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An Investigation into the Suitability of Sulfate-Reducing Bacteria as Models for Martian Forward Contamination

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An Investigation into the Suitability of Sulfate-Reducing Bacteria as Models for Martian Forward Contamination

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Abstract

The NASA Planetary Protection policy requires interplanetary space missions do not compromise the target body for a current or future scientific investigation and do not pose an unacceptable risk to Earth, including biologic materials. Robotic missions to Mars pose a risk to planetary protection in the forms of forward and reverse contamination. To reduce these risks, a firm understanding of microbial response to Mars conditions is required. Sulfate-reducing bacteria are prime candidates for potential forward contamination on Mars. Understanding the potential for forward-contamination of sulfate-reducers on Mars calls for the characterization of sulfate-reducers under Mars atmosphere, temperature, and sulfate-brines.

This study investigated the response of several sulfate-reducing bacteria, including spore formers and psychrophiles. The psychrophile *Desulfotalea psychrophila* was found to inconsistently survive positive control lab conditions, attributed to an issue shipping pure cultures. *Desulfotomaculum arcticum*, a spore-forming mesophilic sulfate-reducer, and *Desulfuromusa ferrireducens,* an iron and sulfate-reducer, were metabolically active under positive control lab conditions with complex and minimal growth medium. A wastewater treatment sulfate-reducing bacteria (SRB) isolate was subjected to sulfate + growth-medium solutions of varied concentrations (0.44 & 0.55% wt. SO_4^{2-}). The wastewater SRB displayed higher cellular light-absorbance levels at delayed rates in 0.55% sulfate solutions, suggesting a greater total culture reproduction, but with increased lag time. Additional SRB were isolated from marine sediments, subjected to a shock pressure of 8.73 GPa, and returned to ideal conditions. The sulfate-concentration patterns in the impacted SRB culture suggests a destruction of culture occurred somewhere during the preparation process. The response of SRB in this investigation to Ca and Na sulfate-brines suggests that Martian sulfate deposits offer a viable energy sink to terrestrial microorganisms, and the studied SRB are capable of replication at

reduced water-activity. Further investigation (i.e. sulfate cations and concentrations, temperature, pressure, etc.) may identify Martian locations at risk to forward contamination.

Contents

1. Introduction

1.1 Planetary Protection & Mars

In March of 2017, the National Academy of Sciences, Engineering, and Medicine published an interim report reviewing the current state of planetary protection policy development at the behest of the United States National Aeronautics and Space Administration (NASA; National Academies of Science, Engineering, 2017). In this report, sample retrieval from the surface of Mars is cited as a goal of NASA's Mars 2020 mission. The 2017 planetary protection review provided the following working definition:

> Planetary protection involves at least three fundamental activities – policy formulation, policy implementation, and compliance and validation. It encompasses three goals, rationales, policies, processes, and substantive requirements that are intended to ensure that any interplanetary space mission does not compromise the target body for a current or future scientific investigation and does not pose an unacceptable risk to Earth (in the case, for example, of sample return missions).

The review offered a further statement as an optional addendum, dependent on pending deliberations regarding the investigation of potential endogenous biologic materials on Mars:

> Further, in the course of ensuring the biological safety of the Earth and other bodies, planetary protection has a role in safeguarding the scientific objectives of future investigations, specifically investigations aimed at ascertaining the possible occurrence and nature of life on other solar system bodies.

Planetary protection is subdivided into three rationales (or goals). The third of these rationales focuses on biologic contamination and its minimization (National Academies of Science, Engineering, 2017). The rationale mandates avoidance of terrestrial organism or organic matter incorporation or contamination in sample material returned from Mars. Terrestrial DNA and proteins were suggested to be unambiguously identifiable, but not so for other organic materials. Organic biomarkers and detection of putative life would be confounded by terrestrial organic matter. Therefore, the final NASA planetary protection rationale states that considerable

effort should be undertaken to prevent such contamination (henceforth referred to as "forward contamination").

1.2 Mars Temperature, Atmosphere, and Radiation

To investigate potential forward contamination on Mars, conditions relevant to (known) biology must be adequately characterized. In the case of the surface and subsurface of Mars, temperature, pressure, radiation, water-availability, and adequate fuel/oxidants are considered the dominant habitability constraints (Kral et al., 2011; Rummel et al., 2014; Schuerger et al., 2013; Tosca et al., 2008).

The surface of Mars has an average temperature of 210 K (NASA Mars Fact Sheet). However, Mars undergoes significant diurnal and seasonal temperature variations. Figure 1 depicts the significant temperature variations experienced diurnally and seasonally on Mars (Pla-Garcia et al., 2017). Surface temperatures on Mars can get as low as 150 K and as high as 303 K as measured by the Curiosity rover (Martín-Torres et al., 2015; Pla-Garcia et al., 2017). Modeling of Mars regolith shows that temperature fluctuations within the subsurface decrease with depth (M. T. Mellon et al., 2004).

A group of scientists collaborated in 2014 (Rummel et al.) in an investigation of Martian habitability. Mars surface and subsurface temperatures were one of the habitability factors considered. The report investigated lab studies on low temperature limits to cell division (findings summarized in table 1) and found two studies (Collins & Buick, 1989; Mykytczuk et al., 2013) that reported cell division at 255 K and 258 K, respectively. Based on the studies in table 1, Rummel et al. (2014) decided on a temperature limit of 240 K for microbial metabolic activity. Low average surface temperatures and significant temperature fluctuations (seasonal and diurnal) make for difficult habitable conditions on Mars. Temperatures can reach more

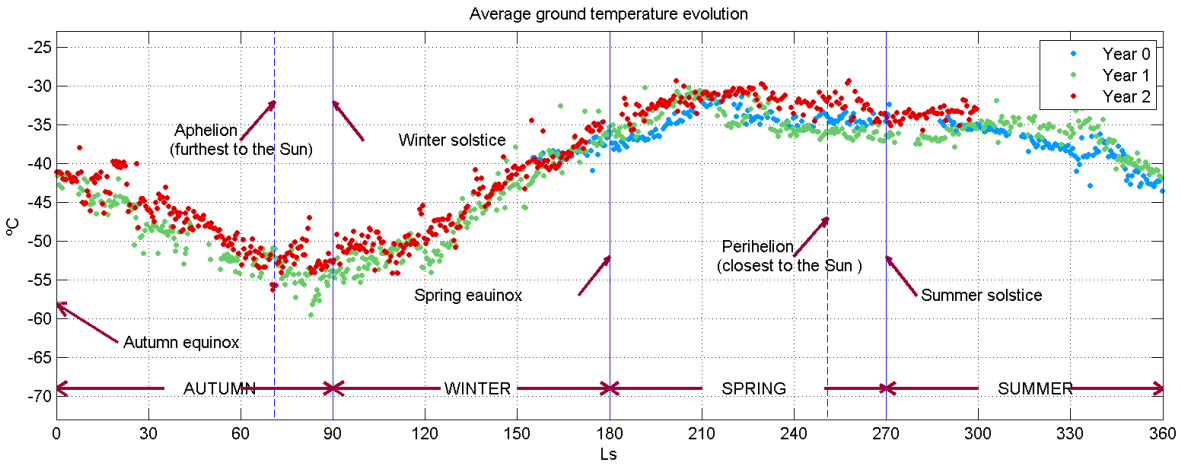

Figure 1a (above): Seasonal evolution of average ground temperature as recorded in Gale Crater by REMS instrument. From Pla-Garcia et al. (2017).

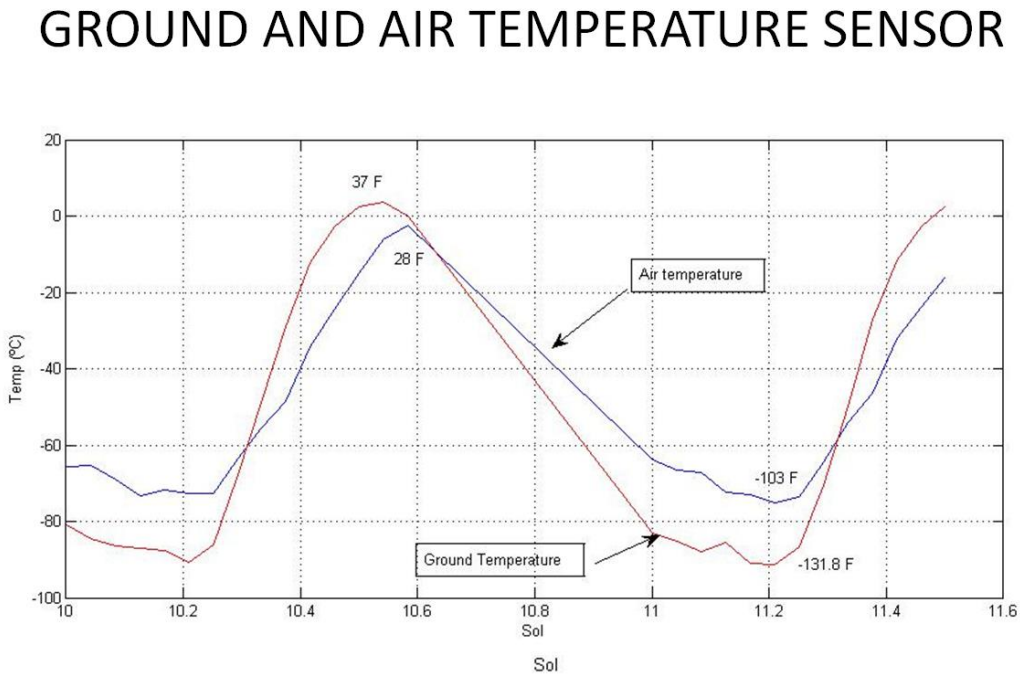

Figure 1b (above): Ground and air temperature recorded using the REMS instrument on Curiosity. Data is from August 2012. From NASA/JPL-CALTECH/CAB(CSIC-INTA).

Table 1: A non-exhaustive literature summary on lower temperature limits for microbial metabolism and survival. Gray reports are from a similar paper to Rummel et al. (2014), published in 2006. Question marks were used to indicate questions regarding what the data represent in the respective study. From Rummel et al. (2014).

