

The Pennsylvania State University

The Graduate School

**ENHANCED EVALUATION OF MARTIAN
HABITABILITY IN THE PAST AND PRESENT: THE
SEARCH FOR VIABLE ELECTRON DONORS**

A Dissertation in

Geosciences and Astrobiology

by

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Abstract

Life requires energy for both growth and metabolism. On Earth, microorganisms have evolved diverse metabolisms to be able to harvest energy from oxidation-reduction (redox) reactions that involve the transfer of electrons from a reduced compound (electron donor) to an oxidized compound (electron acceptor). Because of life's reliance on redox reactions, the search for habitable environments on other planets necessitates an investigation of the available electron donors and acceptors. On Mars, the environment today has abundant electron acceptors, such as sulfate and ferric iron. Possible electron donors on Mars, such as sulfide or reduced carbon, are less common near the surface and in the atmosphere, but represent a critical component of Martian habitability. This dissertation broadly focuses on understanding the distribution and viability of reduced sulfur and carbon as possible electron donors for a putative microbiology on Mars.

Chapter 2 of the dissertation investigates the presence of reduced sulfur in Gale crater, Mars from the Mars Science Laboratory (MSL) Sample Analysis at Mars (SAM) evolved gas analysis (EGA) data. Simple mixtures of Mars-relevant compounds (including sulfides and sulfates) were investigated using laboratory SAM-like EGA. Select gases evolved from these mixtures were compared to SAM data using quadratic discriminant analysis (QDA). This novel analysis of SAM data found that reduced sulfur was likely present in numerous Martian samples from first 2300 sols of the MSL mission, implying a large spatial and temporal distribution of reduced sulfur in Gale crater.

Chapter 3 expands upon Chapter 2 and investigates the distribution of reduced sulfur in the 'clay-bearing' (as seen from orbital data) region of Gale crater. This chapter combines EGA temperature interpretations, QDA comparisons of SAM and laboratory data, and sulfur isotope calculations as complementary methods to identify samples with

reduced S. There is consistent evidence from these analyses to indicate that two samples include reduced sulfur in this area. The presence of sulfide in these samples, which represent different lithologies and times in Martian history, further supports the finding that reduced S was available for putative microbial metabolism in the ancient Martian environment.

Chapter 4 investigates the origins of methane and chloromethane that are observed during thermal decomposition of solid Martian samples. The thermodynamics of potential oven reactions forming these compounds are considered, as are possible isotopic consequences. These estimates will aid future investigations of thermally-released methane and chloromethane on Mars.

Chapter 5 investigates carbon monoxide, which is relatively abundant in the modern Martian atmosphere, as a possible electron donor. Numerous diverse microorganisms on Earth can oxidize CO for energy. We performed thermodynamic calculations and laboratory experiments to assess the plausibility of various CO-based metabolisms. While the thermodynamics of CO oxidation are promising, laboratory experiments suggest that microbial use of CO is likely limited by the desiccated Martian environment.

The results of this dissertation expand the understanding of habitability on Mars in the past and present. Reduced sulfur, which can potentially serve as a microbial electron donor, has been identified on Mars from Curiosity rover data. This dissertation has also explored possible pools of reduced carbon that microbes could exploit for energy. Together, these results will improve interpretations of Martian habitability as exploration of the Red Planet continues.

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PREFACE

Chapter 2 of this dissertation represents published, multi-authored work led by G. Wong. G. Wong designed experiments, performed laboratory research, analyzed data, and wrote the manuscript in consultation and collaboration with coauthors. This chapter was published in the *Journal of Geophysical Research: Planets*. Reproduced/reformatted with permission from John Wiley & Sons. Citation: Wong, G. M., J. M. T. Lewis, C. A. Knudson, M. Millan, A. C. McAdam, J. L. Eigenbrode, S. Andrejkovičová, F. Gómez, R. Navarro-González, and C. H. House. 2020. “Detection of Reduced Sulfur on Vera Rubin Ridge by Quadratic Discriminant Analysis of Volatiles Observed during Evolved Gas Analysis.” *Journal of Geophysical Research: Planets* 125 (8). John Wiley & Sons, Ltd. doi:10.1029/2019JE006304.

Chapter 3 represents a draft manuscript led by G. Wong for submission to the *Journal of Geophysical Research: Planets*. Data was collected in collaboration with the Sample Analysis at Mars and Mars Science Laboratory teams. G. Wong analyzed the data and wrote the manuscript. Coauthors will include at least Heather Franz, Joanna Clark, Amy McAdam, James Lewis, Rafael Navarro-González, and Christopher House. The coauthor list is subject to change with likely additions from the Mars Science Laboratory team.

Chapter 4 represents a section of a larger, multi-authored manuscript in preparation. G. Wong performed all analysis, calculations, and writing of this section in consultation with C. House.

Chapter 5 represents a draft manuscript that we intend to submit to the Astrobiology section of the open access journal, *Life*. G. Wong designed experiments, performed laboratory work, did thermodynamic calculations, and wrote the chapter in collaboration with Z. Zhang and C. House.

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Chapter 1: Introduction

The importance of electron donors for habitability on Mars

Life as we know it is the basis for the search for life elsewhere in the Universe. To that end, it is understood that liquid water, basic elemental building blocks (CHNOPS), and an ability to harvest energy are requirements for life (Mckay 2014). On Earth, organisms have found numerous ways to extract energy from the environment to survive. One of the primary methods of energy extraction takes advantage of oxidation-reduction (redox) reactions (Thauer, Jungermann, and Decker 1977; Martin and Russell 2003). Many are familiar with the redox reaction of aerobic respiration by which we oxidize an organic carbon source (an electron donor, such as sugar) using molecular oxygen (an electron acceptor) to form carbon dioxide and water. This reaction, typified by the simplified formula $\text{CH}_2\text{O} + \text{O}_2 \leftrightarrow \text{CO}_2 + \text{H}_2\text{O}$, releases energy that can be converted into a usable form by our cells. Microorganisms have evolved diverse metabolisms capable of analogous redox reactions using a variety of electron donors and acceptors. For example, some microbes can use compounds, such as CH_4 , NO_2^- , HS^- , and Fe^{2+} , as electron donors with various electron acceptors, such as NO_3^- , O_2 , SO_4^{2-} , Fe^{3+} , and HCO_3^- (Thauer, Jungermann, and Decker 1977). The search for life on other planets thus necessitates a search for suitable electron donors and acceptors in the environment.

Mars in particular has been the focus of exploration for its potential to harbor life and/or habitable environments in the past and present. The surface today would be considered an extreme environment on Earth with freezing temperatures down to -123°C , no liquid water, and a thin (<10 mbar), CO_2 -dominated atmosphere (Clark 1998; Franz et al. 2015). Additionally, the Martian surface environment is bombarded by biocidal

ultraviolet radiation, which would quickly kill even some of the hardiest microbes on Earth if unshielded (Khodadad et al. 2017). In the past, however, Mars was likely significantly more hospitable. Martian geomorphology indicates that liquid water was once abundant, indicating moderate temperatures and a thicker atmosphere, though the water has largely been lost due to atmospheric escape (Kurokawa et al. 2014). Much of Mars exploration has been dedicated to ‘following the water’ in the search for ancient habitable environments. More recent missions, such as the Mars Science Laboratory, have expanded this search to include the other requirements for life as we know it (Grotzinger et al. 2012).

The Mars Science Laboratory (MSL) mission includes a variety of instruments on board the Curiosity rover that were designed to investigate Martian geological/environmental history and potential for ancient habitability (Grotzinger et al. 2012). (See Appendix A for a more detailed description of the MSL mission and its instruments.) The Curiosity rover landed at Gale crater, Mars in August of 2012 to begin its exploration of Aeolus Mons (informally Mt. Sharp), which contains ~5 km of sedimentary stratigraphy that recorded the environmental history of the crater (Grotzinger 2014; Wray 2013). The first analyses of drilled samples (Sheepbed mudstone samples John Klein and Cumberland) in Gale crater revealed evidence for an ancient habitable environment on Mars (Grotzinger et al. 2014). These samples showed evidence for a long-standing, circumneutral lake (from the observations of mudstones and phyllosilicates) with the elemental building blocks of life (Grotzinger et al. 2014; Vaniman et al. 2014). Native organic carbon was also observed in the Cumberland drill sample by gas chromatography-mass spectrometry (Freissinet et al. 2015). Additionally, mineralogical evidence of mixed redox states of iron (as Fe_3O_4) and sulfur (as CaSO_4 and FeS) were reported from these

drill samples (Vaniman et al. 2014), though sulfide minerals were not included in later reports of these samples (Morrison et al. 2018). Still, an analysis of the volatile content of these samples suggested the presence of oxidized and reduced sulfur (Ming et al. 2014), which could represent an amorphous phase or be below the detection limit of the x-ray diffractometer. However, reduced sulfur (observed as H₂S during thermal decomposition of solid samples by evolved gas analysis) can also be formed by reactions during heating and would ultimately not reflect the presence of a native sulfide (McAdam et al. 2014; Sutter, McAdam, et al. 2017; Wong et al. 2020). The presence of both oxidized and reduced iron and sulfur indicates that both electron acceptors and donors were present in the once habitable lake system.

Oxidized compounds (electron acceptors), such as sulfate, perchlorate, nitrate, and hematite have been observed throughout Gale crater (Stern et al. 2018; Stern et al. 2015; Sutter, Quinn, et al. 2017; Rampe et al. 2020). Native electron donors, with the exception of Fe²⁺ in magnetite, are less commonly observed in solid samples. While there have been some indications of sulfide presence in solid samples, it appears to be only a minor contribution to the total sulfur observed (if present at all) (Vaniman et al. 2014; Franz et al. 2017). Thus, there are still open questions about reduced sulfur as a possible electron donor. This dissertation, in part, looks to answer the following questions:

- (1) Do sulfates and sulfides evolve distinct volatiles from solid samples during pyrolysis, and can this be used to detect reduced sulfur?
- (2) Do the volatiles evolved from solid Martian samples during pyrolysis indicate the presence of any reduced sulfur?
- (3) What is the distribution of reduced sulfur in Gale crater?

(4) What are the implications for Martian habitability?

In addition to reduced sulfur, reduced organic carbon is a key factor for the assessment of habitability. Thermal experiments have been conducted on Mars since the Viking landers and have found evidence of chlorinated organic compounds (Biemann et al. 1977). Chemical reactions that occur during heating of complex mixtures can complicate interpretations of reduced carbon observations. It is therefore important to assess reactions that may occur during pyrolysis to aid in interpretations of the source carbon. Furthermore, carbon isotopic compositions of evolved volatiles may help with source identification if they can be determined; however, oven reactions may similarly complicate the isotope interpretations. Therefore, it is important to understand both the oven reactions and their possible isotopic consequences to interpret the source of reduced organic carbon on Mars.

Additionally, the dry, CO₂-rich Martian atmosphere has abundant photochemically-derived carbon monoxide (CO) (Barth 1974; Smith et al. 2009; Krasnopolsky 2015; Krasnopolsky 2017). CO on Earth can act as an electron donor for numerous diverse microorganisms through the energetically-favorable oxidation of CO to CO₂ (Oelgeschläger and Rother 2008). Given the abundance of CO on Mars and its use as an electron donor on Earth, CO has been studied for its potential use by microbes under select Mars-like conditions (King 2015). However, it is still unclear whether CO can act as a viable electron donor on Mars today.

Chapter outline

Chapters 2-5 of this dissertation address the above questions regarding potential Martian electron donors, their identification, and the viability of their use by microbes. The chapters are briefly outlined below.

Chapter 2 primarily focuses on the development and implementation of a novel method used to interpret volatiles evolved from pyrolyzed Martian solid samples to identify samples that contain reduced sulfur. Here, we used quadratic discriminant analysis to statistically compare Martian EGA data to a suite of laboratory analogue materials. This statistical method of sample classification was applied to both Martian aeolian material and drilled rock samples through the first ~2300 sols of the MSL mission to determine where reduced sulfur may be present. The implications for these findings on geologic history and habitability are discussed.

Chapter 3 advances the work of Chapter 2 and takes a multi-faceted approach to identifying samples in the “clay-bearing unit” of Gale crater that contain reduced sulfur. This work explicitly combines analysis of volatile temperature releases, results from quadratic discriminant analysis comparing Mars and laboratory data, and sulfur isotope calculations of evolved sulfur gases. These three methods complement each other to indicate the presence of reduced sulfur with higher confidence than any single method. The application of this combined analysis was used for sulfur in the clay-bearing unit, which is a region of high astrobiological interest to the mission due to the potential for enhanced preservation of organic molecules in phyllosilicates. The implications of our sulfur analysis on this region are explored.

Chapter 4 of this dissertation explores the production of chloromethane and methane during pyrolysis of solid samples on Mars. The thermodynamics of select reactions are considered to determine the plausibility of them occurring during heating. From these reactions, possible isotopic effects on the carbon are explored through isotopic fractionation modeling. These isotopic implications will lead to a more complete understanding of measured carbon isotopes of evolved carbon gases from all Martian samples. This information will ultimately inform about the nature of reduced carbon in solid Martian samples.

Chapter 5 focuses on the investigation of CO as an electron donor, especially for Mars. This chapter has three main objectives. The first is to determine the thermodynamic favorability of potential CO-based metabolisms in a range of environmental conditions. The second is to test whether a microbe that had previously been indicated to oxidize CO under select Mars-like conditions could, in fact, metabolize or even grow on CO. The third objective is to determine whether CO can act as the sole electron donor for microbes in anoxygenic photosynthesis. A variety of laboratory experiments were performed to determine the plausibility of CO as an electron donor on Mars and the implications of these experiments on habitability in the past and present are discussed.

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Chapter 2: Detection of Reduced Sulfur on Vera Rubin Ridge by Quadratic Discriminant Analysis of Volatiles Observed During Evolved Gas Analysis

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Abstract

The Mars Science Laboratory mission investigated Vera Rubin ridge, which bears spectral indications of elevated amounts of hematite and has been hypothesized as having a complex diagenetic history. Martian samples, including three drilled samples from the ridge, were analyzed by the Sample Analysis at Mars instrument suite via evolved gas analysis-mass spectrometry (EGA-MS). Here, we report new EGA-MS data from Martian samples and describe laboratory analogue experiments. Analyses of laboratory analogues help determine the presence of reduced sulfur in Martian solid samples, which could have supported potential microbial life. We used evolved carbonyl sulfide (COS) and carbon disulfide (CS₂) to identify Martian samples likely to contain reduced sulfur by applying a quadratic discriminant analysis. While we report results for 24 Martian samples, we focus on Vera Rubin ridge samples and select others for comparison. Our results suggest the presence of reduced sulfur in the Jura member of Vera Rubin ridge, which can support various diagenetic history models, including, as discussed in this work, diagenetic alteration initiated by a mildly reducing, sulfite-containing groundwater.

Plain Language Summary

The Mars Science Laboratory studied the chemical composition of Vera Rubin ridge in Gale crater, Mars. The Sample Analysis at Mars, a set of scientific instruments designed to study rock chemistry, observed a number of gases released during the heating of Martian drilled samples. The same gases were observed when Mars-relevant minerals were analyzed with similar instruments on Earth. From these two sets of data, we applied statistical analyses to determine which Mars samples on Vera Rubin ridge contained important sulfur compounds. Two samples on the ridge showed evidence for these compounds, which could have supported the energetic requirements for life. The results presented here improve both the understanding of the history of Gale crater and the potential for ancient life to have existed.

Introduction

Mars Science Laboratory Mission and Vera Rubin Ridge

The Mars Science Laboratory (MSL) Curiosity rover has been exploring Gale crater, Mars, since landing in 2012. Gale crater, an ~155 km diameter impact crater, is located near the Martian crustal dichotomy and contains some of the lowest elevations in the southern hemisphere (Wray 2013). Within Gale crater is Aeolis Mons (informally known as Mount Sharp), which hosts ~5 km of stratified deposits that recorded Martian geological and environmental history (Grotzinger & Milliken, 2012). Evidence from orbit indicates a geologic record of ancient aqueous environments and varying mineralogy that include phyllosilicates, sulfates, and hematite (Milliken et al., 2010). Gale crater was the chosen MSL landing site to investigate the variation and extended stratigraphy further.

One of the primary goals of MSL is to investigate Gale crater for evidence of past habitability while characterizing the crater's geology (Grotzinger et al., 2012). To this end, MSL has instrumentation to determine the presence of ancient liquid water, organic carbon, and redox gradients by analyzing the chemistry and mineralogy of Martian samples. Hundreds of meters of Gale's stratigraphy have been explored, and it has been found that there are mineralogically diverse sedimentary rocks that largely consist of mudstones and sandstones (Figure B.1 in Appendix B). Early in the mission, it was determined that a habitable fluvio-lacustrine environment persisted at Gale crater—including fresh liquid water with a circumneutral pH and nonequilibrium mineral assemblages that included oxidized and reduced iron minerals (Grotzinger et al., 2014). As the Curiosity rover has ascended Mount Sharp, additional analyses have found evidence of native Martian organics, including chlorohydrocarbons (Freissinet et al., 2015) and recalcitrant organosulfur compounds (Eigenbrode et al., 2018).

One interesting geomorphological feature of Gale crater is a 200 m wide ridge with a strong spectral signature for hematite, now referred to as Vera Rubin ridge (VRR). Understanding the formation environments for the hematite-capped ridge is important for its implications in the broader context of Martian paleoenvironments, particularly in Gale crater. Previous studies about VRR focused on orbital data and two endmember models for the ridge's formations were derived from these data sets (Fraeman et al., 2013, 2016). The first proposed model by Fraeman et al. (2013) is that the hematite was authigenic and deposited from a redox interface of underlying Fe(II) from groundwater with atmospherically sourced oxidants. Their second model

proposes that the hematite formed through interactions between diagenetic fluids in which dissolved Fe(II) came into contact with a second, oxidized fluid and precipitated out as hematite. These fluids would have been controlled by stratigraphy and porosity. Additional analyses performed by MSL allow for a more detailed understanding of VRR than from orbital data alone.

The Curiosity rover explored VRR from sol 1809 to 2302. VRR has been divided into two informal geologic members: Pettegrove Point member and Jura member (Figure B.1). Jura is stratigraphically above Pettegrove Point and both members consist primarily of finely laminated mudstones. While the VRR members form a ridge, observations by MSL indicate that VRR is a continuation of the Murray Formation, which consists of hundreds of meters of finely laminated mudstones (Fedo et al., 2019). Additional observations indicate that VRR experienced several diagenetic events. For example, the Jura member consists of red and gray patches whose boundaries are not stratigraphically defined, which is suggestive of alteration (Horgan et al., 2019). Variations in Li and Mn content on VRR also suggest the flow of diagenetic fluids through VRR at some point in its history (Frydenvang et al., 2019). Data from the Sample Analysis at Mars (SAM) can be used to better understand diagenesis on VRR, including the ridge's redox history.

SAM Sulfur Volatile Findings

The SAM is an instrument suite on MSL that measures volatile compounds evolved from samples, which complements geological interpretations by other MSL instruments. SAM includes a pyrolysis oven and a gas chromatograph coupled to a mass spectrometer. Using these instruments, SAM can perform evolved gas analysis-

mass spectrometry (EGA-MS) for solid sample analysis and gas chromatography-mass spectrometry (GC-MS) for separation and identification of organic and inorganic molecules (Mahaffy et al., 2012). EGA is a sensitive technique that is able to detect volatiles evolved during the ramped heating of solid samples. Previous EGA work has found evidence of both oxidized and reduced sulfur in the forms of SO_2 and H_2S (and lesser COS and CS_2) in both eolian materials and drilled samples of sedimentary rocks (Archer et al., 2014; Leshin et al., 2013; McAdam et al., 2014; Stern, Sutter, et al., 2018; Sutter et al., 2017). One explanation for the evolution of oxidized and reduced sulfur from a single sample is the presence of a sulfur mineral assemblage that is not in redox equilibrium. Reactions that occur in the oven during EGA can also affect the composition and/or oxidation state of evolved sulfur compounds. Either way, there is an indication that there is a source of reducing power in the Martian samples. While SO_2 is by far the most abundant evolved sulfur-bearing volatile, followed by H_2S , there are other minor sulfur gases that can contain important redox information about a sample, such as carbonyl sulfide (COS) and carbon disulfide (CS_2).

Carbon-Sulfur Volatiles From Sulfides

Carbon-sulfur gases can be telling of sample composition. A variety of carbon-sulfur gases (e.g., COS , CS_2 , CH_3SH , and $\text{C}_2\text{H}_6\text{S}$) observed at high temperature during EGA in SAM have indicated the presence of organosulfur compounds (Eigenbrode et al., 2018). The high-temperature observations of these gases have been interpreted as either breakdown products of larger molecules or the result of oven reactions. Carbon-sulfur gases have also been predicted and observed during coal pyrolysis, particularly at high temperature ($>500^\circ\text{C}$), as a result of reactions between

iron sulfides and carbon species (Attar, 1978). COS has especially been investigated and found to be produced during coal pyrolysis in significant amounts when CO₂ is the carrier gas, but not with N₂ carrier gas (Duan et al., 2009; Frigge et al., 2016). Production of COS can be the result of gas phase interactions in which CO or CO₂ reacts with H₂S, or COS can be produced by a series of solid-gas interactions by reaction between FeS₂ and CO₂ (Arutyunov, 1992; Bhargava et al., 2009). The enhanced production of COS in CO₂ carrier gas (compared to N₂ carrier gas) suggests that CO₂ can serve as a carbon source for carbon sulfur volatiles during pyrolysis. While most production of COS occurs at high temperature during coal pyrolysis, COS has been observed at temperatures below 600°C (Shao et al., 1994) and even as low as 250°C (Wang et al., 2014). Shao et al. (1994) also suggested that the production of COS competes with the production of CS₂ from ~350°C to 900°C. CS₂ can form from gas-phase reactions between COS and COS, H₂S and COS, or C and S₂. While less frequently discussed compared to COS, CS₂ is a common coal pyrolysis product (Attar, 1978) and has also evolved during EGA of numerous Martian samples (Eigenbrode et al., 2018; Leshin et al., 2013; McAdam et al., 2014). Though coal pyrolysis describes different samples than Mars (notably, coal has abundant organic carbon), the production of COS and CS₂ by reduced sulfur and carbon interactions during coal pyrolysis can inform about possible related sulfur reactions during EGA with SAM. The significance of the production of these gases, particularly at lower temperatures, requires further examination. These volatiles can be used to study complex Martian samples and aid in determining the redox history of sites on Mars.

This paper presents a novel analysis of EGA data to aid in the identification of

Martian samples that may contain reduced sulfur. We tested a range of volatile-bearing one or two component mixtures in laboratory SAM-like EGA. The EGA results from those experiments were used as training data for a multivariate analysis in which the relationships among COS, CS₂, SO₂, CO₂, and bisilylated water (BSW; a tracer of the organic derivatization agent N-methyl-N-tert-butyldimethylsilyl-trifluoroacetamide, that is, MTBSTFA, in the SAM) were analyzed to discriminate between samples containing sulfides and sulfates. The laboratory work has direct implications to Mars, especially the presence/absence of reduced sulfur in the drilled samples of VRR, and can inform about the diagenetic history and potential habitability of the ridge.

Materials and Methods

SAM Evolved Gas Analysis-Mass Spectrometry

The operation of EGA on SAM has been described previously (Glavin et al., 2013; Mahaffy et al., 2012; Sutter et al., 2017). Briefly, solid samples—either drilled or scooped—are delivered to a pyrolysis oven. Samples are heated under a constant flow (0.8 ml/min) of helium at 25 mbar from ~30°C to ~850°C at a temperature ramp rate of 35°C/min. Volatiles released from the sample are carried by the He flow to the quadrupole mass spectrometer, which identifies mass-to-charge ratios (m/z) of the ionized volatiles over the entire temperature range. These data are stored and converted into pyrograms that show intensity (counts per second) versus temperature.

Laboratory Experiments

We used a SAM-like EGA instrument setup at the NASA Goddard Space Flight Center to analyze the majority of the samples for this work. This setup used an Agilent

5975T-LTM MS attached to a Frontier PY-3030D pyrolysis oven. Powdered samples (see following section) were contained in organically clean stainless-steel cups. Sample cups were dropped into the pyrolysis oven and kept at 75°C for up to 31 min (depending on the oven program) under a constant flow (~50 ml/min) of He at 30 mbar to allow for the desorption of adsorbed water from the sample. Cups were then heated linearly at 35°C/min until the oven temperature reached at least 850°C (SAM-like). The He flow carried volatiles produced during thermal decomposition to the mass spectrometer. The MS monitored up to m/z 200, which covers the range of masses of the species expected to be detected. While the laboratory instrument setup is not exactly the same as SAM, the conditions used during EGA are selected to be comparable to SAM conditions and different SAM-like setups are frequently used to compare samples to SAM data (e.g., Glavin et al., 2013; McAdam et al., 2014; Ming et al., 2014). The major difference in EGA conditions is the high flow rate and split ratio used in the laboratory, which is necessary when running sulfur- and chlorine-rich samples to preserve instrument integrity.

Table 2.1*Compounds Used in Laboratory EGA*

Sulfur compounds		Nonsulfur compounds for mixtures	
Pyrite ^a	FeS ₂	Halite	NaCl
Troilite ^b	FeS	Calcium chloride	CaCl ₂
Ferric sulfate hydrate	Fe ₂ (SO ₄) ₃ •nH ₂ O	Magnesium chloride	MgCl ₂
Jarosite ^c	KFe ₃ (SO ₄) ₂ (OH) ₆	Calcium perchlorate	Ca(ClO ₄) ₂
Kieserite ^d	MgSO ₄ •H ₂ O	Iron (III) perchlorate	Fe(ClO ₄) ₃
Melanterite	FeSO ₄ •7H ₂ O	Magnesium perchlorate	Mg(ClO ₄) ₂
		Nontronite ^e	(Ca _{0.5} ,Na)Fe ₂ (Si,Al) ₄ O ₁₀ (OH) ₂ •nH ₂ O
		Magnetite	Fe ₃ O ₄
		Siderite	FeCO ₃
		MTBSTFA-DMF	C ₉ H ₁₈ F ₃ NOSi – C ₃ H ₇ NO
		Magnesium acetate	C ₄ H ₆ MgO ₄
		Iron (III) oxalate	C ₆ Fe ₂ O ₁₂
		Myristic (tetradecanoic) acid	C ₁₄ H ₂₈ O ₂

Note. All compounds were synthetic and purchased from Sigma-Aldrich except those marked with exceptions.

^aNatural samples from Ward's Science and synthetic sample from Sigma-Aldrich. ^bAlfa Aesar. ^cSynthesized according to method by Driscoll and Leinz (2005). ^dESTA. ^eNatural, Clay Minerals Society Source Clay NAu-2 from Uley Mine, Australia.

