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Research Paper

The Potential for Lithoautotrophic Life on Mars: Application to Shallow Interfacial Water Environments

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ABSTRACT

We developed a numerical model to assess the lithoautotrophic habitability of Mars based on metabolic energy, nutrients, water availability, and temperature. Available metabolic energy and nutrient sources were based on a laboratory-produced Mars-analog inorganic chemistry. For this specific reference chemistry, the most efficient lithoautotrophic microorganisms would use Fe^{2+} as a primary metabolic electron donor and NO_3^- or gaseous O_2 as a terminal electron acceptor. In a closed model system, biomass production was limited by the electron donor Fe^{2+} and metabolically required P, and typically amounted to ~800 pg of dry biomass/ml (~8,500 cells/ml). Continued growth requires propagation of microbes to new fecund environments, delivery of fresh pore fluid, or continued reaction with the host material. Within the shallow cryosphere—where oxygen can be accessed by microbes and microbes can be accessed by exploration—lithoautotrophs can function within as little as three monolayers of interfacial water formed either by adsorption from the atmosphere or in regions of ice stability where temperatures are within some tens of degrees of the ice melting point. For the selected reference host material (shergottite analog) and associated inorganic fluid chemistry, complete local reaction of the host material potentially yields a time-integrated biomass of ~0.1 mg of dry biomass/g of host material (~10^9 cells/g). Biomass could also be sustained where solutes can be delivered by advection (cryosuction) or diffusion in interfacial water; however, both of these processes are relatively inefficient. Lithoautotrophs in near-surface thin films of water, therefore, would optimize their metabolism by deriving energy and nutrients locally. Although the selected chemistry and associated model output indicate that lithoautotrophic microbial biomass could accrue within shallow interfacial water on Mars, it is likely that these organisms would spend long periods in maintenance or survival modes, with instantaneous biomass comparable to or less than that observed in extreme environments on Earth. **Key Words: Mars—Life—Thin film—Interfacial water—Iron—Phosphorus—Nitrogen—Free energy.** Astrobiology 7(2), 342–354.

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INTRODUCTION

THE MICROBIAL HABITABILITY of Mars is a focal point of astrobiological interest. Central to this issue are questions that address the location and mechanisms for water on Mars and what type of metabolic activity can be supported (*e.g.*, Jakosky, 1998). Below a cryosphere that likely averages several kilometers or more in thickness (Clifford, 1993), aquifers could provide favorable habitats. Hydrothermal activity could thin the cryosphere and provide additional energy for the biosphere. The possibility of chemoautotrophs exploiting deep subsurface niches on Mars was considered by Boston *et al*. (1992). Yet habitable regions with liquid water at or near the surface are still important because of their accessibility to near-future exploration. Fluvial water is highly transient at best, and its presence has been controversial (*e.g.*, Malin and Edgett, 2000; Christensen, 2003). Water ice, however, is abundant in the residual polar caps (*e.g.*, Jakosky, 1985) and in the regolith poleward of ±50° latitude (Feldman *et al.*, 2002). In subfreezing environments, small quantities of "unfrozen" water still persist as interfacial films that separate ice from silicates or other ice crystals. This interfacial water behaves like a liquid: it flows and acts as a solvent (hereafter, we use the term "water" to refer collectively to a liquid-like phase of H_2O). The intermolecular forces that form interfacial water result in adsorption, capillarity, frost heaving, and even freezer burn (for a review, see Davis, 2001). Water thicknesses are generally measured in monolayers (~0.3 nm each), with a smooth transition between the capillary regime (several monolayers or more near the melting temperature) and the adsorption regime (a few monolayers that persist to very low temperature). Nuclear magnetic resonance has confirmed that water thin films in terrestrial soils are mobile at temperatures well below 0°C (*e.g.*, Anderson and Tice, 1973; Watanabe and Mizoguchi, 2002), with a mobile adsorbed fraction detected even down to liquid nitrogen temperature [−196°C (Pearson and Derbyshire, 1974)].

Earth's cold deserts, frozen soils, and ice sheets can serve as model environments for possible life on Mars. Microbes in arctic permafrost have been found metabolizing at temperatures as low as −20°C, possibly exploiting thin films of unfrozen water for transport of ions and nutrients (Gilichinsky *et al.*, 1993; Rivkina *et al.*, 2000; Jakosky *et al.*, 2003). Viable microbes have also been discovered in permanent lake- and deep subglacial-ice from below the Antarctic ice sheet (Priscu *et al.*, 1998, 1999; Karl *et al.*, 1999; Christner *et al.*, 2006), possibly residing in the channels of water that separate ice crystals (Price, 2000; Tung *et al.*, 2005, 2006). There is recent evidence that the psychrophile *Colwellia* 34H can incorporate the amino acid leucine at temperatures well below –80°C (Junge *et al.*, 2006). Literature reviews (Harris, 1981; Beaty *et al.*, 2006) indicate that life is possible in water films as thin as three monolayers.

Jakosky *et al*. (2003) examined some constraints on the habitability of ice in Mars' polar regions where water ice is abundant. They contended that −20°C was a lower limit both to the presence of biologically useful interfacial water and to microbial metabolism. Jakosky *et al*. (2003) calculated that peak summertime polar temperatures can exceed −20°C at obliquities above 40°. Laskar *et al*. (2004) computed a mean Mars obliquity of ~38° over the last 4 billion years, so favorable high-obliquity states may be common. The habitability range of near-surface ice on Mars in terms of water activity and temperature may be extended beyond the limits imposed by Jakosky *et al*. (2003), because three or more monolayers of interfacial water may persist to temperatures many tens of degrees below freezing, depending on soil type and solute concentration (*e.g.*, Wettlaufer, 1999) and there may not be a minimum temperature for metabolism (Price and Sowers, 2004).