T (°C)	*Activity*	*Method*	*Environment*	*Time (days)*	*Reference*
Brines					
−12	Cell division DT 10 days	Turbidity measurement	Culture of sea ice isolate *Psychromonas ingrahamii* in 5% glycerol	42	Breezee *et al.*, 2004
−13.5	Protein synthesis	Uptake of ^{3}H-leucine	Lake Vida samples (188 psu salinity, primarily Cl^{-}, Na^{+}, Mg^{2+})	6–30	Murray *et al.*, 2012
−15	Cell division DT 50 days	Plate counts	Culture of permafrost isolate *Planococcus halocryophilus* Or1 in 18% NaCl, 7% glycerol	200?	Mykytczuk *et al.*, 2013
Ices and frozen environments					
−5	Respiration (maybe cell division, DT 43 days)	CTC reduction, cell numbers, respiration of ^{14}C-acetate, incorporation of ^{3}H-adenine, ^{3}H-leucine	Frozen cultures of glacial ice isolate *Paenisporoarcina* sp. and *Chryseobacterium*	50	Bakermans and Skidmore, 2011a
−10	CH_4 production	Reduction of $H^{14}CO_3^-$	Arctic permafrost	21	Rivkina *et al.*, 2007
−18	Metabolism	Incorporation of $^{14}CO_2$	Frozen cultures of permafrost isolates	90	Panikov and Sizova, 2007
−18	Cell division DT 34 days	Plate counts	*Rhodotorula glutinis* (yeast) inoculated onto surface of frozen peas	200	Collins and Buick, 1989
−20	Metabolism	Incorporation of ^{14}C-acetate into lipids	Permafrost microcosms	550	Rivkina *et al.*, 2000
−20	Protein synthesis	Uptake of ^{3}H-leucine	Frozen culture of sea ice isolate *Colwellia psychroerythraea* 34H	6	Junge *et al.*, 2006
−20	DNA replication	Incorporation of ^{13}C-acetate into DNA	Microcosms of permafrost from Alaska, many bacterial species active	180	Tuorto *et al.*, 2014
−15, −33	Respiration	CTC reduction, respiration of ^{14}C-acetate	Frozen cultures of glacial ice isolates *Paenisporoarcina* sp. and *Chryseobacterium*	200	Bakermans and Skidmore, 2011b
−25	Respiration	Mineralization of ^{14}C-acetate to $^{14}CO_2$	Permafrost microcosms with *Planococcus halocryophilus* Or1 added	200	Mykytczuk *et al.*, 2013
−32	Ammonia oxidation	$^{15}N_2O$ production from ^{15}N-ammonia	Frozen culture of marine isolate *Nitrosomonas cryotolerans*	307	Miteva *et al.*, 2007
−15 to −40	Photosynthesis?	Fluorescence of chlorophyll *a* in photosystem II	Thalli of lichen *Pleopsidium chlorophanum* collected from Antarctica and incubated in Mars simulation chamber	35	de Vera *et al.*, 2014

hospitable ranges (i.e. >273 K), but whether the time at these temperatures is sufficient for microbial survival is a topic of debate (Rummel et al., 2014). The stabilizing effect of the Martian subsurface may offer a solution to the issue of temperature by reducing the amplitude of temperature fluctuation experienced. However this same effect results in a lower maximum temperature (Rummel et al., 2014).

The Rummel et al. (2014) study also investigated pressure limitations to microbial habitability on Mars. According to the NASA Mars Fact Sheet, the mean atmospheric surface pressure on Mars is 6.36 mbar, ranging from 4.0 to 8.7 depending on the season and altitude (Williams, 2010). The atmosphere consists of CO_2 (95.32%), N_2 (2.7%), Ar (1.6%), O_2 (0.13%), CO (0.08%), and low levels (under 300 ppm) of H_2O, NO, Ne, HDO, Kr, and Xe. Rummel et al. (2014) described several studies which exhibited microbial survival at these pressures. A more recent study (Mickol & Kral, 2016) investigated methanogenesis (a metabolic pathway applicable to Mars conditions) at 6 mbar of CO_2 and found microbial methane production to be maintained (compared to higher-pressure controls). Several studies, including Mickol and Kral (2016) have successfully exhibited microbial survival (and in some cases metabolic activity) at Mars surface pressures (Kral et al., 2011; Kral & Travis Aitheide, 2013; Nicholson et al., 2013; Rummel et al., 2014; Schuerger et al., 2013).

Ultra Violet and other forms of radiation can be extreme biocidal factors. The negative effects of UV radiation exposure can range from growth inhibition (Jagger, 1981) to biocidal (Newcombe et al., 2005). The severity of UV exposure is dependent on the wavelength of the incoming radiation and the intensity of the total dose (Jagger, 1981; Newcombe et al., 2005). On Earth, UV dose to surface organisms is attenuated by the atmosphere. However, this is not the case on Mars, where UV doses are expected to be lethal (Newcombe et al., 2005; Rummel et al.,

2014). Experiments subjecting spores (which are more resistant to UV than cells) to Mars level UV radiation demonstrated that unshielded spores were rapidly inactivated within a few minutes to a few hours (Cockell et al., 2005; Newcombe et al., 2005; Schuerger et al., 2003; Tauscher et al., 2006). However, those same experiments also demonstrated that a thin layer (<1mm) of UV-opaque materials, including regolith simulant JSC Mars-1 could effectively shield microbes (Cockell et al., 2005).

1.3 Water Availability & Sulfates on Mars

As more information is gathered on the surface of Mars, the amount of evidence for past liquid water increases (Rummel et al., 2014). More recently, Mars Reconnaissance Orbiter's HiRISE and CRISM instruments have detected polar and subpolar ground ice (Cull et al., 2010). Summertime sublimation of water ice from Mars' polar caps acts as the primary climactic control on global atmospheric humidity, with a smaller component from seasonal exchange with the Martian regolith (Jakosky, 1985; Jakosky et al., 1993). Ground ice appears to be stable at locations where mean annual water-vapor density with respect to ice in the soil pore space equals that of the atmosphere (Mellon et al., 1993). Current ground-ice distribution is at equilibrium with an atmosphere containing 20 precipitable μm of vertically well mixed water vapor (Mellon et al., 2004). Diurnal and seasonal temperature variations, coupled with the stabilizing effect with depth, may allow water vapor in the soil pore space to either build up or be depleted, depending on atmospheric conditions at the time (figure 2).

Martian water vapor alone will not be sufficient to provide habitable conditions (Rummel et al., 2014). Martian surface conditions may intermittently allow for stable liquid water, when conditions exceed its triple point (Rummel et al., 2014). Water-brines however, formed through deliquescence of atmospheric water vapor, interaction with ground ice, or from putative ground

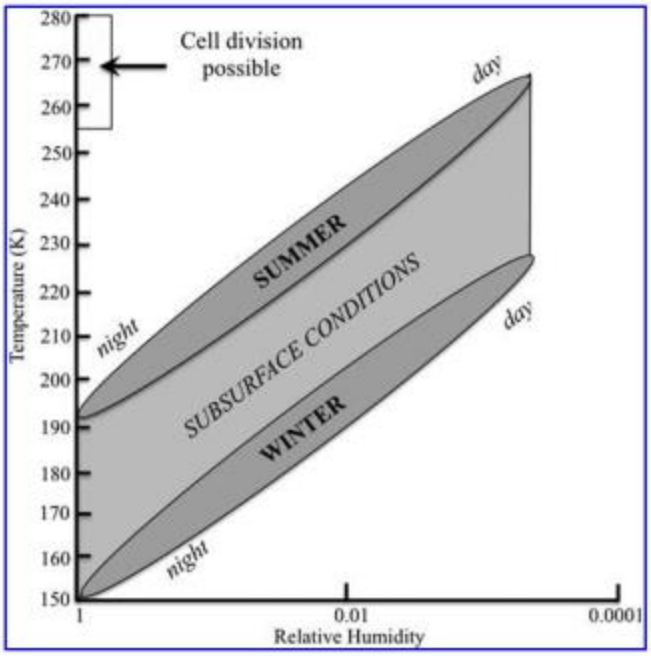

Figure 2: Conceptual shallow subsurface conditions at the PHX landing site. The cell division isolation area is assuming atmospheric humidity to be the only water source. From Rummel et al. (2014).

water (see figure 3; Clark et al., 2005; Knoll et al., 2005; Rummel et al., 2014) have a lower freezing temperature than pure water due to salts in solution, and remain stable for longer periods of time (Altheide et al., 2009; Chevrier et al., 2009; Chevrier & Altheide, 2008; Rummel et al., 2014). Perchlorates and sulfates have been identified on the surface of Mars and can form brines stable at Martian conditions (Chevrier et al., 2009; Chevrier & Altheide, 2008; Rummel et al., 2014; see figures 4 & 5). Brines have a lower freezing point than pure water, but the presence of dissolved salts reduces the water activity of the solution. Terrestrial life utilizes liquid water with a chemical activity above ~0.65 as a solvent (Pappalardo et al., 2013). Rummel et al. (2014) put this water activity minimum at 0.6. These water activity limitations constrain the concentration of putative Martian brines in relation to microbial habitability.

1.4 Biogenic Elements & Energy Sources

Along with liquid water, terrestrial life requires the presence of several key elements, e.g. C, H, N, O, P, and S, as well as various micronutrients (Wackett et al., 2004). On Mars, UV photolysis of surface ice, as well as serpentinization of regolithic minerals, should allow for the presence of H_2 and O_2 (Fisk & Giovannoni, 1999; Rummel et al., 2014). The CO_2 and N_2 present in Mars' atmosphere, despite the low pressures, is considered sufficient for microbial growth (Nier & McElroy, 1977; Rummel et al., 2014). Abiotic or possibly biotic methane (CH_4) has also been detected in the Martian atmosphere (Formisano et al., 2004; Mumma et al., 2009).

As previously mentioned, sulfates have been identified on the surface of Mars, satisfying the requirement for sulfur. Nitrate salts and possibly phosphates have been identified in Yellowknife Bay rocks and Gale Crater using the APXS instrument aboard the Curiosity rover, as well as elevated abundances of sulfur and calcium (Clark et al., 2005; Johnson et al., 2007; Sutter et al., 2017).