Sample Preparation

Six inorganic sulfur-bearing compounds were used, including two sulfides (pyrite and troilite) and four sulfates (kieserite, jarosite, melanterite, and ferric sulfate hydrate). Refer to Table 2.1 for details on all compounds used. Ferric sulfate hydrate, kieserite, melanterite, and one pyrite sample were purchased from Sigma-Aldrich. All other pyrite samples were drilled out from a natural pyrite source (Ward's Science) and confirmed pure by X-ray diffraction (XRD; Terra® by inXitu, Inc., Campbell, CA; crystalline detection limit 1 wt.% or better; Blake et al., 2012). The jarosite was produced according to the protocol of Driscoll and Leinz (2005), and purity was confirmed by Terra XRD. FeS was from Alfa Aesar, stored in a desiccator, and >98 wt.% troilite/pyrrhotite and ~2 wt.% Fe metal according to Terra XRD. All solid samples were sieved to <150 µm grain size to simulate the size fractions analyzed with SAM. Samples were weighed and mixed with an inert, organically clean fused silica

(Conrad et al., 2012; <150 μm) to physically disperse the sulfur minerals to simulate their natural occurrences where they are found within a matrix of other minerals. By mass, the ratio of sulfur compound to fused silica was $\sim 9:1$ in a given mixture. Mixing occurred either in bulk by mortar and pestle for 3 min or in individual sample cups with an inert, organically clean stainless-steel mixing tool. Samples were prepared at room temperature and atmospheric pressure. This is a difference from Martian samples, which are under Martian conditions (i.e., <10 mbar CO_2 -dominated atmosphere and cold temperatures) before delivery to SAM. Additionally, much more sulfur was used in the laboratory analyses (~ 10 wt.%) compared to the sulfur content observed in much more heterogeneous Martian samples ($\sim 2\text{--}5$ wt.% SO_3 ; Sutter et al., 2017). Due to the differences in laboratory and SAM analyses, the results of the laboratory EGA experiments are not exact replicas of Martian samples. Rather, these experiments allow us to explore the products of sulfur pyrolysis from simple, controlled mixtures in order to better describe the complex Martian mixtures.

In addition to the sulfur compounds alone in fused silica, mixtures were made with an $\sim 10:1$ mass ratio of sulfur to nonsulfur components except where otherwise noted. For a given sulfur/fused silica mixture, one of the following compounds was added in aqueous solution: sodium chloride, magnesium chloride, calcium chloride, calcium perchlorate, magnesium perchlorate, or iron (III) perchlorate (see Table 2.1). These Cl-bearing compounds, representing compounds present or potentially present on Mars, tested effects of HCl evolution and O_2 production on the evolution of sulfur-bearing volatiles. Mixtures of nontronite and magnetite with sulfur phases (1:1:1 ratio by mass) were used to test effects of possible H_2 production on sulfur evolution. To

test the effects of carbon/sulfur redox on sulfur volatile evolution, mixtures of oxidized or reduced sulfur with either siderite (1:1) magnesium acetate (1:1), ferric oxalate (1:1), or myristic acid (sulfur to myristic acid by mass, 10:1) were used.

One additional experiment accounts for background MTBSTFA and dimethylformamide (DMF) that is in the SAM system. MTBSTFA is a derivatization compound used for SAM wet chemistry experiments. At least one of the cups containing MTBSTFA leaked into the SAM Sample Manipulation System (SMS) and produces low level background during analysis (Glavin et al., 2013). To investigate the effects of this reduced carbon contamination and potential mass interferences with COS and CS₂, MTBSTFA-DMF (4:1) was added to select samples of oxidized and reduced sulfur in the laboratory in an ~100:1 mass ratio of sulfur to MTBSTFA. MTBSTFA readily reacts with hydroxyl groups and can produce a range of byproducts, including 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyldisiloxane (BSW). BSW is detected in all Martian samples (Eigenbrode et al., 2018) and was used in this work as a tracer for MTBSTFA.

The presence of MTBSTFA acts as a confounding factor in the determination of reduced sulfur presence for two reasons. First, MTBSTFA is a large organic compound that can potentially act as a reducing agent during pyrolysis, increasing the amount of COS and CS₂ through reactions with sulfates. The second reason is that MTBSTFA can produce fragments or reaction byproducts that have interfering m/z values with COS and CS₂. We tested several samples and mixtures with exposure to MTBSTFA to look at the derivatization agent's effects on the relevant volatiles used in the analysis. Included in these experiments were the four melanterite standards

examined by the flight module (see below), the two CB analogue samples (see below), and five SAM-like EGA laboratory samples. These samples addressed the role of MTBSTFA, its byproducts, and its reactions in the QDA.

In addition to the laboratory experiments, we analyzed four samples of melanterite in the SAM flight module and two FeS-bearing mixtures in the SAM testbed. The melanterite standards were analyzed in the SAM flight module prior to launch and have been used as calibration standards for abundance calculations (Archer et al., 2014). The FeS-bearing mixtures were analyzed in the SAM testbed, a complete replica of the SAM flight model housed in a Mars simulation chamber (Malespin et al., 2016). These two samples were from the Cumberland Analogue (Knudson et al., 2018; Stern, Graham, et al., 2018), a mixture of analogue materials that simulates the mineralogy of the MSL Cumberland drill sample from the Sheepbed mudstone (Vaniman et al., 2014).

Data Processing and Analysis

Volatile Areas

EGA data were processed in WaveMetrics IGOR Pro v6.0. Each run was deadtime (time between scans of different masses) corrected followed by a background subtraction. Data were then smoothed twice with an 11-point and subsequent 21-point moving average to reduce noise and ensure peaks are not a result of random background (Eigenbrode et al., 2018). After these corrections and smoothing, negative values were removed. Area under the curve (AUC) was calculated for each of the following volatiles from 75°C to 600°C: SO₂ (m/z 64), CO₂ (m/z 44), COS (m/z 60), CS₂ (m/z 76), and BSW (m/z 147). For the SAM data, SO₂ (m/z 64) and

CO₂ (m/z 44) frequently saturate the detector. Due to this saturation, isotopologues were used: m/z 66 for SO₂ and m/z 45 for CO₂. The amounts of m/z 64 and m/z 44 were calculated by multiplying the areas for the isotopologues by 20.39 and 83.25, respectively, which are the relative ratios of the isotopologues according to the National Institute of Standards and Technology (NIST Mass Spectrometry Data Center & Wallace, 2018). Temperatures for samples from pyrolysis oven 2 used the “oven-2_model-1” model, which differs from some previous work due to ongoing model refinements (see McAdam et al., 2020 for more details). Due to the variation of integrated counts among different mixtures from oven reactions, statistical noise, differences in background subtractions, and smoothing parameters, the errors of associated integrated counts are estimated conservatively to be 20% for any count, consistent with previously reported EGA work (McAdam et al., 2014).

Quadratic Discriminant Analysis

Quadratic discriminant analysis (QDA) is a multivariate, supervised machine-learning technique that is used to cluster unknown samples within a group based on known training variables. Other statistical methods, such as principal component analysis, aim to make interpretation of multivariate data sets simpler by reducing a data set's dimensionality to variables that account for the majority of variation (Härdle & Simar, 2015b). Discriminant analysis, on the other hand, aims to classify new observations (i.e., SAM data) into groups known a priori (i.e., laboratory sulfides and sulfates). QDA, as an extension of linear discriminant analysis, does not assume equal covariance matrices between known classifications and identifies classification regions with quadratic, rather than linear, functions (Härdle & Simar, 2015a). While

discriminant analyses assume multivariate normal distributions of the data, a benefit of QDA is that it is robust to violations of the assumption of normality (as is the case for the EGA data here) and often outperforms other classification techniques (Finch & Schneider, 2006).

QDA is a standard method for classifying unknown data based on a training data set and has found use in a range of disciplines including finance (Altman & Loris, 1976), molecular biology (Zhang, 1997), and geo- chemistry (Wang et al., 2018). Here, SO₂, CO₂, COS, CS₂, and BSW areas from the laboratory EGA experiments were used as training data. Each sample had an assigned “sulfide” or “no sulfide” label. Based on the relationships between the variables and their labels, the QDA clusters the samples in the five-dimensional space and demarcates a region separating sulfides from sulfates. The same variables are used for the Mars samples, which do not have associated labels and are considered the “unknowns.” Based on the values of the five variables and the training data, the QDA predicts whether an unknown sample clusters with the sulfides or sulfates. Posterior probabilities for the Mars samples are calculated to report the likelihood that the prediction is correct compared to the training data. QDA was performed in Python 2.7.14. Statistics and figures were completed with scikit-learn (Pedregosa et al., 2011), Pandas (Mckinney, 2010), NumPy (van der Walt et al., 2011), SciPy (Virtanen et al., 2019), and Matplotlib (Hunter, 2007).

Normalization Methods and Zero Removal

The calculated areas independently underwent various types of normalization. Four different processing methods were used to determine the data set that most

accurately differentiated between sulfide- and nonsulfide-containing laboratory samples. These methods were (1) raw calculated areas, (2) log-transformed areas ($\log_{10}[\text{counts}+1]$), (3) normalized to total sample mass and log-transformed, and (4) normalized to calculated SO_2 evolved from 75°C – 600°C and log-transformed. For the final analysis used here, nondetections and zero values of COS and CS_2 were not included because they do not accurately reflect SAM data and introduce an artificial source of bias in the QDA where all samples with COS and CS_2 are classified as containing sulfide.

We did not normalize to estimated sulfur masses from either the Alpha Particle X-Ray Spectrometer (APXS) or SAM for several reasons. APXS reports the total amount of sulfur within its analysis area. The amount of sulfur observed by APXS is typically higher than that observed by SAM (Knudson et al., 2018). This difference is due to in large part to the ubiquity of CaSO_4 on Mars, which is measured in APXS analyses, but is not observed by SAM because it evolves sulfur volatiles above the temperature range used by SAM. Normalizing to this larger total sulfur value in Mars samples would artificially decrease the normalized amount of SO_2 evolved by SAM. Similarly, we chose not to normalize by estimated sulfur from SAM measurements because estimated sulfur is derived from evolved major SO_2 peaks. The calculation often includes SO_2 evolved at temperatures above the $<600^\circ\text{C}$ used for analysis in this work, due to the presence of Fe- and/or Mg-sulfates, which overestimates the relevant sulfur. It also propagates a second source of error—the mass of sample delivered to SAM and total weight percent of sulfur. These calculations also exclude reduced sulfur volatile (e.g., H_2S , COS, and CS_2) contributions to the weight percent

estimates.

Area Under Curve-Receiver Operating Characteristics

To test which set of training data most accurately predicted samples with sulfides or sulfates, we used area under curve-receiver operating characteristics (AUC-ROC). This metric compares the true positive rate to the false positive rate at different thresholds for classification (e.g., determines true positive rate if using a 90% probability that classification is correct versus 50% probability) during a QDA of training data. These rates can be plotted against each other and an AUC score can be calculated, with an AUC score between zero and one. An AUC score of one is an ideal scenario and means the discrimination method correctly groups samples every time. An AUC score of one-half means the method is no different from random guessing. An AUC score of zero means the method incorrectly predicts groups for every sample.

Using the AUC-ROC metric for the different data processing methods, we chose the log-transformed training data set that normalized samples to the total mass. The AUC score for this training data was 0.98, which suggests a high degree of accuracy in discriminating between sulfides and sulfates. The AUC score for unnormalized training data was also 0.98; however, this similarity in AUC score was likely due to similarity in total sample sizes of the laboratory data (between 5 and 10 mg). For a more accurate comparison to the Mars data, we chose the normalized masses because Mars sample masses ranged from ~20 to 135 mg. Training data sets without log transformation and normalized to low-temperature SO₂ evolution had AUC scores of 0.71 and 0.40, respectively.

Spearman Correlation Coefficient Analysis

Spearman rank-order correlation coefficients (ρ) and two-tailed p -values were calculated between all pairs of volatiles' areas after mass normalization. Data were grouped by laboratory sulfides ($N = 34$), laboratory sulfates ($N = 22$), Mars samples in which QDA identified sulfides ($N = 10$), and Mars samples in which sulfides were not detected (SND) by QDA ($N = 14$). Potentially significant differences between these data subsets' pairs of volatiles' correlations were identified through the following steps. First, Spearman correlation coefficients were transformed to z -values by the Fisher's Z -transformation to ensure a normal distribution. The z -values were then compared to determine a “ Z -observed” by the following formula (Myers & Sirois, 2006):

$$Z_{\text{obs}} = \frac{z_1 - z_2}{\sqrt{\frac{1}{N_1 - 3} + \frac{1}{N_2 - 3}}}.$$

Using a normal distribution, the Z_{obs} was compared to critical values to determine two-tailed p -values to identify potentially significant differences. All calculations were performed using Python SciPy and Microsoft Excel.

Results and Discussion

EGA Comparisons

The EGA profiles of the five variables used in the QDA were produced for eight Martian samples: John Klein 4 (JK, fourth sample of JK), Cumberland 3 (CB, third sample of CB), Oudam (OU), Big Sky (BS), Duluth (DU), Stoer (ST), Highfield (HF), and Rock Hall (RH). JK, CB, and OU are mudstones (JK and CB are samples of the Sheepbed mudstone, and OU is a sample of the Murray mudstone) that had previous

evidence from CheMin and/or SAM isotopes suggesting the presence of reduced sulfur (Franz et al., 2017; Vaniman et al., 2014). BS is a sample of the Stimson sandstone that appears to contain sulfide based on the QDA (see section “QDA results” below). DU is another Murray Formation mudstone that lies stratigraphically just below VRR. The three remaining samples (ST, HF, and RH) are from two members of VRR (ST is a sample from the Pettegrove Point member, HF and RH are gray and red samples of the Jura member, respectively) and were plotted for comparison. See Figure B.1 for additional stratigraphic context. These profiles are shown in Figures 2.1a–2.1e. Profiles were adjusted to show normalized sample sizes of 45 mg with the assumption that larger or smaller samples were homogeneous. Each volatile uses the same scale across samples. These profiles were produced from original data and may differ from previously reported EGA curves due to differences in background subtraction, smoothing, and refinements in the temperature model (see section “Volatile Areas” above). The EGA profiles presented here focus on traces used in the QDA and to show examples that, based on the evolution of different volatiles, may (or may not) be indicative of sulfide.

Figure 2.1a shows the profiles of SO₂ for the eight Martian samples. All profiles of SO₂, except for HF, begin to increase dramatically between 350°C and 550°C. These are the beginnings of the main SO₂ peaks that can be attributed to iron sulfates or iron sulfides. Notably, however, there are small peaks of SO₂ (or corrected *m/z* 66) in all samples except JK at temperatures below the beginning of the main peak. These small, low temperature peaks could be the result of sulfide or elemental sulfur oxidation (McAdam et al., 2020) or, alternatively, sulfonic acids (Franz et al., 2017).

These low temperature evolutions of SO₂ are consistent with SO₂ observed during EGA of troilite in the laboratory. RH and HF have among the largest and longest evolutions of SO₂ in this lowest temperature range, possibly hinting at the presence of reduced sulfur in these samples. Importantly, ST and DU also have low temperature peaks (<200°C), but these correlate with BSW (Figure 2.1e), suggesting the presence of MTBSTFA that could be contributing interfering masses.

Figures 2.1b and 2.1c show the COS and CS₂ profiles of the Martian samples. Interestingly, RH and HF have the most complicated structures of COS and CS₂ with several peaks spanning hundreds of degrees Celsius. The COS peaks in ST and DU largely correspond with the evolution of BSW, suggesting the possibility of interfering masses from MTBSTFA and/or its byproducts. BS has a COS peak around 250°C, but relatively little CS₂ structure. OU has two small peaks of COS (~150°C and 500°C) and one peak of CS₂ (350°C). JK and CB each have two shallow peaks of CS₂ below ~300°C. CB has a clear COS peak, while JK offers little in terms of structure beyond a broad, shallow evolution, though their integrated counts are similar.

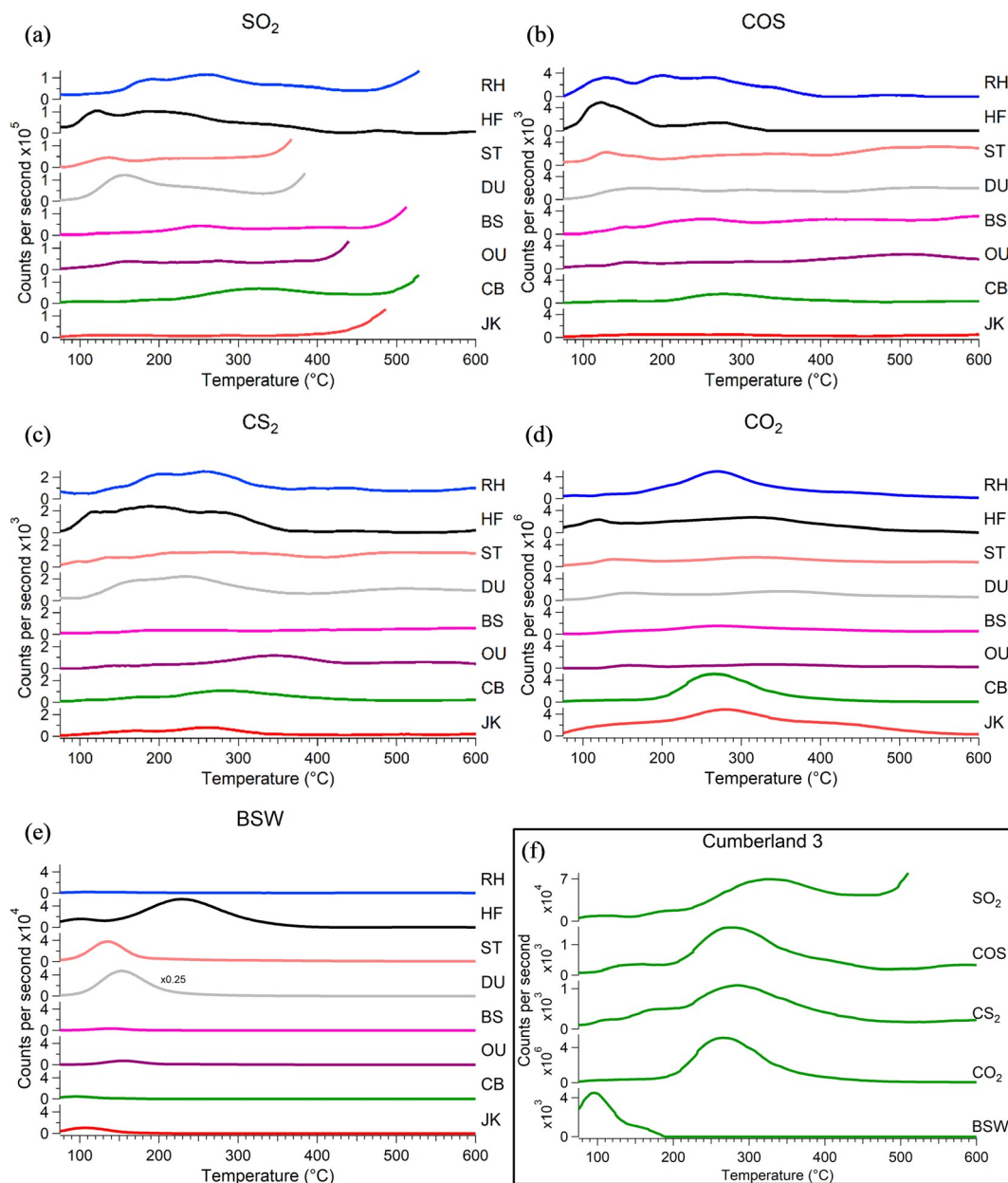


Figure 2.1. (a–e) EGA profiles of SO_2 (m/z 66), COS (m/z 60), CS_2 (m/z 76), CO_2 (m/z 45), and BSW (m/z 147) for select Martian drilled samples. Each profile has been vertically adjusted to represent the relative intensities of the volatile evolutions from a theoretical 45 mg homogeneous sample. Each volatile has the same y-axis scale across samples. The BSW profile for DU has been vertically reduced by a factor of four to fit on the same scale as the other samples. Horizontal axes all range from 75 $^{\circ}\text{C}$ to 600 $^{\circ}\text{C}$, the range used for area calculations. The SO_2 plots focus on the low temperature volatile evolution; the starting tails of the main SO_2 peaks in the samples are seen as inflection points. (f) EGA profiles from CB as an example of the volatiles' (co-)evolutions. Each profile's vertical axis in this plot has been adjusted to emphasize profile structure.

Figure 2.1d shows CO₂ evolution in the select Martian samples. Interestingly, OU, DU, ST, and HF have broadly similar CO₂ evolutions with a shallow peak between 100°C and 200°C and another around 350°C. The lower temperature peaks of CO₂ in these four Martian samples do correspond to peaks of SO₂, COS, and CS₂. The CO₂ also corresponds with BSW in OU, DU, and ST, but not HF. The coevolution of the five volatiles in these three samples may suggest that the reduced sulfur volatile discriminants are a result of interactions with MTBSTFA. The lack of correlation with BSW in HF could be indicative of reactions between a reduced sulfur component and CO or CO₂. JK, CB, and RH, on the other hand, have single, larger peaks of CO₂ between 250°C and 300°C. However, with the exception of CO₂ and CS₂ in JK, the CO₂ does not clearly correspond to any other volatile evolutions.

Figure 2.1e shows the BSW in the eight Martian samples. All Martian samples have some BSW due to the presence of MTBSTFA vapor in the SMS, which reacts with water (Freissinet et al., 2019). However, some samples produce more BSW than others and the differences are likely a function of how much MTBSTFA and water were present. The amount of MTBSTFA vapor present for reactions is variable and affected by factors such as how long a cup has been exposed to the SAM SMS, whether a sample had previously been used in the cup, the number of pyrolysis runs since the full-cup wet chemistry experiment was run (Millan et al., 2019), and the amount of powder in the cup, which acts as a matrix for adsorption of the MTBSTFA vapor from the instrument background. Of the samples shown in Figure 2.1e, DU has 3–5 times as much evolved BSW as HF and ST. The other samples have relatively small peaks of BSW that occur below 200°C.

Figure 2.1f shows a reconsolidated plot of all five volatiles for a single sample: Cumberland 3, which has the strongest case for presence of a reduced sulfur compound. The vertical axes in this panel have been optimized to emphasize the profile structures and demonstrate coevolutions of volatiles. The SO₂ peak around 300°C is consistent with the oxidation of a sulfide or elemental sulfur. At approximately the same temperature as the SO₂ evolution, COS, CS₂, and CO₂ coevolve. The coevolution of these four volatiles is indicative of a reaction or decomposition. It is possible that a reduced sulfur compound is reacting with the CO₂ or CO to form COS and CS₂. It is also possible that the approximate coevolution is the result of the breakdown of a sulfur-containing organic. Relatively little BSW is evolved and its evolution is not correlated with other volatiles used in the analysis. Taken together, the BSW result in CB suggests that MTBSTFA is not a likely complicating factor in the production of the COS and CS₂, prime potential indicators of reduced sulfur.

Similar to CB, RH has relatively little BSW that mostly evolved at low temperature. RH also has coevolving peaks of CO₂, SO₂, CS₂, and COS between 350°C and 400°C. RH has additional COS and CS₂ peak evolutions below 350°C that are not clearly correlated with CO₂ or BSW. DU and ST are unlike CB and RH in terms of these five volatiles. While CO₂, SO₂, CS₂, and COS have coevolving peaks around 150°C, they are accompanied by a large peak of BSW in both DU and ST. The coevolution of BSW, as an MTBSTFA indicator, with the other four volatiles suggests that MTBSTFA, its byproducts, and their fragments are related to these peaks. Interestingly, OU has similar behavior to DU and ST in terms of BSW and the other

volatiles with coevolving peaks around 150°C, though the peak of BSW is much smaller in OU than DU or ST. HF is different from all of these samples in that its main peak of BSW evolves above 200°C and does not clearly coevolve with the other peaks. However, around 120°C, there are coevolving peaks similar to CB. BS has coevolving peaks of SO₂, COS, and CO₂ around 250°C, while BSW and CS₂ evolve shallow peaks at lower temperatures. JK has little structure of SO₂ and COS below 400°C, but its CS₂ and CO₂ peaks approximately coevolve, and it has only a small peak of BSW that evolved before the other volatiles. The volatile comparison profiles for each of these samples can be found in Figure B.2.

Laboratory EGA Results

The various laboratory samples and mixtures were analyzed by EGA and example iron sulfide (pyrite) and iron sulfate (melanterite) volatile products are shown in Figure 2.2. These two sulfur compounds were chosen to demonstrate their similarities and differences in key volatile evolutions. Both compounds evolved SO₂ around the same temperature (550°C – 600°C). SO₂ from sulfates results from sulfate decomposition. Sulfides alone, counterintuitively, consistently evolve SO₂ in EGA experiments (McAdam et al., 2014; Stern et al., 2013) with minor O₂ or H₂O likely serving as oxidants. The decomposition of other compounds, such as oxychlorines, can also provide oxygen for sulfide oxidation. While sulfides can evolve elemental/molecular sulfur ions (i.e., S_n⁺; m/z 32, 64, 96, 128, 160, 192, 224, 256), the mass spectra of the evolved gases are not consistent with major S_n⁺ evolution during our experiments (Table B.1). Additionally, m/z 48 (SO) is a major fragment that tracks m/z 64, which is consistent with SO₂ rather than S₂ evolution.

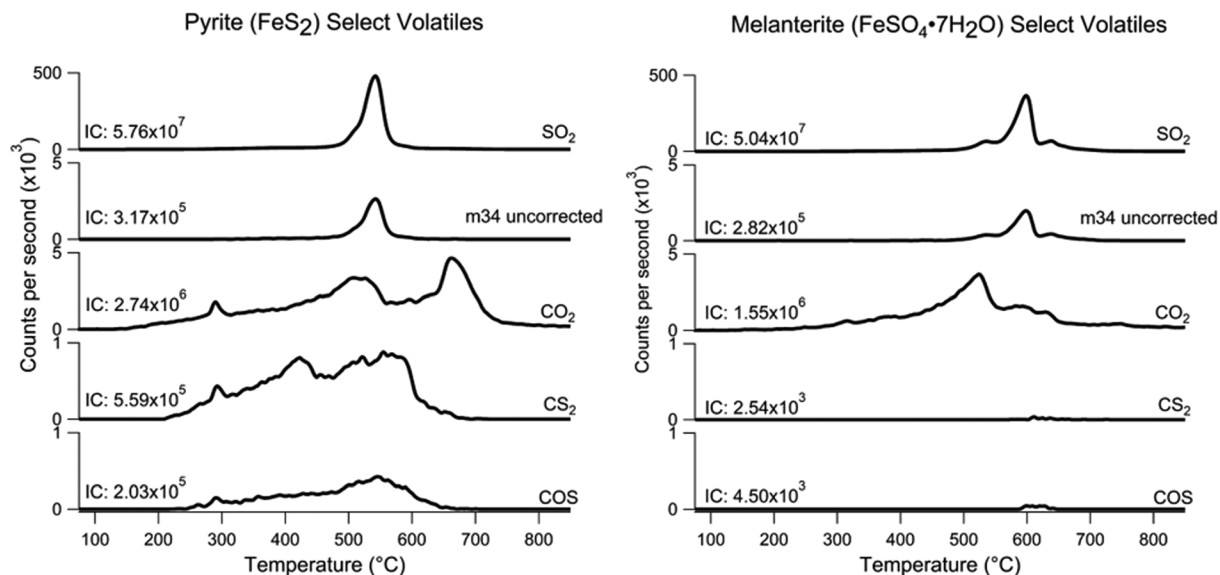


Figure 2.2. Comparison of laboratory EGA results for pyrite (FeS_2 , left) and melanterite ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, right). Each mineral was mixed in a 9:1 ratio of fused silica to S-mineral by mass. From top to bottom, EGA evolutions of SO_2 (m/z 64), m34 uncorrected (overwhelmingly SO_2 fragments, unlikely H_2S), CO_2 (m/z 44), CS_2 (m/z 76), and COS (m/z 60). IC = integrated counts for each mass from 75°C to 850°C . Additional example plots are in the (Figure B.3).