Small quantities of hydrogen at low latitude [equivalent to ~5% (wt/wt) H_2O (Feldman *et al.*, 2002)] could be due to hydrous minerals or adsorbed water. Möhlmann (2005) developed a concept for habitability based on the latter interpretation. He inferred that adsorbed water would reach its greatest thickness during the night and morning hours, during which time microbes may be able to incorporate water using proteins similar to aquaporins (de Groot and Grubmüller, 2001). Metabolism would be carried out during the warmer (approximately −20°C) midday. The temperature in low-latitude regions of Mars, however, frequently drops below the frost point (−75°C), freezing all but the first few monolayers of adsorbed water. In these regions sufficient relative humidity to exceed the two or three monolayers of water [>60–85% (see Möhlmann, 2005)] necessary to support microbes exists only within 1–3°C of the frost point, which occurs for an average of ~20 min/sol distributed between the evening and early hours after sunrise. Although this is an order of magnitude shorter time than indicated by Möhlmann (2005), the possibility of microbes exploiting adsorbed water, at the very surface of the planet, is intriguing.

These findings share a common theme with relevance to the near surface of Mars: the ability of life to survive in extremely cold habitats limited to water in thin films. We explored these environments through a twofold investigation. Because there have been no clear signs of labile organics on Mars to support heterotrophic life (*e.g.*, Biemann and Lavoie, 1979; Benner *et al.*, 2000), we first developed a generalized numerical model to predict lithoautotrophic biosynthesis based on the availability of nutrients and energetically favorable primary/terminal electron donors/acceptors in a specified aqueous system. The model is based on general thermodynamic principles and can be applied to numerous potential environments and water chemistries on Mars or elsewhere. We focused the second part of our study on the habitability of near-surface interfacial water on Mars and developed general relationships among source-material (regolith) properties, metabolic rates, biomass, and longevity.

LITHOAUTOTROPHIC MODEL

Energy and nutrient availability

The first step in developing our model for lithoautotrophic metabolism was to assess the host major-element chemistry from which microbes will draw energy and nutrients. The data from Viking and Mars Global Surveyor (Thermal Emission Spectrometer) indicate an outermost martian crust that is dominated by pristine and altered volcanic rocks of andesitic to basaltic composition and enhanced generally in iron-rich clays, ferric oxides and oxyhydroxides, and sulfate crusts (Toulmin *et al.*, 1977; Banin *et al.*, 1992; Christensen *et al.*, 2001; Zuber, 2001). Spectrometer data from the Mars Rover Spirit in the Gusev crater indicate a range in degrees of rock alteration, represented by varying Fe^{2+}/total Fe ratios, with rocks in the Columbia Hills appearing the most weathered and enriched in mobile elements, including P (Gellert *et al.*, 2006; Ming *et al.*, 2006; Morris *et al.*, 2006). In the Meridiani Planum region, the Mars Rover Opportunity has revealed siliciclastic rocks that contain hematitic concretions, jarosite and sulfates, an observation that points to alteration processes in the presence of acidic groundwaters (Clark *et al.*, 2005; Squyres and Knoll, 2005). Periods in martian history when these geochemical processes were active are poorly constrained and, as Clark *et al.* (2005) pointed out, may not have reached equilibrium yet. A model by Hurowitz *et al.* (2006) indicates that martian soil chemistries observed by Mars Exploration Rover, Pathfinder, and Viking are consistent with chemical alteration in the presence of low pH and water:rock volume ratios, an environment that could be provided by adsorbed thin films of water. Reflectance spectra indicate that the host mineralogy of martian surface units closely match that of basaltic shergottites (McSween, 1985; Hamilton *et al.*, 1997). Bullock *et al.* (2004) reported results from laboratory experiments in which a mineral mix derived from the composition of basaltic shergottites was allowed to react with a simulated Mars atmosphere for 7 months. The major-element (Ca, Mg, Al, S, F, Cl) abundance patterns in the resulting sulfate-chloride solutions were similar to martian fines at the Pathfinder, Viking, and Mars Exploration Rover Spirit (Gusev crater) sites (Brückner *et al.*, 2003; Gellert *et al.*, 2006). Mars appears to be scarce in N, but not P, compounds (Toulmin *et al.*, 1977; Clark, 1982; Brückner *et al.*, 2003; Gellert *et al.*, 2006), in contrast to the laboratory findings of Bullock *et al.* (2004). Despite this apparent discrepancy, which is discussed in more detail below, we believe that this experimentally derived aqueous chemistry is a reasonable Mars analog starting point for assessing sources of lithoautotrophic energy and nutrients. Specifically, the species concentrations for the aqueous chemistry are taken from the experiments of Bullock *et al.* (2004) performed at 3°C.

To identify potential energy sources, we evaluated the Gibbs free energy change (ΔG) values of various redox half reactions known to provide terrestrial microorganisms with primary electron donors or terminal electron acceptors (Table 1). Aqueous concentrations of molecular N_2 and O_2 were estimated using Henry's Law and assuming diffusive equilibrium with the martian atmosphere containing 2.7% N_2 and 0.13% O_2 (Kieffer *et al.*, 1992). This approximation becomes less valid for the higher solution ionic strengths that occur at lower temperatures; nevertheless,

