breakdown of its constituent minerals.

Basalt and rocks of generally basaltic composition are widely found on the Moon and Mars. Basalt and other crustal materials contain a variety of economically useful metals (Table 2). Indeed, seen purely from the point of view of practical use, a lump of basalt contains a vast range of elements that have industrial uses. One might even refer to the hypothetical economy that could be sustained from these elements as the ‘basalt economy’ (accepting that materials other than basalt might also be mined). The extraction of useful metals from this material is currently economically prohibitive on the Earth (Steen & Borg 2002; Pickard 2008), although as rich ores dwindle, the use of crustal material as a source of elements may become more attractive. If rich ores are eventually discovered on the moon or Mars, then crustal material might also be economically less attractive than ores. However, depending on the cost of mining and transportation of localized ores, processes that can extract useful elements from the abundant basalt material on those planetary bodies are worthy of investigation. Crucial investigations are the extent to which these precursor rocks contain elements that are toxic or detrimental to the growth of organisms proposed in biomining operations.

Table 2. *The ‘Basalt Economy’. Examples of elements in basalt and their industrial uses (minor elements are just a few of the elements found in basalts)*

Element	Application
Major elements	
Si	Solar cells, glass, ceramics
Al	Lightweight materials, alloys, varnishes, catalysts
Ca	Alloys, steel production, cements
Mg	Die castings, alloys, batteries
K	Soaps, glassware
Na	Heat transfer agents, lighting, organic syntheses
Minor elements	
V	Carbon dioxide cracking
Ni	Catalysts (e.g. methane production on Mars)
Cu	Catalysts
Zn	Protective coatings, paint, batteries
Li	Desiccants, batteries, polymer production
Sr	Optical materials, refining Zn
Cd	Alloy coatings

Experimental: methods and results

Experiment 1: Biomining from asteroidal material

Biomining organisms can be shown to carry out iron oxidation in asteroidal material. Gronstal *et al.* (2009) reported experiments using the aerobic acidophilic iron-oxidizing bacterium, *Acidithiobacillus ferrooxidans*. The authors obtained the organism from the DSMZ (Deutsche Sammlung von und Zellkulturen GmbH) (Braunschweig, Germany) (DSM 583). *A. ferrooxidans* was cultured in modified K medium at 28 °C (Silverman & Lundgren 1959). Whole rock samples of the Murchison and Cold Bokkeveld (both CM2) carbonaceous chondrites were split and crushed in a Class 100 clean room using an agate pestle and mortar. The Cold Bokkeveld meteorite sample was supplied by the Natural History Museum, London. It fell in South Africa in 1838. The Murchison meteorite fell in Australia in 1969, and was rapidly collected after fall.

Carbonaceous chondrites are just one type of asteroidal material. They are primitive and contain a diverse suite of carbon compounds. Most common are the CM2 type (used here), which contain silicates and chondrules (made from olivine) with a variety of other elements at low concentrations. Although the carbonaceous chondrites represent just one type of material, they are very common in the asteroid belt and

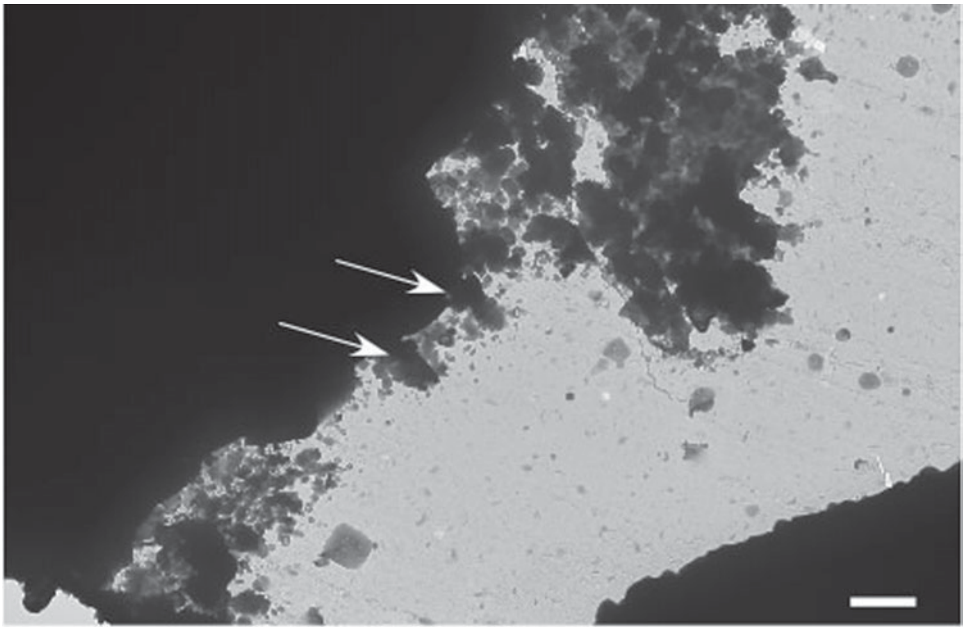

Fig. 2. Asteroidal biomining. *A. ferrooxidans* growing on reduced iron in the Murchison meteorite (black material in background). The rod-shaped organisms (arrows) are covered in particles of meteorite and oxidized iron (scale bar 1 μm). The clear area is the TEM grid on which the material was deposited from the reaction flask.

constitute between 40 and 80% of asteroids, depending on location (Norton *et al.* 1998).