Given the similarity in temperature release and amount of SO_2 evolved, Figure 2.2 highlights the difficulty in using SO_2 as a sole indicator of sulfide versus sulfate presence. Evolved H_2S could be used as a sulfide indicator due to its possible production through reactions between sulfides and H_2 , HCl , or H_2O . However, H_2S can also form from oven reactions between SO_2 and H_2 (McAdam et al., 2014). The H_2S mass-to-charge ratio (m/z 34) is also not necessarily diagnostic of H_2S ; rather, it can be the result of isotopically heavy oxygen ($^{16}\text{O}^{18}\text{O}$) or ^{34}S , either of which could come from fragments of SO_2 . Given these interferences, the laboratory EGA experiments did not produce an appreciable amount of H_2S (m/z 34) from sulfides or sulfates, regardless of mixtures. The observed m/z 34 tracks with SO_2 from sulfides and sulfates and is interpreted as a fragment of SO_2 (Figure 2.2). The lack of

production of H₂S in the laboratory is likely from water background and oxygen leaks in laboratory systems, which are greater on Earth than on Mars and can result in oxidation to SO₂. Similar experiments were performed using a laboratory GC-MS setup and again showed that evolved H₂S is not a strong discriminator between sulfides and sulfates (Figure B.4).

We also investigated the evolution of carbon (as CO₂) given its role in the production of carbon-sulfur volatiles (Figure 2.2). Sources for CO₂ include: the background in the EGA data, as an oxidation product of organics, and/or from decomposition of carbonates. In Figure 2.2, CO₂ evolves in similar amounts over a range of temperatures in both melanterite and pyrite. A search for organic fragments (e.g., *m/z* 55, 58, and 78, indicative of alkyl groups, acetone, and benzene, respectively) in the EGA runs did not indicate the type of carbon compounds that may have been present—most carbon appears to have been oxidized. Importantly, all samples and mixtures evolved CO₂ in variable amounts. While the integrated counts of CO₂ spanned several orders of magnitude for laboratory samples used in the QDA ($\sim 3.3 \times 10^4$ to $\sim 2.8 \times 10^7$), there were no statistically significant ($p < 0.05$) differences in counts of CO₂ across different sulfur minerals. Additionally, all samples that evolved greater than 10^6 integrated counts of CO₂ in the laboratory EGA were intentional mixtures with carbon-containing compounds (Figure B.5). Based on these observations, we conclude that any organic contaminants in the laboratory EGA to be at trace levels.

Despite similarities in profiles and amounts of SO₂, *m/z* 34, and CO₂, significant differences in evolution of COS and CS₂ were observed in sulfides versus

sulfates. Figure 2.2 demonstrates this difference with both COS and CS₂ having evolved approximately two orders of magnitude greater integrated count areas in pyrite compared to melanterite. Both pyrite and troilite (Figure B.3) evolved more COS and CS₂ than any of the sulfates when tested alone, without being mixed with additional volatile-evolving compounds. Troilite typically evolved less COS and CS₂ than pyrite, which is consistent with troilite's relatively greater stability. The smallest difference in COS and CS₂ evolved for minerals on their own was CS₂ evolving about twice as much in troilite as in kieserite. This was an anomalous case, however, and troilite more typically evolved 1 and 2 orders of magnitude more COS and CS₂ than any of the sulfates. Pyrite evolved even more COS and CS₂: usually 2 and 3 orders of magnitude more than the sulfates. These trends held for most mixtures, too, where *m/z* 60 and 76 productions were typically 1–3 orders of magnitude greater with the sulfides compared to the sulfates.

Notable exceptions to the trends in COS and CS₂ described above occurred during EGA of mixtures with high levels of added carbon, such as Mg-acetate, myristic acid, and MTBSTFA-DMF. The elevated production of COS and CS₂ were likely due to the large amounts of reduced carbon in those samples and interfering mass fragments of these larger molecules or their byproducts. It was expected that the addition of large amounts of organic matter would increase the evolved COS and CS₂ of both sulfates and sulfides due to both the increased reducing power available and increased carbon. However, in samples without added carbon sources, sulfides still typically evolved orders of magnitude more COS and CS₂, possibly through direct reactions with evolved CO₂. While such reactions may be slow at temperatures

<600°C, they have, nonetheless, been observed in other works (Shao et al., 1994; B. Wang et al., 2014). It should also be noted that evolved COS and CS₂ are minor components of the total evolved sulfur gases (e.g., SO₂ counts are up to five orders of magnitude greater than COS and CS₂ counts in laboratory analyses). Based on the carbon-containing EGA mixtures, it was estimated that several millimoles of added carbon per mg sample would be required for sulfates to consistently produce comparable levels of COS and CS₂ as sulfides (Figure B.5). This would represent much more carbon than is typically observed in EGA by SAM, which, based on CO₂, has been reported at levels of at most $2,373 \pm 820 \mu\text{gC}_{(\text{CO}_2)}/\text{g}$ ($\sim 0.10 \pm 0.04 \text{ mmol/mg}$; Sutter et al., 2017).

QDA Results

Based on the data processing described above, we used a training data set with 56 samples that included sulfides and sulfates in different mixtures (Table 2.1). From this training data set, we performed a randomized QDA validation test. For this validation, half of the training data sets ($N = 28$) were randomly selected for the QDA fit. Predictions were performed on the other half of the training data. The AUC-ROC score for this data validation and the percentage of correct classifications of the predictions were calculated. The validation was repeated 50 times. From the 50 randomly selected training data sets, samples were correctly classified (i.e., >50% posterior probability) an average of 77.93% (standard deviation of 9.00%) of the time (see Figure B.6), which is comparable to previous work using QDA (Wang et al., 2018). The average AUC score for the 50 validation runs was 0.99 (standard deviation of 0.01), which indicates a high level of discriminating power within this training data

set.

When predictions with the training data were made, several samples stood out as clustering with the sulfide training data (>50% calculated posterior probability of clustering with the sulfide training data). Table 2.2 lists all of the Martian samples analyzed, whether they classified with the sulfides, and their calculated posterior probabilities. Among the samples that were identified as containing reduced sulfur were JK, CB, BS, HF, and RH. Previous work has indicated that JK and CB contain small amounts of pyrrhotite and possibly pyrite (Vaniman et al., 2014), described further in the “Implications” section. HF and RH are two samples that were collected from the Jura member of VRR. Interestingly, DU and ST, which are samples collected from the Blunts Point and Pettegrove Point members of the near-VRR and VRR, respectively, were not identified in the QDA as likely to contain reduced sulfur. The training data and Mars samples are plotted in Figure 2.3. This figure shows the clustering of select Martian samples that were identified as containing sulfides compared to those that were not. While DU, ST, HF, and RH all evolved similar amounts (within an order of magnitude after sample size normalization) of CO₂, COS, and CS₂, HF and RH evolved approximately 10 times less SO₂ than the other two samples. BSW was variable across the four samples, with DU having the highest and RH having the lowest integrated counts. Taken together, this information suggests that evolved SO₂ is an important discriminating factor in the QDA in which less evolved SO₂ is consistent with reduced sulfur, which is logical in an inert/poorly oxidizing environment.

Table 2.2.*QDA Results for 24 Martian EGA Samples*

Mars sample	QDA classification (i.e. “Did this sample cluster with the lab sulfides?”)	Probability of the Mars sample clustering with the lab sulfides
Rocknest 1 (RN1)	No	10%
Rocknest 2 (RN2)	No	3%
Rocknest 3 (RN3)	No	1%
Rocknest 4 (RN4)	No	3%
John Klein (JK)	Yes	52%
Cumberland (CB)	Yes	89%
Windjana (WJ)	No	7%
Confidence Hills (CH)	No	<1%
Mojave (MJ)	No	<1%
Telegraph Peak (TP)	No	<1%
Buckskin (BK)	No	1%
Big Sky (BS)	Yes	78%
Greenhorn 1 (GH1)	No	<1%
Greenhorn 2 (GH2)	Yes	60%
Gobabeb 1 (GB1)	Yes	86%
Gobabeb 2 (GB2)	Yes	57%
Oudam (OU)	No	16%
Marimba (MB)	Yes	74%
Ogunquit Beach 3 (OG3)	Yes	51%
Quela (QL)	No	1%
Duluth (DU)	No	1%
Stoer (ST)	No	3%
Highfield (HF)	Yes	95%
Rock Hall (RH)	Yes	95%

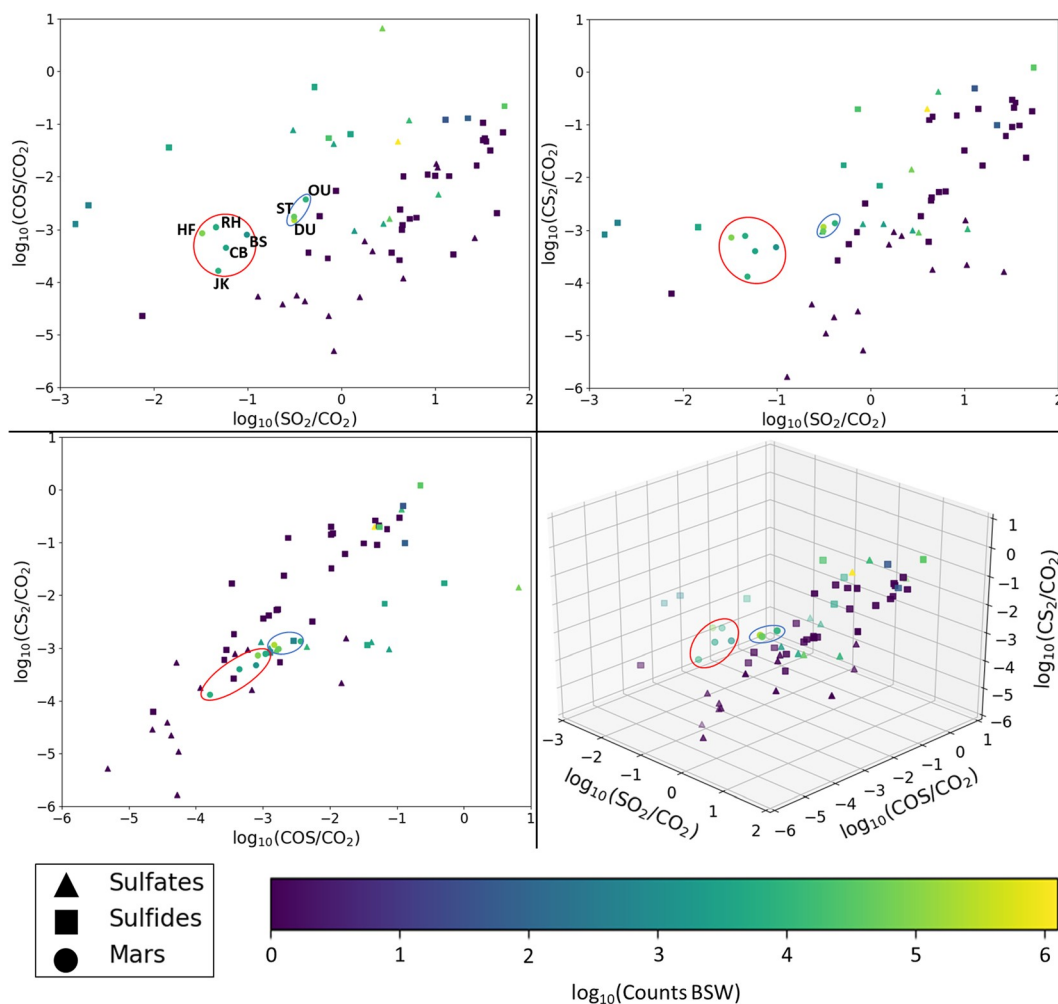


Figure 2.3. This figure shows 2-D and 3-D plotting of the volatiles for the training data and eight select Martian samples. The 2-D plots show face-on views of the 3-D plot from different perspectives. Counts of SO₂, CS₂, and COS have been divided by counts of CO₂ and are shown in the plot as the log₁₀ of these ratios. The log₁₀ of the BSW counts are shown according to the color bar. The ellipses mark out which Martian samples clustered with the laboratory sulfides (red) and which did not (blue) according to the QDA. The ellipses are illustrative only. Refer to Figure B.7 for zoomed-in versions of the 2-D plots.

Spearman Correlation Coefficient Results

The Spearman's ρ between pairs of volatiles can provide insight into both the reactions that may have occurred during volatile coevolution and the differences between subsets of the data. Figure 2.4 shows both the Spearman correlation coefficients for four subsets of the data as well as differences between data subsets

with potential significance marked in bold.

Laboratory Results

The laboratory sulfides show significant correlations between SO₂ and COS ($\rho = 0.40, p < 0.05$), SO₂ and CS₂ ($\rho = 0.56, p < 0.01$), and COS and CS₂ ($\rho = 0.79, p < 0.01$). The sulfides appear to have a weak positive correlation between CO₂ and COS ($\rho = 0.31, p < 0.1$).

The laboratory sulfates are similar to the sulfides in that they show significant correlations between SO₂ and COS ($\rho = 0.65, p < 0.01$), SO₂ and CS₂ ($\rho = 0.69, p < 0.01$), and COS and CS₂ ($\rho = 0.79, p < 0.01$). Also similar are the weaker correlations between CO₂ and COS ($\rho = 0.40, p < 0.1$) and CO₂ and CS₂ ($\rho = 0.40, p < 0.1$). A key difference between the data subsets, however, is the significant correlation between SO₂ and CO₂ ($\rho = 0.67, p < 0.01$).

This difference is reflected in the bottom table and demonstrates that the laboratory sulfides have a significantly lower Spearman's rho than the laboratory sulfates for the SO₂ vs. CO₂ comparison ($\rho_{\text{difference}} = -0.64, p < 0.01$). The difference in correlation coefficients for CO₂ and CS₂ ($\rho_{\text{difference}} = -0.49, p < 0.1$) also tends toward significance. No other significant differences in Spearman's rho were observed. The positive correlation between SO₂ and CO₂ in the laboratory sulfates appears to be largely driven by the sulfate samples that had added reduced carbon (MTBSTFA, acetate, myristic acid). There appears to be a moderate increase in evolved SO₂ when reduced carbon is added to the samples; though the effect seems to be mineral-dependent where melanterite was more affected than jarosite and the sulfates were more affected than the sulfides. Plots of the data are presented in the Figure B.8.

Mars Results

The Mars samples that contain reduced sulfur according to the QDA have few significant correlations between variables. COS and CS₂ are strongly correlated ($\rho = 0.68, p < 0.05$) while SO₂ and CO₂ appear more moderately positively correlated ($\rho = 0.61, p < 0.1$). No significant correlations were observed between BSW and any of the other volatiles. Importantly, it should be noted that these samples were identified in the QDA as likely to contain reduced sulfur, but these samples largely contain sulfate.

The Mars SND samples have three important correlations. COS and CS₂ are again strongly positively correlated ($\rho = 0.85, p < 0.01$). CS₂ and BSW are strongly positively correlated ($\rho = 0.73, p < 0.01$) while COS and BSW are similarly correlated to a lesser extent ($\rho = 0.51, p < 0.1$). Given the interferences MTBSTFA can produce, these correlations suggest that MTBSTFA/BSW may affect the observed m/z 60 and 76 (COS and CS₂) in the Mars SND samples. The other two marked correlations—CO₂ and COS ($\rho = -0.56, p < 0.05$) and SO₂ and CS₂ ($\rho = 0.49, p < 0.1$)—are driven by a sampling effect from the four Rocknest samples (see Figure B.8). These correlations go away when only a single representative Rocknest sample (RN3) is used in the correlation analysis.

The differences in Spearman's rho between the two Martian data subsets are reflected in the third table. SO₂ and CO₂ have a stronger correlation ($\rho_{\text{difference}} = 0.78, p < 0.1$) in the samples with reduced sulfur as identified by QDA. CO₂ and COS have a significantly different correlation in the two subsets ($\rho_{\text{difference}} = 0.89, p < 0.05$): Mars SND had a negative correlation, while Mars QDA sulfides had a weak, nonsignificant positive correlation. While it is tempting to interpret these correlations on Mars as

being the result of sulfides or organic sulfur, the SO₂ is likely driven largely by sulfate decomposition, which makes the interpretation difficult. No other significant differences in Spearman's rho were observed. The relatively few differences could be due to small sample size or the presence of sulfate in the samples that were identified as likely to contain sulfide by the QDA.

Lab Sulfide (N=34)					
	SO ₂	CO ₂	COS	CS ₂	BSW [‡]
SO ₂		0.03	0.40**	0.56***	0.10
CO ₂			0.31*	-0.09	0.27
COS				0.79***	0.47
CS ₂					0.43
BSW					

Mars QDA Sulfide (N=10)					
	SO ₂	CO ₂	COS	CS ₂	BSW
SO ₂		0.61*	0.25	-0.08	-0.49
CO ₂			0.32	-0.12	0.24
COS				0.68**	0.21
CS ₂					0.15
BSW					

Lab Sulfate (N=22)					
	SO ₂	CO ₂	COS	CS ₂	BSW [‡]
SO ₂		0.67***	0.65***	0.69***	0.15
CO ₂			0.40*	0.40*	-0.50
COS				0.79***	0.25
CS ₂					0.57
BSW					

Mars SND (N=14)					
	SO ₂	CO ₂	COS	CS ₂	BSW
SO ₂		-0.17	0.39	0.49*	-0.05
CO ₂			-0.56**	-0.38	-0.05
COS				0.85***	0.51*
CS ₂					0.73***
BSW					

Lab Sulfide vs. Lab Sulfate ($\rho_{\text{difference}}$)					
	SO ₂	CO ₂	COS	CS ₂	BSW
SO ₂		-0.64***	-0.25	-0.13	-0.05
CO ₂			-0.09	-0.49*	0.77
COS				0.00	0.22
CS ₂					-0.14
BSW					

Mars Sulfide vs. Mars SND ($\rho_{\text{difference}}$)					
	SO ₂	CO ₂	COS	CS ₂	BSW
SO ₂		0.78*	-0.14	-0.57	-0.44
CO ₂			0.88**	0.26	0.29
COS				-0.17	-0.30
CS ₂					-0.58
BSW					

Figure 2.4. These tables show the Spearman's rho correlation coefficients for subsets of the data (top four tables) and the differences in the rho values between the either lab or Mars data subsets (bottom two tables). The three tables on the left focus on laboratory data. The three tables on the right focus on Mars data with samples divided based on how they clustered in the QDA. Any correlation or difference in correlation with $p < 0.1$ is bolded. Approximate p -values are represented with asterisks: $*p < 0.1$, $**p < 0.05$, $***p < 0.01$. [†]Significance of $\rho_{\text{difference}}$ calculated by z-score comparison as described in section 3.5. [‡]Only samples with measured m/z 147 were used in calculating these correlations, $N = 9$ for each of the laboratory sulfides and laboratory sulfates.

Implications for Martian Samples and VRR

Using COS and CS₂ as Key Proxy Indicators of Reduced Sulfur

This work provides the first case of using carbon sulfur gases observed in EGA to discriminate between mixed samples with reduced sulfur and those without. Previous work has looked at evolutions of COS and CS₂ as indicators of recalcitrant organosulfur compounds (Eigenbrode et al., 2018). Other EGA studies have also observed total COS and CS₂ and suggested that their presence could be indicative of sulfides (McAdam et al., 2014). However, these studies focused on the high-temperature production of these gases. The work presented here focused on low temperature evolutions of COS and CS₂ as well as the gases' relationships to SO₂, CO₂, and MTBSTFA (using BSW as a proxy). On their own, COS and CS₂ are the strongest discriminators among the five variables used. However, important information is contained within the relationships among the variables. SO₂ evolved at the temperatures analyzed can have mineralogical contributions from oxidized sulfides/elemental sulfur, iron sulfides, and/or iron sulfates. The relative amounts of COS and CS₂ compared to SO₂ play important roles in the QDA. Samples with similar amounts of COS and CS₂, but different amounts of SO₂ are likely to be classified differently. The samples with more SO₂ are less likely to be classified with the sulfides. This is notably exemplified with the different classifications of HF/RH and DU/ST. More COS and CS₂ evolved relative to SO₂ in HF and RH than in DU and ST. The four samples also spanned a range of evolved BSW, indicating that production of COS and CS₂ was not a clear function of MTBSTFA presence. RH and HF have among the lowest and highest integrated counts of BSW, respectively, but they were

both classified as likely to contain reduced sulfur while ST and DU had high BSW and were not likely to include reduced sulfur. This underscores the importance of the relationships among all of the variables. High BSW alone is not enough to disqualify a sample from classification with reduced S. CO₂ was higher for HF and RH than DU and ST, possibly acting as a source of carbon for the COS and CS₂.

Classification of Samples With Previously Identified Sulfides

John Klein, Cumberland, and Oudam have previously been reported as having isotopic and/or XRD evidence consistent with the presence of sulfides. While JK and CB clustered with the sulfides in the QDA, OU did not. The posterior probability of JK clustering with the sulfides was 52%, which is lower than the other samples that clustered with the sulfides. John Klein was reported to contain small amounts of pyrrhotite (1.0 wt.%) and pyrite (0.3 wt.%) by CheMin (Vaniman et al., 2014), though these amounts were at or below the instrument's detection limit, which is 1 and 2 wt.% (Bish et al., 2013). Follow-up analysis of the CheMin data has not indicated the presence of sulfides in John Klein (Morrison et al., 2018). Likewise, reported isotopic evidence suggested that John Klein's SO₂ evolution was not derived from sulfide (Franz et al., 2017). Cumberland, a nearby drilled sample from the same Sheepbed Mudstone as John Klein, did cluster with sulfides in the QDA with a high posterior probability of 89%. Like John Klein, pyrrhotite was originally reported by CheMin near the detection limit at 1.0 wt.% (Vaniman et al., 2014). The iron sulfide was similarly not reported in follow-up work (Morrison et al., 2018). However, unlike John Klein, sulfur isotopic evidence did suggest the presence of reduced sulfur in Cumberland (Franz et al., 2017). Taking this previous evidence into account with the

EGA curves (Figure 2.1) and the results of the QDA, Cumberland is the strongest contender for having a reduced sulfur presence.

Oudam (OU) is a drilled sample from a Murray Formation mudstone. While there have been no reports of crystalline sulfides above CheMin detection limits, sulfur isotopes have indicated the possible presence of reduced sulfur in OU (Franz et al., 2017). There are two possible explanations for the mixed results. OU could be a false negative result in the QDA and actually contain some reduced sulfur. Alternatively, OU may have once had sulfides that were subsequently completely oxidized, but maintained the sulfidic isotopic signature. This latter scenario would align with the CheMin and QDA results. The results of these samples underscore the importance of using all information available from different sources in determining the likelihood of the presence of reduced sulfur.

Implications for Vera Rubin Ridge

The results of the QDA indicate the presence of trace and/or amorphous sulfide in HF and RH, which are both samples of the Jura member on VRR. This in contrast to the nearby samples, ST (Pettegrove Point member on VRR) and DU (Blunts Point member stratigraphically below VRR), which were not identified in the QDA as containing reduced sulfur. The QDA classifications of these four samples are consistent with the samples' EGA profiles in Figure 2.1. This difference in sulfide presence between the stratigraphic members is indicative of differences between Jura and the rest of VRR/near-ridge rocks. Crystalline sulfides were not detected by CheMin in any of these four samples. Thus, the identification of sulfide in HF and RH necessitates that they are either amorphous, S-bearing organics (e.g., methyl sulfides

or thiophenes, which have previously been observed on Mars; Eigenbrode et al., 2018), or below the CheMin detection limit. It is likely that the sulfides are at a trace level given the low integrated counts of sulfur volatiles in the EGA.

The likely presence of reduced sulfur in the Jura member of VRR adds to the complexity of the ridge's diagenetic history. Other observations also require explanation, such as the mobility of manganese, presence of jarosite, and color variations in VRR features. Figure 2.5 shows images of samples on Mars that demonstrate a range of possible alteration effects. Figures 2.5a–2.5d are the four drills holes of the (near) VRR samples: DU, ST, HF, and RH. Notably, Figure 5c shows an example of “gray” Jura member (HF), compared to the other, “red” VRR samples. Other parts of the Murray also show color variations likely caused by diagenetic fluids, as shown in Third Lake in Figure 2.5e. Third Lake shows a distinct red/gray color boundary in the block where the gray coloring appears to be associated with the white sulfate veins. Diagenetic fluids that followed the vein fractures likely altered the rock. Similarly, Figure 2.5f shows evidence of vein-associated alteration. This ChemCam Remote Micro-Imager of a Jura member target, Laphroaig, shows rod-like iron oxide “sticks” that indicate the occurrence of iron mobilization.

Several models have been proposed to explain the ridge's ancient diagenetic history and its timing (Fraeman et al., 2020). Here, we suggest an alternative model that can explain the observations of diagenetic features and presence of reduced sulfur on VRR. We propose that hematite formation was syndepositional and can account for the red coloring. Third Lake (Figure 2.5e), though not on VRR, suggests that red hematite may be from primary deposition and later alteration. Sulfite-containing

groundwater produced from SO₂ dissolution (Halevy et al., 2007; Halevy & Schrag, 2009) could have flowed under a proto-Mt. Sharp. This fluid followed the diagenetic front observed by both Mn enrichments on VRR suggesting downward mobility (Frydenvang et al., 2020) and the ridge apparently conforming to the outline of the proto-Mt. Sharp. Similarly, the iron oxide “sticks” at Laphroaig (Figure 2.5f) suggest that iron was mobile in the water. Sulfite can reduce ferric iron from hematite (Palandri et al., 2005) and result in magnetite production. This magnetite would then be re-oxidized, likely by oxidants such as nitrates (Dhakal et al., 2013) or oxychlorine phases (such as chlorate; Mitra & Catalano, 2019), which appear to have been preserved in RH (McAdam et al., 2020). The resulting oxidized mineral phase would be martite, a pseudomorph of magnetite that is identical to specular hematite in XRD and can account for the gray patches observed on VRR. The reduced sulfur identified in the Jura samples in this work could, in principle, be directly derived from the decomposition/disproportionation of some of the diagenetic sulfite (Matsuzaki et al., 1978; Pryor, 1960). Alternatively, the sulfite could have reacted with preexisting organic material forming organosulfur compounds. A reduced, mineral-bound organosulfur compound may have had a greater chance at preservation than an unbound sulfide (Keil & Mayer, 2013). This “sulfite model” explains the observations on VRR without requiring strongly reducing or hydrothermal fluids.

