SURVIVABILITY OF CHEMOTROPHIC MICROORGANISMS IN MARTIAN-ANALOGUE WATER ACTIVITIES.

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Introduction: Life finds a unique way to adapt to changing and extreme environmental conditions; yet liquid water is required by all organisms on earth as both a solvent and reactant to preform essential biological processes. Water activity (a_w), the measure of chemically unbound water in a substance, determines cellular osmosis via gradients established from higher extracellular water activities. In low a_w environments such as arid or high saline, osmotic stress forces cells to shut off their processes and go dormant or even die [1]. From extensive research in the food safety industry, we know that most of life thrives over a range of a_w from 1.0 (pure water) to .7 for more robust halophiles [2], even .62 in extreme cases [3].

Most of Earth's crust lies between a_w of .98 and 1.0 [4], seawater at .98, and closed basin systems such as the Great Salt Lake ~.75 due to increasing salinities which decrease a_w values (3.5% salt in seawater vs. 23-27% for Great Salt Lake) [2,5]. However little research is available on the necessary water activities in sedimentary settings needed to sustain life in terrestrial settings and planetary analogues. Our goals for this research investigation is to fill in these gaps for both baseline a_w and usage of these values for Martian sedimentary material comparisons.

Motivation: It is estimated that evaporation of Meridiani groundwater in the late Noachian/early Hesperian led to a sustained a_w of 0.78 to 0.86 [4]. These estimates are based on sedimentary analysis of groundwater diagenesis in the Burns Formation at Meridiani Planum [6,7], layering at Gale crater, and global hydrated mineralogy as observed by MRO/CRISM and the OMEGA instrument on Mars Express [8-11]. If life evolved on Mars to the point of utilizing chemical molecules as an energy source (chemotrophy), the adaptation of those organisms to low a_w would be necessary for survival. We seek to replicate Martian analogue water activities in vitro with chemotrophs to determine if life can indeed find a way to survive and carry out metabolic processes in low solar flux and xerophilic conditions that occurred globally on Mars well into the Amazonian period. Ongoing work [12] showed that hydrated mineral evaporites, including modern gypsum clays and halite salts from Great Salt Lake, can preserve and maintain µm-scale habitats and DNA. Preservation was six-fold higher in gypsum clays which naturally precipitate at higher a_w (determined by Usiglio in 1884 [13]), suggesting a correlation between high a_w and the ability to preserve and/or harbor life.

These evaporites occur globally on Mars in areas of past surface and groundwater movement such as Jezero Crater [14]. Furthermore, groundwater downwelling into potential closed basin systems in the Martian subsurface may have provided low, but adequate a_w to sustain drought tolerant life as we know of on Earth. Our goals are to quantify the a_w needed to sustain microbial metabolism.

Methods: Experiments were conducted in our EIS instrument (Figure 1), capable of in-situ electrochemical impedance spectroscopy, variable humidity controls, temperature (-70° to 190°), N_2 purging, and 2 to 4 electrode configurations based on sample type [15]. We used chemotrophic *Bacillus subtilis* (ATCC 6051) and *Deinococcus radiodurans* (ATCC BAA-816D-5) bacteria because of their ability to survive in anoxic, low solar flux environments using Martian occurring organics and inorganics as an energy source. Neither of these organisms are xerophilic in nature, *B. subtilis* preferring a_w around .90 [16], while *D. radiodurans* is hardier to desiccation [17]. Therefore, the microbes ability to adapt to or endure low water activity could be studied.

Prior to microbial introduction and growth, we adjusted EIS chamber humidity to establish our experimental water activities as a function of electrical current in our control (Martian soil analogue MMS-2 [18] and Media: Figure 2A). We then tested *B. subtilis* in and *D. radiodurans* in TGY media, each in two cups at 30°C for 3 hours and 9 hours respectively at a_w's of 1.0, .92, .89, .86, and .83 (Figure 2B).

We used a three diode EIS system to monitor redox currents produced by chemolithotrophs in real time showing instantaneous water activity effects on metabolism. Raman spectroscopy on the inorganic broth before and after experiment determined how much substrate had been oxidized by the bacteria.

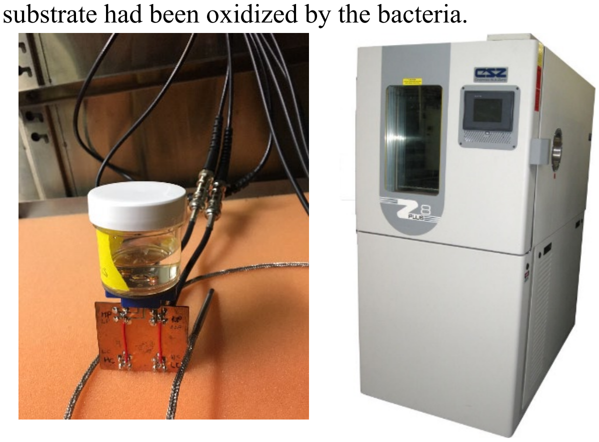

Figure 1: EIS chamber and sample cup with diode configuration capable of measuring current.

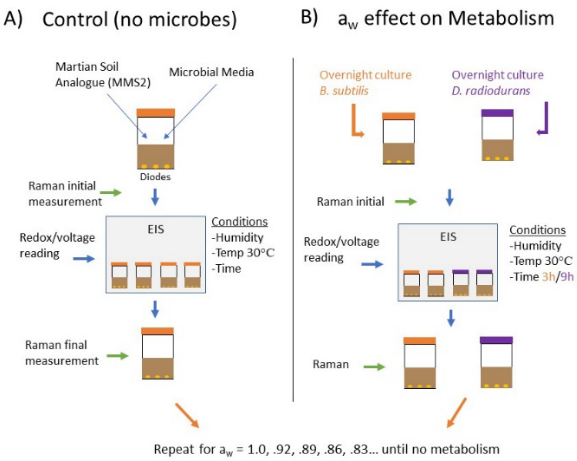

Figure 2: (A) Control setup to establish water activity in the absence of microbes (B) Microbes added and brought to established humidity for water activity.

Discussion: Low water activity presents habitability challenges for both ancient and modern mars. Ancient closed-basin, subsurface aquifers may have supported life that could adapt to the changing conditions; the mystery of RSL's could support the presence of a liquid groundwater system even today [19]. In either case, Mars contains a plethora of inorganics used by chemolithotrophs on Earth such as iron-sulfide reducing or perchlorate-nitrate reducing microbes. If the highest level of a_w available occurred in the subsurface, chemotrophy would be the only sustainable form of metabolism. Therefore, chemotrophs that can survive low a_w may be a proxy for Martian life because of their flexibility in adapting to available ions [12], changing solar conditions, and water burial. Our experiment shows using EIS technology we can study metabolisms in real time for microbes in Martian environmental conditions. After baseline EIS measurements for water activity we will use the MUFFINS water activity sensor [20] for additional instrument comparisons. Future experiments in EIS can include UV conditions and eventually pressure conditions to create a near complete analogue for closed basin aquifers in the Martian subsurface.

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