The first experiment with carbonaceous meteorites (experiment 1) was conducted with 20 mg of meteorite material (the quantity of material procured was such that the experiment was limited to 20 mg) using a 25 μl inoculum of cells ($\sim 1 \times 10^7$ cells/cm^3) in 2 ml glass vials. As there was limited material, the experiment was repeated with the two different meteorites (Murchison and Cold Bokkeveld) to provide replication. Initially the concentration of iron was low in the small amount of material available so that the abiotic rate of oxidation of iron was indistinguishable to the biological rate. The experiment was repeated using 30 mg of meteorite with the H_2SO_4 concentration increased to hold the Fe^{2+} in a reduced state to provide time for biological iron oxidation to occur (experiment 2). In order to increase biological activity a 50 μl inoculum of cells was used. Throughout the experiment the concentration of iron was measured using the ferrozine assay (Stookey 1970).

The amount of carbonaceous chondrite used was such that only small (<112 μM) amounts of reduced iron were released into solution. In experiment 1, after four days the iron in both the control and organism-containing experiments had been oxidized. However, by increasing the concentration of the acid in experiment 2 the reduced iron was sufficiently stable to measure the biotic iron oxidation compared to abiotic control (Gronstal *et al.* 2009). After 9 d, 35% of the control Fe^{2+} from the Cold Bokkeveld had been oxidized compared to 97% in the experiment containing *A. ferrooxidans.* The organisms interacted with the meteorite during the oxidation of its reduced iron (Fig. 2) and appeared to facilitate disaggregation of the material.

Discussion and application of synthetic geomicrobiology

Experiment 1: biomining from asteroidal material

The first step of successful biomining of iron-containing asteroidal material is to demonstrate the ability of iron-oxidizing microorganisms to oxidize reduced iron without the parent material being toxic to the very organisms carrying out this transformation. As shown here, *A. ferrooxidans* can oxidize reduced iron in carbonaceous chondrites without the bulk material being detrimental to the organisms. Meteoritic materials also contain some sulphides (including troilite; FeS), which might provide a source of sulphuric acid to sustain the leaching reactions, similar to sulphidic ores on Earth. Material could be ground into a powder or grains, similar to our experiments, and leached in oxygen-pressurized batch reactors.

Although these experiments show that non-engineered bacteria could probably be used to carry out the necessary biomining operations on asteroidal material, synthetic biology might be used to improve the biological *in situ* resource capacity of microbial strains. Examples of improvements to designer biomining organisms include:

1. Improved rates of sulphide and iron oxidation to accelerate asteroidal material breakdown.
2. Engineering of filamentous growth to improve the access of the organisms into fractures and spaces within crushed asteroidal material.
3. Incorporation of motility into biomining organisms to improve access of organisms into materials.
4. Incorporation of other super-weathering abilities (such as metal sequestration compound production; see above) to facilitate breakdown and biomining of non-sulphide-containing asteroidal materials.

Experiment 2: biomining basalt and other non-sulphidic ores

Experiments show that bacteria can accelerate the weathering of basaltic materials (Barker & Banfield 1996; Rogers *et al.* 1998; Calvaruso *et al.* 2006). As with other materials (see above), any biological process that changes the pH will cause rock weathering. However, more directed approaches to extracting elements from rocks might be useful for future biomining operations whereby microorganisms are engineered to sequester specific elements from rocks.

Studies using microarray technology were able to identify the genes involved in iron sequestration from basaltic rock (Olsson-Francis *et al.* 2010b). The microorganism, *Cupriavidus metallidurans*, which has been found naturally dwelling in volcanic environments (Sato *et al.* 2004), was grown in the presence and absence of basalt where the iron was either supplied in the medium or the organism was forced to acquire it from the basaltic rock. Microarray analysis was used to investigate which genes were upregulated when the organisms were forced to acquire iron from the basaltic rock. The authors reported that no siderophore genes were expressed, consistent with physiological studies and consistent with the fact that most of the iron in basalt is present as Fe^{2+}, not Fe^{3+} (the latter is the target of the siderophore system). Porin genes were upregulated, suggesting acquisition of Fe^{2+} from the leached surface of the basalt through membrane channels. Some active transporters were upregulated, which might indicate active uptake of iron.

Intriguingly, systems for the removal of heavy metal from cells, such as the *czc* operon, were also upregulated in response to the presence of heavy metals found in basaltic rocks. Thus, in acquiring a useful element such as iron, the organisms are also exposed to potentially deleterious heavy metals, and specific sets of genes are upregulated in response to them.