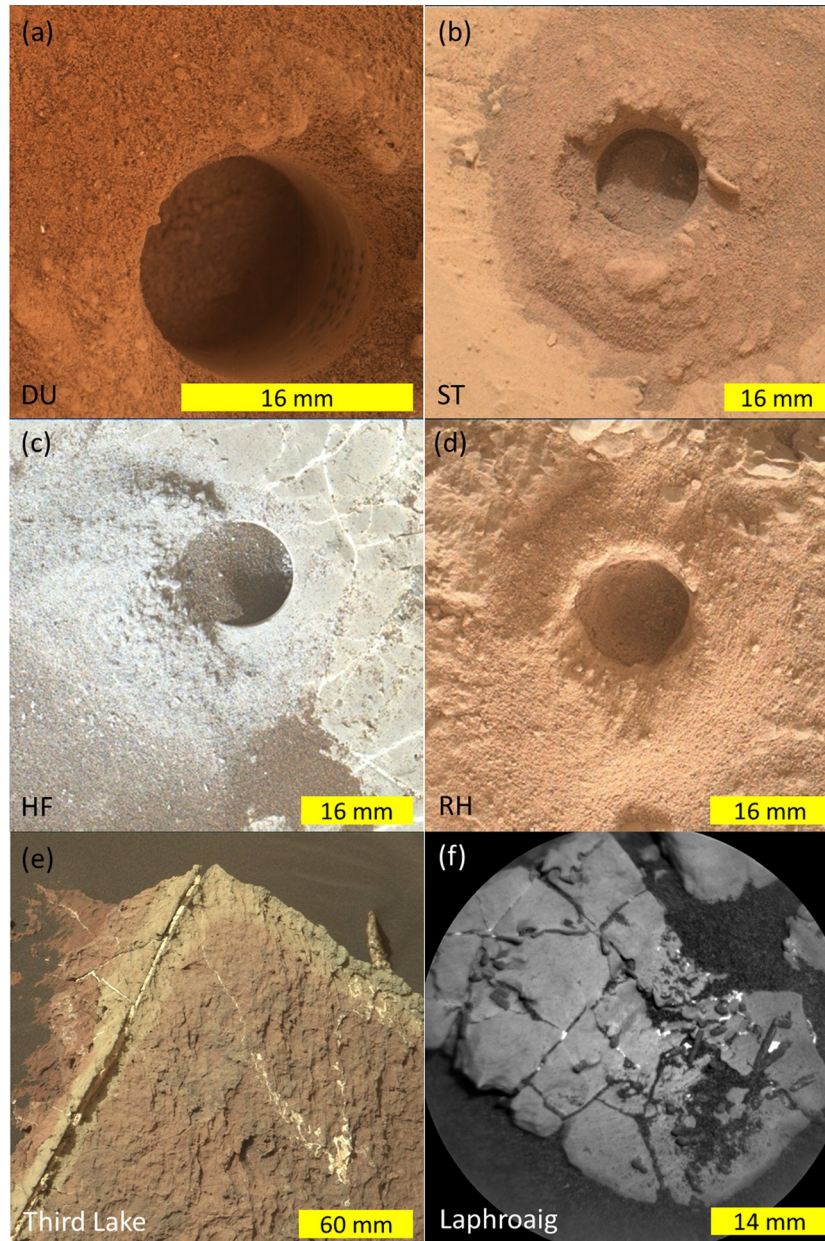


Figure 2.5. (a–d) MAHLI images of the four (near) VRR samples. Drill hole diameter is ~16 mm. (e) A Mastcam image of a Murray Formation bedrock block, Third Lake, that shows a color transition. (f) An image from the ChemCam Remote Micro-Imager of a Jura member target focusing on “sticks,” which are linear iron oxide features. Image identifiers: (a) 2082MH0001220010802084C00, (b) 2154MH0004650010802746C00, (c) 2247MH0004240010803292C00, (d) 2288MH0004240010803600C00, (e) 1612MR0082450010801054E02, (f) CR0_566520230PRC_F0671358CCAM03904L1. Credits (a–e) NASA/JPL-Caltech/MSSS, credits (f) NASA/JPL-Caltech/LANL.

The presence of reduced sulfur in the Jura member of VRR has important implications for the potential habitability of the ridge. Reduced sulfur, in the form of trace crystalline metal sulfides, amorphous sulfides, or organosulfur compounds, could have supported sulfur-oxidizing metabolisms of a possible Martian microbial community. On Earth, various microorganisms have been described as iron sulfide oxidizers depending on Eh and pH conditions. For example, *Metallogenium gen.*, *Acidithiobacillus ferrooxidans*, *Sphaerotilus natans*, and *Gallionella ferruginea* have been reported as iron sulfide oxidizers in the pH ranges between 2 and 5 (Walsh & Mitchell, 1972a, 1972b) and 6 to 9 (Ralph, 1979). Under anaerobic conditions nitrate is the main electron acceptor used for sulfide oxidation by *Acidithiobacillus denitrificans* and *Sulfurimonas denitrificans* (Bosch et al., 2012; Poser et al., 2014). With reduced sulfur in VRR cooccurring with various oxidized species, such as nitrate in RH (McAdam et al., 2019), an energetic barrier to habitability is lifted. Given the once-present diagenetic fluids flowing through the rocks of VRR and likely presence of organic carbon, the conditions supported a habitable environment.

Conclusions

This work provides the first detailed analysis determining the presence of sulfides in Martian drilled samples using COS and CS₂ observed during evolved gas analysis. Using SAM-like EGA with sulfate and sulfide mixtures, we developed a set of training data for QDA. Using COS, CS₂, SO₂, CO₂, and BSW evolved below 600°C in both the training data and Mars data, the QDA classified various Martian samples based on their likelihood of containing reduced sulfur. Two samples, John Klein and Cumberland, were classified as likely to contain sulfide, which is consistent with

previously reported evidence and serves as a positive control for the QDA. On VRR, the Jura member samples, Highfield and Rock Hall, were identified as likely to contain reduced sulfur, while the two stratigraphically lower samples were not. Trace and/or amorphous sulfide in the Jura samples could be explained by several possible alteration models for VRR, including the “sulfite model” described here.

Data Availability Statement

Original SAM data are publicly available in the Planetary Data System (<http://pds-geosciences.wustl.edu/missions/msl/>). The laboratory data used in this work are available on Harvard Dataverse, doi:10.7910/DVN/UOURYF (Wong, 2020).

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Chapter 3: Variable sulfur redox observed by the Sample Analysis at Mars during the Glen Torridon campaign at Gale crater, Mars

This chapter is a draft manuscript that is intended to be submitted to the Journal of Geophysical Research: Planets in a special issue focusing on the Glen Torridon campaign performed by the Mars Science Laboratory. G. Wong is the lead author of this work. Coauthors will include at least Heather Franz, Joanna Clark, Amy McAdam, James Lewis, Rafael Navarro-González, and Christopher House. The coauthor list is subject to change with likely additions from the Mars Science Laboratory team.

Introduction

The Mars Science Laboratory mission has been exploring Gale crater, Mars since August 2012 to assess the region's geologic history, present environment, and evidence of past habitability (Grotzinger et al. 2012; Grotzinger et al. 2015). Gale crater, an ~155 km diameter impact crater of Noachian age, is located near the Martian equator at the crustal dichotomy (Grotzinger and Milliken 2012). It is the topographic low within ~1000 km radius. Gale crater also features ~5 km of exposed stratigraphy that forms Aeolus Mons, informally referred to as Mt. Sharp (Wray 2013). From orbit, it has been observed that there are a variety of rocks and spectral features in Gale crater along Mt. Sharp that were key targets for exploration by the Curiosity rover. Among these were high thermal inertia units (representative of well-cemented sedimentary rocks), elevated spectral signatures of hematite along a ridge, a clay-bearing unit, and an overlying sulfate-bearing unit (Fraeman et al. 2016). The records of environmental change along these sedimentary strata and potential for preserved evidence of habitability provide the MSL mission excellent opportunities for exploration. Starting on sol 2300, the rover has explored the clay-bearing (Glen Torridon) unit of lower Mt. Sharp in what has been named the Glen Torridon (GT) campaign.

Exploration of the clay-bearing unit was a key mission goal because of elevated spectral signatures for phyllosilicates, which both indicate the ancient presence of near-

neutral water and have the potential for enhanced preservation of organic compounds. The clay-bearing unit underlies a sulfate-bearing unit and represents a time in Martian geological history before a major environmental shift (Grotzinger and Milliken 2012). The exploration of the Glen Torridon unit is important to understand the history of Mars before its transition to a more acidic and then drier planet, especially as the rover continues its investigations of the clay-sulfate transition. Its exploration provides a continued understanding of changes in the lacustrine environment that was once within Gale crater and a framework to understand the environmental changes that allowed for the formation of sulfate-rich strata (Fox et al. 2020). The locale also allows for the exploration of ancient habitable environments (Sutter et al. 2020) with the possibility of greater organic preservation in the phyllosilicates (Keil and Mayer 2013)

The Glen Torridon unit, while having elevated spectral signatures for smectites, is a continuation of the several-hundred meters thick Murray Formation (Fox et al. 2020). The GT unit has been divided into the Jura member (stratigraphically equivalent to Vera Rubin ridge Jura), the Knockfarril Hill (KHm) member, and the fractured intermediate unit (FIU) between Jura and KHm (Fedo et al. 2020; O’Connell-Cooper et al. 2020). APXS data has shown that the GT unit is chemically similar to median Murray composition (O’Connell-Cooper et al. 2020). CheMin XRD has shown elevated levels of phyllosilicates, as expected from orbital data, and lower amounts of hematite compared to the Vera Rubin ridge (Thorpe et al. 2020). The Sample Analysis at Mars (SAM) evolved gas analysis (EGA) has indicated the presence of Fe-sulfate/sulfide, Mg-sulfate, and chlorides with the notable absence of abundant nitrate or oxychlorine phases (A. C. McAdam et al. 2020; Sutter et al. 2020). SAM gas chromatography-mass spectrometry has

identified various high molecular weight compounds and sulfur organics in GT samples (Millan et al. 2020)

Sulfur is of particular interest related to habitability on Mars. Sulfur is able to exist in a range of redox states from S^{2-} to S^{6+} to form various minerals and organic compounds. Such variable redox allows for sulfur to be either an electron donor or acceptor depending on its state. Oxidized sulfur in the form of sulfate is ubiquitous on Mars near the surface. Sulfates have been observed over much of the surface from orbit and in situ measurements (King and McLennan 2010). Ca-sulfate is also obvious from ground observations in the form of white veins throughout Gale crater rocks (Kronyak et al. 2019; Gasda et al. 2020). Various crystalline sulfates (i.e. gypsum, bassanite, anhydrite, and jarosite) have also been observed throughout Gale crater by CheMin, with combined Ca-sulfates occasionally reaching over 20 wt% of the crystalline fraction of the sample (Rampe et al. 2020). Other sulfates, such as Mg-sulfates and Fe-sulfates have been inferred from data from the Sample Analysis at Mars (SAM) evolved gas analysis (EGA) (Sutter et al. 2017; Stern et al. 2018). SAM EGA records the volatiles released by compounds in solid samples during thermal decomposition. It is a highly sensitive technique that can be especially useful for identifying compounds that are either X-ray amorphous or below the CheMin detection limit.

While oxidized sulfur is observed in every sample on Mars due to the ubiquity of sulfates, reduced sulfur is less commonly detected. The first apparent detection of sulfide was in the Sheepbed mudstone samples John Klein and Cumberland, which were collected early in the mission. These two samples showed evidence for pyrrhotite and/or pyrite in CheMin, though the observations were near the instrument's detection limit and subsequent

interpretations of the XRD data have not reported crystalline sulfide detections (Vaniman et al. 2014; Morrison et al. 2018). However, SAM analyses of the Cumberland drill sample in particular suggest the presence of trace and/or amorphous sulfide based on sulfur isotope analysis and quadratic discriminant analysis comparing the sample to laboratory data (Franz et al. 2017; Wong et al. 2020). Other EGA interpretations have suggested the presence of sulfide in certain samples based on the observations of certain evolved volatiles, such as H_2S (Leshin et al. 2013; McAdam et al. 2014; Ming et al. 2014; Sutter et al. 2017). However, the evolution of H_2S may also occur due to oven reactions between oxidized sulfur and H_2 , which has also been observed in EGA work, particularly at high temperature (McAdam et al. 2014). Other analyses of SAM EGA data have found various organic sulfur compounds, such as thiophene, evolving at high temperature (Eigenbrode et al. 2018). Taken together, the various SAM studies of solid Martian samples suggest the presence of reduced sulfur in various places throughout the rover's exploration of Gale crater.

Here, we present complementary methods of analyzing EGA data to detect whether Glen Torridon campaign samples contain sulfide, particularly at trace or amorphous levels. We use SO_2 peak evolution temperatures, which can indicate the type of sulfur in a sample along with its metal cation in the case of sulfate salts. We also use multivariate statistical comparisons of Mars EGA data to laboratory data to identify samples that are likely to contain sulfide. Finally, we look at the sulfur isotope values calculated from evolved SO_2 to potentially identify which SO_2 peaks are derived from the oxidation of a sulfide or decomposition of a sulfate. This combined analysis represents a novel approach to identifying sulfide in Martian samples from EGA data that is more powerful than any single

method, providing greater confidence for any detection. Based on the detection of sulfide in Glen Torridon samples, we also discuss implications for habitability and geologic history of the clay-bearing unit in Gale crater.

Methods

SAM EGA

The SAM EGA process has been described in detail previously (Mahaffy et al. 2012; Sutter et al. 2017; McAdam et al. 2020). Briefly, solid samples from the drill or scoop is delivered to a quartz sample cup. The cup is then sealed in one of the two pyrolysis ovens. Under a constant He flow of 0.8 cm³/min at 25 mbar, the sample is heated to ~850°C with a temperature ramp rate of 35°C/min. For evolved gas analysis, volatiles resulting from thermal decomposition are carried by the He flow directly to the quadrupole mass spectrometer (QMS) for analysis. The QMS records the ionized volatiles as a mass-to-charge (m/z) ratio over time. The relative intensity for each m/z is recorded as counts-per-second (cps) for the m/z range from 2 to 535. The time is converted to sample temperature with a pyrolysis temperature model. EGA plots showing intensity versus temperature for any given m/z can be created.

Laboratory EGA

The laboratory EGA conditions have also been described previously in detail (Glavin et al. 2013; Wong et al. 2020), but are briefly discussed here. The laboratory EGA conditions have been set up to produce SAM-like results, though there are some differences. The EGA work used for the quadratic discriminant analysis comparisons was all performed at Goddard Space Flight Center with an Agilent 5965T-LTM MS attached to a Frontier PY-3030D pyrolysis oven. Samples were first kept at 75°C for up to 31

minutes under a constant He flow rate of 35°C/min (50 mL/min at 30 mbar) to allow for the desorption of volatiles within the sample material without thermal decomposition. Samples were heated in stainless steel pyrolysis cups in the oven to either 850°C or 1050°C with the same flow conditions. The He flow is routed to the MS for analysis of evolved volatiles from m/z range up to 200. To ensure safety of the system while running sulfur- and chlorine-rich samples, the relatively high flow rate and a split ratio of 100:1 are used, which are differences from the SAM EGA conditions.

Sample preparation

A variety of sulfur compounds and two-component mixtures were analyzed on the GSFC SAM-like EGA system for use in the quadratic discriminant analysis. These samples included oxidized (ferric sulfate hydrate, jarosite, kieserite, and melanterite) and reduced (pyrite and troilite/pyrrhotite) sulfur along with mixtures of various chlorine-, water-, or carbon-bearing phases. All solid compounds were mixed with inert fused silica (9:1 fused silica:sulfur by mass) to act as a non-volatile-bearing matrix to simulate the bulk Martian sample. Solid mixtures were either mixed together by an organically clean steel mixing tool or ground by mortar and pestle for three minutes. Compounds were sieved to a size fraction <150 μm . The same set of samples were used for statistical comparisons to Mars samples through the Vera Rubin ridge and have been described in detail by Wong et al. (2020). The data for these laboratory samples are all publicly available (Wong 2020).

Quadratic discriminant analysis

Select evolved gases from SAM EGA were compared to known compounds analyzed by laboratory EGA. The statistical comparison was quadratic discriminant analysis (QDA) of the integrated counts of evolved volatiles SO_2 , COS , CS_2 , CO_2 , and bi-

silylated water (BSW) from 75°C-600°C. QDA is a supervised multivariate machine learning statistical method by which variables from a known source (volatiles evolved from sulfide or sulfate during laboratory EGA) are compared to the same variables with an unknown classification (Mars samples with unknown sulfur redox). This method is commonly used to classify unknowns into one of two categories and has been used previously on Mars for identifying Martian samples likely to contain reduced sulfur (Wong et al. 2020). All integrated counts were normalized to total sample size and then log transformed for comparisons. The posterior probabilities of the classifications are also calculated – reported here are the probabilities of clustering with the laboratory sulfides. All QDA work was performed in Python 2.7.14 as described previously (Wong et al. 2020)

Sulfur isotope calculations

Sulfur isotopes (reported as $\delta^{34}\text{S}$ V-CDT) are calculated from evolved peaks of $^{32}\text{SO}_2$ (m/z 64) and $^{34}\text{SO}_2$ (m/z 66) after dead time and background corrections. Ratios of $^{34}\text{SO}_2/^{32}\text{SO}_2$ are calculated for each simultaneously collected m/z 66 and m/z 64 during the evolution of and SO_2 peak. The average ratio of heavy to light SO_2 is calculated for the values under the peak when the ratio appears stable and the associated error with this average is carried through calculations. The $^{34}\text{SO}_2/^{32}\text{SO}_2$ is then corrected for interfering isotopologues (i.e. $^{32}\text{S}^{16}\text{O}^{18}\text{O}$, $^{33}\text{S}^{17}\text{O}^{16}\text{O}$, and $^{32}\text{S}^{17}\text{O}^{17}\text{O}$) that would artificially increase the apparent ratio. This subtraction effectively yields a ratio of $^{34}\text{S}/^{32}\text{S}$, which is compared to the Vienna-Canyon Diablo Troilite (V-CDT) standard ratio to calculate a delta value such that:

$$\delta^{34}\text{S} = 1000 \times [({}^{34}\text{R}_{\text{sample}}/{}^{34}\text{R}_{\text{V-CDT}}) - 1],$$

$$\text{where } {}^{34}\text{R}_{\text{sample}} = {}^{34}\text{S}/{}^{32}\text{S} = m66/m64 - 2x^{18}\text{R} - 2x^{17}\text{R}x^{33}\text{R} - {}^{17}\text{R}^2$$

$$\text{and } ^{18}\text{R} = ^{18}\text{O}/^{16}\text{O}, ^{17}\text{R} = ^{17}\text{O}/^{16}\text{O}, \text{ and } ^{33}\text{R} = ^{33}\text{S}/^{32}\text{S}$$

to account for the isotopologues contributions mentioned above. In this work the following assumptions about the oxygen and sulfur isotope ratios were made:

- (1) An assumed $\delta^{18}\text{O}$ of +50‰ was used to calculate ^{18}R (Webster et al. 2013),
- (2) ^{17}R was calculated from Martian meteorites $\Delta^{17}\text{O}=+0.32\text{‰}$ where $\Delta^{17}\text{O}=\delta^{17}\text{O}-0.52\times\delta^{18}\text{O}$ (Franchi et al. 1999),
- (3) ^{33}R was assumed to be equal to the V-CDT standard (Franz et al. 2017).

These sulfur isotope calculations follow the extended methods described by Franz et al. (2017). As a general rule, enriched values $\delta^{34}\text{S}$ represent sulfates, depleted values are consistent with sulfide, and $\delta^{34}\text{S}$ near 0 are consistent with mantle-derived sulfide or sulfate.

Results

The seven drilled samples since the VRR campaign showed variable sulfur redox states based on EGA, QDA, and $\delta^{34}\text{S}$ analyses. The samples represent a variety of lithological units and levels of stratigraphy (Figures 3.1 and 3.2). Figure 3.1 shows the stratigraphic column for the MSL mission through December 2019. Figure 3.2 shows Mars Hand Lens Imager (MAHLI) images of the drill holes in the Glen Torridon region and on top of the Greenheugh pediment unconformity. Drill holes in Figure 3.2 are ordered according to their relative stratigraphy and labeled with their associated member/unit.

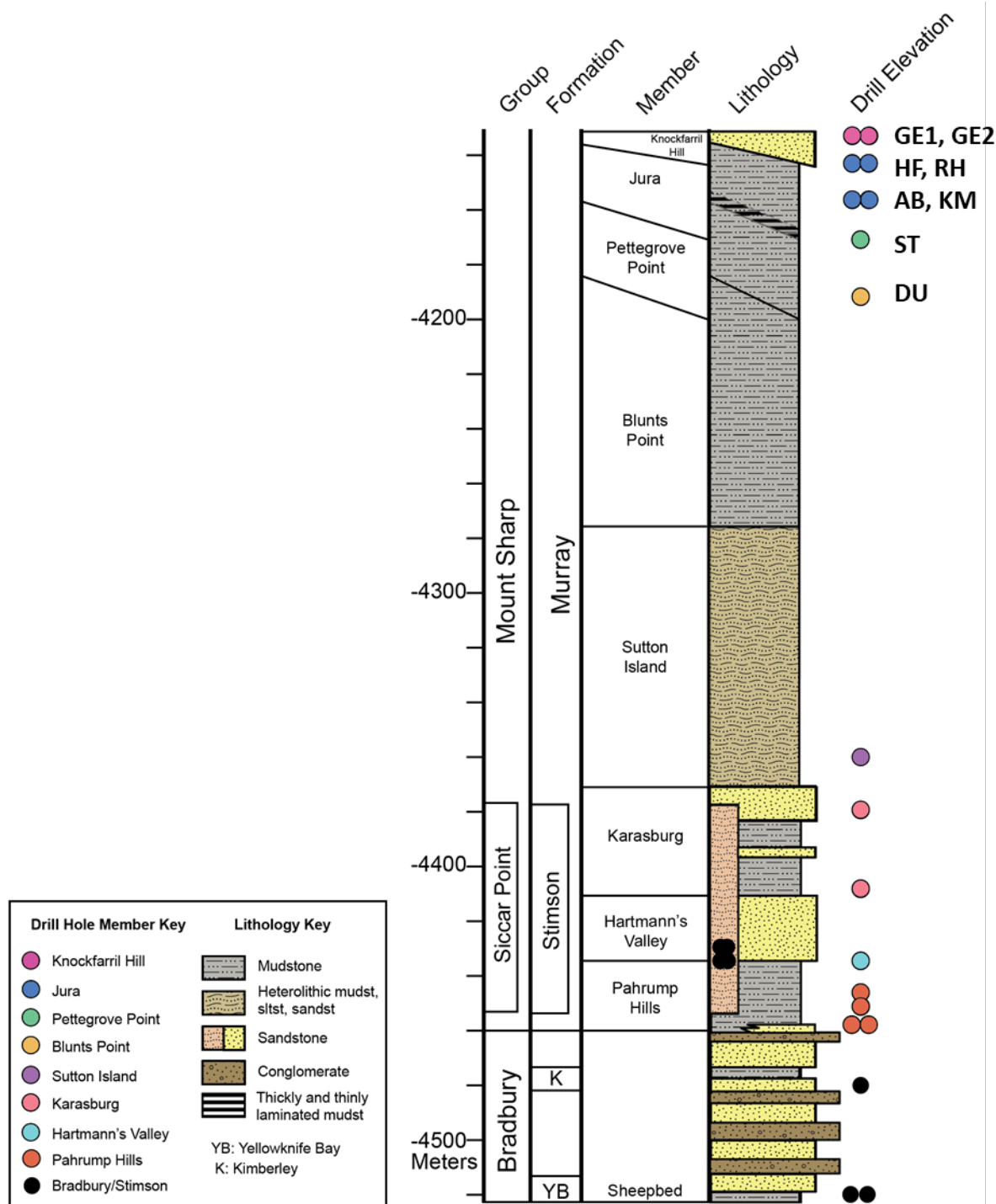


Figure 3.1. Stratigraphic column of the rocks explored by MSL and associated drill holes through December 2019. More recent drill holes are labeled. DU: Duluth, ST: Stoer, AB: Aberlady (no EGA), KM: Kilmarie, HF: Highfield, RH: Rock Hall, GE1 and GE2: Glen Etive drill holes 1 and 2. See Chapter 2 for discussion about DU, ST, HF, and RH. Not shown are the four most recent drill holes, which are stratigraphically above the limits of this stratigraphic column (see Figure 3.2). Column credit: MSL Sed-Strat Working Group.

