

**ENERGY DEMAND, NOT SUPPLY, DICTATES MICROBIAL SUBSTRATE PREFERENCE.** M. A.

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**Introduction:** Microorganisms drive global biogeochemical cycles and therefore exert strong control on the habitability of Earth, both today and in the geological past. Consequently, a central goal in astrobiology research is to predict the distributions and activities of microorganisms in natural environments on Earth, with application toward predicting the habitability of other planets. The application of thermodynamic calculations to geochemical data makes it possible to predict and infer the variety and abundance of chemical energy available to microbial populations [1, 2]. Such approaches have been widely used to rationalize the observed distribution of microbial populations and their activities in a variety of analog environments on Earth [3, 4].

Thermodynamic-based approaches rank assemblages of redox reactions according to the amount of energy that can be dissipated, with the distribution of microorganisms and their activities presumed to follow this sequence of reactions due to competition for energy-rich substrates [3-5]. However, thermodynamic-based predictions often fall short of accurately describing the distribution of microorganisms and metabolic processes in natural [6, 7] and engineered systems. To provide insight into why these predictions fail to play out in natural systems and to improve our ability to accurately predict patterns of substrate usage, we characterized the bioenergetics and biomass yields of the metabolically flexible, thermoacidophilic crenarchaeote *Acidianus* DS80 when grown autotrophically with hydrogen (H<sub>2</sub>) or elemental sulfur (S<sup>0</sup>) as electron donors and S<sup>0</sup> or ferric iron (Fe<sup>3+</sup>) as electron acceptor.

**Results:** Thermodynamic calculations indicate that S<sup>0</sup>/Fe<sup>3+</sup> and H<sub>2</sub>/Fe<sup>3+</sup> should be preferred redox couples in cultures of *Acidianus* DS80, yielding three- and four-fold respectively more energy per mol electron transferred than the H<sub>2</sub>/S<sup>0</sup> couple. However, biomass yields in autotrophic cultures provided with H<sub>2</sub>/S<sup>0</sup> were four- and eight-fold greater than when provided S<sup>0</sup>/Fe<sup>3+</sup> or H<sub>2</sub>/Fe<sup>3+</sup>, respectively, indicating the H<sub>2</sub>/S<sup>0</sup> redox couple is preferred. Indeed, cells provided with all three growth substrates (H<sub>2</sub>, Fe<sup>3+</sup>, and S<sup>0</sup>) grew preferentially by reduction of S<sup>0</sup> with H<sub>2</sub>. Given that the only difference in culture conditions was the source of electron donor and acceptor, these observations indicate that energy demands of electron transfer reactions rather than energy supplies dictate substrate preference.

The results of these experiments raise questions about why the energy demands differ when using these

reactions, and where and how electron transfer takes place in cells that grow preferentially with H<sub>2</sub>/S<sup>0</sup> over S<sup>0</sup>/Fe<sup>3+</sup> or H<sub>2</sub>/Fe<sup>3+</sup>. Respiration of S<sup>0</sup> with H<sub>2</sub> in *Acidianus* is mediated by a short electron transfer chain comprising a membrane-associated [NiFe]-hydrogenase and a membrane-associated sulfur reductase complex, linked by a quinone cycle. Despite the absence of a characterized Fe<sup>3+</sup> reduction pathway DS80 cells grown with Fe<sup>3+</sup> as oxidant, had numerous hair-like protrusions extending from the membrane that resembled “nanowires” [8]. Such structures were not observed when S<sup>0</sup> was provided as an oxidant, suggesting a role for these structures in Fe<sup>3+</sup> reduction. In addition to the additional energy cost associated with synthesis of pili-like structures, it is possible that energy is lost as heat during successive electron transfers along the array of electrically conductive components leading to a lower thermodynamic efficiency per electron transported. Energy loss through this mechanism would represent additional energetic costs to cells, which could otherwise contribute to biomass synthesis and more efficient cellular yields [9].

**Conclusions:** Collectively, these observations indicate that the preferential use of electron donors and acceptors in metabolically versatile strains, such as DS80, cannot be understood in terms of energetic supplies based on thermodynamic analysis or on measurements of T<sub>n</sub> alone. Rather, these data show that combining thermodynamic calculations with measurements of biomass yields provides a metric to describe the physiology of cells in a way that more accurately captures the physiological nuances experienced by cells that dictate differences in energy demands attending substrate utilization. We suggest that a combined approach, as outlined above, could be used to develop more accurate models for describing the distribution of microorganisms and their activities in natural and engineered environments

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