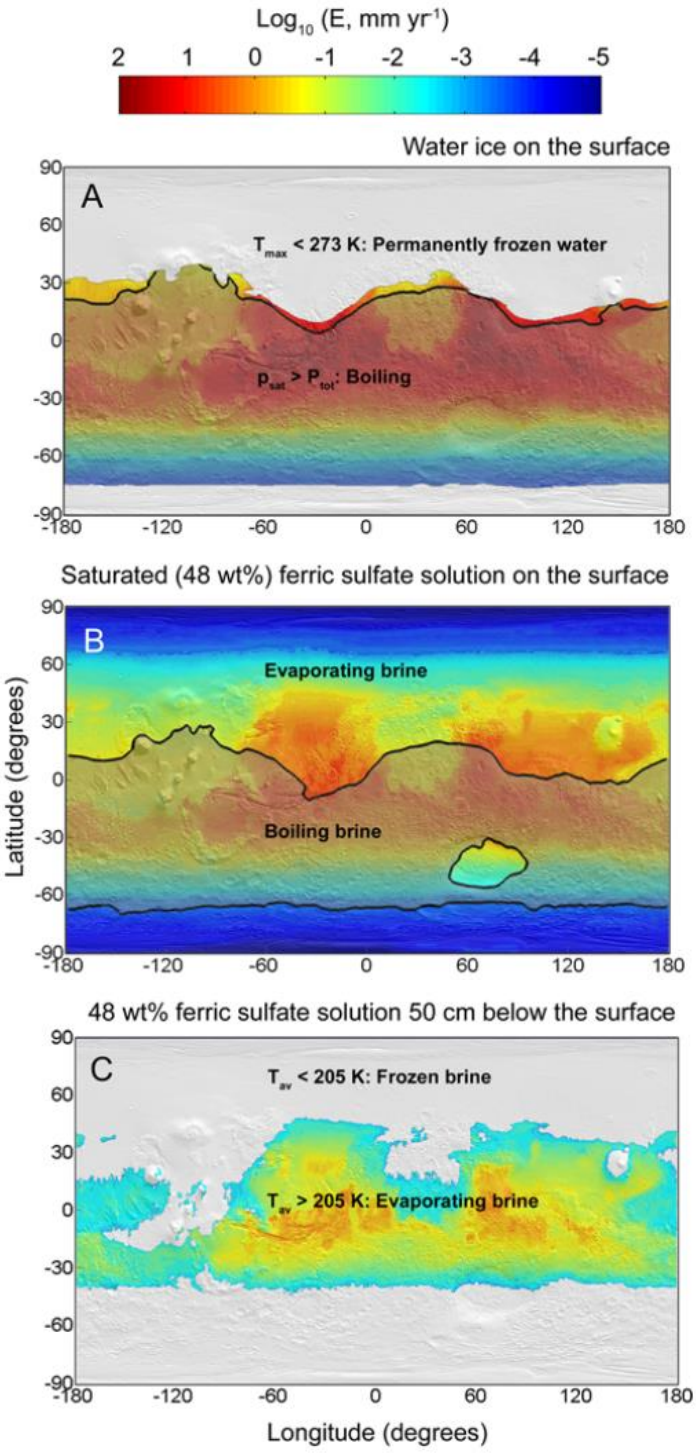

Figure 3: Maps of water ice sublimation (a) and saturated ferric sulfate brine evaporation rates (b and c) on Mars, projected on a MOLA shaded relief map. Light gray indicates freezing conditions (temperature below the eutectic) and the shadowed areas limited by thick black lines indicate boiling conditions (where the water equilibrium vapor pressure is above the atmospheric ambient pressure). (a) Pure water ice can only melt in the colored area, where the maximum temperatures reach 273 K, but high sublimation rates and boiling prevent it from being present for significant periods of time. (b) Saturated (48 wt.%) ferric sulfate solution on the surface. Ferric sulfate can melt anywhere because maximum temperatures are always above 205 K. However, for maximum temperatures, boiling can occur in the equatorial regions. (c) Saturated ferric sulfate solution 50 cm below the surface. Maximum surface temperatures do not reach such depth, so average temperatures were used. From Chevrier and Altheide (2008).

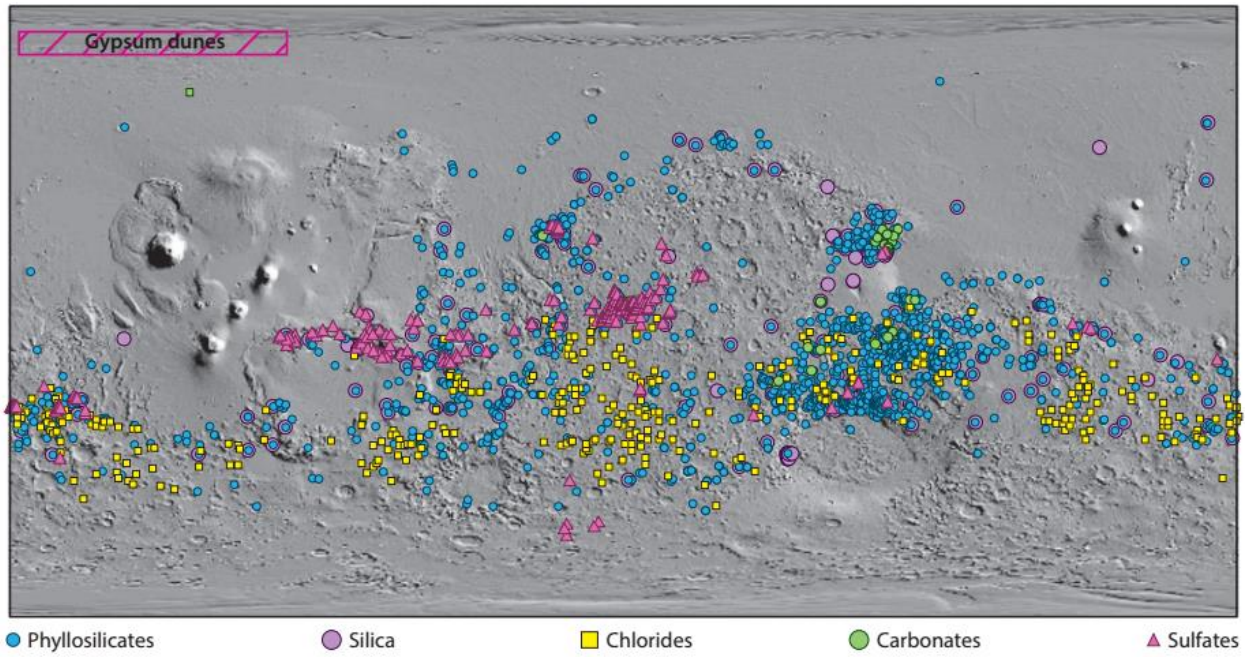

Figure 4: Global distribution of the major classes of aqueous minerals on Mars. From Ehlmann and Edwards (2014).

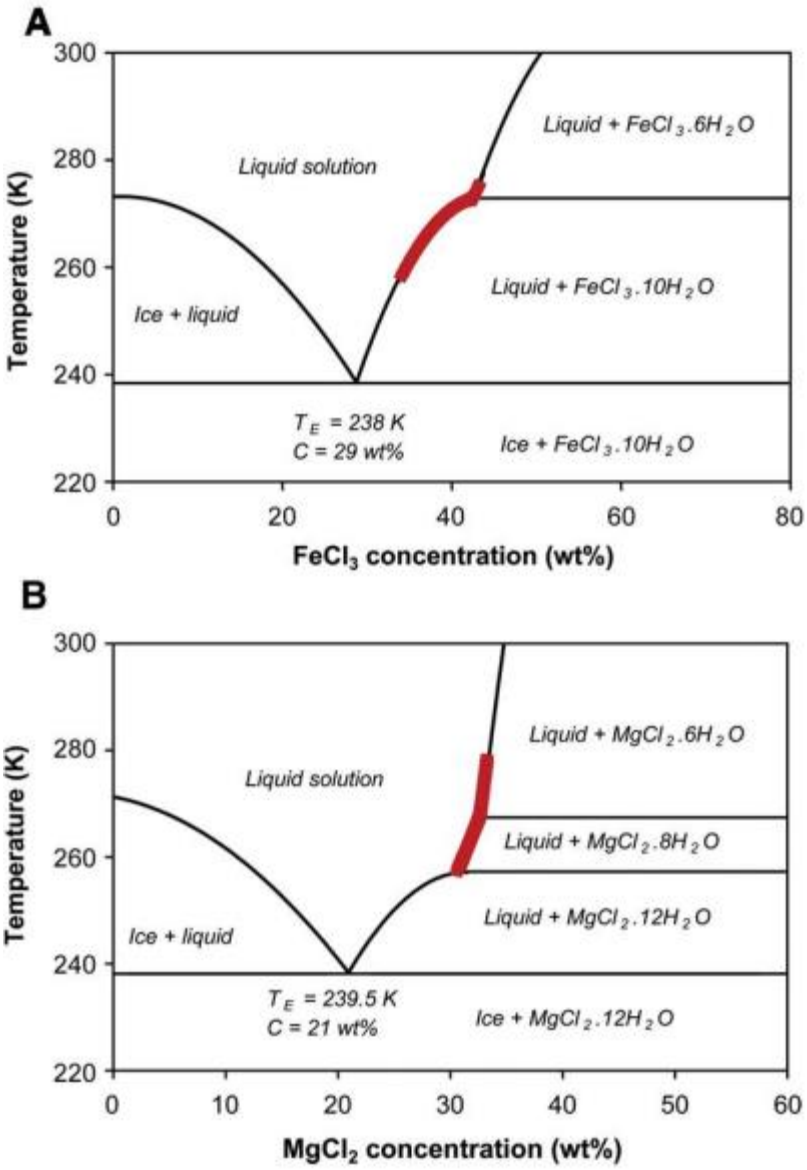

Figure 5: Phase diagrams of Fe (a) and Mg (b) chlorides as a function of temperature and concentration. From Altheide et al. (2009).

Organic molecules have also been detected on Mars in trace amount (Freissinet et al., 2015). However, extensive chemical oxidation is expected to prevent the preservation of any organic carbon not below the surface, either embedded in minerals or as metastable organic salts (Rummel et al., 2014). Martian meteorites have been shown to contain reduced macromolecular carbon phases (including in one case polycyclic aromatic hydrocarbons) of abiotic/igneous origin (Steele et al., 2012). Other important nutrients, such as Mg, Na, and K have been identified by the MECA instrument aboard Phoenix (Hecht et al., 2009).

1.5 *Sulfate-brines as Potential Habitable Zones on Mars*

The goal of the (2014) Rummel et al. investigation was to review and refine Mars "special regions:"

> A region within which terrestrial organisms are likely to replicate [or] any region which is interpreted to have a high potential for the existence of extant Martian life forms.

Sulfate-brines (water and Ca, Mg, Fe(II), or Fe(III) SO_4^{2-}) in the surface or subsurface were determined to be one of these regions (Rummel et al., 2014). Furthermore, the study identified knowledge gaps needing further study, including microbial activity at low temperature, pressure, and water activity, and microbial activity under multiple extreme factors (low temperature, pressure, etc.).

1.6 Sulfate and Iron (III) Reducing Bacteria

Sulfate and iron reduction are considered to be among the earliest metabolic pathways to arise on Earth (Archer & Vance, 2006; Wagner et al., 1998). Phylogenetic studies of the dissimilatory sulfite reductase (DSR) gene suggest a common origin of the sulfate-reduction gene between archaea and bacteria (Wagner et al., 1998).