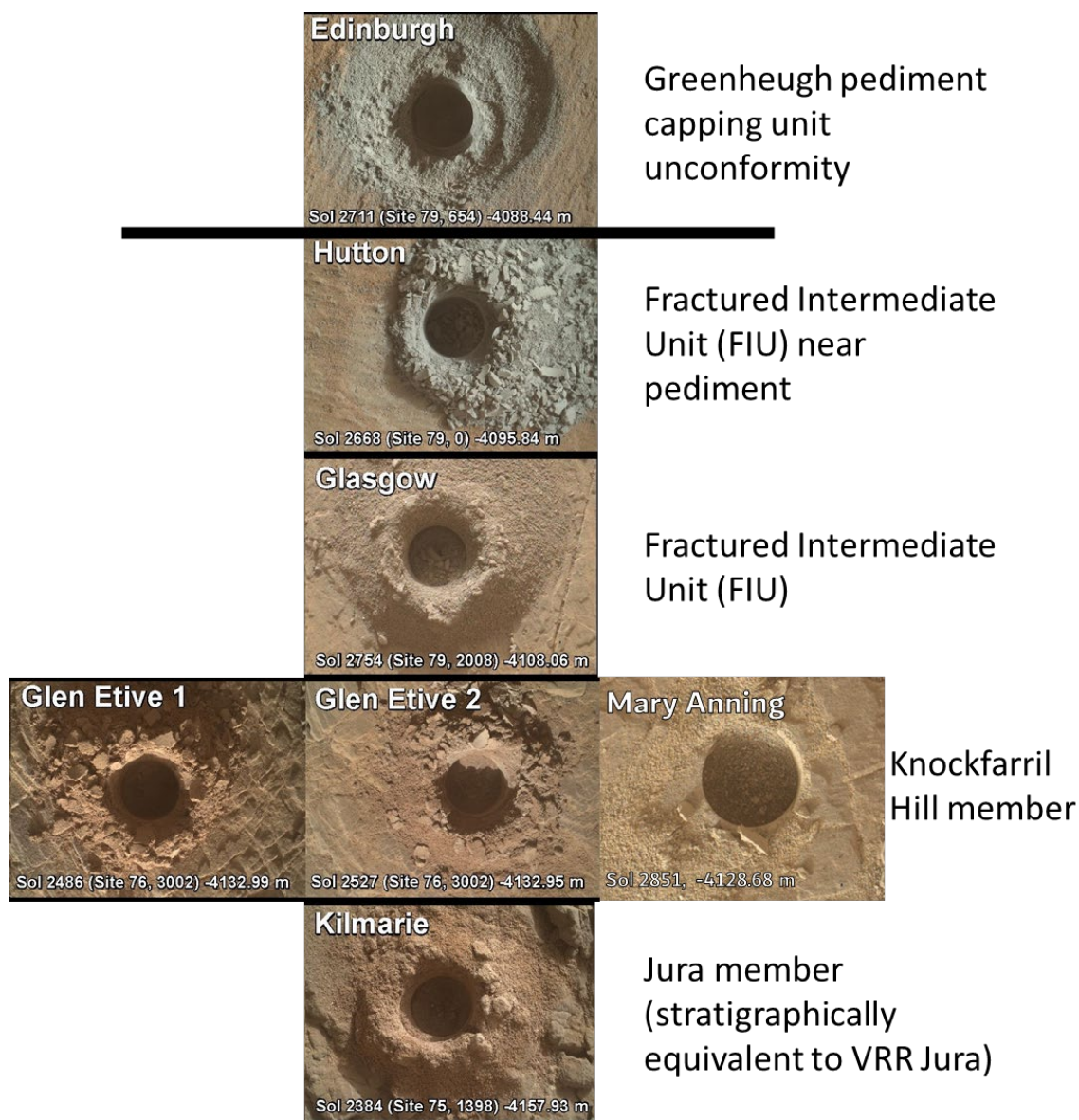


Figure 3.2. MAHLI images of the drill holes from the GT and Greenheugh pediment campaigns. Drill holes are ordered according to stratigraphy. Glen Etive 1 and 2 were drilled from the same rock for two sets of experiments. Each hole is ~1.6 cm in diameter. Courtesy of NASA/JPL-Caltech/MSSS.

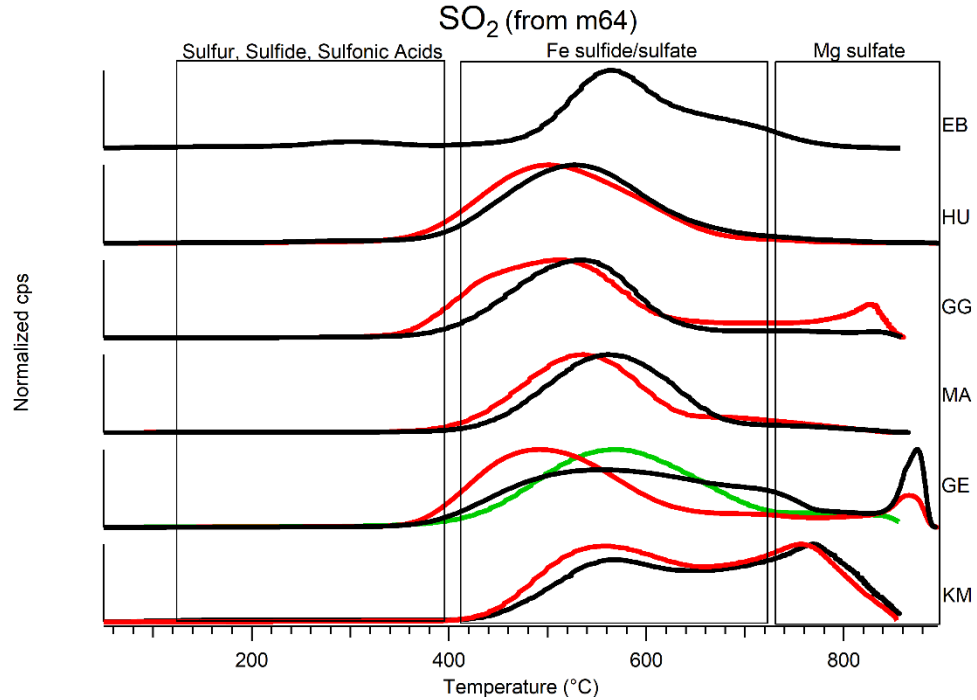


Figure 3.3. SO₂ EGA traces for each sample during the GT campaign and the pediment capping unit mini-campaign. Each run has been normalized to the maximum intensity of evolved SO₂ during EGA. Different colors represent different EGA runs of the same sample (black=first, red=second, green=third).

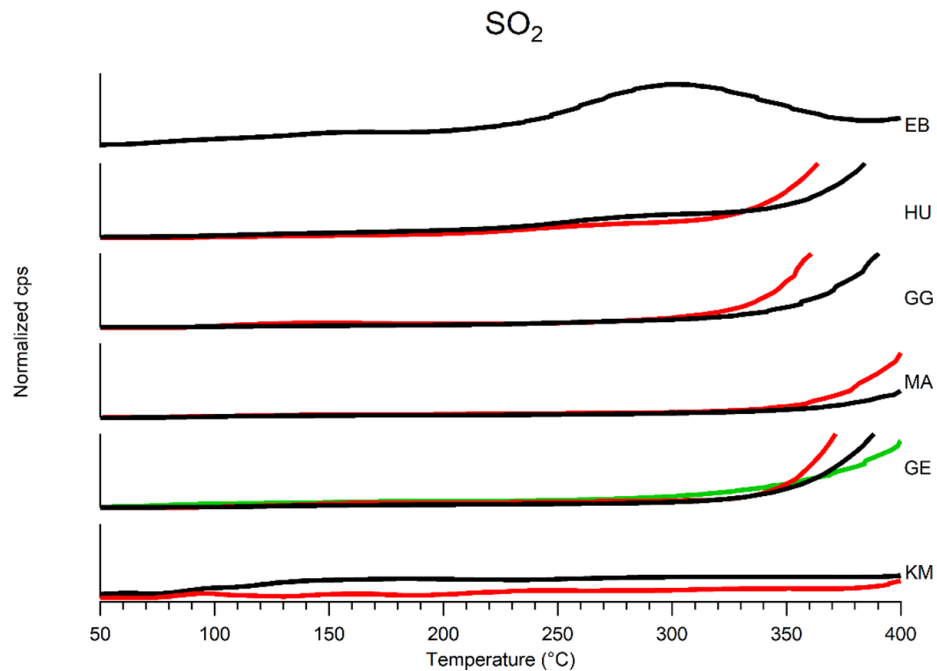


Figure 3.4. Low temperature evolution of SO₂ from 50°-400°C. Vertical axes have been adjusted to show the lower intensity SO₂ releases in this temperature range compared to Figure 3.3. EB is the only sample with a clear SO₂ peak. Black=1st sample, red=2nd sample, green=3rd sample.

Kilmarie (KM, drilled on sol 2384) was the first sample analyzed by SAM during the GT campaign. KM was chosen as a representative of the ‘smooth’ clay-bearing unit and was determined to be part of the Jura member. The Jura member also had two samples (Highfield and Rock Hall) analyzed by SAM on Vera Rubin ridge, both of which were determined by QDA to be likely to contain reduced sulfur (Wong et al. 2020). Two subsamples of KM (KM1 and KM2) were analyzed by EGA and showed consistent SO₂ evolution profiles (Figure 3.3). Both KM samples evolved SO₂ peaks consistent with Fe-sulfate/sulfide (~560°C) and Mg-sulfate (~770°C). Based on QDA, the posterior probabilities of KM1 and KM2 clustering with the laboratory sulfides were 99% and 84%, respectively. These are consistent with previously reported Jura member samples and other Mars samples, such as Cumberland, which has several lines of evidence suggestive of sulfide presence. The $\delta^{34}\text{S}$ value of KM1 was consistent with a sulfide, with $\delta^{34}\text{S} = -21 \pm 19\text{‰}$, while KM2 was effectively unconstrained, with $\delta^{34}\text{S} = 0 \pm 20\text{‰}$.

Two adjacent Glen Etive (GE) samples from the same rock were drilled (sols 2486 and 2527) after KM as a representative of the ‘fractured’ clay-bearing unit, part of the Knockfarril Hill member. GE1 and GE2 represent EGA work from the first drill hole while GE3 is from the second drill hole. Mary Anning (MA), drilled on sol 2838, is also a representative of the Knockfarril Hill member. MA was chosen as a drill target primarily for its apparent similarity to GE for a wet chemistry derivatization experiment. Three subsamples of GE and two of MA have had EGA performed on them. Despite the apparent similarities of GE and MA, their SO₂ profiles were distinct (Figure 3.3). All of the GE analyses showed broad SO₂ releases consistent with Fe-sulfide/sulfate. GE1 and GE2 also had high temperature SO₂ releases consistent with Mg-sulfate. Both MA samples, on the

other hand, had generally narrower peaks consistent with Fe-sulfide/sulfate and no Mg-sulfate. The quadratic discriminant analysis was not consistent with laboratory sulfides in any of the GE or MA samples analyzed by SAM. For QDA, the highest posterior probability was 11% for GE2, which is less than the 50% cutoff for being considered likely to contain reduced S. The GE and MA samples did show a range of $\delta^{34}\text{S}$ values from $-14 \pm 5\text{‰}$ (GE3) to $20 \pm 4\text{‰}$ (GE1). While GE3 does have a moderately depleted $\delta^{34}\text{S}$, other evidence is not consistent with a sulfide.

Glasgow (GG), drilled on sol 2754, was another GT sample chosen for MSL analysis as a representative of the fractured intermediate unit (distinct from the smooth and fractured units). Two samples of GG were analyzed by EGA. Both GG1 and GG2 had SO_2 evolutions consistent with Fe-sulfate/sulfide while only GG2 had an appreciable peak consistent with Mg-sulfate. Quadratic discriminant analysis suggests that neither sample is consistent with the laboratory sulfides. The calculated $\delta^{34}\text{S}$ values from the Fe-sulfide/sulfate peak were highly enriched at $5 \pm 9\text{‰}$ and $-5 \pm 9\text{‰}$ for GG1 and GG2, respectively. These isotope values are also consistent with a sulfate.

During the GT campaign, there was an opportunity to approach and scale a pediment unconformity that represents more recent strata. This Greenheugh pediment mini-campaign was performed to sample rocks that may provide insight into diagenetic fluids that may have altered the Glen Torridon rocks. It also provided the chance to sample strata that are planned to be encountered later in the mission. Two samples were associated with this pediment mini-campaign: Hutton (HU, drilled sol 2668) and Edinburgh (EB, drilled sol 2711). Two SAM analyses were performed on HU, which was chosen as a drill target due to its proximity to the unconformity between the fractured intermediate unit and

the pediment. Only a single sample was analyzed from EB, which was chosen as a representative sample of the Greenheugh pediment capping unit, which is a different lithology from the underlying Glen Torridon rocks. The sulfur results differ substantially. Both HU samples generally resemble GG and MA SO₂ evolutions, with only Fe-sulfate/sulfide peaks. HU was not consistent with laboratory sulfide according to QDA. The $\delta^{34}\text{S}$ values of HU1 and HU2 were enriched at $18 \pm 6\text{‰}$ and $21 \pm 4\text{‰}$, suggestive of a sulfate formed from equilibrium fractionation. While HU sulfur redox results were largely consistent with other GT samples, EB had an entirely different result. The primary SO₂ peak evolution of EB was again consistent with Fe-sulfate/sulfide (Figure 3.3). However, there was also a significant peak of SO₂ at $\sim 300^\circ\text{C}$, which may be consistent with sulfonic acids, elemental sulfur, or oxidation of sulfide. Such a low temperature peak was not observed in any of the other GT samples (Figure 3.4). The SO₂ EGA profile of EB is comparable to laboratory-run FeS (Appendix Figure C.1). Furthermore, the quadratic discriminant analysis clustered EB with the laboratory sulfides, suggesting the current presence of a sulfide reacting with carbon gases and oxidizing. The $\delta^{34}\text{S}$ was also highly depleted at $-27 \pm 7\text{‰}$, consistent with a sulfide formed during equilibrium fractionation or hydrothermal alteration. The low temperature SO₂ observed in EB is too small to calculate a $\delta^{34}\text{S}$ value.

A summary of the results for the 12 (sub-)samples analyzed by EGA is provided in Table 3.1. EB has consistent evidence for the presence of a sulfide from the three methods of analysis. KM also appears likely to contain reduced sulfur.

Table 3.1. Summary of sulfur EGA, QDA, and S isotope results for all drilled samples in the GT campaign and pediment mini-campaign. “Low temperature” is considered to be <400°C. A sample is considered to contain sulfide if the posterior probability is >50%. The S isotopes are only calculated for the main SO₂ peak in the Fe sulfate/sulfide region.

SAMPLE	PEAK TEMP. OF IRON SULFATE/SULFIDE SO ₂ RELEASE (°C)	LOW TEMP. SO ₂ RELEASE (°C)	SULFIDE QDA POSTERIOR PROBABILITY	QMS S ISOTOPES ($\delta^{34}\text{S}$ V-CDT; ‰)
EB	562	297	73%	-27 ± 7
HU1	529	N/A	<1%	18 ± 6
HU2	503	N/A	<1%	21 ± 4
GG1	534	N/A	1%	5 ± 9
GG2	514	N/A	<1%	-5 ± 9
MA1	563	N/A	1%	8 ± 5
MA2	538	N/A	1%	11 ± 6
GE1	555	N/A	7%	20 ± 4
GE2	490	N/A	11%	-6 ± 7
GE3	568	N/A	5%	-14 ± 5
KM1	568	N/A	99%	-21 ± 19
KM2	557	N/A	84%	0 ± 20

Discussion

Sulfide likely in some samples from the GT campaign

Most of the samples analyzed during the GT campaign did not have results consistent with sulfide. Given the ubiquity of sulfate on Mars and the oxidizing conditions near the surface, this was reasonable to expect. Interestingly, two of the samples did have multiple lines of evidence for the presence of reduced sulfur: KM and EB. These two samples had EGA, QDA, and/or S isotope evidence consistent with a sulfide. KM1, KM2, and EB are shown as a distinct group in QDA-variable space in Appendix Figure C.2.

The QDA clustering and depleted $\delta^{34}\text{S}$ values in KM strongly suggested the presence of a sulfide in this Jura member sample of the GT campaign. The main release of SO₂ during EGA was consistent with either a sulfide or sulfate. The presence of a sulfide in KM is also consistent with previously reported results from the Vera Rubin ridge where

two Jura member samples, Highfield and Rock Hall, also clustered with laboratory sulfides in QDA (Wong et al. 2020). In this previous work, it was hypothesized that the reduced sulfur on VRR was the result of mildly reducing, sulfite-containing diagenetic fluids in which the sulfite decomposed in disproportionation reactions to form reduced S. Given that KM is also a sample of the Jura member, it is possible that its reduced sulfur is from the same source. This would suggest that the lower strata of the Glen Torridon were altered by these fluids along with the upper strata of VRR. Lower strata on VRR and higher strata in GT do not have compelling evidence for reduced sulfur.

The Edinburgh sample had some of the strongest evidence for presence of reduced sulfur. The SO₂ evolution in EB was consistent with laboratory-run troilite and pyrrhotite samples, which have similar EGA profiles (Figure C.1). However, the temperatures of the peaks were shifted ~100°C higher in the SAM analysis compared to the laboratory results. Still, EB had a distinct SO₂ peak around 300°C, which is consistent with the oxidation of sulfide. Similar small low temperature SO₂ peaks have been observed in numerous samples on Mars, including Cumberland, Highfield, and Rock Hall, all of which have other evidence for reduced sulfur. Quadratic discriminant analysis and S isotopes also agreed that a sulfide was likely in the EB sample. EB represents the pediment capping unit unconformity, which is a sandstone that appears similar to the previously sampled Stimson sandstone on lower Mt. Sharp. This overlying lithological unit was deposited after the Murray formation mudstones that have been typical of many of the observed rocks. The sulfide present on the capping unit may have been derived from equilibrium fractionation between a sulfide and sulfate or hydrothermal alteration, which would allow for the preservation of the depleted sulfur signal. Importantly, the EB stratigraphy will be

encountered again later in the mission as the rover continues up Mt. Sharp, which will allow for additional analyses of similar rocks. If reduced sulfur is widespread on these more recent strata, that would remove an energy hurdle for habitability in the area.

Complementary sulfur redox analyses

This work presents numerous avenues to aid in determining whether Martian samples contain oxidized or reduced sulfur. SO₂ release is among the first possible indicators of sulfur redox. While SO₂ is readily produced from the thermal decomposition of sulfates during EGA, it can also form during the decomposition and oxidation (by water or oxygen) of sulfides. Based on laboratory data, SO₂ peaks around 500°C-600°C are generally consistent with either Fe-sulfate or Fe-sulfide decomposition in the sample. Furthermore, low temperature peaks near 300°C may be consistent with sulfide, elemental sulfur, or sulfonic acids. Taken together, there is not a unique solution from EGA temperature alone, especially given that multiple sulfur compounds from laboratory analyses may match the SO₂ release in a Martian sample.

Quadratic discriminant analysis takes EGA a step further and investigates multiple evolved volatiles compared to known laboratory data at one time to make a determination of whether sulfide is likely to be present. From the laboratory analyses, it was clear that sulfides consistently evolved the reduced carbon-sulfur gases COS and CS₂, which were broadly interpreted as reaction products between the sulfide and CO/CO₂ in the system (Wong et al. 2020). These gases are key variables in the QDA and can be used to identify which Martian samples are likely to presently contain reduced sulfur. However, the amount and type of reduced sulfur cannot be determined from this method, though it is most likely

that it is in the form of trace and/or amorphous iron sulfide. This method also does not reveal anything about the history of sulfur in the sample.

Calculated sulfur isotope compositions can provide valuable information about the redox state of sulfur. The history/type of sulfur can also sometimes be inferred from these values. Highly depleted values are consistent with the sulfide formed during equilibrium fractionation of sulfide/sulfate over time. Depleted values may also be consistent with sulfide formed from the disproportionation of mid-valence sulfur compounds, such as sulfite. Delta values near zero suggest that the sulfur was derived from mantle material. This may be consistent with either igneous sulfides or sulfate formed from volcanic SO₂ rainout. More enriched sulfur isotopic values are consistent with sulfate formed during equilibrium fractionation, disproportionation reactions, or atmospheric processing. Despite the benefits in learning about sulfur redox from their isotopes, there remain some challenges. The $\delta^{34}\text{S}$ values near zero can be ambiguous in that they could still represent either sulfide or sulfate. Additionally, some peaks are too small for accurate isotope values to be calculated due to the relatively low abundance of ³⁴S. The signal-to-noise ratio of an *m/z* 66 peak can be too low for accurate calculation. On the other hand, sometimes the SAM QMS is inundated with SO₂, causing saturation of the *m/z* 64 peak and preventing accurate sulfur isotope calculations from SO₂.

Each method of analyzing samples and their sulfur redox has strengths and weaknesses that complement each other well in different situations. EGA temperatures can provide quick insight into the presence of an iron-bearing sulfur compound. QDA can help distinguish whether that compound is a sulfide or sulfate. S isotopes can confirm the QDA finding if the $\delta^{34}\text{S}$ shows a strong enrichment or depletion. Alternatively, QDA can suggest

whether an ambiguous $\delta^{34}\text{S}$ result represents a present sulfide or not. As the MSL mission continues to probe Gale crater for signs of habitability, it would be prudent to use all three analyses in conjunction to identify sulfur redox states. These analyses further complement chemical and mineralogical work performed by the other rover instruments, such as CheMin and APXS to gain a more complete picture of Martian habitability.

Conclusion

The Glen Torridon campaign accomplished a long-held goal of the MSL mission: to explore the clay-bearing region of Gale crater and assess its potential for ancient habitability. The work here specifically investigated the possible presence of reduced sulfur in the clay-bearing unit and nearby rocks using a combination of complementary methods analyzing EGA data. Most of the samples did not have strong evidence for reduced sulfur except for Kilmarie and Edinburgh. Kilmarie may have undergone similar diagenetic alteration responsible for likely sulfide in other Jura member samples observed on the Vera Rubin ridge. It is not yet clear why Edinburgh appears to contain reduced sulfur, though it presents an opportunity for further study when the rover encounters the same strata later in the mission. The observation that most GT samples did not contain sulfide suggests that the GT region has been largely oxidized, consistent with most of the underlying Murray Formation samples. Sulfide in the two samples, however, suggests that reduced S may have been more available as an electron donor in the ancient environment with reducing conditions at different times in Martian history.

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Chapter 4: Exploration of methane and chloromethane observed during evolved gas analysis of Martian solid samples with possible constraints on their isotopic compositions

This chapter has been written as a draft section for a larger SAM team manuscript. This chapter represents work performed by G. Wong in consultation with C. House, who might lead the larger manuscript.

Introduction

The search for reduced organic carbon on Mars is a key factor in assessing Martian habitability. Chloromethane was among the first reported organic molecules observed on Mars by the Viking lander when solid samples were heated; however, it was written off at the time as terrestrial contamination from solvents (Biemann et al. 1977). With the discovery of perchlorates on Mars approximately 30 years later by the Phoenix lander (Hecht et al. 2009; Kounaves et al. 2010), the question of native organics seen by Viking was reconsidered. Laboratory and modeling work found that, when heated, a mixture of perchlorates and organics can release chloromethane as well as CO₂ and other single-carbon volatiles (Navarro-González et al. 2010).

The Mars Science Laboratory's (MSL) Sample Analysis at Mars (SAM) instrument suite has also detected perchlorates and chlorinated organics during its thermal analyses of solid Martian samples (Glavin et al. 2013). It has been speculated that these chlorinated compounds are the result of chlorine-bearing gases released during thermal decomposition reacting with organics – either native to Mars or from background contamination on SAM (Leshin et al. 2013). Chloromethane in particular is observed in all SAM analyses (Eigenbrode et al. 2018, supplementary information). Additionally, methane is observed in SAM evolved gas analysis experiments, though it is generally attributed to the on-board derivatization agent, N-tert-butylmethylsilyl-N-methyltrifluoroacetamide (MTBSTFA)

(Eigenbrode et al. 2018, supplementary information; Stern et al. 2015, supplementary information). It is important to investigate reactions that form both methane and chloromethane during pyrolytic heating to better understand the possible sources of these reduced carbon compounds.

Carbon isotopes are a potentially useful tool to help determine the origins of the reduced carbon in methane and chloromethane. If the carbon is from cleaning solvents, as had been speculated for the Viking chloromethane, a comparison of the carbon isotopic composition between chloromethane and the solvents could, in principle, be used to distinguish between contamination and native Martian carbon (Table D.1). Such a comparison is not necessarily straightforward, though. Martian solid samples are complex mixtures that release a variety of gases during pyrolytic heating. Therefore, in addition to the reactions that may occur during heating, possible effects on the carbon isotope compositions of methane and chloromethane must be considered. It is especially important to understand the effects from thermal decomposition experiments given their past (Viking and Phoenix), present (Mars Science Laboratory), and potential future use on Mars (ExoMars) (Biemann et al. 1977; Smith et al. 2008; Mahaffy et al. 2012; Goesmann et al. 2017).

Here, we investigate reactions that may occur to form methane and chloromethane during the thermal decomposition of Martian solid samples. We also model scenarios that could affect the carbon isotope compositions of the resultant methane/chloromethane. The results of this work can be used to help constrain the origins of these single-carbon reduced compounds observed in Martian pyrolysis work.

Methods

Thermodynamic modeling

We investigated chemical reactions that could occur during thermal analyses based on their thermodynamics. These calculations establish possible reaction products and their equilibrium constants at representative temperatures during pyrolysis. All equations are from Atkins and de Paula (2014). This information establishes possible sources of methane and, depending on the system's equilibrium state, could provide conditions for equilibrium fractionation to occur. All calculations were performed in Python. Standard Gibbs free energy of reaction ($\Delta_r G^\circ$) for a given reaction was calculated from tables of standard Gibbs free energy of formation ($\Delta_f G^\circ$) with the formulation:

$$\Delta_r G^\circ = (\sum \nu \Delta_f G^\circ)_{\text{products}} - (\sum \nu \Delta_f G^\circ)_{\text{reactants}}, \quad (\text{Eq. 4.1})$$

where ν are the stoichiometric coefficients. All standard Gibbs free energies are calculated with the units kJ/mol at 25°C.

Determining the equilibrium constant (K_{eq}) at various temperatures is useful for comparisons to EGA data. The equilibrium constant is defined by the equation:

$$\Delta_r G^\circ = -RT \ln(K_{eq}), \quad (\text{Eq. 4.2})$$

where $\Delta_r G^\circ$ is the standard Gibbs free energy at 298 K, R is the ideal gas constant, T is the temperature in K, and K_{eq} is the equilibrium constant for the given temperature. Given the calculated standard Gibbs free energy in Eq. 4.2, the equilibrium constant can be calculated for a given temperature, T , with the assumption that $\Delta_r G^\circ$ is constant at the temperature of interest. K_{eq} is a unitless value by definition and can alternatively be calculated according to the following equation:

$$K_{eq} = \prod_j a_j^{\nu_j} \quad (\text{Eq. 4.3})$$

where J is the species in the reaction, a is the activity of species J at equilibrium, and v is the stoichiometric coefficient of species J . The instantaneous reaction quotient, Q , can be calculated the same way as Eq. 4.3, but with activities at any given point in a reaction.

Isotope models

It is critical to understand the range of possibilities of isotopic fractionation during pyrolysis. To explore these possibilities, we used two isotope models: an equilibrium fractionation model of a reversible reaction in a closed system and a model of fractionation for an irreversible reaction in a closed system. These could represent a range of reactions that may occur during pyrolysis. These models and outputs were produced in Microsoft Excel. Equations are from Hayes (2004). The relevant equations used in the isotopic modeling are described below.