Table 1. Inorganic Redox Reactions for Metabolic Energy in the Model

Half reactions considered, oxidized species on left side of each couple

Redox couple	*References*	*Reference information*
Fe^{3+}/Fe^{2+}	1, 3–6, 8, 12, 13	1. Thauer *et al.* (1977)
Mn^{3+}/Mn^{2+}	3, 4, 8	2. Price (2000)
SO_4^{2-}/S^0	1, 4, 5, 6, 10	3. Varnes *et al.* (2003)
SO_4^{2-}/SO_3^-	1, 4, 5, 13	4. Madigan *et al.* (2003)
SO_4^{2-}/HS^-	1, 3–5, 6, 8–10, 12	5. Decker *et al.* (1970)
NO_3^-/NO_2^-	1, 4, 5, 13	6. McCollom and Shock (1997)
NO_3^-/NO	1, 4	7. Stevens and McKinley (1995)
NO_3^-/N_2	1, 4, 5, 11, 12	8. Fisk and Giovannoni (1999)
CO_2/CH_3COO^-	4	9. Shock (1997)
CO_2/CH_4	3, 4, 6–10, 12	10. Stetter and Huber (2000)
$CO_2/CHOO^-$	2, 13	11. Smith and Hoare (1977)
O_2/H_2O	1, 4–6, 8, 11, 12	12. Peck (1968)
H^+/H_2	1, 4, 6–13	13. Koch and Schmidt (1991)

Balanced redox reactions used for metabolic energy sources

Number	*Redox reaction*
1	Fe^{2+} (aq) + 0.25 O_2 (aq) + H^+ (aq) → Fe^{3+} (aq) + 0.5 H_2O (l)
2	Fe^{2+} (aq) + 0.2 NO_3^- (aq) + 1.2 H^+ (aq) → Fe^{3+} (aq) + 0.1 N_2 (aq) + 0.6 H_2O (l)
3	Fe^{2+} (aq) + 0.125 SO_4^{2-} (aq) + 1.125 H^+ (aq) → Fe^{3+} (aq) + 0.125 HS^- (aq) + 0.5 H_2O
4	0.5 H_2 (aq) + 0.25 O_2 (aq) → 0.5 H_2O (l)
5	0.5 H_2 (aq) + 0.2 NO_3^- (aq) + 0.2 H^+ (aq) → 0.1 N_2 (aq) + 0.6 H_2O (l)
6	0.5 H_2 (aq) + 0.125 SO_4^{2-} (aq) + 0.125 H^+ (aq) → 0.125 H^- (aq) + 0.5 H_2O (l)
7	0.5 H_2 (aq) + 0.125 CO_2 (aq) → 0.125 CH_4 (aq) + 0.25 H_2O (l)

The upper portion of the table presents all redox half reactions and references considered. The lower portion of the table contains favorable redox reactions for metabolic energy under martian conditions. Selection of the seven redox reactions is based on the redox couples considered in the upper portion of the table and the laboratory-simulated Mars geochemistry in Bullock *et al.* (2004).

Henry's Law provides a reasonable baseline for the near-freezing conditions of the experiments of Bullock *et al.* (2004). The seven balanced redox reactions found to be most exergonic (*i.e.*, lowest ΔG values) were used as potential metabolic energy sources for our model (lower portion of Table 1).

In addition to redox energy, biosynthesis on Earth requires macronutrients such as N and P, as well as a host of trace elements (Madigan *et al.*, 2003). The abundance of atmospheric CO_2 on Mars provides a readily available carbon source for lithautotrophic organisms. A comparison of elements abundant on Mars and in terrestrial bacteria (Todar, 2004) indicates that elements N and P should be considered above others as limiting macronutrients on Mars. The experimental data of Bullock *et al.* (2004) indicate that NO_3^- and PO_4^{3-}, the most common sources for N and P in terrestrial microorganisms, may form from water–rock–atmosphere interaction and become available for metabolism and protein formation. Therefore, we focused on CO_2, N (as NO_3^-), and P (as PO_4^{3-}) as the primary C and macronutrient supplies for potential martian life. Again, we note that NO_3^- compounds have not been detected in previous missions to Mars. However, in consideration of known abiotic N_2-fixation mechanisms on Earth, which include lightning (Navarro-Gonzáles *et al.*, 2001), photochemical reactions on dust (Yang *et al.*, 1994), and even ion partitioning due to freezing (Takenaka *et al.*, 1992), the presence of NO_3^- on Mars seems enigmatic but likely. Therefore, we feel it is reasonable to include NO_3^- in our model following concentrations in the dataset of Bullock *et al.* (2004).

Numerical implementation

A numerical model, hereafter referred to as BIOFORM 1.1, was developed to quantify the energy and nutrients, at user-specified initial conditions, conducive to microbial biomass formation in a chemically closed system. Therefore, the flux of aqueous species arising from complexities in hydrology, water–rock–atmosphere, and possible biotic, interaction processes is not captured, but is noted as a general requirement for sustained life.

The cellular C:P and C:N for balanced microbial growth are adjustable model parameters, set in the present study to 100 and 10, respectively, to reflect values found in terrestrial bacteria (*e.g.*, Goldman and Denett, 2000; Vrede *et al.*, 2002; Todar, 2004). Based on these ratios, the composition of biomass is represented by the formula $C_{10}H_{19}O_3NP_{0.1}$. The element- (though not charge-) balanced biosynthesis relation for building dry biomass is:

$$10CO_2\ (aq) + 9.5H_2O\ (l) + NO_3^-\ (aq) + 0.1PO_4^{3-}\ (aq) \xrightarrow{\text{Energy}} C_{10}H_{19}O_3NP_{0.1} + 14.95O_2\ (aq)$$