Sulfate-reducing bacteria utilize sulfate (SO_4^{2-}) as a terminal electron acceptor during anaerobic respiration (figure 6; Archer & Vance, 2006). Current understanding divides sulfate-

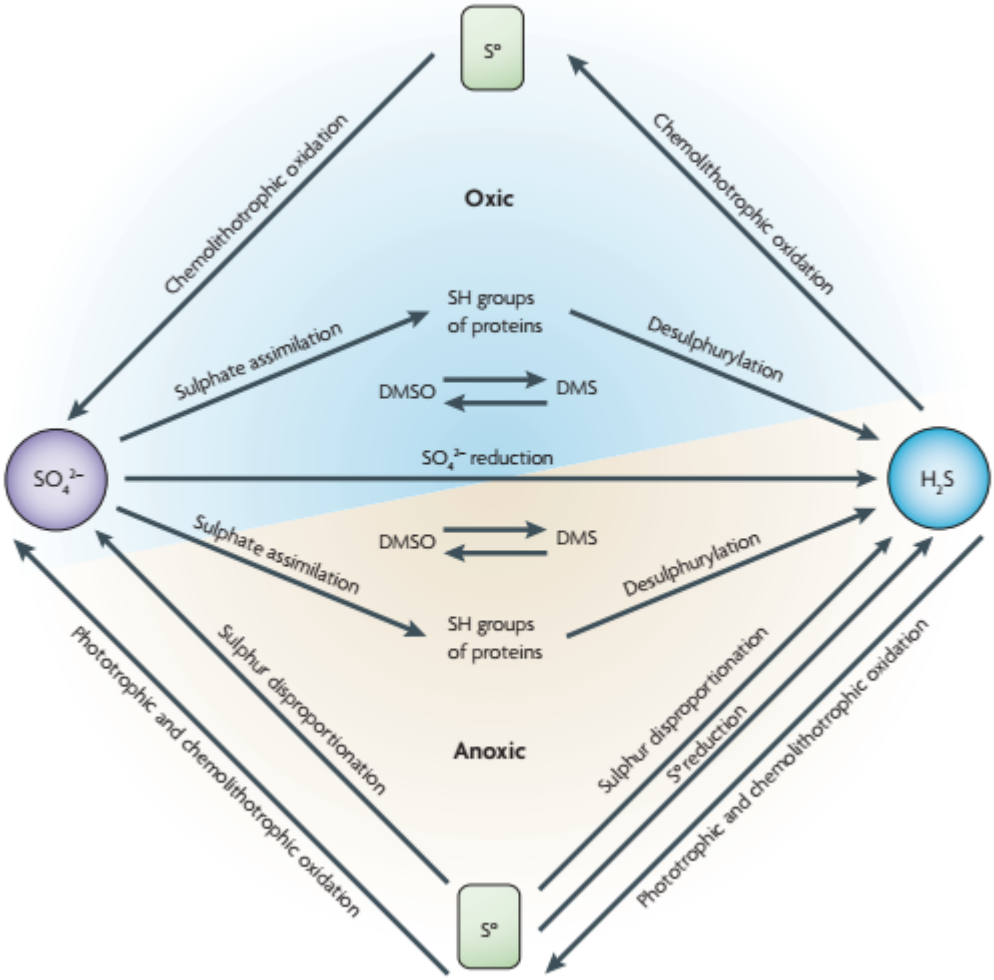

Figure 6: Microbial metabolic sulfur transformation pathways. Sulfate-reducing bacteria (SRB) use sulfate (SO_4^{2-}) as a terminal-electron acceptor in the degradation of organic matter, which results in the production of hydrogen sulfide (H_2S). Subsequently, the sulfide can be oxidized through a variety of metabolic pathways via sulfur-oxidizing bacteria to elemental sulfur (S°) and SO_4^{2-}. Other transformations carried out by specialized groups of microorganisms result in sulfur reduction and sulfur disproportionation. Organic sulfur compounds (i.e. dimethyl sulfoxide; DMSO) can be transformed into dimethyl sulfide (DMS) and vice versa by several groups of microorganisms. From Muyzer and Stams (2008).

reducers into two categories: those that degrade organic compounds incompletely to acetate, and those that degrade organic compounds completely to CO_2 (Muyzer & Stams, 2008). Sulfate-reducers have been found which are capable of growth on one-carbon compounds, such as methanol, carbon monoxide, and methanethiol (Muyzer & Stams, 2008). Sulfate-reducing bacteria have also been shown to grow via dismutation of thiosulfate, sulfite, and sulfur, resulting in the formation of sulfate and sulfide (Muyzer & Stams, 2008). The typical product of microbial sulfate-reduction is hydrogen sulfide (H_2S), which can be subsequently oxidized by sulfur-oxidizing bacteria (figure 6; Muyzer & Stams, 2008). Furthermore, psychrophilic (cold-temperature tolerant) and spore-forming sulfate-reducing bacteria have been successfully isolated from nature and cultured in laboratory settings (C Knoblauch et al., 1999; Muller et al., 2014; Vandieken et al., 2006a). Endospores have been found to be more resistant to biocidal factors (compared to bacterial cells) relevant to Mars (i.e. UV radiation, cold temperatures, and low pressures), making them a primary concern for planetary protection (Benardini et al., 2003; Fajardo-Cavazos et al., 2007; Horneck et al., 2001, 2012; Schuerger et al., 2003; Tauscher et al., 2006). Several genera (i.e. *Desulfovibrio)* of sulfate-reducing bacteria have also been shown to reduce iron (Fe^{3+}), producing iron sulfide (FeS), although whether this process is utilized for energy is unclear.

The metabolic reduction of sulfate and iron by sulfate-reducing bacteria produces traceable compounds and alterations to solution concentrations. Changes in H_2S concentration in solution and gas phase can be utilized as a tracer of SRB metabolic activity, but this has only been lightly studied (Aharon & Fu, 2000; Reese et al., 2011). Changes in FeS can be utilized in the same fashion with SRB shown to reduce Fe (III) and/or elemental sulfur (Fossing & Jrgensen, 2016;

Vandieken et al., 2006b). Change in solution sulfate concentration can also provide a means of SRB growth tracking (Silver, Berger, et al., 2018).

2. Experiment Design

2.1 Scientific Question

The Rummel (2014) special regions review, in combination with NASA planetary protection protocols, identified knowledge gaps regarding microbial survival in sulfate-brines on the Mars surface or in the subsurface. This study investigated the capability of microbes believed to be best suited to Mars conditions (psychrophilic sulfate-iron-reducing bacteria; some sporulating) to survive (and potentially grow) in simulated Mars conditions, including temperature, atmospheric composition and pressure, and putative sulfate-brines.

2.2 Experiment Methods

Sulfates relevant to Mars: were selected for investigation: $CaSO_4$, $MgSO_4$, $Fe^{2+}SO_4$, and $Fe^{3+}{}_2(SO_4)_3$ (Christensen et al., 2004; Johnson et al., 2007; Lane et al., 2008). The concentrations of sulfates in solution were chosen based on the respective sulfate's eutectic point under Mars conditions (see Figure 7 and table 2).

Strains of sulfate-reducing bacteria (and one sulfate and iron reducing bacterium) were obtained from a commercial microbial repository, the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Two psychrophilic strains (see table 3) and one mesophilic (optimal temperature of 37°C) strain were selected for study. Despite being mesophilic, *Desulfotomaculum arcticum* is a valid species for study as it produces endospores and was isolated from permanently cold arctic marine sediment (Vandieken et al., 2006a). The DSMZ provides recipes for solutions defined as ideal for growth of the respective organism (referred to as "optimal growth medium"). These recipes are typically based on the publication

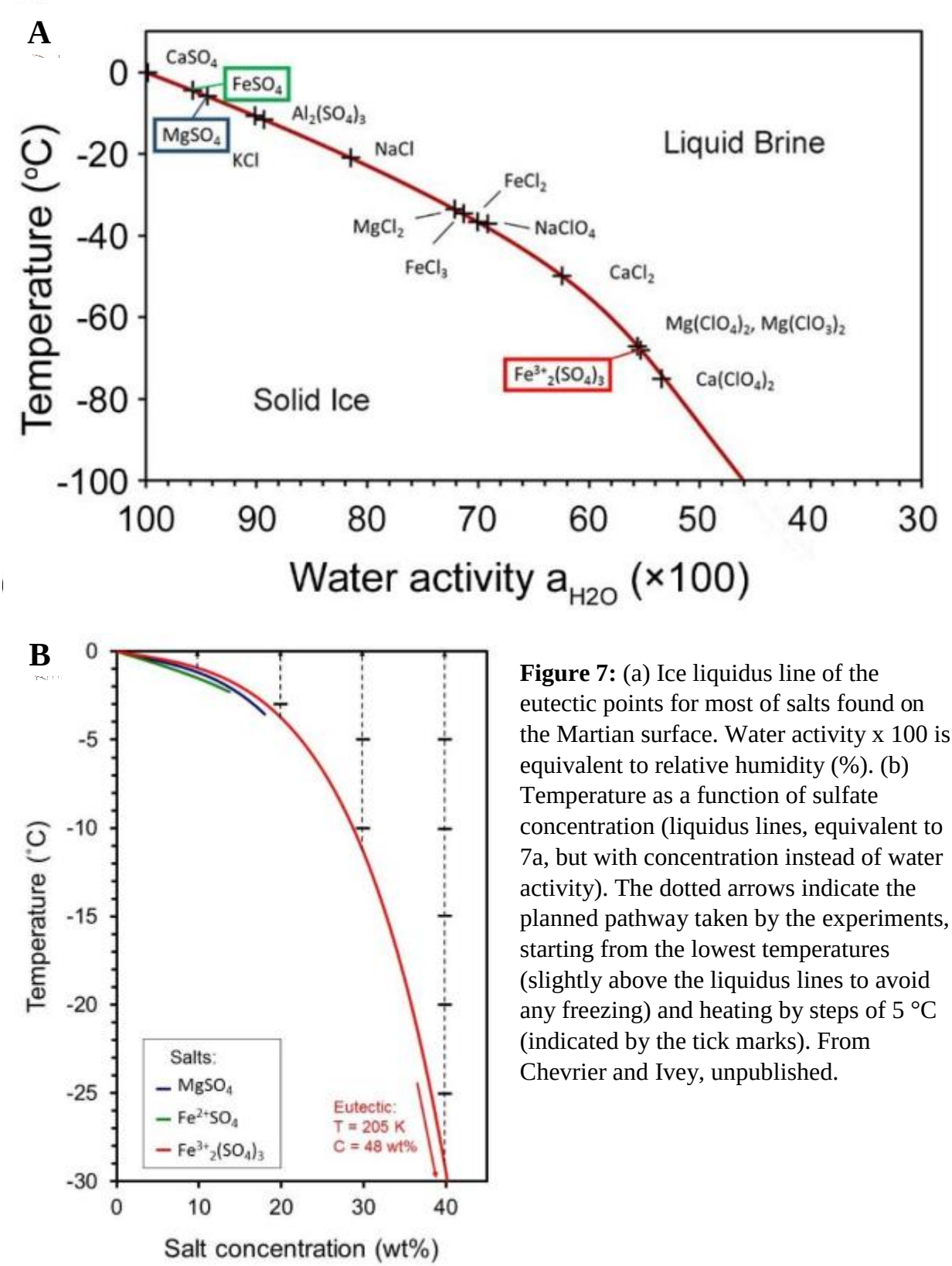

Figure 7: (a) Ice liquidus line of the eutectic points for most of salts found on the Martian surface. Water activity x 100 is equivalent to relative humidity (%). (b) Temperature as a function of sulfate concentration (liquidus lines, equivalent to 7a, but with concentration instead of water activity). The dotted arrows indicate the planned pathway taken by the experiments, starting from the lowest temperatures (slightly above the liquidus lines to avoid any freezing) and heating by steps of 5 °C (indicated by the tick marks). From Chevrier and Ivey, unpublished.