Discussion and application of synthetic geomicrobiology

Experiment 2: biomining basalt and other non-sulphidic ores

The experiments on the interactions between a microbe and the surface of basalt using microarray technology reported by the authors as above and the large literature of other metal cycling pathways (e.g. Holmes *et al.* 2009; Stanliand *et al.* 2010), illustrate a simple idea – that with knowledge of genetic pathways of elemental extraction and sequestration from raw materials such as basalt, it should be possible to engineer microorganisms to preferentially sequester desired elements from basalt in a type of 'directed biomining'. One way of approaching this would be to engineer an organism that had a genetically engineered preference for an element of industrial use and grow it in the presence of crushed basalt. The organism would extract and sequester the element from the rock. The rock leachate with organisms would then be collected, the organisms removed by centrifugation and the element recovered from the organisms.

However, another conceptual approach to this is illustrated in Fig. 3. Here, I refer to this idea as Multiple Elemental Extraction using Engineering Microorganisms (ME^{3}M Technology). In this scheme, synthetic microorganisms are created that have a preference for the weathering and sequestration of particular elements. Within each organism is a gene for the expression of a specific colour of fluorescent protein, i.e. each element-sequestering organism fluoresces a different colour. The organisms are added to the ground basalt; weathering and biomining proceeds. The leachate is then collected and passed through an industrial-scale flow cytometer (cell-sorter), which sorts the cells based on fluorescent protein colour, i.e. according to different elements. Each separate solution is collected and the relevant element extracted from the organisms. Either the organisms are ground up and the element recovered by some chemical means or the organisms are engineered so that the addition of a compound in their media induces them to secrete the elements that they have sequestered (this approach would depend on the solubility of the element that they have sequestered).

The characteristics of the synthetic microorganisms used in this scheme can be stated as follows:

(1) Genes engineered to give the organisms a highly specific preference for the uptake and sequestration of particular elements.
(2) Addition of fluorescent protein to each organism with high expression to allow effective cell sorting.
(3) Engineered element sequestration mechanisms to maximize the ease of element extraction from whole cells following the biomining procedure (i.e. elements not strongly bound to organic ligands and other cell components).
(4) Cells engineered to produce low concentrations or no polysaccharide to prevent them clumping (to maximize efficiency of cell sorting).
(5) High longevity and activity in presence of rock to maximize their effectiveness at biomining.

Regolith hardening

Background

Regolith on any planetary surface is subject to long-term pulverization or 'gardening' of the surface by impact events, resulting in fine dust and small fragments (e.g. Lee 1995; Kahre *et al.* 2006). This material is a pervasive problem for machines and humans. In machines, it causes wear-and-tear and can interfere with sensitive and fragile equipment. In humans, it might be toxic or cause respiratory complications.

A challenge in planetary exploration is to control this material and bind it together, minimizing its movement. There are two biological approaches to this problem. The material could be bound together using minerals produced by microorganisms. DeJong *et al.* (2006) show how carbonate-producing bacteria can be used to solidify ground by binding mineral grains in carbonates. The bacteria (*Bacillus* sp.) are injected into the ground, fed appropriate nutrients and a source of carbon (urea) to generate alkaline conditions, which cause precipitation of carbonate minerals. In the study reported it was suggested that they might be used to solidify