The BIOFORM 1.1 algorithm (Fig. 1) consists of six steps, four repeating, situated around a main loop. In step 1, each of the seven redox reactions listed in Table 1 are activated if exergonic under martian conditions. In step 2, moles of reducing agent, n_i (where i corresponds to redox reaction index numbers in Table 1) are calculated using the bisection method to carry each redox reaction forward to an equilibrium state ($\Delta G = 0$). The molar increments δn_i (the primary variables) are determined by dividing n_i by an arbitrarily large integer. The main loop incrementally generates biomass by way of δn_i variables until all energy sources or a nutrient becomes depleted. In step 3, an energy increment, δG, is calculated by summing over energy increments $\delta G_i(\delta n_i)$ from activated redox couples, meanwhile withdrawing species from the aqueous system. An energy increment useful for biosynthesis, δE, is calculated by scaling down δG by the "thermodynamic efficiency" parameter, ν, where $\delta E = \nu\ \delta G$. The ν values for terrestrial microorganisms generally vary between 0.1 and 0.5, depending on the nature of metabolic electron donors and acceptors (Decker *et al.*, 1970; Thauer *et al.*, 1977; McCollom and Shock, 1997). An intermediate ν value of 0.2 is used in the present study. The energy increment, δE, is converted to a molar increment of ATP, δATP, using the relation $\delta ATP = \delta E/U_{\text{ATP}}$, where U_{ATP} is the energy required to form 1 mole of ATP. The value of U_{ATP} generally varies from 41.8 to 50.2 kJ/mol (Thauer *et al.*, 1977). Following the convention in McCollom and Shock (1997), we set the U_{ATP} value to 42 kJ/mol. In step 4, an increment in biomass, δB, is generated using the relation $\delta B = \delta ATP \times Y_{\text{ATP}}$, where Y_{ATP} is the quantity of dry biomass (in g) generated per mole of ATP consumed. We assume the typical Y_{ATP} value of 10 g/mol for terrestrial bacteria (Decker *et al.*, 1970; Thauer *et al.*, 1977). If the quantity of δG, C, N, or P in the system becomes equal to zero, the main loop exits in step 5, and the routine finishes. Otherwise, ion activity coefficients are updated in step 6 using the Davies equation (Faure, 1991; Gill, 1996), and the main loop repeats.

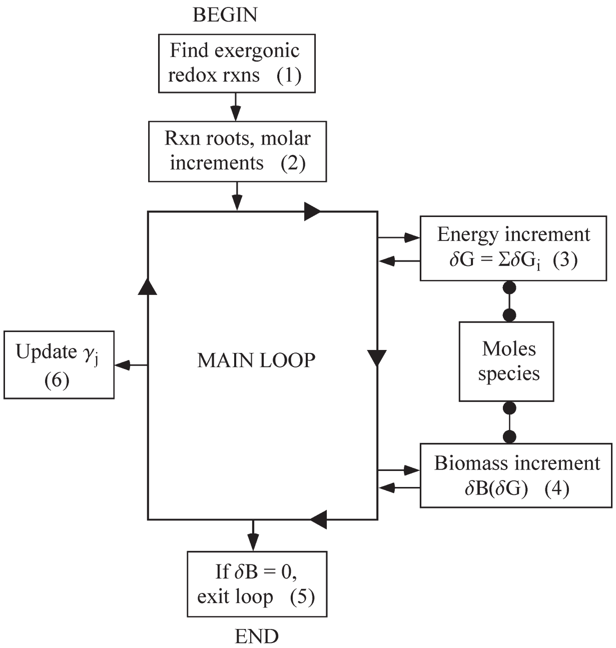

FIG. 1. Flow chart of BIOFORM 1.1 model. The algorithm consists of six main steps situated around the main loop. δG_i = increment in Gibbs energy from i^{th} redox reaction, δG = increment in Gibbs energy as a sum over the i redox reactions, δB = increment in biomass, γ_j = activity coefficient of j^{th} ion.

RESULTS

BIOFORM 1.1 identified two exergonic redox reactions for the selected martian inorganic chemistry: Fe^{2+} oxidation–O_2 reduction (Table 1, reaction 1) and Fe^{2+} oxidation–NO_3^- reduction (Table 1, reaction 2). The ΔG values of these reactions are listed in Table 2 for each experimental time in Bullock *et al.* (2004). The average values for Fe^{2+} reduction of O_2 and NO_3^- are −53 and −47 kJ/mol of electrons, respectively. Note that all times reported in this section are those of the inorganic chemistry reaction progress (*i.e.*, days after adding water to simulated martian en-

TABLE 2 GIBBS ENERGY CHANGE, ΔG_{RXN} (KJ/MOL OF ELECTRONS), OF FERROUS IRON REDUCTION OF O_2 AND NO_3^-, BASED ON THE EXPERIMENTAL TIME SERIES IN BULLOCK *ET AL*. (2004) AT 3°C

	ΔG_{rxn}	
Time (days)	*O_2 reduction*	*NO_3^- reduction*
1	−46.6	−41.2
21	−53.9	−47.8
42	−55.0	−48.5
84	−56.0	−49.4
168	−54.5	−48.0
198	−52.7	−46.4
Average	−53.1	−46.9

vironment) and are unrelated to microbial metabolism.

Numerical results from BIOFORM 1.1 for water of intermediate chemistry (Bullock *et al*., 2004) (84 days of inorganic reaction progress) are shown in Fig. 2. The electron donor Fe^{2+} became depleted when the total dry biomass reached ~800 pg/mL (Fig. 2d). Most of the biomass generation (63%) was fueled by Fe^{2+} reduction of O_2; the remaining 37% was supported by Fe^{2+} reduction of NO_3^- (Fig. 2b). Changes in the electron donor Fe^{2+} exceeded corresponding changes in C (as CO_2), the nutrients P and N, and the electron acceptor O_2 (compare Fig. 2c and d). The total dry biomass values generated from the aqueous chemistries at each of the inorganic reaction-progress times from Bullock *et al.* (2004) are plotted in Fig. 3. For experimental times less than 198 days, Fe^{2+} was the limiting quantity with O_2 respiration providing the most fuel for metabolism (Fig. 3). No biomass was generated after 198 days of reaction progress in the inorganic chemistry owing to P deficiency in the system.

