MICROBE SURVIVAL IN SULFATE BRINES OF VARIED CONCENTRATIONS, CHARACTERIZED VIA OD_{590}. M. Silver[1]; S. Mora[2], M. Ivey[2], and V. Chevrier[1]. [1]*Arkansas Center for Space and Planetary Sciences at the University of Arkansas. 332 N Arkansas Ave., Fayetteville, AR 72701.* [2]*The University of Arkansas Department of Biological Sciences. 1 University of Arkansas, Fayetteville, AR 72701.*

Introduction:

Significant sulfate mineral exposures have been identified on the Martian surface by the Viking era missions and NASA Mars Exploration Rovers [1,2] including Mg, Fe, and Ca sulfates. Investigation of Martian conditions indicates that liquid water in the form of brines may form and remain stable in the shallow subsurface for extended lengths of time [2-4]. According to the second Mars Exploration Program Analysis Group (MEPAG) Special Regions Analysis Group (SR-SAG2), these sulfate brines on the Martian surface or shallow subsurface need to be considered "special regions": regions where terrestrial organisms are likely to replicate and/or have a high potential for the existence of extant Martian life forms [5]. For these reasons, cellular growth and reproduction was characterized in organisms which utilize sulfates as metabolic energy sinks (SO_4^{2-} as terminal electron acceptor).

Experiment:

To assess the effect of potential subsurface Martian brines on microbial growth, sulfate-reducing bacteria (SRB) were collected from a local waste-water treatment facility and selected using medium as previously described [6]. Successful isolation of SRB was determined through PCR using primers isolating the dsrAB operon [7].

Experiments were performed with unmodified medium from [6] (~0.44 wt.% Na_2SO_4) and with an additional 0.1% (wt.) $CaSO_4$. All samples were kept at 32°C. Replicates of each experiment were kept uninoculated as negative controls. Samples were prepared anaerobically and kept in sealed serum vials 1cm in diameter (outer) with 0.5 bar of 80% H_2 + 20% CO_2 (by volume) gas mix comprising one half of the serum vial volume (headspace). Samples were then inoculated with 10% (wt.) exponential growth phase waste-water SRB culture. Samples were then allowed to incubate for either 22 hours (unmodified medium) or 42 hours ($CaSO_4$ modified) before beginning analyses. Headspace gas was replenished weekly.

Microbial growth was characterized through optical density (absorbance) measurements at 590 nm (OD_{590}) using a WPA CO 7500 Colorimeter utilizing negative controls for reference. Optical density measurements fluctuated by ±0.01 depending on the amount of time used to analyze a sample. Therefore, a minimum error of ±0.01 was assumed for all data. Sample pH was also measured 1) during media preparation, and 2) at the end of each experiment. Cultures supplied with unmodified medium incubated for a total of 308 hours, and cultures supplied with additional sulfate incubated for 166 hours. All samples were kept in an incubator, but the start of incubations was staggered. After 166 and 308 hours (modified and unmodified medium cultures, respectively), there was a failure in temperature control and the experiments were ended.

Results:

Absorbance in cultures supplied with unmodified medium ("Medium S1" and "Medium S2", respectively) increased between 22-44 hours of incubation (Fig. 1). Rates of absorbance-increase then raised in Medium S1 and Medium S2, respectively, until 69 hours of incubation. Medium S1 then decreased in absorbance until 186 hours of incubation. Medium S2 decreased in absorbance until 165 hours, then did not