For equilibrium fractionation in a closed system such that $A \leftrightarrow B$, we used the equations:

$$\delta_A = \frac{\alpha_{A/B}\delta_\Sigma + f_B\varepsilon_{A/B}}{\alpha_{A/B}(1-f_B) + f_B} \quad (\text{Eq. 4.4})$$

$$\delta_B = \frac{\delta_\Sigma - (1-f_B)\varepsilon_{A/B}}{\alpha_{A/B}(1-f_B) + f_B} \quad (\text{Eq. 4.5})$$

where δ_A , δ_B , and δ_Σ are the permil abundances of a given isotope in component A, B, the weighted average of A and B compared to a standard, f_B is the fraction of material that is B, $\alpha_{A/B}$ is the fractionation factor between A and B, and $\varepsilon_{A/B}$ is defined as $\alpha_{A/B} - 1$. The fractionation factor $\alpha_{A/B}$ is defined as R_A/R_B , where R is the isotope ratio of rare to abundant isotope (e.g. $^{13}\text{C}/^{12}\text{C}$ abundances for carbon).

For the isotope calculations of an irreversible reaction $R \rightarrow P$, the following equations were used:

$$\delta_R = \delta_{R,0} + \varepsilon_{P/R} \ln f \quad (\text{Eq. 4.6})$$

$$\delta_P = \delta_{R,0} - \left[\frac{f}{1-f} \right] \varepsilon_{P/R} \ln f \quad (\text{Eq. 4.7})$$

where $\delta_{R,0}$, δ_R , and δ_P are the permil delta values of the initial reactant, the final reactant, and the pooled product, respectively, f is the fraction of reaction that has occurred, and $\varepsilon_{P/R}$ is defined as $\alpha_{P/R} - 1$ where $\alpha_{P/R} = \frac{R_{P'}}{R_R}$. P' is the instantaneous product and will differ from the unreacted reactant pool based on the isotope effect/fractionation factor.

Evolved gas analysis

Evolved gas analysis-mass spectrometry (EGA) is useful for identifying relative amounts of gases that are produced during thermal decomposition of solid samples and is used on the Sample Analysis at Mars (SAM). The details of EGA are described elsewhere in this dissertation (Chapter 2). Briefly, a solid sample of drilled or scooped material is heated in a pyrolysis oven to $>800^\circ\text{C}$ under a constant flow of ~ 30 mbar He. Volatiles evolved during decomposition are carried by the He to the quadrupole mass spectrometer (QMS), where they are ionized and recorded as mass-to-charge (m/z) ratios. The QMS scans for m/z ratios from 2 to 535 on SAM. From the m/z ratios, volatile gases can be inferred and plotted against time or temperature.

Results

Formation and possible equilibration of CH_3Cl and CH_4

Several reactions are possible to form methane or chloromethane during heating. Navarro-González et al. (2010) determined with a chemical kinetics model that CH_4 and CH_3Cl are readily produced from a precursor organic carbon in Viking-like heating conditions. Here, we investigated a few example reactions in terms of their thermodynamic

equilibrium constants as functions of temperature and their standard Gibbs free energies of reaction. The reactions and their standard Gibbs free energies are listed in Table 4.1:

Table 4.1. List of reactions and their standard Gibbs free energies explored in this work.

Reaction #	Reaction	ΔG° (kJ/mol)
1	$2\text{CH}_3 + \text{Cl}_2 \leftrightarrow 2\text{CH}_3\text{Cl}$	-411
2	$2\text{CH}_3 + \text{H}_2 \leftrightarrow 2\text{CH}_4$	-397
3	$2\text{CH}_3 + \text{HCl} \leftrightarrow \text{CH}_4 + \text{CH}_3\text{Cl}$	-308
4	$\text{CH}_4 + \text{Cl}_2 \leftrightarrow \text{CH}_3\text{Cl} + \text{HCl}$	-102
5	$\text{CH}_4 + \text{HCl} \leftrightarrow \text{CH}_3\text{Cl} + \text{H}_2$	89
6	$2\text{CH}_4 + \text{Cl}_2 \leftrightarrow 2\text{CH}_3\text{Cl} + \text{H}_2$	-13

Reactions 1-3 use CH_3 as the carbon reactant. CH_3 may derive from the decomposition of larger organic compounds, methoxyl-bearing organics, or MTBSTFA, and it could be form CH_4 and CH_3Cl (Navarro-González et al. 2010; Keppler et al. 2014; Stern et al. 2015). Reactions 1-3 support these hypotheses – production of methane and chloromethane is highly favored from CH_3 under standard conditions. Furthermore, the formation of CH_3Cl and CH_4 from these reactions is strongly favored at a wide range of temperatures, including those reached in the SAM oven (Figure 4.1). See Appendix A4 for additional plots of K_{eq} vs. temperature.

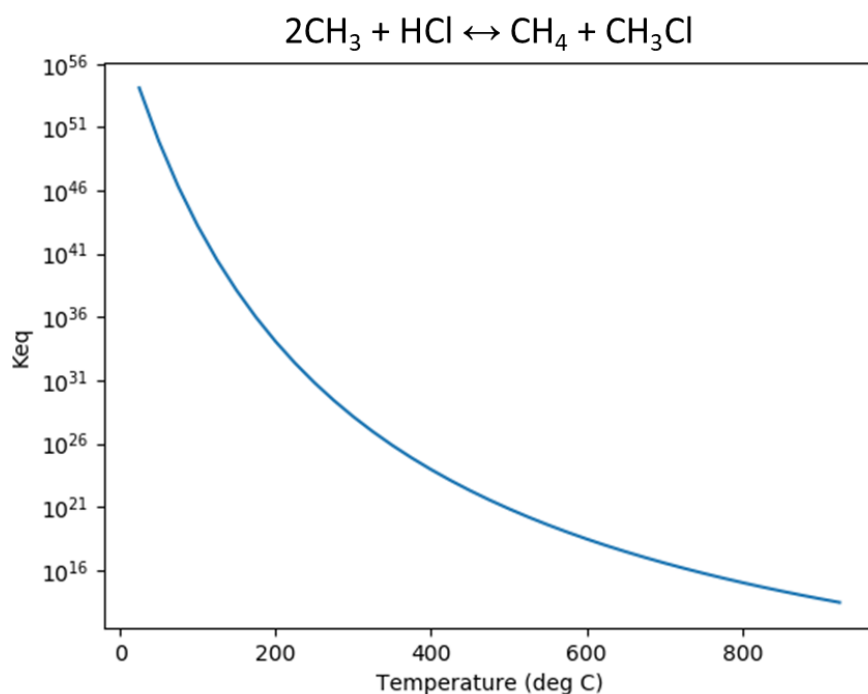


Figure 4.1. Equilibrium constant as a function of temperature for Reaction 3 in Table 4.1. The equilibrium constant indicates that products are strongly favored over the entire SAM temperature range.

Reactions 4-6 in Table 4.1 investigate the relationships between methane and chloromethane more directly. Reaction 6 is the ‘net’ reaction of Reactions 4 and 5. This net reaction has a ΔG° of -13 kJ/mol, suggesting that the favorability of reactant vs. product formation has a relatively strong dependency on the presence of constituent volatiles and the temperature. CH_3Cl and H_2 are favored throughout the SAM oven temperature range, though only to a small degree (Figure 4.2).

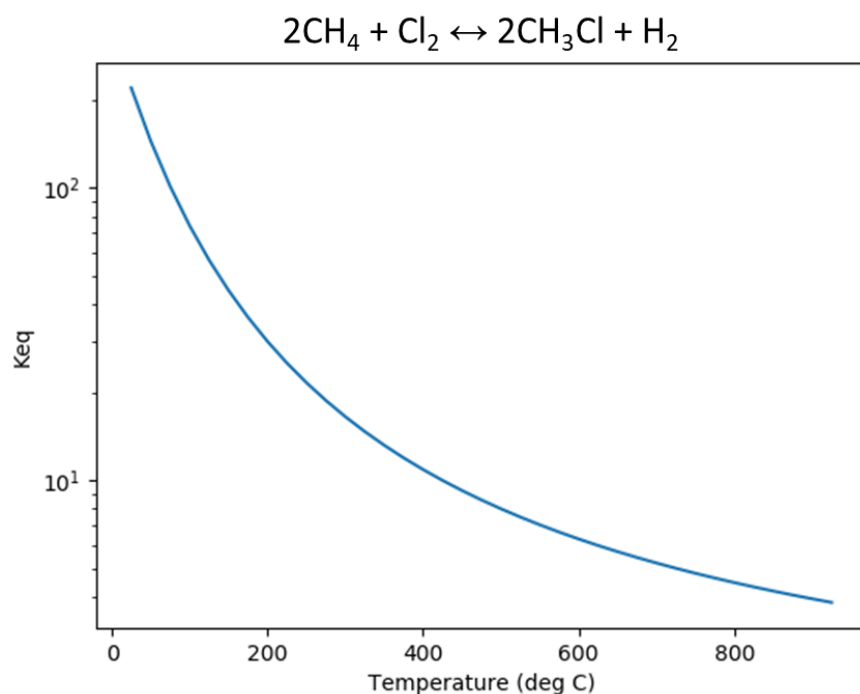


Figure 4.2. Plot of K_{eq} vs. temperature for Reaction 6. Reaction products CH_3Cl and H_2 are slightly favored over the SAM temperature range.

Together, these possible reactions set up a range of possibilities for methane and chloromethane formation during SAM analyses. Reactions 1-3 in Table 4.1 suggest that CH_3Cl and CH_4 would be readily formed by reactions involving CH_3 produced during decomposition of organic precursors. The formation of methane and chloromethane would be favorable in essentially all temperature conditions during pyrolysis. These gases could also react with HCl , Cl_2 , and H_2 , where the favorability of formation would be determined by temperature and relative volatiles available for reaction. Depending on the gases present, their flow, and time, methane and chloromethane may be able to equilibrate (Reaction 6).

Kinetic fractionation forming chloromethane

The results of isotope fractionation modeling for an irreversible reaction from precursor carbon to chloromethane are shown in Figure 4.3. For possible fractionation factors, it was assumed that the precursor carbon would behave like methoxyl-functional group carbon as investigated by Keppler et al. (2014). They investigated the isotopic effects of pyrolyzed meteorite material mixed with chlorine-bearing compounds and found that chloromethane had $\Delta^{13}\text{C}$ (defined as $\delta^{13}\text{C}_{\text{precursor}} - \delta^{13}\text{C}_{\text{product}}$) between -10 and -30‰ relative to native methoxy-bearing organics. Therefore, fractionation factors $\alpha_{\text{P/R}}$ of 0.99 and 0.97 were used, which can result in fractionation of the chloromethane by up to 10‰ and 30‰, respectively, lower than the precursor carbon depending on the CH_3Cl yield. Chloromethanes typically comprise of only a small fraction of the carbon volatiles observed during Martian pyrolysis experiments (Glavin et al. 2013; Freissinet et al. 2015). Small yields of CH_3Cl from a precursor carbon would result in large relative depletions in $\delta^{13}\text{C}$. Assuming a generous yield of 20% CH_3Cl from a precursor carbon, the $\Delta^{13}\text{C}$ of CH_3Cl would be $\sim -9\text{‰}$ for $\alpha=0.99$ and $\sim -27\text{‰}$ for $\alpha=0.97$ (Figure 4.3). This depleted chloromethane could theoretically then be available for equilibration with methane as discussed in the following section.

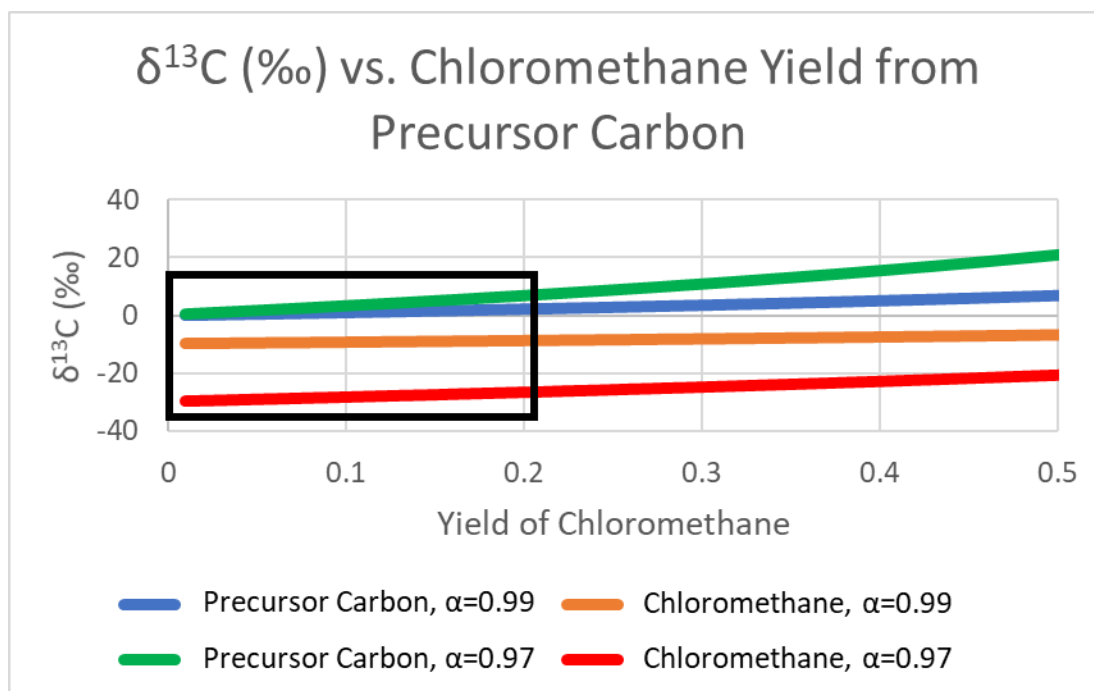


Figure 4.3. Irreversible Reaction Precursor C $\rightarrow$ CH₃Cl with $\delta^{13}\text{C}=0\text{‰}$ starting material, $\alpha=0.99$ or 0.97 . The black box denotes the range of possible yields of chloromethane and the resulting isotopic compositions of the precursor and CH₃Cl.

Equilibrium fractionation between methane and chloromethane

Depending on the conditions of any given pyrolysis experiment, chloromethane and methane could equilibrate and result in important isotopic effects, which are generally modeled in Figure 4.4. For this fractionation modeling, it was assumed that a reversible reaction between methane and chloromethane with a total $\delta^{13}\text{C}$ of 0‰ would occur in a closed system. An assumed fractionation factor $\alpha_{\text{CM/Methane}}$ of 1.018 was used, resulting in the relative depletion of methane. This equilibrium fractionation factor is based on calculations by Gropp, Iron, and Halevy (2020, preprint) for the equilibration of CH₃OH and CH₄ at 50°C in enzyme-catalyzed biological systems. Methanol was chosen as an approximation for chloromethane in the absence of chloromethane fractionation data due to the similarity of -Cl and -OH functional groups. The redox similarity of the two molecules may make them fractionate similarly with methane. However, CH₃Cl has a

longer C-Cl bond length (1.785 Å) compared to the C-O bond length (1.427 Å) in CH₃OH (Johnson III (ed.), 2020). The longer bond length in CH₃Cl would may result in smaller fractionations due to the smaller relative difference between ¹³C and ¹²C.

A few equilibration scenarios are explored here with the assumptions described above. In a case where CH₃Cl and CH₄ instantaneously equilibrate after their formation from a precursor carbon and are present in equal amounts, the resulting isotopic compositions would deviate by ~ +9‰ and ~ -9‰, respectively (Figure 4.4, red box). In a case where methane and chloromethane were able to equilibrate according to the reaction $2\text{CH}_4 + \text{Cl}_2 \leftrightarrow 2\text{CH}_3\text{Cl} + \text{H}_2$ (Reaction 6 in the previous section), their relative abundances would be under redox control. In a more reducing environment, methane would be the favored carbon compound and would not have a large isotope deviation while CH₃Cl would be enriched by nearly 20‰ (Figure 4.4, blue dashed box). Alternatively, in a more oxidizing environment, CH₃Cl would be favored and the small amounts of methane could be depleted by nearly 20‰ relative to the chloromethane (Figure 4.4, black box). These scenarios may be largely sample-dependent, especially if redox controlled.

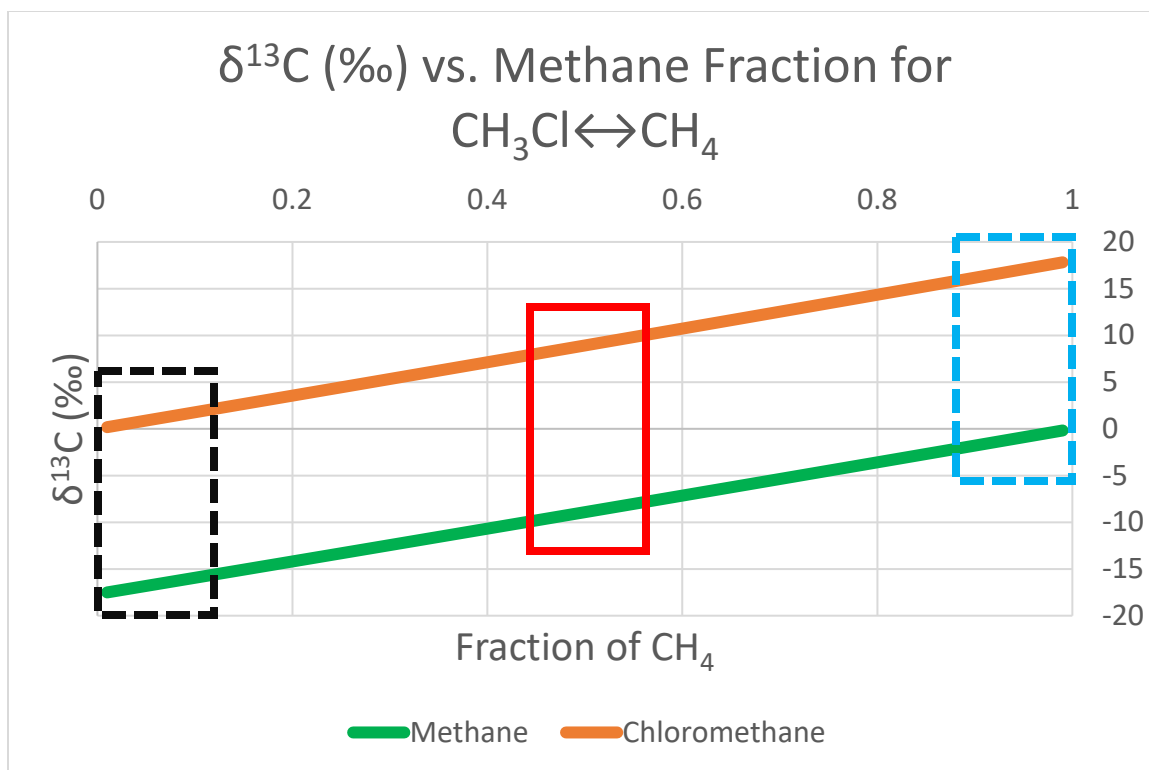


Figure 4.4. Carbon isotopic effects of a reversible reaction in a closed system for the equilibration of $\text{CH}_3\text{Cl} \leftrightarrow \text{CH}_4$ with an overall isotopic composition of $\delta^{13}\text{C} = 0\text{‰}$ (e.g. the composition of the original pyrolyzed carbon) with $\alpha_{\text{CM/Methane}} = 1.018$. Boxes represent three different equilibration scenarios. Red box represents the instantaneous equilibration of CH_3Cl and CH_4 after their formation where they are present in equal abundances. Dashed boxes represent more extreme cases of equilibration with the black box (left) representing a more oxidizing environment and the blue box (right) representing a more reducing environment.

Taken together, the overall change in carbon isotope composition of methane or chloromethane compared to a precursor carbon could, in principle, be quite large. In a kinetic fractionation, the $\delta^{13}\text{C}$ value of chloromethane may be depleted by up to 30‰ relative to the precursor carbon in an extreme case. However, a simple methyl cleavage and subsequent chlorination may only impart an $\sim 10\text{‰}$ observed depletion in the chloromethane relative to the precursor carbon. If this chloromethane were to then equilibrate with methane, the CH_3Cl may act as a (relative) ^{13}C reservoir. In a case where CH_3Cl and CH_4 instantaneously equilibrate in equal fractions, the net fractionation (kinetic

plus equilibration, assuming a 10‰ depletion from kinetic fractionation) may yield $\Delta^{13}\text{C}_{\text{chloromethane}}=0\text{‰}$ and $\Delta^{13}\text{C}_{\text{methane}}=-20\text{‰}$. If the methane and chloromethane had the opportunity to equilibrate in a redox-controlled environment, the net effect could be that $\Delta^{13}\text{C}_{\text{chloromethane}}$ is between -10‰ (oxidized environment) and +10‰ (reducing environment). Likewise, $\Delta^{13}\text{C}_{\text{methane}}$ could be between -10‰ and -30‰. These effects could be increased by up to ~20‰ if the kinetic fractionation results in CH_3Cl that is depleted by 30‰ (rather than 10‰) relative to the precursor carbon. A case study of possible reactions and the isotopic constraints is discussed in the following section.

Discussion

Cumberland 3 case study

The Cumberland 3 drill sample was among the earliest drill samples from the MSL mission. It is a well-characterized sample from the Sheepbed mudstone in Gale crater and was identified as containing all of the chemical requirements for habitability (Grotzinger et al. 2013). Still, the wealth of data collected by the various rover instruments necessitates continued analysis of the sample. Here, a few select volatiles from EGA are plotted along with the temperature cut during which gases were sent to the tunable laser spectrometer (in addition to the QMS for analysis). In keeping with the previous reaction examples, the plot focuses on evolved CH_4 , H_2 , HCl , Cl_2 , and CH_3Cl . The EGA plot (Figure 4.5) shows the relative amounts of each gas with $[\text{H}_2]>[\text{HCl}]>[\text{CH}_4]>[\text{CH}_3\text{Cl}]>[\text{Cl}_2]$ in the temperature cut. Integrated counts of these volatiles are listed in Table 4.2.

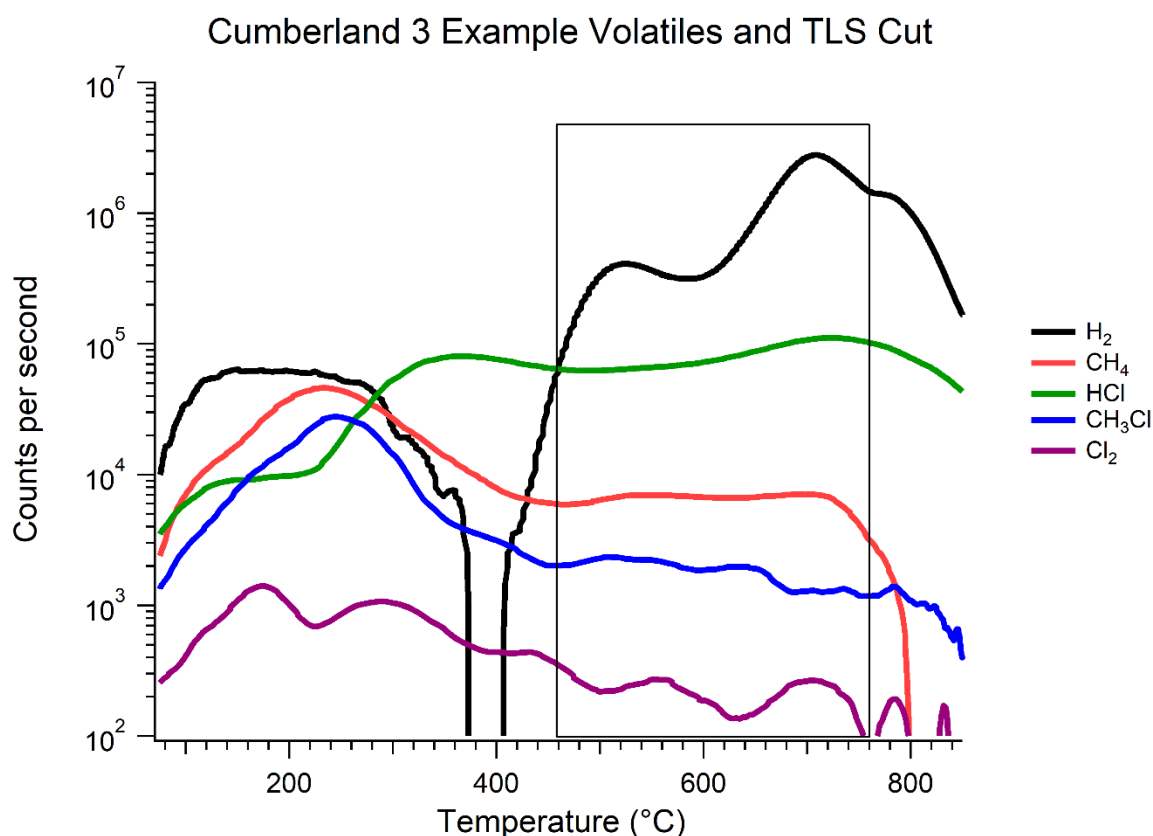


Figure 4.5. EGA plot showing counts per second vs. temperature in degrees Celsius for six example volatiles. The box marks off the temperature range 455°C-755°C used for the TLS cut.

Table 4.2. Integrated counts of the volatiles shown in Figure 4.5 during the TLS temperature cut.

Volatile	Integrated count
H ₂	2.59E+08
HCl	9.22E+06
CH ₄	7.12E+05
CH ₃ Cl	1.95E+05
Cl ₂	2.36E+04

Native organics above SAM background levels have been detected in the Cumberland 3 drill sample (Freissinet et al. 2015). Perchlorates were also inferred from this sample (Ming et al. 2014). The observed chloromethane could have formed from reactions between native organics (or MTBSTFA per Freissinet et al. 2015) and the chlorine from perchlorate decomposition. This chloromethane formation may have resulted in an ~20‰ ¹³C depletion relative to the precursor carbon. Based on the evolved volatiles

from the Cumberland 3 sample in the TLS temperature cut range, the reaction quotient Q is ~ 830 , which would favor the formation of CH_4 (Table 4.3). This is largely due to the large amount of evolved H_2 . If the gases had the opportunity to equilibrate, the favored formation of methane would cause the ^{13}C of methane to approximate that of the CH_3Cl before equilibration ($\sim -20\%$ relative to the precursor) while equilibrated chloromethane may actually become relatively enriched. These effects could be tested if the carbon isotopes of methane and chloromethane could be determined. While the signal-to-noise-ratio is too small for isotope calculations from mass spectrometer data, the tunable laser spectrometer (TLS) can, in principle, determine $^{13}\text{C}/^{12}\text{C}$ ratios of methane if abundant enough (Webster and Mahaffy 2011).

Table 4.3. Summary of the minimum and maximum K_{eq} values, the reaction quotient Q (based on the evolved volatiles in Figure 4.5/Table 4.2, and an indication of which direction the written reaction would proceed for three possible reactions.