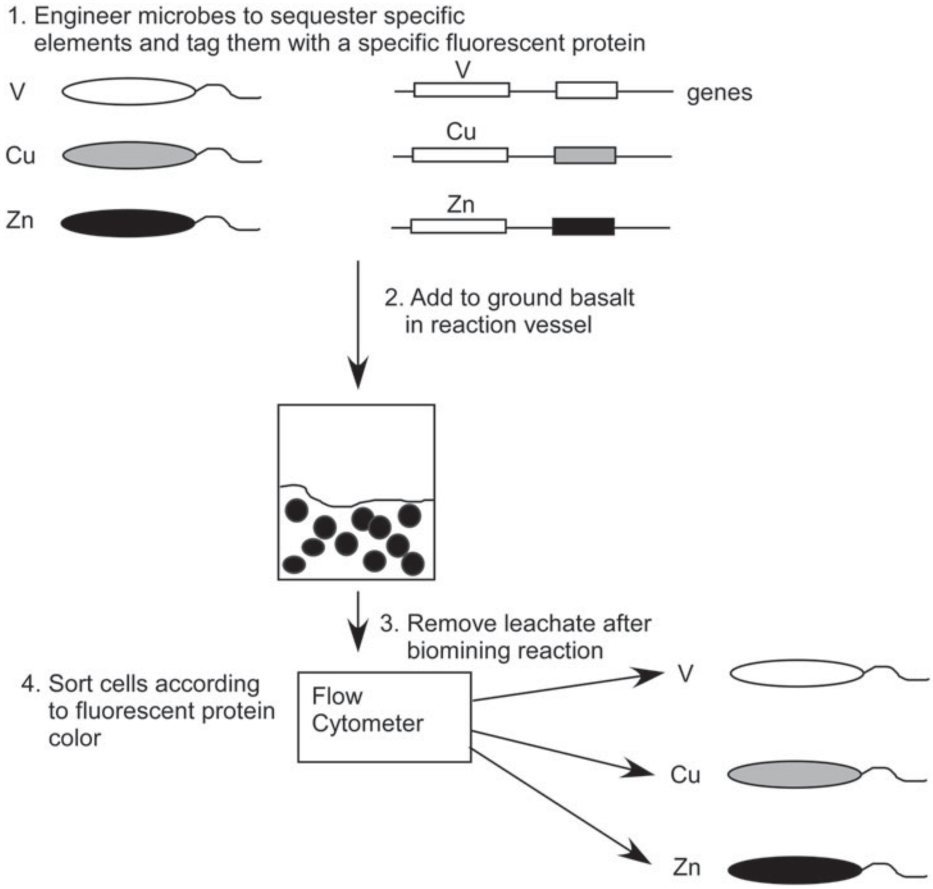

Fig. 3. Principle of a possible 'directed biomining' concept referred to here as ME^3M technology. Synthetic microorganisms are used to selectively extract and sequester economically important metals from raw rock material.

ground in earthquake-prone regions. Similar principles were described and applied to the protection of the outside of buildings by Metayer-Levrel *et al.* (1999). In extraterrestrial environments, one obvious application of this approach is to solidify ground and bind regolith. The bacteria cannot be grown under ambient conditions on the surface of asteroids, the moon or Mars, and so the ground would initially have to be covered in an atmosphere-tight enclosure to allow for liquid water to persist and the bacteria to grow and produce minerals within the regolith. Once the ground is solidified, the enclosure could be removed.

A second approach to binding surface material and minimizing the lofting of dust is to bind regolith minerals together using the 'matting' habit of microorganisms (Liu *et al.* 2008). This approach uses the tendency of some microorganisms to grow across the surface of substrates and bind grains within the polysaccharide exuded by the organisms. Unlike the former approach, it is better suited to the binding of dust and mineral grains in greenhouse-like environments where permanent conditions can be created for microbial growth. In addition to binding the existing dust and surface regolith, by flowing air over the microbial communities, the atmosphere could be cleansed of particles. In this way, the crust can be used as an air filtration method.

Experimental: methods and results

Liu *et al.* (2008) reported experiments in which they collected soil crusts from the Shapotou region in China (Tengger Desert). Five phototrophs were cultured from the crusts: four filamentous cyanobacteria: *Microcoleus vaginatus* Schk., *Phormidium tenue* (Menegh.) Gom., *Scytonema javanicum* (Kutz.) Born. et Flah., and *Nostoc* sp. and a single-celled green alga: *Desmococcus olivaceus* (Pers. ex Ach.) Laundon. They were cultured in 200 ml bottles, and then in 5-litre bottles in BG11 at a temperature of 25 ± 2 °C and an irradiance of 50 μmol photon m^{-2} s^{-1}. They were bubbled with ambient air. After 20 days of growth, the organisms were harvested by filtration.

To prepare crusts for wind tunnel experiments, rectangular trays (30 × 40 × 2.8 cm) were filled with sterilized sand from Shapotou to a height of 2.3–2.4 cm, soaked with water and levelled. Cultured cyanobacteria were harvested by filtering. The organisms were spread into a thin layer, air dried and ground to pass through a mesh sieve. The dried cells were rehydrated and sprayed onto the surface of the sand in the trays as homogeneously as possible. In all experiments, four replicates were used for each treatment. Trays were kept in the greenhouse and in the field at Shapotou, where the highest air temperature recorded was 43 °C, with a surface sand temperature of 8–38 °C. The trays were watered with a fogger at 8:30 AM and 17:30 PM (100 ml at each time for each tray). At 11:30 AM and 14:10 PM, the trays were also sprayed with BG11 medium under the same conditions.

After 15 days of growth cultivation, the trays were put into the wind tunnel to test the strength of the crusts. Wind-tunnel experiments were conducted in the wind-tunnel laboratory of the Lanzhou Institute of Desert Research, Chinese Academy of Sciences as described by Hu *et al.* (2002). Trays were level with the bottom of the tunnel to maintain laminar, non-turbulent flow. Wind speed was measured with a pitot tube. Wind speeds of 5, 6, 10, 12, 15, 20, 25 $m\,s^{-1}$ were examined. Five-minute exposure times were used to study the crusts. The threshold friction velocity, i.e. the surface velocity at which erosion of the crust begins to occur, was determined as a measure of the strength of the crust.