Table 2: Sulfate-brine concentrations (in solution, by weight) used for investigation, separated by cation.

$CaSO_4$	$MgSO_4$	$Fe^{2+}SO_4$	$Fe^{3+}{}_2(SO_4)_3$
0.1%	10%	10%	10%
	18%	14%	20%
			30%
			40%
			48%

Table 3: Sulfate (& iron in the case of *Desulfuromusa ferrireducens*) reducing bacteria strains received from the DSMZ microbial repository. Optimal growth temperature, temperature growth range, and pH range are from the respective organism's initial publication: *D. psychrophila* (Knoblauch et al., 1999); *D. arcticum* (Vandieken, 2006); and *D. ferrireducens* (Vandieken et al., 2006b).

Organism	Optimal Growth Temperature	Temperature Growth Range	pH Range
Desulfotalea psychrophila LSv54	10°C	-1.8 to 19°C	7.3 to 7.6
Desulfotomaculum arcticum 15^T	44°C	26 to 46.5°C	7.1 to 7.5
Desulfuromusa ferrireducens 102^T	14°C	-2 to 23°C	6.5 to 7.9

first isolating, identifying, and culturing said species or strain. However, in some cases the optimal growth medium prescribed by the DSMZ is an industry standard, such as sulfate-reducing bacteria medium published by Widdel and Bak (1992) or Postgate (1984). The exact source of a strain's DSMZ "optimal growth medium" is unclear, so experimentation was performed (detailed in sections 3 and 4) to determine the strain's true optimal growth medium. Table 3 provides a summary of each strain's ideal growth conditions based on the respective parent publication (Christian Knoblauch et al., 1999; Vandieken et al., 2006a, 2006b).

Once strains of SRB were obtained from the DSMZ, controls were established. Initial controls involved growth of SRB strains under respective ideal conditions (see table 3) using optimal growth medium. Then solutions under ideal conditions with optimal growth medium augmented with sulfates (as outlined in table 2; defined in sections 3 and 4) were tested.

2.3 Analytical Methods

Analytical techniques included Polymerase Chain Reaction (PCR), optical density (OD)/absorbance, phase-contrast microscopy, gram-staining, and ion chromatography. Polymerase Chain Reaction (PCR) synthesizes short-lengths of single-stranded DNA (oligonucleotides) which are used to direct target-specific synthesis of new DNA copies using DNA polymerase (Jones et al., 2002). Two oligonucleotides, complementary to opposite strands of the target DNA segment, specifically amplify the region between them (Jones et al., 2002). The product of one polymerase activity is added to the pool of template (oligonucleotide strands used) for the next round of replication (Jones et al., 2002). Use of specific oligonucleotides (referred to as "primers") allows for the isolation and amplification of specific DNA segments (Jones et al., 2002). In this study, several sets of primers were utilized (Silver et al., 2017); 1) primers designed to isolate genes universal to bacteria (16S rDNA gene) and 2) primers designed

to isolate genes universal to sulfate-reducing bacteria (dsrAB gene; Daly et al., 2000; Dar et al., 2005). Once PCR was completed, the amplified DNA was subjected to gel electrophoresis. Gel electrophoresis of PCR products allows for the separation of DNA segments by size and electrical charge (Rodney, 2016). When PCR products are subjected to gel-electrophoresis alongside a commercially available standard, the DNA segment sizes can be determined and compared to the anticipated PCR product (based on primers used; Rodney, 2016).

Absorbance (also known as optical density, or turbidity) was also utilized to characterize cellular growth. Absorbance is a measurement of the amount of light scattered or refracted by a suspension of bacterial cells with the use of a colorimeter (Reddy, 2007). The change over time in the absorbance of a sample, compared to a control solution without microbes, can be used to measure the change in cell concentration (Reddy, 2007; Silver, Mora, et al., 2018), where the amount of light scattered is proportional to the concentration of cells.

Phase-contrast microscopy is also a useful tool for microbial analysis. Phase-contrast microscopy utilizes slight differences in the refractive index of various cell components (and the cell suspension solution) which are transformed into differences in the intensity of transmitted light (Slonczewski & Foster, 2014). These differences in transmitted light intensity are then shifted out of phase to reveal the causal refractive index differences as patterns of light and dark (see figure 8; Slonczewski & Foster, 2014).

Microscopy can also be used in tandem with cell-staining, which allows for differentiation between different types of bacteria within a sample (Slonczewski & Foster, 2014). This study utilized Gram-staining, a process involving a series of chemical stains, binding agents, decolorizers, and counter-stains to stain one type of cell (Gram-positive) a violet color, and the other (Gram-negative) a red color (Slonczewski & Foster, 2014). The color retained by a cell

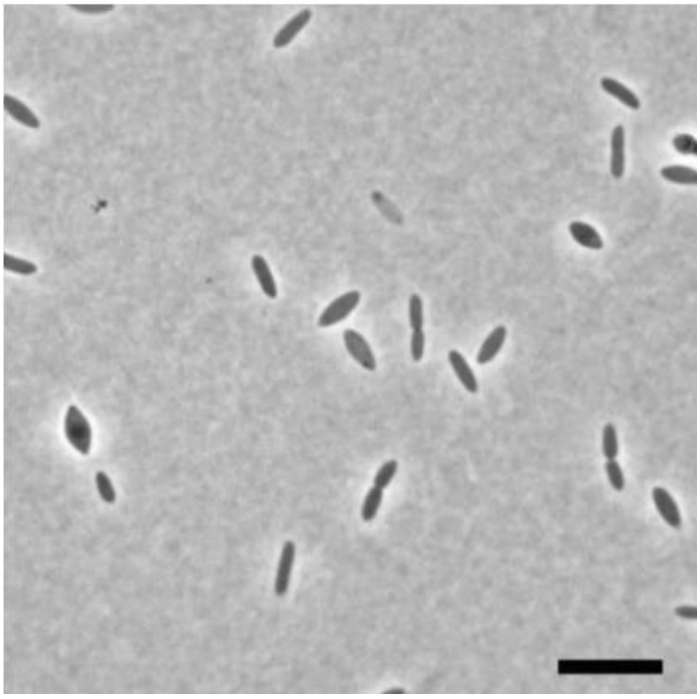

Figure 8: Phase-contrast micrograph of *Desulfotomaculum arcticum* strain 15^T. The black bar is 10 µm in length. From Vandieken et al. (2006a).

(violet or red; G +/-) is dependent on the layers of peptidoglycan (sugar-chains cross-linked by peptides) within the cell wall (Slonczewski & Foster, 2014). Primary publications on the isolation of our SRB species of interest (see table 3) characterized the Gram-stain retention (positive or negative) of the respective species. Therefore, gram-staining of samples can be compared to literature results (see table 3) to verify the presence of SRB species.

Column chromatography, a form of adsorption chromatography, has become a widely used analytical tool in biochemistry (Rodney, 2016). Adsorption chromatography usually consists of a solid stationary phase and a liquid mobile phase (Rodney, 2016). Column chromatography confines the stationary phase to a glass or plastic tube while the mobile phase is allowed to flow through the solid adsorbent (Rodney, 2016). The sample of interest enters the column of adsorbing material and the molecules present are distributed between the mobile phase and stationary phase (Rodney, 2016). The various components in the sample have different affinities for the two phases and move through the column at different rates (Rodney, 2016). Ion chromatography is a form of column chromatography which separates ions and polar molecules based on their affinity to the solid phase (ion exchanger; Bak et al., 1991). Ion chromatography can be utilized to determine the concentration of SO_4^{2-} in sample at biologically relevant levels (Bak et al., 1991). Sulfate-concentration is an accurate tracker of SRB metabolic activity so long as controls are effectively designed (Bak et al., 1991).

3. Investigation One: Growth of Organisms in Optimal Growth Medium & Sulfate-Brines

3.1 Introduction

Investigation of sulfate-reducing bacteria under simulated Mars conditions required a series of well-characterized controls. This experiment examined the effect of sulfate-brines under

otherwise ideal conditions on sulfate-reducing bacteria best suited for Mars surface and subsurface conditions.

3.2 Organisms Investigated

Two sulfate-reducing bacteria strains (*Desulfotomaculum arcticum 15^T* and *Desulfotalea psychrophila LSv54)* and one sulfate and iron reducing bacteria strain (*Desulfuromusa ferrireducens 102^T)* were received from the DSMZ (see section 3.2 and table 3). These represent psychrophilic, chemolithoautotrophic, heterotrophic, and sporulating organisms. *Desulfuromusa ferrireducens 102^T* was utilized to test for the combination of iron reduction and sulfate reduction in ferrous(Fe^{2+})/ferric(Fe^{3+}) sulfates.

3.3 Methods

Each organism was initially subjected to ideal conditions as defined in the literature (see section 3.2 and table 3): optimal growth medium, ideal growth temperature, optimal pH range, and an atmosphere of N_2 within sealed serum vials. Optimal growth media were prepared anaerobically (Postgate, 1984; Widdel & Bak, 1992) in sealed serum vials, flushed with N_2, and brought to ideal temperature. Replications of these solutions were made with additional sulfates (see table 2). Samples were then inoculated with SRB culture as received from the DSMZ in an anaerobic glove bag. Samples were returned to ideal growth temperature conditions and allowed to incubate for approximately five months.

After one month of incubation, sample aliquots were periodically purified using a suite of commercially available MOBIO DNA Isolation Kits. PCR was performed on the purified DNA using a series of primers based on the 16S rRNA of six phylogenetic groups of sulfate-reducing bacteria (see section 3.3). Gel electrophoresis was then performed using the SYBR Green or SYBR Safe dyes. Double stranded DNA concentrations were also quantified prior to PCR using

a Qubit High Sensitivity Fluorometer. Separate from DNA analyses, aliquots of samples were analyzed via phase-contrast microscopy as well as gram-staining.

3.4 Results

Amplification of the 16S rDNA gene or the dsrAB operon of *D. psychrophila*, *D. arcticum*, and *D. ferrireducens* cultures in optimal growth media and media supplemented with additional sulfates (of varied concentrations) was inconsistent (see figure 9**)**. The initial hypothesis was an issue with the PCR process. However, dsDNA concentrations yielded through Qubit fluorometry were consistently low (<25 µg/mL) in *D. psychrophila* and *D. ferrireducens* samples, and inconsistent in *D. arcticum* samples (see table 4**)**. The presence of black compounds was observed in some samples and were suggested to be FeS, but these were not analyzed (see figure 10).