	$\text{CH}_4 + \text{Cl}_2 \leftrightarrow \text{CH}_3\text{Cl} + \text{HCl}$	$\text{CH}_4 + \text{HCl} \leftrightarrow \text{CH}_3\text{Cl} + \text{H}_2$	$2\text{CH}_4 + \text{Cl}_2 \leftrightarrow 2\text{CH}_3\text{Cl} + \text{H}_2$
Minimum K_{eq}	2.80E+04	2.92E-16	3.82
Maximum K_{eq}	7.52E+17	1.37E-04	220
Reaction Quotient Q	108	7.72	830
Reaction toward?	Right	Left	Left

Implications, limitations, and future directions

Chloromethane is commonly observed during the thermal decomposition of solid samples on Mars. This chloromethane has largely been attributed to reactions between Cl-bearing volatiles released during oxychlorine decomposition and carbon from MTBSTFA (Glavin et al. 2013) or native organics (Navarro-González et al. 2010). Here, we have shown chloromethane formation is thermodynamically favorable for all pyrolysis oven temperatures. Such reactions are most favored at lower temperatures, which is consistent with the largest CH_3Cl peaks observed in EGA. These reactions could readily occur in the SAM oven during sample heating and impart a kinetic fractionation that results in a

depleted ^{13}C relative to the precursor. Further, if such a pool of depleted CH_3Cl was able to equilibrate with CH_4 , there could be an additional fractionation that may be redox controlled. In the case study with Cumberland 3, however, equilibration would likely favor little fractionation in methane, and a net enrichment in CH_3Cl .

An underlying assumption of this work is that carbon is only from a general precursor organic, in chloromethane, or in methane. Other forms of carbon are present in Martian samples, and reactions with them may impart different isotopic effects. Chief among these is evolved CO_2 , which has been shown to have a wide range of $\delta^{13}\text{C}$ values from $-25\pm 20\text{‰}$ to $+56\pm 11\text{‰}$, indicating various phases of native carbon (Franz et al. 2020). This native carbon could serve as a precursor and would affect the final carbon isotope compositions of methane and chloromethane. Alternatively, the evolved CO_2 could be reduced in the oven to form methane, which would likely impart a large isotopic depletion during its reduction. Such possible reactions would need to be considered for each individual sample with both CO_2 and CH_4 abundances and carbon isotope values.

Additionally, the exact isotopic effects of a CH_3Cl pool on $^{13}\text{CH}_4$ could be explored more directly in future work. While approximations of isotopic effects were investigated here, more direct modeling and laboratory work should be undertaken. Molecular modeling using density functional theory could help elucidate the magnitude of fractionation between methane and chloromethane in a potential equilibrium. Results of such modeling could be tested in the laboratory using SAM-like pyrolysis of methane- and chlorine-evolving compounds to be analyzed by an isotope ratio mass spectrometer.

Conclusion

This chapter investigated relationships between select volatiles observed during the thermal decomposition of Martian solid samples. With observations of chloromethane dating back to thermal volatilization experiments on Viking and its continued presence in SAM analyses today, it is important to understand pertinent oven reactions. Further, determining constraints on the isotopic composition of chloromethane (and methane) is important in determining the origin of the reduced carbon. The investigations here indicated that oven reactions can produce methane with a depleted ^{13}C composition relative to a precursor carbon source. An extreme case under the right conditions may see ^{13}C depletions in methane of up to 50‰, but would most likely be <30‰. Similarly, oven reactions may result in chloromethane with a ^{13}C composition that is most likely within 10‰ of the precursor carbon. Understanding these values is especially useful for future isotopic analysis of these thermally-released reduced gases as pyrolysis experiments on Mars continue.

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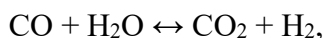
Chapter 5: Carbon monoxide as a microbial energy source on Mars

This chapter represents a draft manuscript that we intend to submit to the Astrobiology section of the open access journal, *Life*. Authors will be G. Wong, Z. Zhang, and C. House.

Introduction

CO background

Carbon monoxide (CO) is infamous for its danger to humans. However, despite its toxicity to us, the gas can actually be a source of energy for diverse microorganisms if they have the right molecular machinery. CO contains a carbon atom with an intermediate oxidation state of +2 (carbon in CO₂ has an oxidation state of +4 while carbon in CH₄ has an oxidation state of -4). This intermediate oxidation state of the carbon means that the carbon has two electrons that can be donated during chemical reactions to oxidize the carbon to a +4-oxidation state. This CO oxidation is typified in the canonical gas-shift reaction:



in which CO is oxidized by water to form CO₂ and molecular hydrogen, typically catalyzed by a metal. This reaction is thermodynamically favorable with a $\Delta G^\circ_{\text{rxn}} = -20$ kJ/mol. The thermodynamic favorability of CO oxidation gives microorganisms the opportunity to exploit this type of reaction for their metabolic energy requirements.

Numerous and diverse microorganisms have been identified as being CO-oxidizers (carboxydotrophs) on Earth despite CO typically existing at low levels (<1 ppm) in the atmosphere (Cheiney et al. 2011). CO-oxidizing microbes have been identified in various environments including soils (Bartholomew and Alexander 1979; Bender and Conrad 1994), alkaline lakes (Hoeft et al. 2007), hot springs (Sokolova et al. 2004), salt marsh sediments (King 2007), and volcanic deposits (Weber and King 2009). These microbes are

metabolically and phylogenetically diverse, including anaerobic/aerobic metabolisms and representatives from bacteria and archaea (Oelgeschläger and Rother 2008). Key to the oxidation of CO is the enzyme carbon monoxide dehydrogenase (CODH). CODH is an iron- and nickel- or molybdenum-containing enzyme common to all carboxydotrophs that catalyzes CO oxidation analogously to the gas-shift reaction (Ragsdale 2004). CODH has been hypothesized as being an ancient enzyme that may have contributed to life's origins (Martin and Russell 2007). Given the diversity and potentially long evolutionary history of CO-oxidizing microbes, they represent interesting case studies in astrobiology.

CO is released from volcanoes on Earth and would have been outgassed by ancient active volcanism on Mars, potentially contributing to a significant fraction of the atmospheric composition (Batalha et al. 2015). Additionally, CO can form through the photodissociation of CO₂ by UV light in terrestrial atmospheres and is expected to be present at high levels on dry planets (Hu, Seager, and Bains 2012). Indeed, CO is relatively abundant on Mars, which is currently an extremely dry terrestrial planet with a thin atmosphere comprised of ~96% CO₂ (Mahaffy et al. 2013) with a surface pressure around 740 Pa (Gómez-Elvira et al. 2014) at Gale crater. Ground-based and orbital measurements of CO on Mars have estimated global averages of ~700 ppm, though the mixing ratio changes spatially and seasonally (Krasnopolsky 2015; Smith et al. 2009). Furthermore, in situ measurements by the Mars Science Laboratory at Gale crater have been determined via the rover's quadrupole mass spectrometer and reported a mixing ratio of 749 ± 2.6 ppm (Franz et al. 2015). Previous modeling work has suggested that Mars could theoretically support a CO-based biomass, though, if present, the putative metabolisms would be limited (Weiss, Yung, and Nealson 2000; Sholes, Krissansen-Totton, and Catling 2019). Given the

prevalence of CO on Mars (and likely other dry, CO₂-rich planets) and its ability to be oxidized for metabolic energy by Earth microbes, the question of whether life as we know it could use CO on such planets is an open question to be investigated.

CO-based microbial survival in Mars-like environments

Alkalilimnicola ehrlichii MLHE-1 is one model organism to study in the context of astrobiology because of its ability to survive in stressful environments. This Gammaproteobacterium was originally identified in the anoxic bottom waters of Mono Lake, a soda lake in California with pH=9.8 and salinity ranging from 70 to 90 g/L (Oremland et al. 2002). *A. ehrlichii* MLHE-1 is a metabolically flexible organism that is capable of surviving and growing in a range of environmental conditions. It is a haloalkaliphilic facultative anaerobe, facultative chemoautotroph able to grow heterotrophically on a variety of organic acids or lithotrophically with a variety of electron donors including H₂, sulfide, and As(III) (Hoeft et al. 2007). Interestingly, Hoeft et al. (2007) also showed that *A. ehrlichii* MLHE-1 was able to consume CO more readily than its close relatives, though CO oxidation was not coupled to increases in cell density. Still, their finding on CO consumption was suggestive of the microbe's exploitation of CO oxidation for at least metabolic energy purposes.

While interest in *A. ehrlichii* MLHE-1 started because of its arsenic utilization, its curious ability to oxidize CO and its ability to survive a range of environmental stressors has led to its use in studies on Martian habitability. In particular, one study focused on the ability of *A. ehrlichii* MLHE-1 to oxidize CO under select Mars-like conditions (King 2015). This study found that the microbe could oxidize CO down to 6°C, at low water potential, in oxic/suboxic/anoxic environments, in the presence of perchlorate or sulfate

salts, in different headspace compositions, and at 10 mbar pressure, though a combination of these ‘Mars-like’ conditions was not investigated. However, it remained unclear how *A. ehrlichii* MLHE-1 was consuming CO in the hypobaric environment (water was not addressed). Also, the microbe’s ability to grow in a Mars-like environment was not investigated; only CO consumption was recorded.

CO-based photosynthesis

Photosynthesis was a significant evolutionary development relatively early in life’s history. This process allowed for the capturing of solar energy by microorganisms to reduce inorganic carbon for metabolic use. Photosynthesis requires that electrons be replenished by some sort of chemical reducing agent. Water serves as a reducing agent for oxygenic (i.e. O₂ is released as a byproduct) photosynthesis in plants and cyanobacteria. Oxygenic photosynthesis was a globally significant evolutionary development that transformed the Earth’s atmosphere to allow for the buildup of molecular oxygen. However, oxygenic photosynthesis evolved from anoxygenic (i.e. O₂ is not produced) photosynthesis, which is a simpler process and uses a wide variety of electron donors. Electron donors for anoxygenic photosynthesis include nitrite, reduced sulfur, molecular hydrogen, arsenic, and ferrous iron (Griffin, Schott, and Schink 2007; Kulp et al. 2008; Bryant and Frigaard 2006). CO is not currently recognized as an electron donor in anoxygenic photosynthesis, however. Given the low redox potential of the CO/CO₂ couple, diversity of electron donors used in anoxygenic photosynthesis, and the presence of CO on planets like Mars, it is worth investigating whether CO can be used as a source of reducing power in anoxygenic photosynthesis.

Interestingly, several microorganisms are able to oxidize CO through the CODH enzyme and, separately, perform anoxygenic photosynthesis. For example, *Rubrivivax gelatinosus* (formerly *Rhodopseudomonas gelatinosa* and *Rhodocyclus gelatinosus*) is a purple nonsulfur bacterium that typically operates as a photoheterotroph using organic acids as its source of electrons and carbon. *R. gelatinosus* also has the CODH enzyme and there has been evidence suggesting the use of CO in photosynthesis (Hirsch 1968; Uffen 1983). These studies focused on the growth of *R. gelatinosus* and incorporation of CO into biomass rather than use of CO as an electron donor in photosynthesis. Evidence was presented that isotopically labeled CO was incorporated into biomass at an elevated rate when grown photosynthetically (Uffen 1983), though it was unclear whether this was direct CO incorporation or CO that had oxidized to CO₂ and then been incorporated. Some of this work was later continued through the lens of hydrogen production as a source of biofuels. These more recent experiments similarly found that *R. gelatinosus* grew better in light with a 20% CO headspace compared to an N₂ headspace (Maness et al. 2005). Although there is tantalizing evidence that *R. gelatinosus* can grow photosynthetically with CO, the question of whether CO donates its electrons for use in anoxygenic photosynthesis.

Objectives

Given the open questions remaining with *A. ehrlichii* MLHE-1 and *R. gelatinosus*, we aimed to investigate the broad use of CO across these species. In particular, we focused on three objectives – the first two were based on wet laboratory experiments and the third was to use thermodynamic modeling to more broadly investigate possible CO metabolisms. Specifically, the objectives were as follows:

1. To determine the plausibility of CO-based growth on present-day Mars (with *A. ehrlichii* MLHE-1).
2. To determine whether CO is a viable electron donor for anoxygenic photosynthesis as a novel metabolism that could be used by putative microbes Mars or an exoplanet (using *R. gelatinosus*).
3. To model the thermodynamics of a variety of CO oxidation reactions in different environmental conditions to understand where different CO metabolisms may be most favorable.

Methods

Objective 1: determining the plausibility of CO-based growth on present-day Mars

The type strain of *A. ehrlichii* MLHE-1 was procured from the German Collection of Microorganisms and Cell Cultures (DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen). Initial cultures were proliferated according to provided DSMZ protocols. Additionally, after initial culturing, aliquots were concentrated and stored for future use in a -80°C freezer.

The standard medium for *A. ehrlichii* MLHE-1 was DSMZ medium 1457. This medium contains, per liter of water: 0.10 g (NH₄)₂SO₄, 0.025 g MgSO₄, 60.00 g NaCl, 0.15 g K₂HPO₄, 0.08 g KH₂PO₄, 10.60 g Na₂CO₃, 4.20 g NaHCO₃, 0.30 mL of 0.1 mM tungstate, 5.00 mL of DSMZ trace element solution SL-10, and 1.00 mL of a mixed vitamin solution. Additionally, 10 mL of 1 M Na-acetate solution is added as the electron donor per L water. The medium is brought to a pH of 9.8. Cultures were typically grown aerobically between 30°C and 32°C with shaking to encourage mixing and growth.

One experiment focused on whether *A. ehrlichii* MLHE-1 could survive for a long period of time on CO in a dry, hypobaric environment. For this experiment, *A. ehrlichii* MLHE-1 was initially grown in full DSMZ medium 1457 (without vitamin solution). Turbid cultures were washed via three rounds of centrifugation at 8000 rpm for 10 min to remove remaining acetate. Half of these washed cells were mixed back into 5 mL DSMZ medium 1457 while the other half was mixed into 5 mL of a modified DSMZ 1457 medium that did not contain acetate. These cell slurries were then distributed to sterile 25 mL butyl-stoppered bottles with 0.2 mL going to each bottle. Cell counts with a hemocytometer indicated that the density of the slurries ranged from $\sim 1.0 \times 10^9$ - 1.2×10^9 cells/mL. Control bottles underwent the same treatment, but uninoculated medium was used. All bottles were freeze-dried over three days with 0.2 μ m filters serving as a barrier between the bottles and freeze-dryer environment to prevent contamination. To test desiccation, 21 of the 36 bottles were reconstituted with 800 μ L sterile DI water through a 0.2 μ m filter. To each bottle that did not contain acetate, 3.5 mL CO at 1.5 bar were added ensuring sterility. Finally, the bottles marked for low pressure experiments were evacuated using a gas manifold system to an average pressure of 3.5 mbar. Each experimental condition, outlined in the table below, was made in triplicate. After bottles were prepared, they were incubated at 30°C for from May 2018 to October 2019 without agitation.

Table 5.1. Summary of the conditions used for the various sets of bottles. An ‘X’ indicates the condition was used in that set of bottles. Each type of bottle was made in triplicate. CO=CO added to headspace, no acetate in the medium. Acetate=no CO added (air headspace only), acetate in the medium. Dry=medium was not reconstituted. Wet=medium reconstituted. LP=bottle evacuated to <10 mbar. HP=bottle kept at atmospheric pressure. Mic=microbes in medium. Con=uninoculated control bottle.

	CO	Acetate	Dry	Wet	LP	HP	Mic	Con
Set 1	X		X		X			X
Set 2	X		X		X		X	
Set 3	X		X			X	X	
Set 4	X			X	X			X
Set 5	X			X	X		X	
Set 6	X			X		X	X	
Set 7		X	X		X		X	
Set 8		X	X			X	X	
Set 9		X		X	X			X
Set 10		X		X	X		X	
Set 11		X		X		X	X	
Set 12		X		X		X		X

After incubation, bottle headspaces were sampled. Bottles that started at atmospheric pressure were sampled directly. The bottles that were evacuated to low pressure were re-pressurized to atmospheric pressure ultra-high purity helium. Sample headspaces were analyzed over two days on a 5890 Series II gas chromatograph with a thermal conductivity detector (GC-TCD). A 15-foot Carboxen 1000 column maintained at 30°C using a He carrier flowing at 12 cm³/min at 22 psi was used for this analysis. CO and CO₂ were measured for each sample and reported as percentages of the sample headspace.

A separate experiment investigated whether *A. ehrlichii* MLHE-1 could grow on CO even in otherwise ideal conditions. For this experiment, four sets of modified DSMZ medium 1457 were prepared (none contained vitamin solution) and varied in their amounts of acetate. The ‘complete’ medium includes 10 mM acetate. The other three types of media included either 5 mM acetate, 2.5 mM acetate, or no acetate. 40 mL of media was

distributed to 125 mL glass butyl-stoppered bottles with air headspace so that six bottles were prepared with each type of acetate medium. To three of each medium bottle, 10 mL CO at 1.5 bar were added. All bottles were autoclaved for 15 min at ~117°C and allowed to cool fully before inoculation. 200 µL of prepared stock *A. ehrlichii* MLHE-1 were added to each of the six 10 mM acetate bottles initially. Two of the CO-containing and two of the air-only bottles were incubated with shaking at 120 rpm between 30°C and 32°C. The remaining 10 mM acetate bottles were kept at room temperature without shaking for slower, but less stressful growth. Inoculated medium was aseptically sampled (1 mL) immediately after inoculation and absorbance at 680 nm through the aliquot was measured with a UV-Vis spectrophotometer for each bottle. Spectrophotometer measurements were recorded regularly. When samples reached absorbance values greater than ~0.150, 200 µL of the culture was transferred to a bottle with the same headspace composition, but next lower acetate concentration (10 mM to 5 mM to 2.5 mM to 0 mM). Again, absorbance measurements were recorded immediately after transfer to determine a baseline value. All cultures were eventually transferred to the media that did not contain acetate. Absorbance measurements continued on these bottles, though values were recorded infrequently over several months due to the COVID-19 pandemic. All results are reported here.

Objective 2: determining whether CO acts as an electron donor in anoxygenic photosynthesis

The type strain of *Rubrivivax gelatinosus* was purchased from the DSMZ and initial proliferation followed DSMZ recommended protocols. The primary medium used for *R. gelatinosus* was the DSMZ *Rhodospirillaceae* medium 27. The base medium 27 contains the following per L water: 0.30 g yeast extract, 1.00 g sodium succinate, 0.50 g ammonium

acetate, 5.00 mL 0.1% ferric citrate solution, 0.50 g KH_2PO_4 , 0.40 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.40 g NaCl, 0.40 g NH_4Cl , 0.05 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.40 mL vitamin B₁₂ solution (10 mg in 100 mL water), 1.00 mL trace element solution SL-6, and 0.30 g L-cysteiniumchloride. The medium's pH was adjusted to 6.8 and it was bubbled with pure N₂ to remove oxygen. Bottles (125 mL glass butyl-stoppered) were filled anaerobically and then autoclaved for 15 min at 121°C.

CO-based photosynthesis was tested with various growth experiments that followed similar protocols. Four experimental conditions were developed: CO headspace in light, CO headspace in the dark, N₂ headspace in the light, and N₂ headspace in the dark. Headspaces contained 100% of their respective gases at 1.5 bar total pressure. 'Dark' samples were kept in the same incubation conditions (30°C with 120 rpm shaking), but completely covered in aluminum foil to block incident light in the incubator. Growth was recorded via UV-Vis spectrophotometry using absorbance measurements at 660 nm wavelength. These growth experiments modified the medium used to systematically remove possible electron donors (succinate was the primary electron donor for *R. gelatinosus* in this medium). The effects of light/dark and CO/N₂ were also observed.

Bottles testing the effects of cysteinium chloride on growth all contained 5% of the nominal amount of yeast extract. Bottles contained either normal amounts of cysteinium chloride (high), 20% of the nominal concentration (low), or 1% of the full amount (trace). Three bottles of each modified medium were filled with 1.5 bar N₂ headspace. Three trace cysteinium chloride bottles were filled with 1.5 bar CO. Absorbance measurements at 660 nm were taken approximately every three days. All bottles were incubated in light between 30°C and 32°C with 180 rpm shaking.

Objective 3: modeling the thermodynamics of various CO metabolisms

A Python script was developed to investigate the thermodynamics of different CO metabolisms in various environments. This code calculates the Gibbs free energy of reaction for chemical equations involving CO oxidation with various oxidants, CO partial pressures, total pressure, water activity/relative humidity, and a range of temperatures. Different plots of Gibbs free energy can be produced as a function of temperature, pressure, or CO availability. Python scripts were written in Python 2.7.14 and used Numpy and Matplotlib modules (Van der Walt, Colbert, and Varoquaux 2011; Hunter 2007).

At its basic level, the code calculates Gibbs free energy of reaction from the equation:

$$\Delta G = \Delta G^\circ - RT \ln(Q),$$

where ΔG is the Gibbs free energy of reaction, ΔG° is the standard Gibbs free energy of reaction, R is the ideal gas constant (0.008314 kJ/mol•K), T is temperature in Kelvin, and Q is the reaction quotient. Henry's Law was used to calculate concentrations of dissolved gases in water according:

$$H = c/p,$$

where c is the concentration of dissolved gas, p is the partial pressure of the gas, and H is the gas-specific Henry's Law constants (Sander 2015). Dissolution of gases is temperature dependent and concentrations were adjusted using the van't Hoff equation:

$$H(T) = H^\circ \exp \left(\frac{-\Delta_{sol}H}{R} \left(\frac{1}{T} - \frac{1}{T^\circ} \right) \right),$$

where $H(T)$ is the Henry's Law constant as a function of temperature, H° is the Henry's Law constant at 298.15 K, $\Delta_{sol}H$ is the enthalpy of dissolution, R is the ideal gas constant, T is the temperature, and T° is the reference temperature (Sander 2015). All constants and

enthalpies of dissolution are tabulated by Sander (2015). There is an assumption that the temperature ranges used in the thermodynamic calculations do not significantly affect the enthalpies of dissolution.

For photosynthetic growth, it was assumed that the electron acceptor to CO would be a cellular electron transport molecule, such as ubiquinone, used in anoxygenic photosynthetic microbes. A representative ratio (0.53) of oxidized to reduced ubiquinone was used for free energy calculations (Klamt et al. 2008). The standard free energy of oxidized/reduced ubiquinone was calculated from the couple's redox potential.

Results from the thermodynamic model are included in the results sections for each of the other objectives to show the plausibility of microbial use of CO under relevant conditions.

Results

CO-based growth on present-day Mars

The thermodynamics of CO oxidation by water without considering photosynthesis were investigated for a range of temperatures with both Earth-like and Mars-like conditions (Figure 5.1). For 'Earth' the conditions were: CO=0.2 ppm, $a_w=1$, CO₂=400 ppm, H₂=1 ppm, and total pressure=10⁵ Pa. For 'Mars' the conditions were CO=1000 ppm, $a_w=0.1$, CO₂=95%, H₂=15 ppm, and total pressure=10³ Pa, which approximate observations from on Mars (Krasnopolsky and Feldman 2001; Martín-Torres et al. 2015). The standard Gibbs free energy was calculated and plotted for comparison. The temperature range was chosen to represent the upper limit of temperatures that may be experienced on Mars today during the day near the equator. The calculated Gibbs free energies for Earth and Mars were similar for this reaction in this range of temperatures, though the Mars-like condition was

slightly more favorable than Earth. Thus, it is plausible that such a reaction could proceed for microbes and be coupled to microbial metabolism.

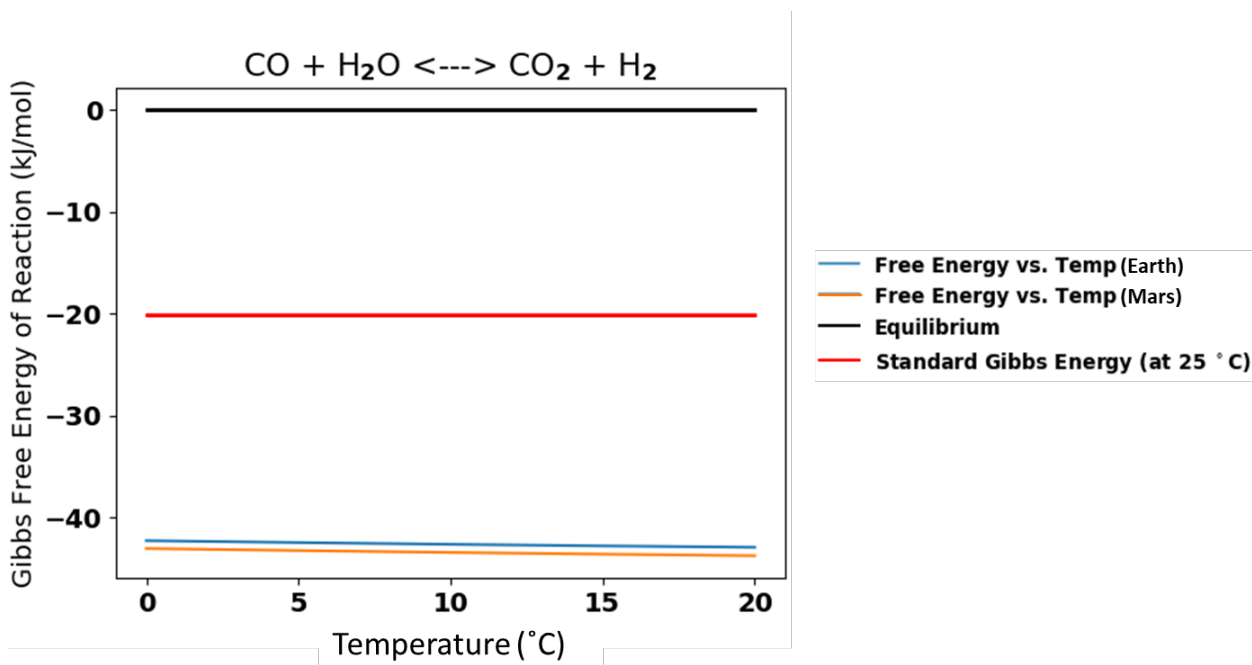


Figure 5.1. Calculations of Gibbs free energy for the reaction $\text{CO} + \text{H}_2\text{O} \leftrightarrow \text{CO}_2 + \text{H}_2$ for a range of temperatures. Water was considered a liquid while other components were calculated as gases dissolved in water. The black line denotes the free energy at equilibrium. The red line represents the standard Gibbs free energy for this reaction. The blue and orange lines represent the calculated Gibbs free energy for Earth- and Mars-like conditions, respectively (see text for details).

The key result from the long-term CO experiment suggested that CO could only be consumed by *A. ehrlichii* MLHE-1 in environments with sufficient water. Figure 5.2 summarizes this finding (additional results plots are shown in Appendix E). In the low-pressure bottles that were not rehydrated, the CO and CO₂ percentages were similar for the uninoculated controls and bottles with *A. ehrlichii* MLHE-1. By comparison, the low-pressure bottles that were rehydrated showed substantial differences in their gas compositions. Notably, the inoculated bottles did not have detectable amounts of CO (detection limit 0.06%) compared to the control bottles that had CO and CO₂ levels similar to the other low-pressure controls. While the CO₂ concentrations in the control and inoculated wet bottles were similar within error, the non-detectable CO in the bottles with

microbes strongly suggests that CO was consumed by the microbe. The relative lack of change in CO₂ could be to its dissolution in the water and/or additional incorporation into biomass given that *A. ehrlichii* MLHE-1 can survive as an autotroph in some situations (Hoeft et al. 2007). The lack of change could also be the result of only small amounts of starting CO available in the low-pressure headspace, which would result in smaller increases of CO₂ due to microbial oxidation.

