ANAEROBIC NITROGEN FIXERS ON MARS. B. G. Lewis, Dept. of Civil Engineering, Northwestern University, Evanston, IL 60208. Email: b-lewis@northwestern.edu

The conversion of atmospheric nitrogen gas to the protein of living systems is an amazing process of nature. The first step in the process is biological nitrogen fixation, the transformation of N_2 to NH_3. The phenomenon is crucial for feeding the billions of our species on Earth. On Mars, the same process may allow us to discover how life can adapt to a hostile environment, and render it habitable.

Hostile environments also exist on Earth. For example, nothing grows in coal refuse piles due to the oxidation of pyrite and marcasite to sulfuric acid. Yet, when the acidity is neutralized, alfalfa and soybean plants develop root nodules typical of symbiotic nitrogen fixation with *Rhizobium* species possibly living in the pyritic material. When split open, these nodules exhibited the pinkish color of leghemoglobin, a protein in the nodule protecting the active nitrogen-fixing enzyme nitrogenase against the toxic effects of oxygen. Although we have not yet obtained direct evidence of nitrogenase activity in these nodules (reduction of acetylene to ethylene, for example), these findings suggested the possibility that nitrogen fixation was taking place in this hostile, non-soil material. This immediately raises the possibility that free-living anaerobic bacteria which fix atmospheric nitrogen on Earth, could do the same on Mars. The Martian atmosphere includes 2.7 % N_2 and 0.13% O_2 [1] -- if N-fixing anaerobes can adapt or be engineered to thrive in Martian "soil", one can postulate an eventual build up of organic nitrogen for subsequent use by other forms of life. Anaerobic photosynthetic bacteria that fix atmospheric nitrogen might, ideally, also build up oxygen in the Martian atmosphere, but the intense UV radiation reaching the Martian surface would preclude survival outside of a light-transparent shield. Sulfate-reducing bacteria such as *Desulfovibrio,* living beneath the surface with possible access to water adsorbed on fine particles, seem more promising in this regard. Free-living anaerobic diazotrophs on Earth include *Archaeoglobus* [2], the bacillaceae *Clostridium, Desulfatomaculum,* and *Desulfovibrio* [3] and the photosynthetic bacteria *Thiorhodaceae, Chlorobacteriaceae,* and *Athiorhodaceae* [4].

N_2-fixing organisms on Earth, whether free-living or symbiotic, have a common enzyme, nitrogenase, that mediates the following reaction:

$$N_2 + 8e^- + 8H^+ + nATP \Rightarrow 2NH_3 + nADP + nP_i$$

Splitting of the N_2 molecule is an energy-intensive process; 8 to 16 moles of ATP are required to fix 1 mole of N2. This value is not easy to determine because the partitioning of electrons between the two electron acceptors H^+ and N_2 depend on conditions such as ATP concentration, pH, substrate and substrate concentration. The electron donor in many of the systems studied is ferredoxin; where iron is deficient, flavodoxin has been found to substitute. Nitrogenase is a two-protein enzyme consisting of an Fe fraction and an FeMo fraction. The initial steps in the action of nitrogenase consist of the reduction of the Fe-protein, activation of the Fe-protein by Mg-ATP, followed by electron transfer between the nitrogenase proteins [5]. Under some conditions, V can substitute for Mo. Thus for starters, the N_2-fixing sulfate reducers require Fe, Mo, Mg, and an oxidant (sulfate or sulfite on Earth), N_2 in the atmosphere, and the absence of oxygen.

Elemental analyses of the Martian surface indicate an iron concentration (as Fe_2O_3) of 18 mass % and Mg as MgO of 8

mass % [1]; molybdenum and vanadium are possibly present, estimated to be about 1.7 ppm and 162 ppm, respectively [6], within the range of their occurrence in terrestrial soils. Sulfur is present at about 5 mass % (expressed as SO_3) in the "soil" from Pathfinder data [1]. Sulfite reduction is more thermodynamically favorable than sulfate reduction. In fact, reduction of sulfate by sulfate-reducing bacteria will not occur without initial activation by ATP and the formation of the intermediate adenylyl sulfatase [5]. Peroxides (whose presence in the Martian surface is inferred from interpretation of the Viking lander life-detection experiments) may serve as oxidants, albeit rather strong ones. These conditions, and effects on N_2-fixation can be tested experimentally in laboratory microcosms.

Another crucial component for survival of anaerobes is a carbon source. For *Desulfovibrio* and other N-fixing microorganisms, organic acids (e.g., malate, succinate, pyruvate, and lactate) and amino acids serve the purpose on Earth. On Mars, however, atmospheric CO_2 is the only abundant source of carbon known to be present (about 95% of its atmosphere). There is conflicting evidence that at least one species of *Desulfovibrio* could use CO_2 as a carbon source, but this result has more recently been attributed to mixotrophy, a coupled reaction [7]. Here, again, is an avenue for experimentation.

Peroxides in the Martian soil, intense UV radiation, extreme cold, and the absence of liquid water bode ill for the survival and evolution of Earth-like organisms on Mars. Yet, even on Earth we find microorganisms in the most unlikely places: in the core of a nuclear reactor, in a concentrated sulfuric acid copper solution, in thermal springs, in vents of volcanoes, and in Antarctica. Study of the physiology and biochemistry of anaerobic microorganisms, particularly the sulfate reducers, in a simulated Martian environment can demonstrate whether such life, or genetically engineered versions thereof, could survive and grow on Mars. Nitrogen-fixers on Earth have evolved several methods for protecting the enzyme nitrogenase against toxic oxygen: development of internal membranes, incorporation into plant nodules, formation of heterocysts, utilization of oxygen scavengers and reductants, and buffers such as leghemoglobin. On Mars, where O_2 is essentially absent, the N_2-fixers may find Heaven.

References: [1] Lodders, K. and Fegley, B., Jr. (1998). The Planetary Scientists' Companion. Oxford University Press, N.Y. [2] Thauer, R.K. and Kunow, J. (1995). In Barton, L. Sulfate-Reducing Bacteria, Biotechnology Handbooks 8. Plenum Press, N.Y. [3] Silvester, W. and Musgrave, D. (1991 In Dilworth, M.J. and Glenn, A.R. Biology and Biochemistry of Nitrogen Fixation. Elsevier, N.Y. [4] Mulder, E.G. (1975) in Stewart, W.D.P. Nitrogen fixation by free-living microorgansms, Cambridge University Press, 3-28. [5] Cypionka, H.. (1995) Chapter 6 in Biotechnology Handbook 8. [6] Morgan, J. and Anders, E. 1979. *GCA* 43:1601- 1610. [7] Postgate, J.R. (1984). The Sulphate-Reducing Bacteria. Cambridge University Press.