A THERMODYNAMIC MODEL FOR ANCIENT PERCHLORATE-DRIVEN ECOSYSTEMS ON MARS.
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Introduction: Perchlorate (ClO_4^-) was detected on Mars by the 2008 Phoenix lander mission [1] and has since been proposed to have played a role in maintaining putative ancient Martian brines in a liquid state through deliquescence [2] as well as providing a source of metabolic energy to potential ancient Martian microorganisms [3], [4], [5], [6]. Hanley (2013) describes eutectic values for perchlorate salts ranging from 270 K to 204 K (temperatures that fall below average annual temperatures on present-day Mars and may have consistently been exceeded in ancient Martian climates) [2]. The high oxidation state of chlorine in perchlorate (+7) gives it a high redox potential in league with that of oxygen, and like oxygen, it can be used as an effective terminal electron acceptor in cellular respiration [7]. Many species of perchlorate reducing microbes (PRM) have been identified on Earth and characterized phylogenies span both bacterial and archaeal domains. The presence of this powerful oxidant on Mars paired with the availability of reduced species known to support biological perchlorate reduction as electron donors, may provide the necessary conditions to meet the energetic constraints of life on the Red Planet.

Perchlorates on Earth

On Earth, perchlorate and its reduced ion chlorate (ClO_3^-) (collectively referred to as (per)chlorates) are generated naturally in the stratosphere through photochemical reactions. Both ions accumulate in arid environments via dry and wet deposition where they are consistently found to co-occur [8], [9], [10], [11]. Synthetic (per)chlorates are also widely distributed as environmental contaminants with anthropogenic sources such as waste discharge from industries that make use of synthetic (per)chlorate salts [11]. The global presence of perchlorate and chlorate on Earth has provided ample opportunity to study environments of naturally occurring (per)chlorates and their biological relevance. Microbial dissimilatory perchlorate reduction has been widely implemented for bioremediation of (per)chlorate contaminated sites and metabolic pathways are well conserved for perchlorate and chlorate reduction using a variety of electron donors [12]. The astrobiological relevance of (per)chlorates has motivated research investigating the biological limits of environments of naturally occurring (per)chlorates (NOP) as a basis for understanding the potential for perchlorate-driven ecosystems elsewhere in the solar system.

Pilot Valley, Utah as a Martian Analog

The goal of this research was to investigate the energetic potential for active biological perchlorate reduction in Pilot Valley, Utah (PV). PV is a paleolake basin analogous to those found on Mars in terms of aqueous mineral composition, and has special astrobiological relevance because like Mars, it contains naturally occurring perchlorate salts [5]. PV is also home to the first reported instance of perchlorate reducing microbes (PRM) co-occurring with natural sources of perchlorate. Studies on the geochemistry of the Pilot Valley environment found that perchlorate was mysteriously absent from flux pathways where the highly mobile anions were expected to exit the system [5]. This otherwise unexplained disappearance of perchlorate, points to a transformation of the oxidant via biological processes. 16S rRNA gene analysis of DNA from PV putative microbial induced sedimentary structures (MISS) revealed multiple taxonomic groups that harbor known canonical perchlorate and chlorate reducers in addition to nitrate reducing bacteria may also be capable of dissimilatory perchlorate reduction. While PV sediments have lower perchlorate content than soils measured on Mars, the coexistence of PRB and NOP is an important phenomenon that shines a spotlight on analogous Martian basin environments as possible sites for perchlorate-driven ecosystems beyond Earth.

Methods: The feasibility of biological perchlorate reduction in PV was evaluated by calculating the thermodynamic favorability of metabolic perchlorate reduction using various electron donors available in the environment, as well as some known reductants on Mars not directly measured in PV. Perchlorate reduction occurs by a three-step process [7]

$$ClO_4^- + 2H^+ + 2e^- \rightarrow ClO_3^- + H_2O \quad 1.189\ V \quad (1)$$

$$ClO_3^- + 2H^+ + 2e^- \rightarrow ClO_2^- + H_2O \quad 0.330\ V \quad (2)$$

$$ClO_2^- \rightarrow Cl^- + O_2 \quad (3)$$

While the final step (equation 3) is a disproportionation of ClO_2^- and does not yield any energy, several molecular strategies have been reported for recycling the oxygen product to drive aerobic cellular processes in anaerobic environments [13]. While this oxygen product likely contributes to the energetic favorability of reducing perchlorate in PV, an oxygen reduction step was not included in Gibbs free energy calculations, making predictions of favorability somewhat conservative. The reductants H_2, H_2S, HS^-, Fe^{2+}, CH_3COOH, CH_4, and CO were evaluated for their energetic contributions as electron donors in major respiratory pathways expected to occur in PV. The oxidants nitrate (NO_3^-) and sulfate (SO_4^{2-})(both prominent in PV) were also evaluated in terms of their energetic usefulness to gain perspective on the relative importance of perchlorate to native microbes, as well as to develop further insight into PV biogeochemical processes.