The experiments showed that crusts artificially formed with cyanobacteria could resist wind strengths of up to 15 $m\,s^{-1}$ and formed coherent crusts within 15 days. The strength of the crusts demonstrates the effectiveness with which the organisms grew through the mineral matrix and bound the soil grains. The organisms constructed a hard crust that was not easily perturbed by surface wind. Contributory factors to the effectiveness of the soil crusts included *inter alia*: ability of filamentous organisms to grow through the mineral substrate, production of copious polysaccharides by the cyanobacteria that bound minerals, fixation of nitrogen by organisms, providing nitrogen for other organisms that contribute to the soil crust strength and community integrity. The soil crusts were not axenic (containing only the cyanobacterial strains without bacterial contaminants), and indeed the production of crust is probably enhanced by the presence of other organisms contributing to biogeochemical cycling within the crust. Therefore, in an extraterrestrial environment, crust production would be best achieved with a mixture of organisms using those naturally associated with cyanobacteria.

Discussion and application of synthetic geomicrobiology

Artificial soil crusts can be used to perform many functions in extraterrestrial settlements. One of their primary uses could be to control regolith within enclosed spaces and to act as a filter, removing dust particles entrained within the atmosphere of a habitat by passing atmospheric gas over or through microbial crusts.

However, crusts might also perform other functions. It has for a long time been recognized that plant growth provides astronauts with a psychological distraction from everyday chores. Although the notion of ‘extraterrestrial gardening’ might seem frivolous, for long-term planetary stations nurturing plants and microbial crusts might be an important pastime for people within these environments.

The experiments described above show that many natural organisms obtained from terrestrial deserts can be used to effectively create crusts of different varieties. However, synthetic geomicrobiology might be used to manipulate individual organisms to be more effective crust components. Synthetic engineered attributes of microbial crust organisms could include:

(1) Enhanced production of copious polysaccharides by the organisms to more effectively bind dust and grains into a crust.
(2) Incorporation of some super-weathering attributes (see above) to improve weathering of regolith into useful products that can be used by plants grown near the crust and the microbial consortium within the crust itself.
(3) Improve the strength and consolidating properties of the crust to improve its longevity against surface perturbation and deterioration, for example, by engineering the propensity of diverse phototrophs to grow in non-linear filaments that improve their tendency to form an intertwined microbial mat.
(4) Crust organisms might be engineered to produce useful drugs. If the organisms are to be grown in the crust for regolith consolidation, they might as well be additionally used to produce products of pharmaceutical or nutrient value. Thus, crusts could be harvested periodically and processed for drugs.

Synthetic geomicrobiology – the common attributes of a designer geomicrobiology organism

In the experiments discussed above, the possible application of synthetic geomicrobiology was discussed for each case and examples were provided of how organisms might be engineered to improve their attributes. However, examination of these three applications and a range of other applications of organisms in microbe–mineral interactions (Cockell 2010) allows a set of attributes of synthetic or engineered microorganisms to be identified that might be used to improve microbe–mineral interactions in general. Specifically engineered characteristics can then be added to these ‘core’ synthetic geomicrobiology attributes. These core characteristics include, but are not limited to:

(1) Tolerance of extremes. Extremophilic rock-interacting microorganisms, such as *Chroococcidiopsis* sp., are generally slow growing. Although extremophilic microorganisms are not required for many applications, they are beneficial because they: (1) provide redundancy in the case of system failure or depressurization in a growth unit, and (2) provide the ability to store cells in extraterrestrial conditions without continuous culturing to maintain a population. The ideal soil-producing microorganisms should combine rapid growth rate with tolerance of extremes associated with extraterrestrial environments. These extreme conditions should include: tolerance to ionizing radiation associated with a given location or mission in space (or an engineered generic tolerance to high ionizing radiations), desiccation tolerance and tolerance of extreme temperature fluctuations. All these extreme tolerances are to be found in terrestrial organisms and, therefore, the natural genetic resources to artificially produce them already exist.

(2) Tolerance of metals found in regolith rocks, particularly volcanic rocks (e.g. Zn, Cu and Ni).
(3) Ability to fix nitrogen. Although nitrogen can be provided in an inorganic solid form, the use of nitrogen gas provides flexibility in culturing methods.
(4) Non-fastidious growth conditions. Ability to grow under a wide diversity of chemical and physical conditions such as pH, temperature etc, to minimize technical requirements for maintaining exact growth conditions.
(5) Minimal elemental requirements. The organisms should have minimal element needs to allow for growth in nutrient-poor rock environments.
(6) Robust resting states (spores, akinetes or dried vegetative cells) that can be used for long-term storage in planetary stations or during interplanetary transfer.

Conclusions

Experiments using microorganisms to perform functions in space settlement and exploration allow us to identify useful attributes that could be improved or augmented in synthetic geomicrobiology. Although a great diversity of extremophiles have been isolated and identified that have characteristics of use in applications such as extraterrestrial soil formation, biomining and regolith hardening, none of these organisms have the desired combined optimal characteristics. However, as discussed here, laboratory experiments allow us to explore the limits of these organisms and to propose new attributes of organisms and technological processes that can use molecular and synthetic biology in space applications.

References

Adams, D.G. & Carr, N.G. (1981). Heterocyst differentiation and cell division in the cyanobacterium *Anabaena cylindrica*: effect of high light intensity. *J. Cell Sci.* **49**, 341–352.