Biomass sustainability

Implementation of BIOFORM 1.1 for a selected Mars-analog inorganic chemistry [3°C and 84 days reaction progress in Bullock *et al.* (2004)] yielded a dry biomass of 800 pg/ml of thin film, which amounted to ~8,500 cells/ml assuming

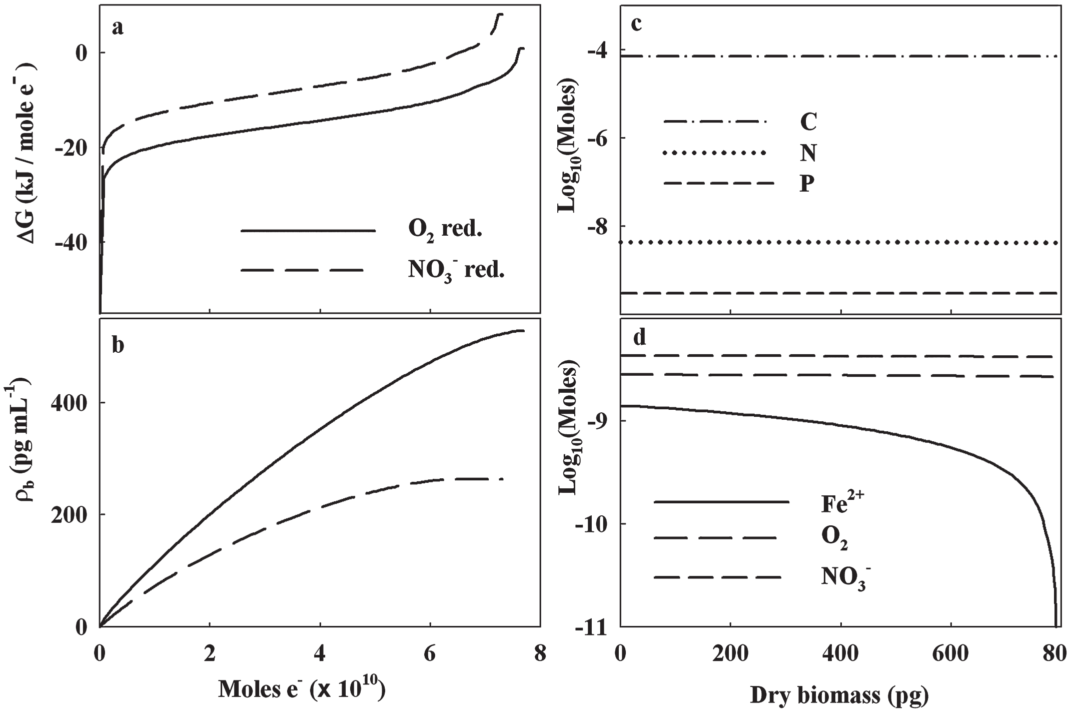

FIG. 2. BIOFORM 1.1 results per ml of thin-film solution of martian-simulated chemistry in Bullock *et al.* (2004) at 3°C and 84 days. The electron (e^-) donor in all redox reactions is Fe^{2+}. (**a**) Gibbs energy change for O_2 and NO_3^- reduction (kJ/mol of e^-). (**b**) Picograms of dry biomass (ρ_b) versus moles electrons transferred for O_2 (solid) and NO_3^- (dashed) reduction. (**c**) Moles of C (as CO_2), N (as NO_3^-) and P (as PO_4^{3-}) versus dry biomass. (**d**) Moles of metabolic e^- donors/acceptors versus dry biomass. For this chemistry, the limiting quantity was Fe^{2+} after generation of ~800 pg of dry biomass. For reference, 1 pg of dry biomass equals ~10 microbial cells (Phelps *et al.*, 1994).

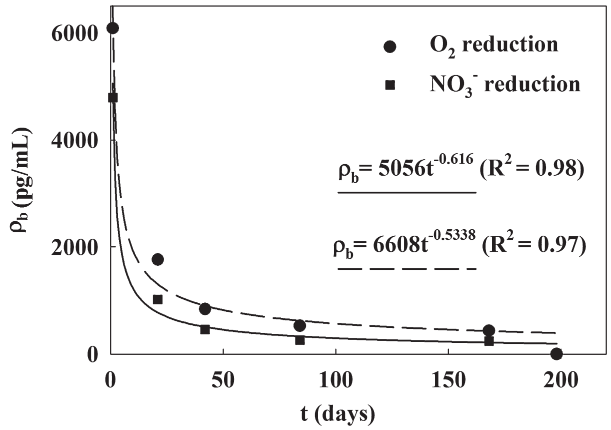

FIG. 3. Picograms of dry biomass per milliliter of thin film, ρ_b, from BIOFORM 1.1. Abscissae values correspond to progress of the inorganic reaction in Bullock *et al.* (2004) and here simply reflect different initial conditions, not microbial growth curves. No biomass generation occurs after 198 days of inorganic reaction progress because of P deficiency. Curve fits represent Fe^{2+}-limiting portion of biomass production and neglect the last data point.

0.47 pg of C/g of dry biomass, 0.12 pg of C/μm^3 of cell, and a cellular volume of 0.37 μm^3 (Nagata and Watanabe, 1990). Because this is a closed-system calculation, continued biological sustainability requires one of the following scenarios: (1) dissolution of the host rock is renewed because of replacement of pore fluid or changes in solution chemistry caused by the biochemical reactions; (2) influx of additional energy- and nutrient-providing solutes from elsewhere; or (3) motile or advective transport of microbes to new, fecund locations.

An upper limit to the total potential (time-integrated) biomass can be calculated for Scenario 1 independent of other assumptions, but predictions in biomass at any point in time for Scenarios 1 and 2 require additional hydrogeological parameters and inorganic/organic reaction rates. Scenario 3 could be reciprocal to Scenario 2 if both are controlled by mass-conservative advection in a closed system.