Samples were subjected to gram-staining and phase-contrast microscopy. Gram-staining was inconclusive, as incorporation of either stain was rare. Initially, motion was observed in some samples of *D. arcticum*, mistaken for microbial locomotion. Upon review, the observations were determined to be Brownian motion. Potential *D. arcticum* spores were observed, but these did not incorporate any gram-stains. Similar experiments were performed by Mora (2017) using different growth media. Mora (2017) tested the growth of *D. arcticum*, *D. psychrophila*, and *D. ferrireducens* in a complex growth medium (DSMZ) and a minimal growth medium (Widdel & Bak, 1992), characterized through amplification of the 16S rDNA gene. The SRB species *D. arcticum* and *D. ferrireducens* were found to grow only in minimal medium containing H_2 as an electron donor and CO_2 as a carbon source (Mora, 2017). Amplification of the 16S rDNA gene in samples of *D. psychrophila* was unsuccessful (Mora, 2017).

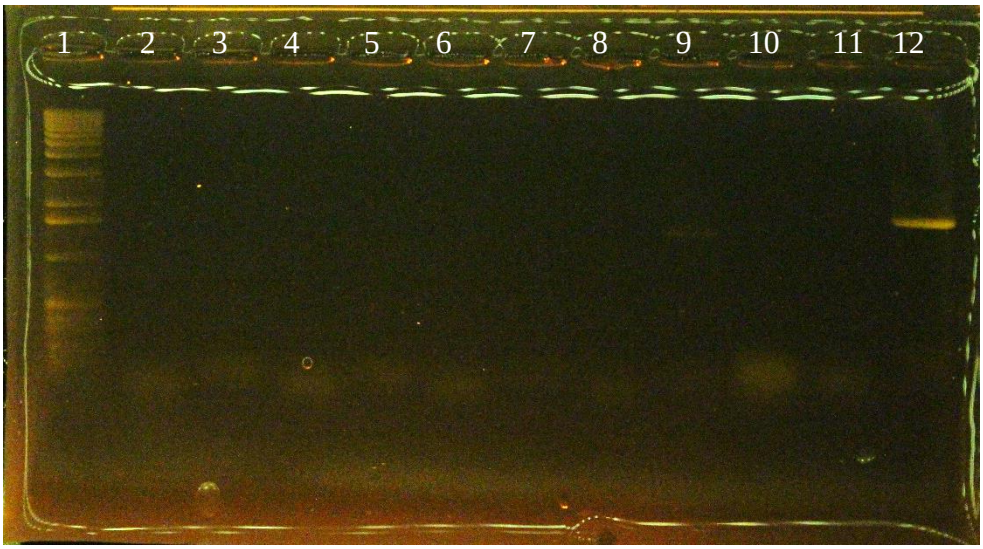

Figure 9: Electrophoresis gel of *Desulfotalea psychrophila*, *Desulfuromusa ferrireducens*, and *Desulfotomaculum arcticum* DNA, cultured in DSMZ optimal growth medium, as well as concentrated *Bacillus cohnii* DNA as a positive control. Sample DNA was purified using a MoBIO DNA isolation kit prior to PCR. The additional *B. cohnii* DNA control was used which was not purified. Two sets of primers were used during PCR: A) dsrAF5 + dsrR1m-RC producing an amplicon of approximately 328 bp, based on the dsrAB gene; and B) 27F + 16sr1 producing an amplicon of approximately 1465 bp, based on 16S rDNA gene.

Well contents: 1) Gibco 1Kb ladder; 2) *D. psychrophila* with primer set A; 3) *D. psychrophila* with primer set B; 4) *D. ferrireducens* with primer set A; 5) *D. ferrireducens* with primer set B; 6) *D. arcticum* with primer set A; 7) *D. arcticum* with primer set B; 8) purified *B. cohnii* DNA with primer set A; 9) purified *B. cohnii* DNA with primer set B; 10) unpurified *B. cohnii* DNA with primer set A; 11) unpurified *B. cohnii* DNA with primer set B; 12) Promega 25 bp ladder.

Table 4: QuBit high-sensitivity fluorometry measurements of sample dsDNA concentrations. DNA concentrations (ng/mL) are presented at times after initial inoculation (i.e. amount of time sample had been incubating).

Desulfotalea psychrophila samples	[DNA] 6 weeks	[DNA] 19 weeks
DSMZ medium +0.1% $CaSO_4$	<0.05	9.40
DSMZ medium +10% $MgSO_4$		7.83
DSMZ medium +18% $MgSO_4$		3.76
DSMZ medium +10% $Fe^{2+}SO_4$		89.37
DSMZ medium +14% $Fe^{2+}SO_4$		23.43
DSMZ medium +10% $Fe^{3+}(SO_4)_3$		3.95
DSMZ medium +20% $Fe^{3+}(SO_4)_3$		6.77
DSMZ medium +30% $Fe^{3+}(SO_4)_3$		7.52
DSMZ medium +40% $Fe^{3+}(SO_4)_3$		2.74
DSMZ medium +48% $Fe^{3+}(SO_4)_3$		3.05
Original shipment solution		4.80
Desulfotomaculum arcticum samples		
DSMZ medium +0.1% $CaSO_4$	15.6	6.74
DSMZ medium +10% $MgSO_4$		11.04
DSMZ medium +18% $MgSO_4$		18.73
DSMZ medium +10% $Fe^{2+}SO_4$		191
DSMZ medium +14% $Fe^{2+}SO_4$		8.55
DSMZ medium +10% $Fe^{3+}(SO_4)_3$		3.22
DSMZ medium +20% $Fe^{3+}(SO_4)_3$		3.45
DSMZ medium +30% $Fe^{3+}(SO_4)_3$		5.20
DSMZ medium +40% $Fe^{3+}(SO_4)_3$		3.08
DSMZ medium +48% $Fe^{3+}(SO_4)_3$		4.24
Original shipment solution		5.04
Desulfuromusa ferrireducens samples		
DSMZ medium +0.1% $CaSO_4$	<0.05	3.16
DSMZ medium +10% $MgSO_4$		2.99
DSMZ medium +18% $MgSO_4$		2.73
DSMZ medium +10% $Fe^{2+}SO_4$		3.57
DSMZ medium +14% $Fe^{2+}SO_4$		2.43
DSMZ medium +10% $Fe^{3+}(SO_4)_3$		4.56
DSMZ medium +20% $Fe^{3+}(SO_4)_3$		2.46
DSMZ medium +30% $Fe^{3+}(SO_4)_3$		2.44
DSMZ medium +40% $Fe^{3+}(SO_4)_3$		3.76
DSMZ medium +48% $Fe^{3+}(SO_4)_3$		2.89
Original shipment solution		22.1

Figure 10: Anaerobic serum tubes containing cultures of *Desulfotomaculum arcticum*¸ displaying possible FeS precipitation. (a) *D. arcticum* in DSMZ medium + 10% $Fe^{2+}SO_4$; (b) *D. arcticum* in DSMZ medium + 14% $Fe^{2+}SO_4$.

3.5 Discussion

Although inconsistent, amplification of the 16S rDNA gene and dsrAB operon in *D. arcticum* and *D. ferrireducens* samples in minimal medium, combined with observations of increased sample turbidity over time, suggests that the two SRB species were incapable of growth using the prescribed DSMZ complex optimal growth medium. Initial studies of *D. arcticum* and *D. ferrireducens* found the organisms capable of utilizing complex medium as well as minimal medium (Vandieken et al., 2006a, 2006b), although growth times were extended utilizing carbon sources more complex than CO_2. The extended growth time reported in solutions with complex carbon sources may account for the observed inconsistent gene amplification: some experiment cultures may have had insufficient time to achieve cell counts sufficient for PCR gene amplification. Mora (2017) attributes the unsuccessful amplification of the 16S rDNA gene and dsrAB operon in *D. psychrophila* to insufficient incubation time. However, after attempts to culture *D. psychrophila* for an extended period (2 months), amplification of the genes was unsuccessful. Therefore, it is likely that mishandling by the investigatory team or issues during transport resulted in the loss of viability in *D. psychrophila* cultures. Successful growth of *D. arcticum* and *D. ferrireducens* in minimal medium suggests that these cultures survived transport, but the presence of H_2 and CO_2 are required for growth.

3.6 *Conclusion*

Two strains of sulfate-reducing bacteria and one strain of sulfate and iron reducing bacteria were tested for growth under ideal conditions or ideal conditions with sulfate brines. The presence of bioturbation in cultures and intermittent DNA banding in gels indicates metabolic activity in some optimal growth medium samples. The observed lack of consistent DNA banding in PCR gels may be a result of 1) insufficient concentration of DNA in PCR amplicons, 2)

insufficient DNA shearing during PCR, 3) the use of inappropriate primers, or 4) human errors during DNA purification and/or PCR. If the issue was an insufficient concentration of DNA, the cause may have been inadequate incubation time or inadequate growth medium. The successful PCR gene amplification of two strains cultured under alternate growth medium suggests that the cause of inconsistent DNA banding was inadequate growth medium.

4. Investigation Two: Growth of Sulfate-reducers in Sulfate-Brines Verified and Characterized

4.1 Introduction

Inconclusive findings from the first investigation created questions regarding the validity of preparation and analytical methods. Therefore, sulfate-reducing bacteria in an exponential growth phase were isolated and subjected to growth medium or growth medium supplemented with additional sulfate. Afterwards, a suite of analytical tools was utilized to characterize microbial growth as well as to verify methods.

4.2 Organisms Investigated

Sulfate-reducing bacteria were collected from the Springdale, Arkansas Wastewater Treatment Facility. Sulfate-reducing bacteria were isolated from the collection sample through repeated incubations in growth medium designed to select for sulfate-reducing bacteria (Postgate Medium C; see section 3). Isolation of SRB was confirmed through PCR utilizing primers isolating the dsrAB operon. Isolated sulfate-reducing bacteria were assumed to be mesophilic, as gastrointestinal microbiota are typically mesophilic, and SRB are a common component of human gut microbiota.

4.3 Methods

Two sets of growth medium were prepared. The first set provided a positive growth control and consisted of Postgate (1984) growth medium, containing 0.44% (wt.) Na_2SO4. The second set of growth media consisted of Postgate (1984) medium, with an additional 0.1% (wt.) $CaSO_4$. Additional duplicate samples of each set were prepared and left uninoculated to provide a negative control. Sulfate with Ca cations was chosen for investigation due to its relevance to Mars (see section 2). Solutions were prepared anaerobically in sealed screw-top serum vials with a 1 cm outer diameter. Serum-vial headspaces were then filled with an overpressure (0.5 bar) of 80% H_2 + 20% CO_2. Samples were kept at 32°C throughout the experiment. Positive control and experiment samples were then inoculated with 10% (wt.) exponential growth phase wastewater SRB cultures. Samples were then allowed to incubate for either 22 hours (positive controls) or 42 hours (experiment samples) before analyses began. Positive growth control incubations were started prior to experiment samples (containing $CaSO_4$) and incubated for a total of 336 hours. A technical issue caused a loss of temperature control, resulting in the abrupt end to all incubations. As a result, experiment samples incubated for a total of 166 hours.