Barker, W.W. & Banfield, J.F. (1996). Biologically versus inorganically mediated weathering reactions: relationships between minerals and extracellular microbial polymers in lithobiontic communities. *Chem. Geol.* **132**, 55–69.

Benzerara, K. & Menguy, N. (2009). Looking for traces of life in minerals. *C. R. Palevol.* **8**, 617–628.

Billi, D., Friedmann, E.I., Hofer, K.G., Grilli Caiola, M. & Ocampo-Friedmann, R. (2000). Ionizing radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. *Appl. Environ. Microbiol.* **66**, 1489–1492.

Billi, D. & Grilli Caiola, M. (1996a). Effects of nitrogen limitation and starvation on *Chroococcidiopsis* sp. (Chroococcales). *New Phytol.* **133**, 563–571.

Budel, B., Weber, B., Kuhl, M., Pfanz, H., Sultemeyer, D. & Wessels, D. (2004). Reshaping of sandstone surfaces by cryptoendolithic cyanobacteria: bioalkalization causes chemical weathering in arid landscapes. *Geobiology* **2**, 261–268.

Busch, M. (2004). Profitable asteroid mining. *J. Br. Interplan. Soc.* **57**, 301–305.

Calvaruso, C., Turpault, M. & Frey-Klett, P. (2006). Root-associated bacteria contribute to mineral weathering and to mineral nutrition in trees: a budgeting analysis. *Appl. Environ. Microbiol.* **72**, 1258–1266.

Certini, G. & Scalenghe, R. (2010). Do soils exist outside Earth? *Planet. Space Sci.* **58**, 1767–1770.

Christensen, P.R., Wyatt, M.B., Glotch, T.D., Rogers, A.D., Anwar, S., Arvidson, R.E., Bandfield, J.L., Blaney, D.L., Budney, C., Clavin, W.M. *et al.* (2004). Mineralogy at Meridiani Planum from the mini-TES experiment on the Opportunity Rover. *Science* **306**, 1733–1739.

Clark, C.A. & Norris, P.R. (1996). Oxidation of mineral sulphides by thermophilic microorganisms. *Miner. Eng.* **9**, 1119–1125.

Cockell, C.S. (2010). Geomicrobiology beyond Earth: microbe–mineral interactions in space settlement and exploration. *Trends Microbiol.* **18**, 304–314.

Cockell, C.S., Schuerger, A.C., Billi, D., Friedmann, E.I. & Panitz, C. (2005). Effects of a simulated Martian UV flux on the cyanobacterium, *Chroococcidiopsis* sp. 029. *Astrobiology* **5**, 127–140.

Dahlgren, R., Shoji, S. & Nanzyo, M. (1993). Mineralogical characteristics of volcanic ash soils. In *Volcanic Ash Soils Genesis, Properties, and Utilization*, ed. Shoji, S. & Nanzyo, M., pp. 101–143. Elsevier, Amsterdam.

DeJong, J.T., Fritzges, M.B. & Nüsslein, K. (2006). Microbial induced cementation to control sand response to undrained shear. *J. Geotech. Geoenviron. Eng.* **132**, 1381–1392.

Deng, M.D. & Coleman, J.R. (1999). Ethanol synthesis by genetic engineering in cyanobacteria. *Appl. Environ. Microbiol.* **65**, 523–528.

Dong, H. & Yu, B. (2007). Geomicrobiological processes in extreme environments: a review. *Episodes* **30**, 202–216.

Erhlich, H.L. & Newman, D.K. (2009). *Geomicrobiology*. CRC Press, Boca Raton, FL.

Gadd, G.M. (2010). Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology-SGM* **156**, 609–643.

Gislason, S. & Oelkers, E. (2003). The mechanism, rates, and consequences of basaltic glass dissolution. II. An experimental study of the dissolution rates of basaltic glass as a function of pH at temperatures from 6 °C to 150 °C. *Geochim. Cosmochim. Acta* **67**, 3817–3832.

Glowa, K.R., Arocena, J.M. & Massicotte, H.B. (2003). Extraction of potassium and/or magnesium from selected soil minerals by *Piloderma*. *Geomicrobiol. J.* **20**, 99–111.

Greenhagen, B.T., Lucey, P.G., Wyatt, M.B., Glotch, T.D., Allen, C.C., Arnold, J.A., Bandfield, J.L., Bowles, N.E., Hanna, K.L.D., Hayne, P.O. *et al.* (2010). Global silicate mineralogy of the moon from the Diviner Lunar Radiometer. *Science* **329**, 1507–1509.

Gronstal, A.L., Pearson, V., Kappler, A., Anand, M., Poitrasson, F., Kee, T.P. & Cockell, C.S. (2009). Laboratory experiments on the weathering of iron meteorites and carbonaceous chondrites by iron-oxidising bacteria. *Meteorit. Planet. Sci.* **44**, 233–248.