It is also conceivable that electron donors/acceptors could be renewed in interfacial water at the planetary surface by external processes. Ultraviolet radiation can reduce ferric oxide to Fe^{2+} by the "photo-Fenton" process (Möhlmann, 2004). Although photo-Fenton reactions can be a source for redox constituents, nutrients and C must also be replenished to support ongoing metabolic activity. A reservoir of nitrogen exists in the martian atmosphere, 2.7% N_2 by volume (Kieffer *et al.*, 1992), which could support N_2-fixing bacteria if they are present. However, N_2-fixation is energetically expensive and requires large amounts of P (for ATP biosynthesis) to reduce N_2 to intracellular ammonium and, eventually, amino acids (*e.g.*, Lehninger, 1971). Moreover, although it is possible that N_2 may have played a key role in the early evolution of life on Mars when partial pressures were thought to be higher, it has been suggested that present levels are inadequate to support bacterial N_2 fixation unless selective pressures produced high-affinity enzyme systems capable of fixing N_2 at current pressures (Klingler *et al.*, 1989). Shallow ground penetration of UV radiation poses an additional restriction to microbes at the very surface (Schuerger *et al.*, 2003), but should not inhibit metabolic activity beyond a depth of a few millimeters.

In situ *nutrient consumption*

The total potential, or time-integrated, biomass that can be developed from inorganic host materials is simply given by the unit biomass produced by BIOFORM 1.1 multiplied by the water:host (*i.e.*, water:rock) volume ratio necessary to exhaust the host (Scenario 1). As discussed above, Fe and P were limiting constituents in the model. The shergottite-analog mineral mixture of Bullock *et al.* (2004) contains 7.1% (wt/wt) Fe and 0.092% (wt/wt) P, and we take 1.6 g/cm^3 as the dry bulk density of the regolith (Moore *et al.*, 1978). The partially reacted inorganic reference solution contains 79 ppb Fe and 9 ppb P. Therefore, the water:rock volume ratios to exhaust Fe and P are 1.4×10^6 and 1.6×10^5, respectively (in ml/cm^3). With P as the limiting constituent, the upper limit for potential biomass accumulation is 1.4×10^9 cells/cm^3 of regolith ($\sim 9 \times 10^8$ cells/g of regolith).

The maximum density of bacteria in terrestrial soils is $\sim 10^9$/g (Barnes and Nierzwicki-Bauer, 1997), which is an "instantaneous value" at a given point in time. Predictions of instantaneous biomass density and lifetime from the results of our model require specification of an instantaneous water:rock volume and a characteristic time scale. For this application, the former is the interfacial water volume. Detailed characterization of interfacial water for a variety of Mars-analog host materials and fluids, using nuclear magnetic resonance and electrical properties, is a subject of ongoing investigation (Grimm *et al.*, 2006). Here, we assume simply that microbes can function in water films 1–2 nm thick [three to seven monolayers (Siegel, 1979; Harris, 1981; van der Wielen *et al.*, 2005; Beaty *et al.*, 2006)]. For specific surface areas of 5–20 m^2/g, estimated for the uppermost regolith of Mars (Ballou *et al.*, 1978), the water:rock volume is 1–7%. Therefore, we take 3% as the representative volume fraction (effective porosity) of unfrozen water under biologically favorable conditions near the surface of Mars. BIOFORM's 8,500 cells/ml of fluid then translates to 260 cells/cm^3, or 160 cells/g, of regolith. Using the previously stated limit to potential biomass of 1.4×10^9 cells/cm^3 of regolith after P depletion, $\sim 5 \times 10^6$ pore volumes of fluid would need to be regenerated to exhaust the source material. One possible time scale is the reaction progress of the inorganic reference chemistry; because the experiments of Bullock *et al.* (2004) did not reach steady state, ongoing precipitation of Fe (energy source) from the solutions led to diminishing potential biomass formation with increasing inorganic reaction progress (Fig. 3). Indeed, precipitation of P (a macronutrient) by 198 days during the inorganic reaction progress (Bullock *et al.*, 2004) would permit no further biomass formation. If solutes must be replaced on time scales of ~100 days—before reactions associated with inorganic leaching of host materials reach completion—then phosphorus will be exhausted in (5×10^6 pore volumes) × (100 days/pore volume) × $\sim 5 \times 10^8$ days, or ~1.5 million years. Because reaction progress in interfacial films may be limited by the small quantities of solvent, this time scale should probably be viewed as a strong lower bound.

Alternatively, metabolic rates can be used to assess the time scales for sustainability of biomass produced by the BIOFORM model. Price and Sowers (2004) found that the temperature dependence of prokaryotic metabolic rates derived from numerous sources can be fit with a single Arrhenius activation energy. We adopt −20°C as representative of favorable environments on Mars (Jakosky *et al.*, 2003; Möhlmann, 2005), though Price and Sowers (2004) find no specific evidence for a minimum temperature for metabolism. At −20°C, time scales, t_u, for turnover of cellular carbon for growth, maintenance, and survival (macromolecular repair only) are ~1 year, 1,000 years, and 1 million years, respectively [reciprocals of metabolic rates in Price and Sowers (2004)]. The activation energy of 110 kJ/mol given by Price and Sowers (2004) translates approximately to a factor of 10 decrease in t_u per decadal increase in temperature. The instantaneous biomass, M, sustainable over a characteristic time t_c, can be easily scaled using the relation $M = M_T t_u / t_c$, where M_T is the total potential biomass extrapolated from BIOFORM 1.1, $\sim 10^9$ cells/g of regolith. Using the above relation for M, a density of $\sim 10^9$ cells/g of regolith, in growth metabolism mode, could be sustained for t_c = 1 year; a density of 1 cell/g of regolith could be sustained for $t_c = 10^9$ years (Fig. 4a). At the other extreme, a biomass of $\sim 10^6$ cells/g could survive solely by macromolecular repair for $t_c = 10^9$ years.