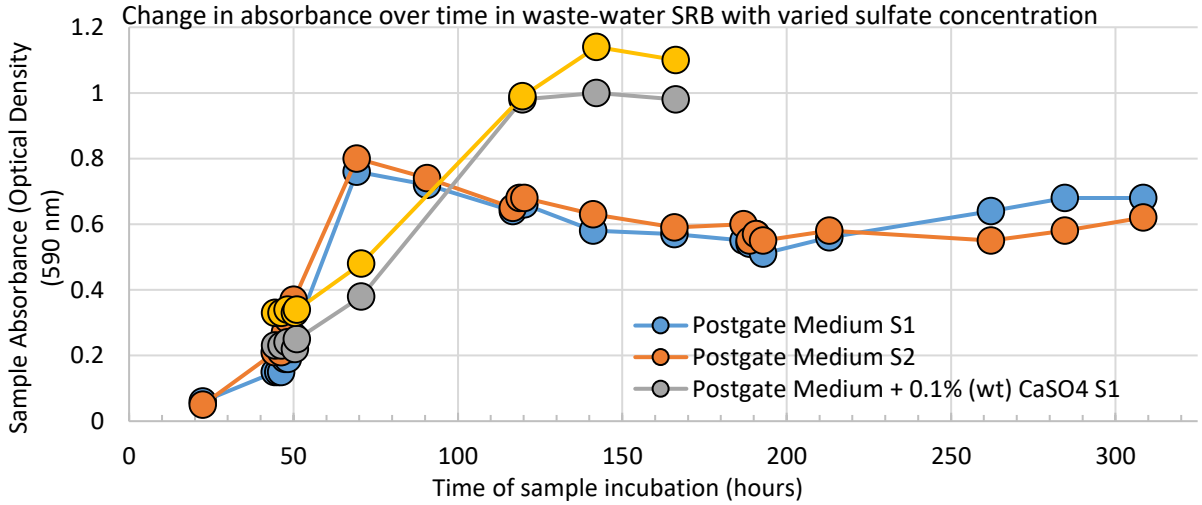

Figure 1: change in sample absorbance measured at 590 nm. Samples were prepared either with medium as described by Postgate (1984) or medium plus additional $CaSO_4$ and then inoculated with exponential growth phase waste-water sulfate-reducing bacteria culture. Measurement deviations (±0.01) are shown, but are obscured by the data points.

show appreciable change until 186 hours. Starting at 186 hours of incubation and continuing until 193 hours, both samples dropped in absorbance by 0.04 and 0.05, respectively. Medium S1 then increased in absorbance until 285 hours of incubation, and then displayed no change in absorbance. Between 193 and 213 hours of incubation, Medium S2 increased in absorbance, then decreased until 262 hours of incubation. From 262 hours until the end of the experiment, Medium S2 increased in absorbance.

Absorbance in cultures with medium supplemented with an additional 0.1% (wt.) $CaSO_4$ ("Medium+$CaSO_4$ S1" and "Medium+$CaSO_4$ S2," respectively) did not change until 46 hours of incubation, then increased in absorbance until 70 hours of incubation (Fig. 1). From 70 to 120 hours of incubation, both samples increased in absorbance at higher rates. At 120 hours, Medium+$CaSO_4$ S1 increased in absorbance, until 142 hours after incubation. From 120 to 142 hours, Medium+$CaSO_4$ S2 increased in absorbance. From 142 hours until the end of the experiment, both samples decreased in absorbance.

All four samples had similar optical density/absorbance values of 0.22-0.37 after 50 hours of incubation. However, samples without additional $CaSO_4$ exhibited greater rates of absorbance increase than those supplemented with 0.1% (wt.) $CaSO_4$ starting after 50 hours. Un-supplemented samples saw peak absorbance after 69 hours of incubation at values of 0.76 Medium S1) and 0.80 (Medium S2), while supplemented samples did not reach peak absorbance until 142 hours, but at greater values (1.00 and 1.14; Medium+ $CaSO_4$ S1 and S2, respectively).

During medium preparation, samples Medium S1 and S2 were brought to pH=7.08 and samples Medium+$CaSO_4$ S1 and S2 to pH=6.85. At the end of the experiment, the pH of Medium S1 and S2 were 6.86 and 6.80, respectively. Samples Medium+$CaSO_4$ S1 and S2 had final pH values of 7.08 and 7.17, respectively.

Discussion

Uninoculated duplicates were used as absorbance references for their respective cultures and thus should account for any abiotic alteration to sample absorbance. Therefore, absorbance changes observed in the reported samples are likely caused by biotic processes [8](e.g. cell reproduction, growth, or death).

The increased sulfate concentrations in the supplemented samples (0.54% vs 0.44%; wt.) decreases the concentration of biologically available water, which may be the cause of the delayed peak-absorbance times compared to un-supplemented samples. If this was the case, SRB in Medium+$CaSO_4$ samples would have spent more initial time adapting to their conditions than Medium samples.

The increased sulfate concentrations in Medium+$CaSO_4$ S1 and S2 may be responsible for the increased peak-absorbance values over Medium S1 and S2, allowing for greater growth/reproduction in SRB. This would imply that sulfate concentration was the dominant growth-limiting factor in samples with unmodified medium. Alternatively, the increased sulfate concentrations may have offset the buildup of biocidal metabolic products in samples Medium+$CaSO_4$ S1 and S2. If biocidal biproducts accumulated in Medium S1 and S2, they may account for the decrease in pH observed. However, it is likely that samples Medium+$CaSO_4$ S1 and S2 would also increase in acidity over time as sulfate-reduction metabolic biproducts accumulate [6], eventually reaching Medium S1 and S2 values.

Due to the abrupt end of the supplemented samples, it is not possible to adequately compare decreasing absorbance phases between the supplemented and un-supplemented samples. However, absorbance trends in Medium S1 and S2 resemble exponential growth and lag phases predicted for typical growth curves[9].

Conclusion:

Cultures of sulfate-reducing bacteria were isolated from local waste-water and provided medium or medium supplemented with Ca-sulfate ($CaSO_4$) to test the effect of sulfate concentration on microbes. Samples were incubated under conditions ideal for mesophilic sulfate-reducing bacteria and analyzed periodically for absorbance/optical density. In samples with increased sulfate concentrations (0.55 wt.%), peak absorbance values were 0.3-0.38 greater than those with lower sulfate concentrations (0.44 wt.%), but peak absorbance was delayed by 94 hours. The increased sulfate concentration may have caused a greater lag time in cellular reproduction (compared to lower sulfate concentration cultures), but allowed for a higher total cell count.

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