Gudbrandsson, S., Wolff-Boenisch, D., Gislason, S.R. & Oeklers, E.H. (2008). Dissolution rates of crystalline basalt at pH 4 and 10 and 25–75 °C. *Miner. Mag.* **72**, 155–158.

Hendrickx, L. & Mergeay, M. (2007). From the deep sea to the stars: human life support through minimal communities. *Curr. Opin. Microbiol.* **10**, 231–237.

Holmes, D.S., Cardenas, J.P., Valdes, J., Quatrini, R., Esparza, M., Osorio, H., Duarte, F., Lefimil, C. & Jedlicki, E. (2009). Comparative genomics begins to unravel the ecophysiology of bioleaching. *Biohydrometall. Adv. Mater. Res.* **71–73**, 143–150.

Hu, C.X., Liu, Y.D., Zhang, D.L., Huang, Z.B. & Paulsen, B.S. (2002). Cementing mechanism of algal crusts from desert area. *Chin. Sci. Bull.* **47**, 1361–1368.

Janssen, P.J., Morin, N., Mergeay, M., Leroy, B., Wattiez, R., Vallaeys, T., Waleron, K., Waleron, M., Wilmotte, A., Quillardet, P. *et al.* (2010). Genome sequence of the edible cyanobacterium *Arthrospira* sp. PCC 8005. *J. Bacteriol.* **192**, 2465–2466.

Kahre, M.A., Murphy, J.R. & Haberle, R.M. (2006). Modeling the Martian dust cycle and surface dust reservoirs with the NASA Ames general circulation model. *J. Geophys. Res.* **111**, E06008.

Kappler, A. & Newman, D.K. (2004). Formation of Fe(III)-minerals by Fe (II)-oxidizing photoautotrophic bacteria. *Geochim. Cosmochim. Acta* **68**, 1217–1226.

Knauss, K.G., Nguyen, S.N. & Weed, H.C. (1993). Diopside dissolution kinetics as a function of pH, CO_2, temperature, and time. *Cosmochim. Geochim. Acta* **57**, 285–294.

Konhauser, K. (2007). *An Introduction to Geomicrobiology*. Blackwell Publishers, Oxford.

Kryzanowski, T. & Mardon, A. (1990). Mining potential of asteroid belt. *Can. Min. J.* **111**, 43.

Lee, L.H. (1995). Adhesion and cohesion mechanisms of lunar dust on the Moon's surface. *J. Adhes. Sci. Technol.* **9**, 1103–1124.

Lehto, K., Kanervo, E., Stahle, K., Lehto, H., Tammi, M. & Virtanen, J. (2007). Photosynthetic life support systems in the Martian conditions. In *ROME: Response of Organisms to the Martian Environment*, ed. C. Cockell & G. Horneck, ESA Special Publication, AP-1299. Paris.

Liu, Y.D., Cockell, C.S., Wang, G., Hu, C.X., Chen, L. & De Philippis, R. (2008). Control of Lunar and Martian dust–experimental insights from artificial and natural cyanobacterial and algal crusts in the desert of Inner Mongolia, China. *Astrobiology* **8**, 75–86.

McGuire, M.M., Edwards, K.J., Banfield, J.F. & Hamers, R.J. (2001). Kinetics, surface chemistry, and structural evolution of microbially mediated sulphide mineral dissolution. *Geochim. Cosmochim. Acta* **65**, 1243–1258.

Metayer-Levrel, G., Castanier, S., Orial, G., Loubiere, J.F. & Perthuisot, J.P. (1999). Applications of bacterial carbonatogenesis to the protection and regeneration of limestones in buildings and historic parsimony. *Sediment. Geol.* **126**, 25–34.

Ming, D.W. & Henninger, D.L. (1994). Use of lunar regolith as a substrate for plant growth. *Adv. Space Res.* **14**, 435–443.

Miot, J., Benzerara, K., Morin, G., Kappler, A., Bernard, S., Obst, M., Férard, C., Skouri-Panet, F., Guigner, J.-M., Posth, N. *et al.* (2009b). Iron biomineralization by anaerobic neutrophilic iron-oxidizing bacteria. *Geochim. Cosmochim. Acta* **73**, 696–711.

Norris, P.R., Burton, N.P. & Foulis, N.A.M. (2000). Acidophiles in bioreactor mineral processing. *Extremophiles* **4**, 71–76.

Norton, O.R., Ort, K. & Norton, D.S. (1998). *Rocks from Space: Meteorites and Meteorite Hunters*. Mountain Press Publishing Company, Missoula, MT.

Oelkers, E.H. & Schott, J. (2001). An experimental study of enstatite dissolution rates as a function of pH, temperature, and aqueous Mg and Si concentration, and the mechanism of pyroxene/pyroxenoid dissolution. *Geochim. Cosmochim. Acta* **65**, 1219–1231.

Olsson-Francis, K. & Cockell, C.S. (2010). Use of cyanobacteria for in-situ resource use in space applications. *Planet. Space Sci.* **58**, 1279–1285.