Because it is unlikely that all, or even a large fraction, of energy and nutrient compounds in the host material can be metabolized, the biomasses in Fig. 4a should be considered upper bounds. Maintenance biomasses for million-year time scales are therefore likely $<<10^6$ cells/g of regolith. Extremely dry environments on Earth support as few as 100 cells/g (*e.g.*, Navarro-Gonzalez *et al.*, 2003).

SUBSURFACE NUTRIENT TRANSPORT

Lithoautotrophic microorganisms may receive fresh inorganic solutes from elsewhere (Scenario 2 above), which would eliminate the constraints on biomass due to locally available resources. This of course assumes that such fluids are not depleted by organisms or inorganic reactions in their own source locations. For microbes in the shallow subsurface of Mars, solutes can diffuse between zones of different concentration, or they can be advected by groundwater flow, driven either by elevation head or cryosuction.

Solute diffusion coefficients D in the Earth are 10^{-11}–10^{-10} m^2/s (Ingebritsen and Sanford, 1998) and can be as low as 10^{-15} m^2/s in solid ice. The time, t_d, required to diffuse solutes over length scale L is $t_d = L^2/D$. Because solute diffusion is driven by solute gradients, movement can be either horizontal or vertical. The time scale for biomass turnover, t_u (given above for growth, maintenance, or survival), divided by the diffusion time, t_d, is the number of pore volumes that are replenished. The instantaneous biomass, M, fol-

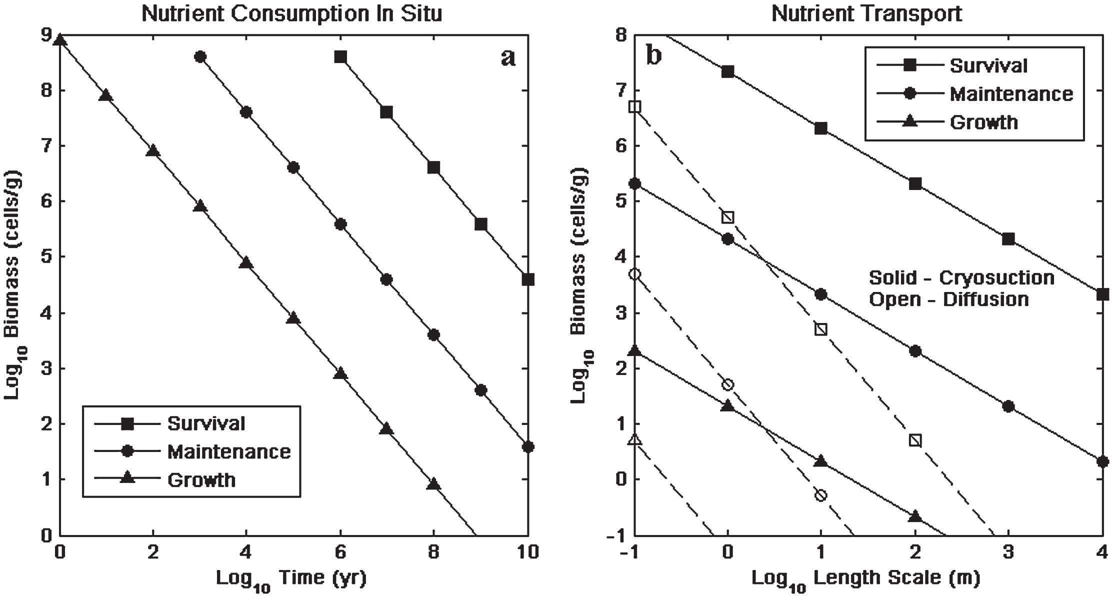

FIG. 4. Biomass extrapolated from BIOFORM applied to near-surface interfacial water. (**a**) Total biomass that can be developed by completely utilizing necessary local inorganic material, using growth, maintenance, and survival metabolic rates defined by Price and Sowers (2004) at −20°C. Curves show different allowable biomass-lifetime outcomes, *i.e.*, the slope does not represent a growth rate. (**b**) Sustainable biomass where nutrient resupply is by advection (cryosuction with effective permeabilty 10^{-16} m^2) or diffusion (10^{-11} m^2/s). Biomass decreases with length scale because greater time is required to deliver nutrients but specified metabolic rates must be maintained. Gravity-driven advection can overcome matric forces only for elevation differences appropriate to large length scales (approximately kilometers) and is an order of magnitude less efficient than cryosuction.

lows by multiplying the number of pore volumes by the biomass per pore volume (160 cells/g of regolith, calculated above). Allowable biomass decreases with increasing length scale because nutrient transport requires more time for a given metabolic rate of t_u^{-1}. For $D = 10^{-11}$ m^2/s, solute diffusion could sustain growing lithoautotrophic microbes only at remarkably low densities, though habitable regions 1–100 m in extent might preserve $>10^2$ cells/g of regolith in long-term maintenance or survival modes (Fig. 4b). Diffusion into broader habitable zones (tens to hundreds of meters or more) is so slow that even microbial densities in survival mode would be very small.