Microbial growth was characterized through absorbance (optical density; OD) measured at a 590 nm wavelength using a WPA CO 7500 Colorimeter utilizing negative controls (see section 5.3) for reference. Sample measurement of OD took approximately 5 minutes and was performed at room temperature (22 ± 2°C). Use of negative controls as optical density references account for changes in sample optical density not caused by the presence or metabolic activity of microbes. 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 sample preparation, and 2) at the end of each experiment.

Analysis of sample pH at the end of the experiment required transfer to new containers and exposure to ambient conditions (temperature, air composition, etc.).

4.4 Results

Absorbance measurements in cultures supplied with unmodified Postgate growth medium (positive controls 1 and 2) began after 22 hours of incubation. In samples with medium supplemented with an additional 0.1% (wt.) $CaSO_4$, measurements began after 44 hours. The results of absorbance measurements are presented in figure 11.

All samples (positive controls and $CaSO_4$ samples) had similar absorbance values of 0.22-0.37 after 50 hours of incubation. However, positive controls exhibited greater rates of absorbance increase than $CaSO_4$ samples starting after 50 hours. Positive controls saw peak absorbance after 69 hours of incubation at values of 0.76 (positive control 1) and 0.80 (positive control 2), while $CaSO_4$ samples did not reach peak absorbance until 142 hours, but at greater values (1.00 and 1.14; $CaSO_4$ samples 1 and 2, respectively).

During growth media preparation, positive control samples were brought to pH=7.08 and $CaSO_4$ samples to pH=6.85. Postgate (1984) growth medium contains $NaHCO_3$ and CO_2, which work in tandem to regulate pH, facilitating the range of starting pH values in our samples. At the end of the experiment, the pH in positive controls 1 and 2 were 6.86 and 6.80, respectively. $CaSO_4$ samples 1 and 2 had final pH values of 7.08 and 7.17, respectively.

4.5 Discussion

Uninoculated sterile 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 (e.g. cellular reproduction, growth, or death; Koch, 1970).

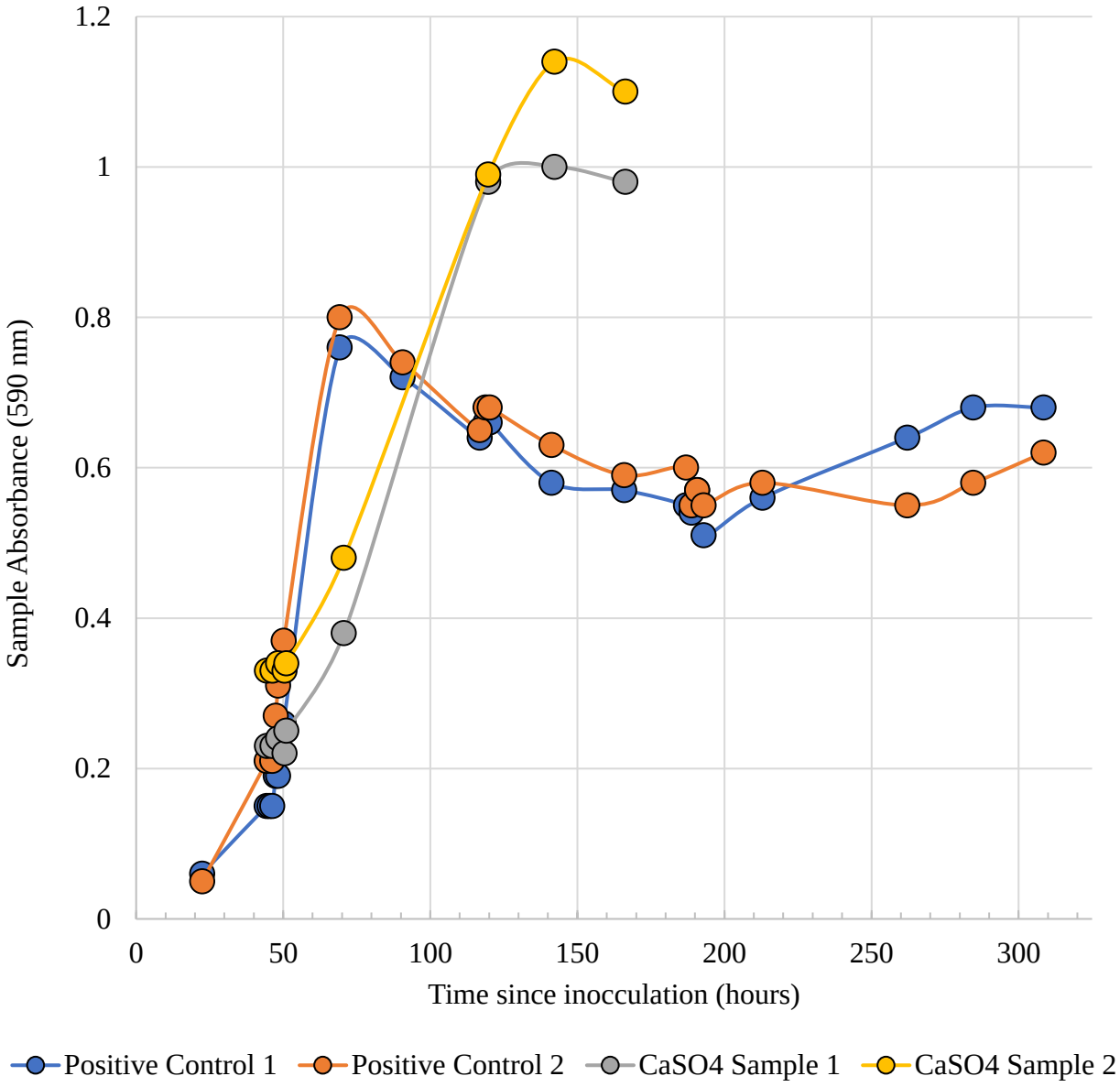

Figure 11: Change in sample absorbance measured at 590 nm. Samples were prepared either with growth medium as prescribed by Postgate (1984; Positive Controls 1 and 2) or growth medium plus an additional 0.1% (wt.) $CaSO_4$ and then inoculated with exponential growth phase wastewater sulfate-reducing bacteria culture. Measurement deviations (±0.01) are shown but obscured by data points.

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

The increased sulfate concentrations in the $CaSO_4$ samples may be responsible for the increased peak-absorbance values over the positive controls, allowing for greater growth/reproduction in SRB cultures. If this were true, it would imply that sulfate concentration or the presence of the Ca^{2+} cation was the dominant growth-limiting factor in positive control samples. If biocidal biproducts (i.e. H_2S) accumulated in positive control samples, they may account for the observed fall in pH. However, it is likely that $CaSO_4$ samples would also accumulate H_2S and decrease in pH over time (Postgate, 1984), eventually reaching positive control end pH values.

Due to the abrupt end of the $CaSO_4$ sample incubations, it is not possible to adequately compare the decreasing absorbance phases between the positive controls and $CaSO_4$ samples. However, absorbance trends in the positive control samples resemble the exponential growth and lag phases predicted for typical growth curves (Prescott et al., 2002).

4.6 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) while allowing for a higher total cell count.

5. Investigation Three: Sulfate-Reducing Bacteria Respond to Impact Shocks

5.1 *Introduction*

During the late heavy bombardment (LHB; ~3.9 Ga) the Earth received an impact influx with a delivered mass of 1.8-2.2 x 10^{20} kg (Abramov & Mojzsis, 2009; Willis et al., 2006). Disagreement persists on the effect these impacts had on potential pre-existing microbial life or impactor-transported microbes (Horneck et al., 2008; Meyer et al., 2011; Willis et al., 2006), including the potential transport of putative Martian microbes to Earth (Fajardo-Cavazos et al., 2007). Experimental work has demonstrated that sterilization could be avoided if impacts occurred in aqueous environments, as water-saturated impact surfaces are capable of dissipating heat and reestablishing habitable conditions post-impact more quickly than unsaturated surfaces (Abramov & Mojzsis, 2009). Therefore, the ability of sulfate-reducing bacteria to survive an impact under aqueous conditions was investigated.

> *Note:* due to the nature of this study, the *Organisms Investigated* and *Methods* sections have been combined.

5.2 Organisms Investigated and Methods

Sulfate-reducing bacteria were harvested from Galveston Bay, Texas marine sediments. Two sediment cores measuring ~32 inches and ~29 inches, respectively, were removed while submerged in ~24 inches of Galveston Bay seawater. Cores were laid onto an open sterile whirl-

pack bag™, separated into thirds (top, middle, and bottom), where the top third is closest to the sediment-water interface. Samples were stored in individual sterile whirl-pack bags™ or 50 mL centrifuge tubes. Water present in each core tube was collected in sterile centrifuge tubes (core-associated water). Sediment and core-associated water were stored at 4°C and were transported to Johnson Space Center (NASA JSC). Three 30g aliquots were taken from each core section and were supplemented with 60g sterile growth medium designed to isolate sulfate-reducing bacteria as described in Muller et al. (2014). A fourth set of aliquots was taken from each core section, sterilized, in an autoclave at 121°C for 30 minutes and then supplemented with media to provide a sterile negative control (see table 5). Cultures (unsterilized samples in growth medium) and controls were prepared anaerobically as described in (Muller et al., 2014) and stored in an anaerobic glove bag (by volume: 84.9% N_2; 10.1% H_2; and 5% CO_2) at 32°C. The remaining sediment was stored at -80°C and core-associated water samples were stored at -20°C.

Aliquots of cultures and controls were taken at time zero (the point of growth medium supplementation), 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 168 hours, and weekly thereafter. Aliquots and core-associated water were analyzed for sulfate concentration using a Dionex ICS-2000 Ion Chromatograph (IC). IC standards were prepared from Dionex sulfate standard stock solution, as well as from dilutions of sterile medium. Sulfate-reduction rates from all core sections were analyzed to determine: 1) sections with the strongest SRB community; and 2) time of peak-exponential growth phase.

Based on results from the first set of measurements, a fresh set of cultures and controls were prepared from the selected core sections and incubated for 8 days before lyophilization. The samples were then stored at -80°C until preparation for impact.

Table 5: Impact experiment (section 6) sample names.

Sample	Treatment	Purpose
Experiment Sample	Unsterilized Shocked	Test effect of shock pressure on microbial activity.
Positive Control	Unsterilized Un-shocked	Ensure sample handling did not kill microbes. Characterize microbial changes to sulfate under normal-lab conditions.
Negative Control	Sterilized Shocked	Characterize abiotic changes due to impact.
Process Control	Sterilized Un-shocked	Ensure sample handling did not introduce new microbes.