Olsson-Francis, K., de la Torre, R. & Cockell, C.S. (2010a). Isolation of novel extreme-tolerant cyanobacteria from a coastal rock-dwelling microbial community using exposure to low Earth orbit. *Appl. Environ. Microbiol.* **76**, 2115–2121.

Olsson-Francis, K., Van Houdt, R., Mergeay, M., Leys, N. & Cockell, C.S. (2010b). Micro-array analysis of a microbe–mineral interaction. *Geobiology* **8**, 446–456.

Pickard, W.F. (2008). Geochemical constraints on sustainable development: can an advanced global economy achieve long-term stability. *Global Planet. Change* **61**, 285–299.

Pronk, J.T. & Johnson, D.B. (1992). Oxidation and reduction of iron by acidophilic bacteria. *Geomicrobiol. J.* **10**, 153–171.

Rawlings, D.E. (2005). Characteristics and adaptability of iron- and sulphur-oxidising microorganisms used for the recovery of metals from minerals and their concentrates. *Microbial. Cell Factories* **4**, 1–15.

Rogers, J.R., Bennett, P.C. & Choi, W.J. (1998). Feldspars as a source of nutrients for microorganisms. *Am. Miner.* **83**, 1532–1540.

Ruzicka, A., Snyder, G.A. & Taylor, L.A. (2001). Comparative geochemistry of basalts from the moon, earth, HED asteroid, and Mars: implications for the origin of the moon. *Geochim. Cosmochim. Acta* **65**, 979–997.

Sato, Y., Nishihara, H., Yoshida, M., Watanabe, M., Rondal, J.D. & Ohta, H. (2004). Occurrence of hydrogen-oxidizing Ralstonia species as primary microorganisms in the Mt. Pinatubo volcanic mudflow deposits. *Soil Sci. Plant Nut.* **50**, 855–861.

Schippers, A., Breuker, A., Blazejak, A., Bosecker, K., Kock, D. & Wright, T.L. (2010). The biogeochemistry and microbiology of sulfidic mine waste and bioleaching dumps and heaps, and novel Fe(II)-oxidizing bacteria. *Hydrometallurgy* **104**, 342–350.

Schroeter, A.W. & Sand, W. (1993). Estimations on the degradability of ores and bacterial leaching activity using short time microcalorimetric tests. *FEMS Microbiol. Rev.* **11**, 79–86.

Silverman, M.P. & Lundgren, D.G. (1959). Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. *J. Bacteriol.* **77**, 642–647.

Solisio, C., Lodi, A. & Veglio, F. (2002). Bioleaching of zinc and aluminium from industrial waste sludges by means of *Thiobacillus ferrooxidans*. *Waste Manage.* **22**, 667–675.

Sonter, M.J. (1997). The technical and economic feasibility of mining the near-Earth asteroids. *Acta Astronaut.* **41**, 637–647.

Stanliand, S., Coppock, M., Tuffin, M., van Zyl, L., Roychoudhury, A.N. & Cowan, D. (2010). Cobalt uptake and resistance to trace metals in *Comamonas testosteroni* isolated from a heavy-metal contaminated site in the Zambian copperbelt. *Geomicrobiol. J.* **27**, 656–668.

Steen, B. & Borg, G. (2002). An estimation of the cost of sustainable production of metal concentrates from the Earth's crust. *Ecol. Econ.* **42**, 401–413.

Stookey, L.L. (1970). Ferrozine – A new spectrophotometric reagent for iron. *Ann. Chem.* **42**, 779–781.

Stott, M.B., Sutton, D.C., Watling, H.R. & Franzmann, P.D. (2003). Comparative leaching of chalcopyrite by selected acidophilic bacteria and archaea. *Geomicrobiol. J.* **20**, 215–230.

Templeton, A. & Knowles, E. (2009). Microbial transformations of minerals and metals: recent advances in geomicrobiology derives from synchrotron-based X-ray spectroscopy and X-ray microscopy. *Annu. Rev. Earth Planet. Sci.* **37**, 367–391.

Welch, S.A., Taunton, A.E. & Banfield, J.F. (2002). Effect of microorganisms and microbial metabolites on apatite dissolution. *Geomicrobiol. J.* **19**, 343–367.

Wogelius, R.A. & Walthier, J.V. (1991). Olivine dissolution at 25 °C: effects of pH, CO_2 and organic acids. *Geochim. Cosmochim. Acta* **55**, 943–954.

Wolff-Boenisch, D., Gislason, S.R. & Oelkers, E.H. (2006). The effect of crystallinity on dissolution rates and CO_2 consumption capacity of silicates. *Geochim. Cosmochim. Acta* **70**, 858–870.

Wu, L., Jacobson, A.D., Chen, H. & Hausner, M. (2007). Characterisation of elemental release during microbe–basalt interactions at $T = 28$ °C. *Geochim. Cosmochim. Acta* **71**, 2224–2239.