Cryosuction is a powerful force that moves water down a temperature gradient from saturated to unsaturated regions. For a geothermal gradient of 10°C/km (McGovern *et al.*, 2002), the cryosuction pressure ~1.1 MPa/°C (*e.g.*, Davis, 2001) is equivalent to a hydraulic-head gradient (height/length) of ~3 at Mars gravity. Cryosuction can act laterally or vertically like diffusion. For comparison, lateral hydraulic-head gradients on Earth are typically $\sim 10^{-2}$. We estimate the advective transport time under cryosuction using Darcy's Law. A reference permeability of 10^{-13} m^2 is appropriate to silty sands (Freeze and Cherry, 1979). This must be reduced for the unsaturated thin-film state, which approximately follows the cube (Ingebritsen and Sanford, 1998) of the ratio of the interfacial water volume (~3%) to saturated volume (~30%), resulting in an effective permeability of $\sim 10^{-16}$ m^2. The conversion to hydraulic conductivity is also an order of magnitude less efficient on Mars than Earth due to lower gravity and temperature-dependent viscosity. Under these assumptions, the groundwater velocity, v, is 10^{-8} m/s. The characteristic solute advection time over distance L is then $t_a = L/v$. Allowable biomass can again be computed as a function of length scale and metabolic rates (Fig. 4b). Greater biomass and/or sizes of habitable regions can be sustained where solutes are replaced by cryosuction advection compared to diffusion because transport times vary as L versus L^2, respectively. Although biomass under growth conditions remains very small, sizeable maintenance or survival biomass is sustainable over any practical length scale. This scenario requires, however, that ground-ice movement is ongoing throughout the lifetime of the lithoautotrophic microorganisms. We note that this simple cryosuction model successfully reproduces the overnight growth of needle ice on Earth for a reference permeability of 10^{-15} m^2. This is a low permeability but within the range of fine-grained soils (*e.g.*, Freeze and Cherry, 1979) that can express needle ice (see Davis, 2001).

Lateral flow of groundwater on Mars can be driven by topography, as it is on Earth. Interfacial water would migrate downhill, with recharge from and discharge to atmospheric vapor occurring at higher and lower elevations, respectively. Matric forces in interfacial water could exceed several meters equivalent pressure head, however, so elevation differences $>>$10 m are required to move interfacial water. Where this is satisfied, we can approximate the hydraulic gradient to be equal to the topographic gradient. Using the same permeability as the cryosuction model, a surface slope of even 10° [regional slopes are typically ~1° (Aharonson *et al.*, 2001)], yields $v = 6 \times 10^{-10}$ m/s. The 17-fold decrease in nutrient-resupply rate compared to cryosuction leads to a similar decrease in sustainable biomass (displacing cryosuction curves 1.2 log units downward in Fig. 4b). At 10° slope, elevation heads greatly exceed matric heads at length scales much greater than tens of meters, where Fig. 4b shows that allowable growth or maintenance biomasses would again be minimal.

CONCLUDING REMARKS

Using a published Mars-analog inorganic chemistry for energy and nutrient supplies (Bullock *et al.*, 2004), and the thermodynamic model BIOFORM 1.1, we found that reduced Fe^{2+} is the most favorable electron donor for CO_2 fixation during chemoautotrophic respiration of O_2 and NO_3^-. These results imply that diffusive exchange of oxygen between the martian atmosphere and near surface thin films could provide exergonic electron acceptors for microbial metabolism and possibly lead to biogenic iron oxide precipitation (Fortin and Langley, 2005) and biosignatures analogous to those sought in recent studies on Earth (Souza-Egipsy *et al.*, 2006). In the reference inorganic solution, the two limiting bioresources calculated by BIOFORM 1.1 were Fe^{2+} and P. Under intermediate initial conditions, the model generated 800 pg/ml of lithoautotrophic dry biomass/ml of thin film, equivalent to ~8,500 cells/ml. If continued leaching allows all useful energy and nutrient sources of the host soil to be consumed, P alone is the limiting constituent, and the total potential (time-integrated) biomass is ~10^9 cells/g of regolith. Using estimated biomass turnover times, t_u, at −20°C (Price and Sowers, 2004), biomass exceeding the minimum for the Atacama Desert [100 cells/g (Navarro-Gonzalez *et al.*, 2003)] can reproduce only for several million years (Fig. 4a). Over the same time scale ~10^8 cells/g survive at reduced metabolic levels sufficient only to repair macromolecular damage. If lithoautotrophic microorganisms cannot completely utilize the full volume of soil minerals, solute transport could sustain metabolism. The sustainable biomass then depends on the size of the habitable region and its feeding region. Diffusion or gravity-driven advection of solutes can support only a small biomass ($<$100 cells/g) in survival mode. Cryosuction can move somewhat greater nutrient quantities but applies only to areas that are actively undergoing ground-ice segregation. All told, no solute-transport mechanism appears to be reliable for near-surface interfacial water, so *in situ* consumption of host materials is favored for energy and nutrients.

This exercise depends on many assumptions of processes and parameter values. The reference inorganic chemistry of Bullock *et al.* (2004) is just one of many that can be investigated, but represents a reasonable baseline for shallow martian environments. In future work, alternative redox couples will be identified for different inorganic chemistries. These fluid and host compositions may dictate alternative paths to maximum biomass that were unapparent using the simplifications of the present study. The amount of interfacial water and its ability to leach solutes from host materials requires additional laboratory and theoretical study. The nutrient–transport curves scale directly with diffusion coefficient and permeability, and more formal advection–diffusion calculations are necessary. Nonetheless, results of the baseline model reported here suggest that lithoautotrophic microbial growth, maintenance, or survival in the shallow subsurface of Mars would be most efficient through continued reaction of local Fe- and P-bearing host materials rather than transporting energy and nutrients through interfacial water. Due to the predicted difficulty in sustaining metabolic rates that would yield actual growth, organisms would likely spend much time in maintenance or survival modes, even at “favorable” temperatures near $-20°C$, with biomasses comparable to or less than dry environments on Earth.

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ABBREVIATION

ΔG, Gibbs free energy change.

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