Samples were loaded into stainless-steel containers and stored at 4°C for ~ 8 hours. The containers were then mounted in the flat-plate accelerator (see figure 12) at NASA JSC and impacted at 7.9 km/s (experiment sample) and 7.52 km/s (negative control; 9.26 and 8.73 GPa, respectively). A target of 10 GPa shock pressure was chosen for initial investigation based on survival events recorded in cyanobacteria mounted in dry sandstone (Meyer et al., 2011). After impact, samples were collected and massed. A portion of the sample was saved for DNA analysis, and the remainder of the sample was supplemented with fresh growth medium, incubated, and sampled at the time intervals previously specified. IC analyses were performed.

5.3 Results

Initial incubations of sediment SRB were found to reach exponential growth after 8 days. New incubations were prepared and subjected to a flat-plate accelerator pressure of ~10 GPa, the results of which are depicted in table 6 and figure 13. Post-impact, the negative control and experiment sample showed increases in sulfate concentrations within 48 hours of incubation. The negative control reached peak sulfate concentration after 12 hours and the experiment sample after 48 hours. After peak concentration was achieved, both samples show a similar trend: sharp sulfate concentration decreases followed by gradual sulfate concentration increases.

The process control increased in sulfate concentration initially, while the positive control sample initially decreased in sulfate concentration, which was followed by an increase in sulfate concentration to greater levels than the process control. After reaching peak sulfate concentrations, the sulfate levels in both the positive and process controls decreased in concentration.

Figure 12: The flat-plate accelerator located at the Experimental Impact Laboratory at NASA Johnson Space Center. Three stainless-steel sample containers are visible in the bottom right quadrant of the image.

Table 6: Sulfate concentration (mg/L) change over time in samples containing Galveston Bay, Texas sediment, growth medium, and 1) unsterilized active native microbial communities, or 2) sterilized sediment. Samples were either kept at lab conditions (positive and process controls) or subjected to impact with a flyer-plate at a pressure of ~10 GPa (experiment sample and negative control).

Sulfate concentration (mg/L)				
Time (hours)	Experiment Sample	Positive Control	Negative Control	Process Control
0	3888.2	3903.8	3859.6	3842.1
6	3757.3	3744.0	3882.5	4035.3
12	3848.0	4218.0	4185.5	4121.1
24	3928.5	4093.4	4022.8	4148.2
48	4110.4	3463.6	3566.6	4082.8
72	3801.9	3927.5	3659.7	3534.6
336	3976.5	3841.7	3739.0	3914.3

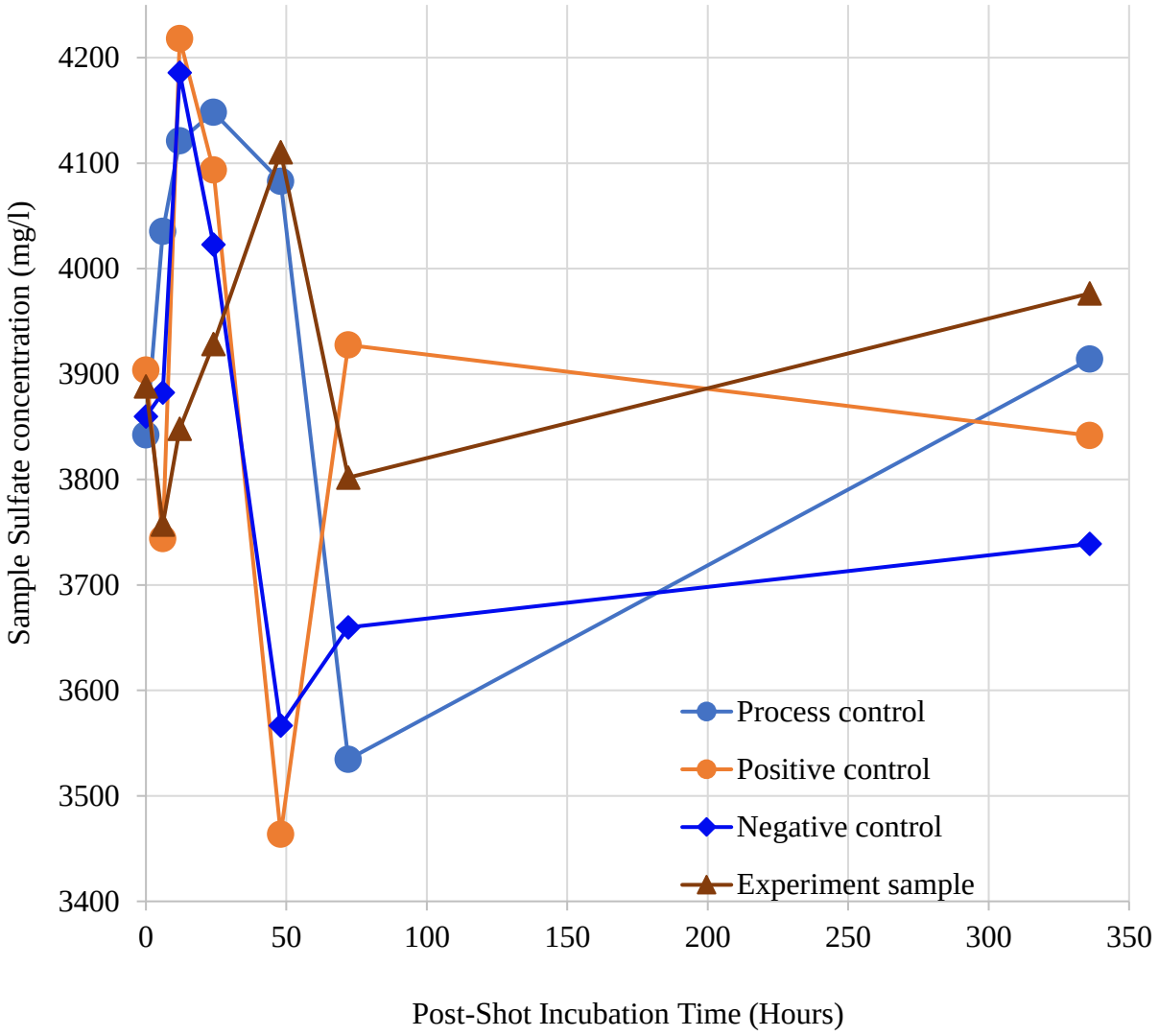

Figure 13: Sulfate concentration (mg/L) change over time in samples containing Galveston Bay, Texas sediment, growth medium, and unsterilized active native microbial communities or sterilized prior to incubation. Samples were either kept at lab conditions (positive and process controls) or subjected to impact with a flyer-plate at a pressure of ~10 GPa (experiment sample and negative control).

5.4 Discussion

The initial increase in sulfate concentration in the negative control and experiment samples suggests an abiotic process (e.g. desorption) may be responsible. The 36-hour delay in peak concentration times between the negative control and experiment sample may be due to microbial sulfate-reduction offsetting the sulfate desorption in the experiment sample. The delay in peak concentration may also be a result of sulfate interaction with shot-lysed cells or pre-shot sulfate reduction metabolic biproducts (e.g. sulfite, elemental sulfur) freed from shot-lysed cells. The pattern of sharp sulfate concentration decreases followed by gradual sulfate concentration increases in both the experiment sample and the negative control indicates an abiotic process, such as absorbance of sulfate back into the remaining sample sediment and desorption into the sample-liquid phase.

The immediate increase in sulfate concentration in the process control suggests desorption of sulfate from sediment sulfate into the medium/water-liquid phase. The initial decrease in sulfate concentration in the positive control may be due to microbial sulfate reduction, but this would require initial microbial sulfate-reduction rates to overcome rates of sulfate desorption. Then, either sulfate desorption rates increase, overwhelming microbial sulfate reduction, or microbial sulfate reduction rates decrease, falling beneath desorption rates. This would then have to be followed by microbial sulfate reduction returning to a rate greater than sulfate desorption.

The trend of sulfate-concentration increasing and then sharply decreasing, in all samples, suggests an abiotic mechanism. The positive control's increase in sulfate-concentration after 48 hours may be due to microbial activity (e.g. sulfur oxidation by a sulfur-oxidizing microbial colony), but the observation of similar trends in the sterilized controls suggests otherwise.

5.5 Conclusion

Sediment cores were retrieved with indigenous microbial communities from Galveston Bay, Texas. These were prepared and provided medium to select for sulfate-reducing bacteria. Bacterial growth was characterized by measuring aqueous sulfate concentration with an Ion Chromatograph. Core segments with the greatest decreases in initial sulfate concentration were identified, and duplicate incubations were subjected to shot impact with a flat-plate accelerator at 8.76-9.23 GPa. Sulfate-concentration changes in experimental samples and all controls were similar, with varied lag-times. It is unclear whether the tested microbes survived the sample handling and impact process. Additional experiments will attempt to clarify the issue.

6. Conclusion

The NASA Planetary Protection policy requires interplanetary space missions do not compromise the target body for a current or future scientific investigation and do not pose an unacceptable risk to Earth, including biologic materials. Robotic missions to Mars pose a risk to planetary protection in the forms of forward and reverse contamination. To reduce these risks, a firm understanding of microbial response to Mars conditions is required. Sulfate-reducing bacteria are prime candidates for potential forward contamination on Mars. Understanding the potential for forward-contamination of sulfate-reducers on Mars calls for the characterization of sulfate-reducers under Mars atmosphere, temperature, and sulfate-brines.

This study investigated the response of several sulfate-reducing bacteria, including spore formers and psychrophiles. The psychrophile *Desulfotalea psychrophila* was found to inconsistently survive positive control lab conditions, attributed to issues shipping pure cultures. *Desulfotomaculum arcticum*, a spore-forming mesophilic sulfate-reducer, and *Desulfuromusa ferrireducens*, an iron and sulfate-reducer, were metabolically active under positive control lab

conditions with complex and minimal growth medium. A wastewater treatment sulfate-reducing bacteria (SRB) isolate was subjected to sulfate + growth-medium solutions of varied concentrations (0.44 & 0.55% wt.). The wastewater SRB displayed higher absorbance levels, at delayed rates in 0.55% sulfate solutions, suggesting a greater total culture reproduction, but with increased lag time. Additional SRB were isolated from marine sediments, subjected to a shock pressure of 8.73 GPa, and returned to ideal conditions. The sulfate-concentration patterns in the impacted SRB culture suggests a destruction of culture occurred somewhere during the preparation process. The response of SRB in this investigation to varied concentration sulfate-brines offers credence to the suggestion that Mars sulfate-deposits could offer an energy sink to terrestrial microorganisms. Further investigation (i.e. sulfate cations and concentrations, temperature, pressure, etc.) may identify Martian locations at risk to forward contamination.

7. References

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