The standard Gibbs free energy change (ΔG^o) of each reaction was calculated from the change in redox potential:

$$\Delta G^o = -nF\Delta E^o \quad (5)$$

Reaction stoichiometry and calculated species activities were then used to model the thermodynamic favorability of each reaction under the geochemical conditions of Pilot Valley by solving for the real-world ΔG:

$$\Delta G = \Delta G^o + RT\ln Q \quad (6)$$

Geochemical Modelling

PHREEQC geochemical modeling software, version 3, was used to calculate the effective concentration, or activity of species in a PV matrix solution using the Pitzer ion interaction approach for high salinity. The pitzer.dat database was updated with species definitions, reactions, thermodynamic values and Pitzer parameters for a $Na - K - NH_4^+ - Mg^{2+} - Ca^{2+} - Fe^{2+}$-$Fe^{2+} - H^+ - Cl^- - ClO_4^- - ClO_3^-$- ClO_2^--SO_4^{2-}–SO_3^{2-}-H_2S-NO_3^-–NO_2^--NH_4^+-OH^-–CH_3COO^--HCO_3^-–CO_3^{2-}–CO_2–O_2–CO-CH_4–NH_3–H_2O system and speciation calculations were run for an isolated solution. A Pilot Valley matrix with ion concentrations estimated from PV brine and sediment measurements and enriched with 0.1 wt% perchlorate and chlorate were used for an initial solution composition. Calculations were performed for a PV solution in equilibrium with an anaerobic chamber gas phase in order to simulate the anaerobic conditions under which most perchlorate reduction occurs. Additional simulations modeled wet lab microcosm environments as part of ongoing research characterizing PV microbial ecology and geomicrobiology. Simulated microcosm experiments included perchlorate-enriched PV matrix solutions supplemented with a single electron donor in order to individually study species effects on PV microbial growth. The feasibility of using reductants available on Mars such as CO and CH_4 for electron donors was evaluated in this way. Energetic predictions of microcosm environments will lend support to future findings.

Results and Discussion: The updated Pitzer database successfully performed speciation calculations while accounting for redox chemistry and ion interactions. Calculated pH and pe values for simulated solutions fell within the expected range for PV and the charge balance percent error of cation and anion distribution was reported below 12%. Figure 1 illustrates the most favorable microbial redox reactions in terms of calculated real-world Gibbs free energy under the simulated chemical conditions of PV.

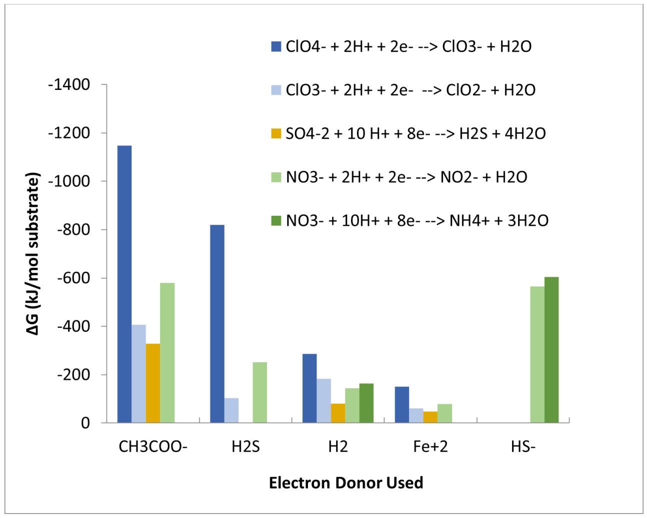

Figure 1: The estimated real-world favorability of microbial respiratory pathways under simulated geochemical conditions of Pilot Valley, Utah using native electron donors.

In terms of Gibbs free energy output alone, perchlorate is the most energetically valuable terminal electron acceptor in PV, providing canonical perchlorate reducers with an advantage and likely incentivizing nitrate reducers to use cryptic or symbiotic methods of perchlorate reduction as suggested in previous work [5].

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