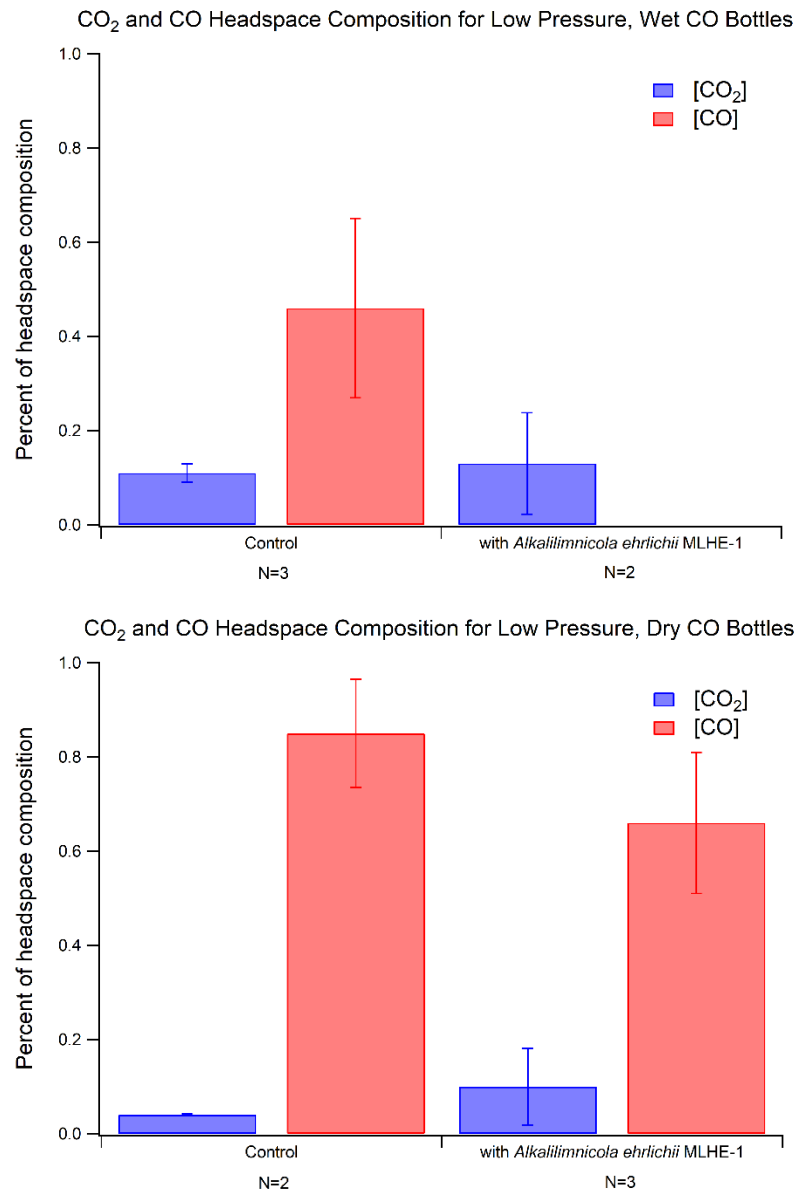


Figure 5.2. CO₂ (blue) and CO (red) percent of bottle headspaces of bottles incubated at low pressure with added water (top) or dry environments (bottom). Control bottles (no inoculated microbe) are shown on the left. Bottles with inoculated *A. ehrlichii* MLHE-1 are represented on the right. Error bars represent one standard deviation of measured percentages from bottle replicates.

The experiment investigating whether *A. ehrlichii* MLHE-1 could grow on CO has yielded inconclusive results. While growth with acetate and a CO-supplemented atmosphere did occur, growth seemed to be impeded in the CO atmospheres when acetate was present (Figure 5.3). When acetate was not available in the medium, no growth

appeared to occur in either headspace within the first few days as it had with acetate. However, after longer incubation (on the order of months), some growth in all bottles apparently occurred (Figure 5.4). Interestingly, the bottles with added CO were generally slightly more turbid than the bottles that did not have CO, possibly suggesting low amounts of CO-based growth in these conditions, particularly for bottles without shaking at ambient temperature (Figure E.4). The differences in turbidity and sample sizes were small, though, and the apparent growth increase in both headspaces could be due to slow growth on residual acetate from the third transfer.

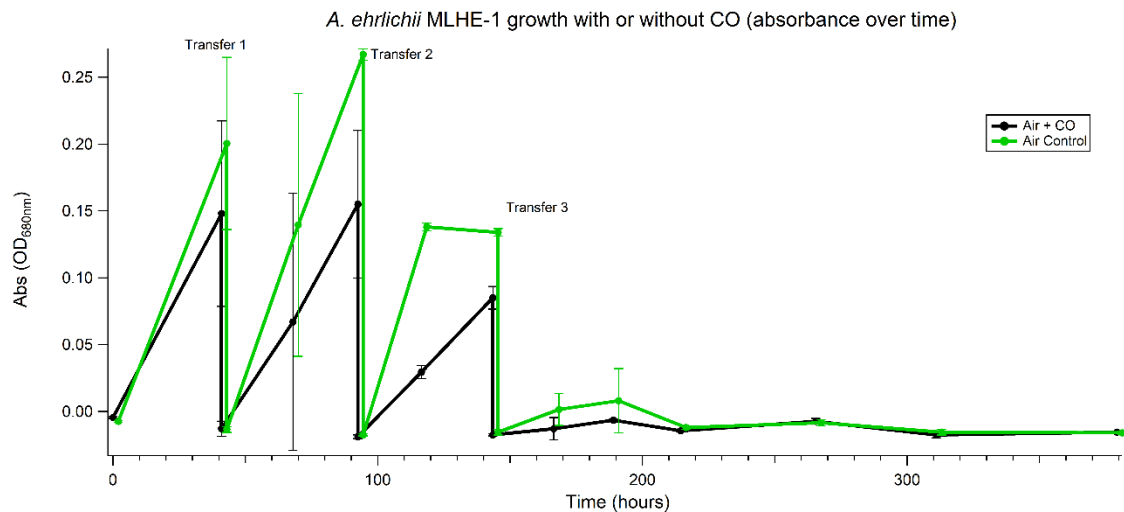


Figure 5.3. *A. ehrlichii* MLHE-1 growth over time with transfers from high acetate to no acetate for cultures grown in an incubator with shaking. Growth was measured by absorbance (680 nm). Transfer 1: 10 mM acetate to 5 mM acetate. Transfer 2: 5 mM acetate to 2.5 mM acetate. Transfer 3: 2.5 mM acetate to no acetate. Black line represents air plus added CO to headspace. Green line represents air headspace without CO. Each data point represents the mean of two measurements of separate samples, error bars are the standard deviation of those measurements.

A. ehrlichii MLHE-1 extended growth over time

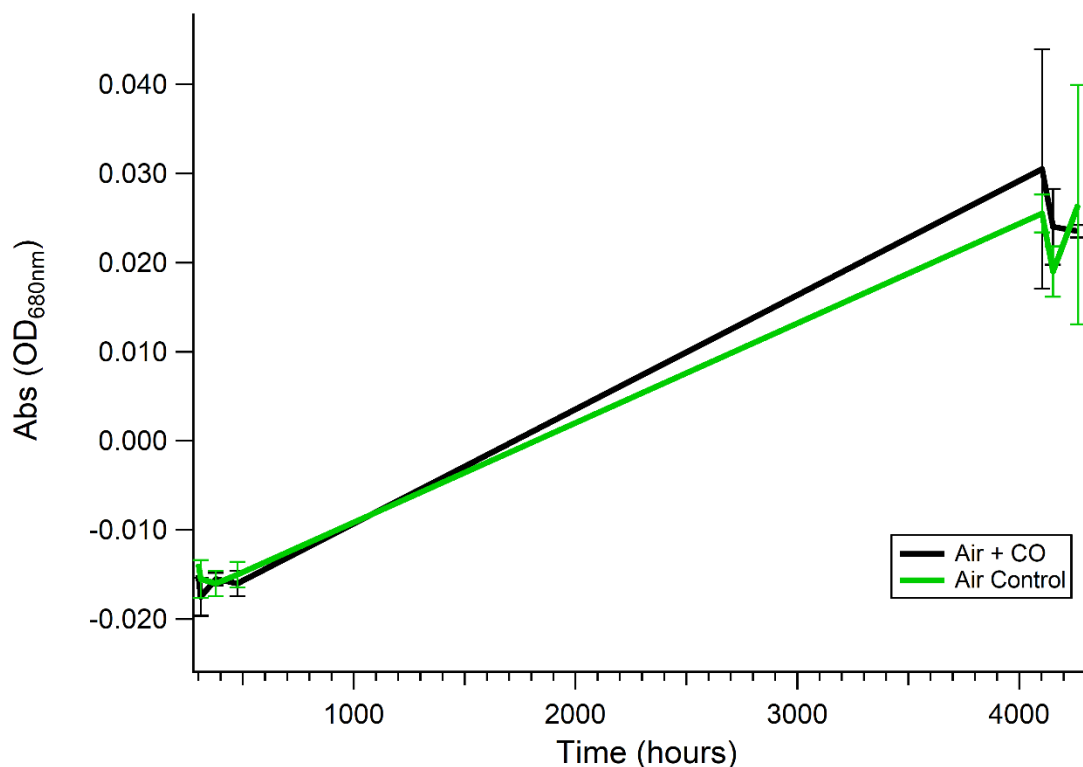


Figure 5.4. Extended growth of *A. ehrlichii* MLHE-1 in air or CO+air headspaces with no acetate added to the media. This plot shows the continuation of data collection from Figure 5.3, which was paused due to COVID-19. Note the change in scale of the y-axis.

CO-based anoxygenic photosynthesis

Like the non-photosynthetic case, the thermodynamics of anoxygenic photosynthesis were investigated under Earth-like and Mars-like environments (Figure 5.5). For ‘Earth’ the conditions were: CO=0.2 ppm, $a_w=1$, CO₂=400 ppm, and total pressure=10⁵ Pa. For ‘Mars’ the conditions were CO=1000 ppm, $a_w=0.1$, CO₂=95%, and total pressure=10³ Pa. Both Earth and Mars used an oxidized to reduced ubiquinone ratio of 0.53 for a hypothetical microbe. The oxidized ubiquinone would act as the electron acceptor for CO and could then shuttle the electrons into the process of cyclic photophosphorylation. The temperature range here was chosen to encompass the majority

of the range at which life can survive and represents a variety of environments that are found on Earth and were likely present on an ancient Mars. In both cases, the Gibbs free energy of reaction is less favorable than the standard Gibbs free energy. Further, while the reaction is favorable for both the Earth- and Mars-like cases, it is slightly less favorable in the Mars-like conditions. Still, the reaction should reasonably proceed to the right based on the thermodynamics in a range of conditions, suggesting that the direct use of electrons from CO in anoxygenic photosynthesis should be plausible.

Additionally, Figure 5.5 (right) shows the calculated Gibbs free energy over the same range of temperatures using succinate as the external electron donor to ubiquinone. Succinate is a known electron donor used in anoxygenic photosynthesis and acts as the primary source of electrons in DSMZ medium 27 used to cultivate *R. gelatinosus* here. Using the same ratio of reduced to oxidized ubiquinone and a ratio of 1 for fumarate to succinate, the Gibbs free energy of reaction was calculated to be slightly more negative than the CO-based reaction over the same range of temperatures. The standard Gibbs free energy for the succinate reaction was closer to equilibrium than the CO reaction.

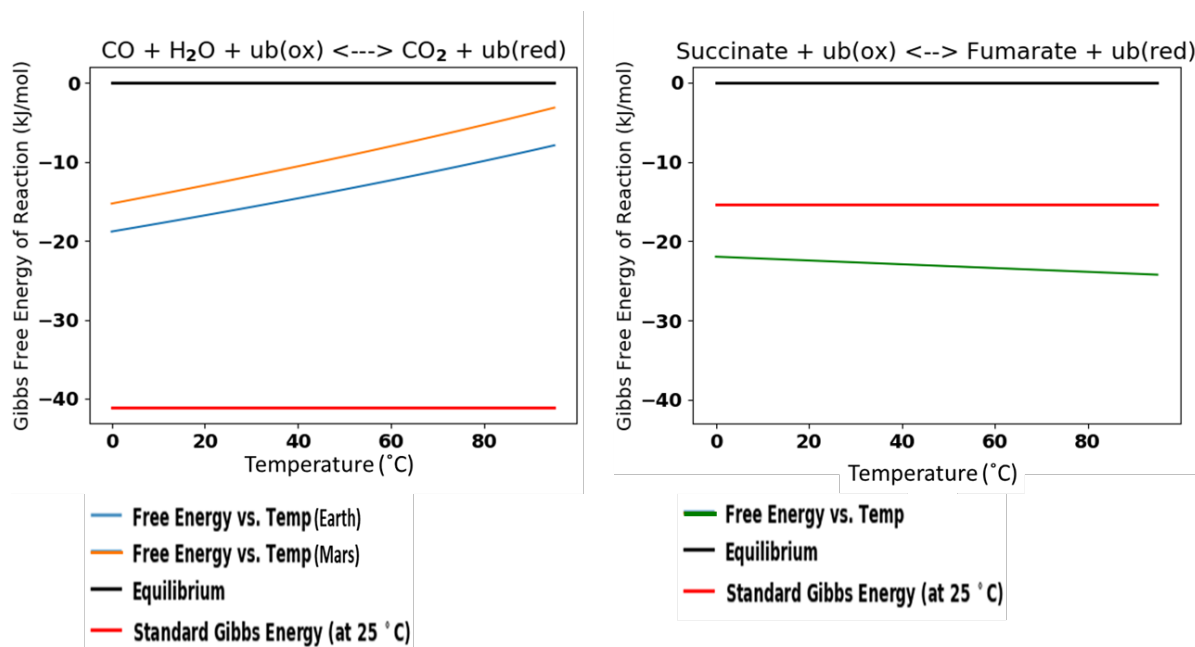


Figure 5.5. (Left) Calculations of the Gibbs free energy for the photosynthetic reaction $\text{CO} + \text{H}_2\text{O} + \text{ub(ox)} \leftrightarrow \text{CO}_2 + \text{ub(red)}$. Here, ub(ox) and ub(red) stand for oxidized and reduced ubiquinone, respectively, as representative redox-sensitive electron carriers involved in anoxygenic photosynthesis. Blue and orange lines represent the calculations for Earth- and Mars-like conditions, respectively (see text). (Right) Analogous free energy calculation for the reduction of ubiquinone by succinate. The green line is the calculated free energy of reaction versus temperature while the black line marks equilibrium and the red line represents the standard free energy. The same ratio of ub(red)/ub(ox) was used as in the CO reaction. A fumarate/succinate ratio of 1 was used following Klamt et al. (2008).

While the thermodynamics of CO donating its electrons to an anoxygenic photosynthetic electron carrier, such as ubiquinone, were promising, the growth experiments of *R. gelatinosus* did not support these calculations. When cultivated in the full DSMZ medium 27, *R. gelatinosus* successfully grew in the light in both 100% CO and 100% N₂ headspaces (Figure 5.6). The growth in the CO headspace may have been dampened relative to the N₂ headspace. No growth was observed in either headspace in the dark-incubated cultures. Interestingly, after an extended incubation period, the cultures grown in a CO headspace were qualitatively darker and redder compared to browner shades in the N₂ headspace cultures (Figure 5.7), possibly due to changes in pigment expression or enhanced lysis of pigmented cells in the CO environment. This color pattern was

frequently observed during prolonged incubations in CO. Cell counts were performed and the color change was not due to an apparent difference in cell density. Nitrogen-headspace bottles had an average cell density of $1.1 \times 10^7 \pm 1.4 \times 10^6$ cells/mL while the CO bottles had nearly a nearly identical average cell density of $1.0 \times 10^7 \pm 1.6 \times 10^6$ cells/mL.

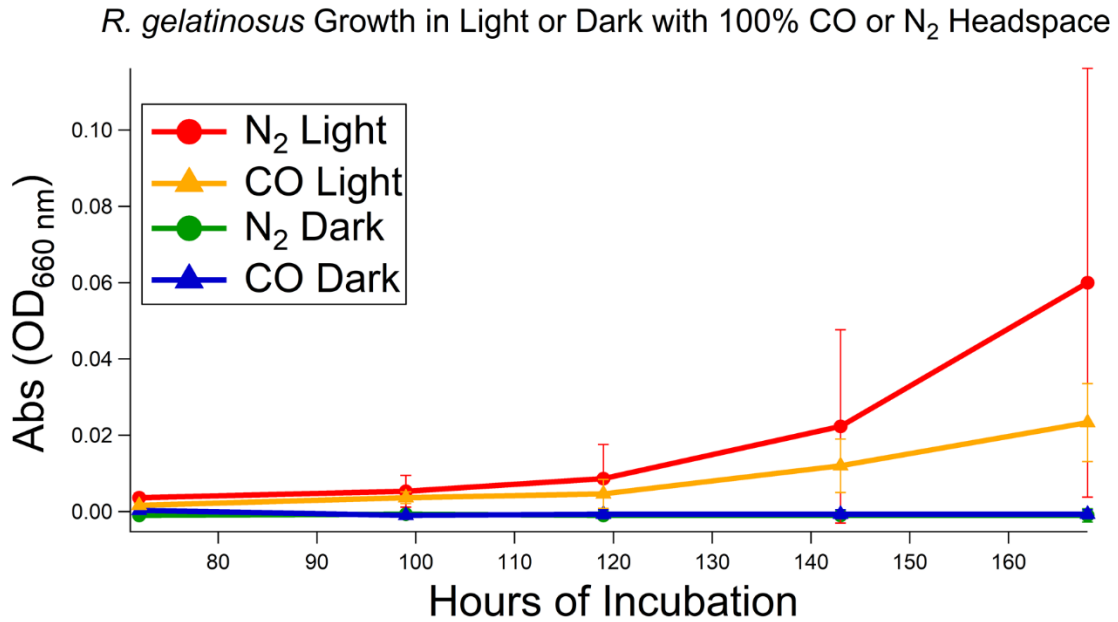


Figure 5.6. Measurements of absorbance (660 nm) over time for *R. gelatinosus* grown in 100% N₂ or CO headspace half a bar over pressure in the light or dark. Media was the full DSMZ medium 27 as described. Each data point represents three replicate measurements. Error bars represent one standard deviation.

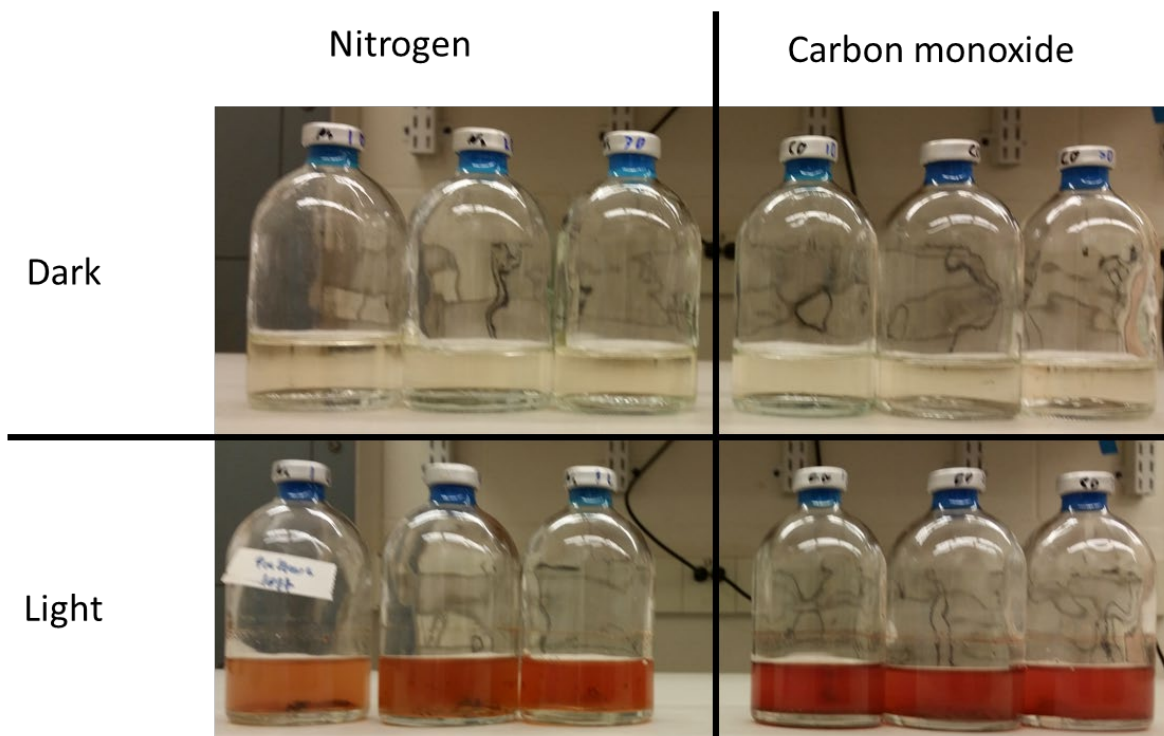


Figure 5.7. Images of *R. gelatinosus* cultures in full DSMZ medium 27 after more than two months of incubation in light or dark with 100% 1.5 bar N₂ or CO headspace.

Recognizing the other possible electron donors in the medium, succinate (the primary source of electrons and carbon in DSMZ medium 27) was removed and the experiment was repeated (Figure 5.8). A similar pattern was observed such that cultures cultivated in the dark did not exhibit visible growth, but both CO and N₂ headspaces allowed for growth. Again, after an extended period of incubation, the CO-exposed cultures were shown as darker and redder than their N₂-grown counterparts. However, while both headspaces initially allowed for cultures to grow at similar rates, it appears that after 220 hours of incubation, the CO-exposed cultures substantially slowed their growth compared to the N₂ cultures, which continued to grow.

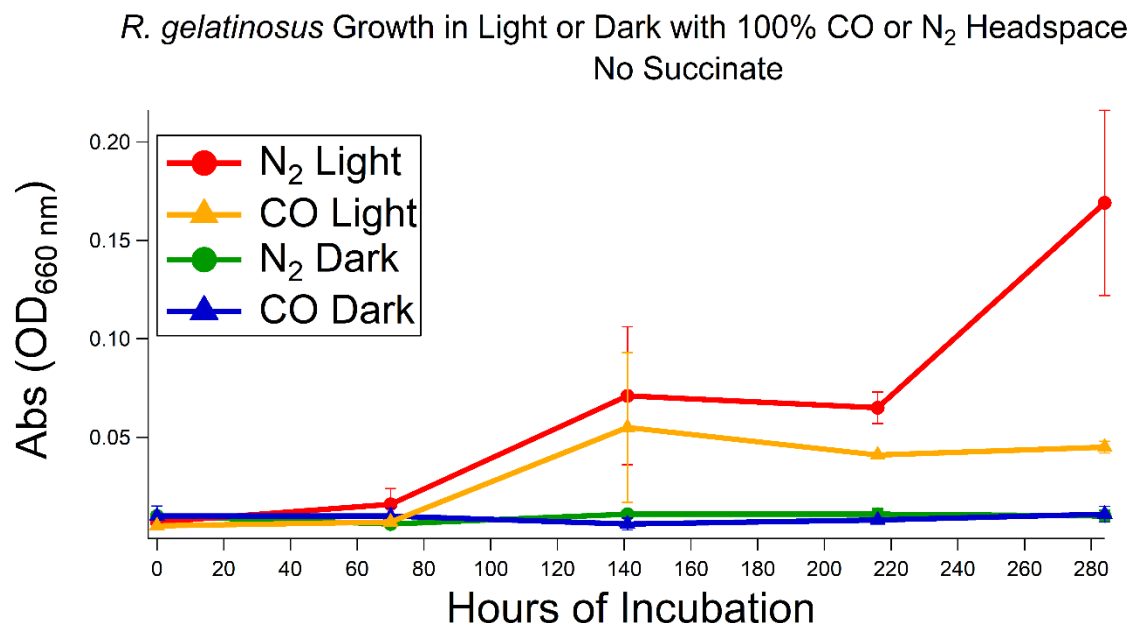


Figure 5.8. Measurements of absorbance (660 nm) over time for *R. gelatinosus* incubated in the light or dark with either 1.5 bar 100% N₂ or CO headspace. Medium was modified from DSMZ medium 27 to remove the succinate, though other organic acids (i.e. acetate and citrate) were still present. Each data point represents three replicate measurements. Error bars represent one standard deviation.

Subsequent growth experiments removed other possible electron (i.e. acetate and citrate) donors from the modified DSMZ medium 27. Growth occurred in bottles with yeast extract, but no growth was observed once yeast extract was removed. It is possible that the yeast extract provided the necessary carbon and nitrogen for *R. gelatinosus* to grow. In the yeast extract-containing medium, cysteinium chloride was still present as a reducing agent, which could have been used as the source of photosynthetic reducing power. Thus, another experiment was performed to test if *R. gelatinosus* could grow photosynthetically in the minimal medium with small amounts of yeast extract while varying cysteinium chloride.

The results of the experiment testing whether cysteinium chloride acts as an electron donor are presented in Figure 5.9. This experiment removed all organic acids from the medium, reduced the amount of yeast extract to 10% of the nominal, and used either the nominal amount (high), 20% (low), or 1% (trace) of cysteinium chloride. Three bottles

each of high, low and trace media were filled with N₂ (labeled NH, NL, and NT, respectively) and an additional three bottles of trace media were filled with CO (labeled CO) at half a bar over pressure. Figure 5.9 shows the spectrophotometer measurements and cell counts of *R. gelatinosus* over time in the different conditions. Interestingly, the bottles with 20% of the nominal amount cysteinium chloride and nitrogen headspace (NL) showed the most robust growth from absorbance measurements while the other bottles had comparable absorbance measurements for the first ~25 days. The first two cell count measurements largely supported this observation, though the both the NH and NL bottles exhibited more growth than either the NT or CO bottles. However, after around Day 30, the spectrophotometer measurements of the NH bottles caught up with those of the NL bottles. Cell counts showed the continued proliferation of *R. gelatinosus* in the NH bottles, but a decline in the NL bottles. No significant growth was observed in either the NT or CO bottles by counts or spectrophotometer measurements. Combined, this experiment indicated that CO was not supporting growth. However, the cysteinium chloride may have been serving as a source of reducing power.

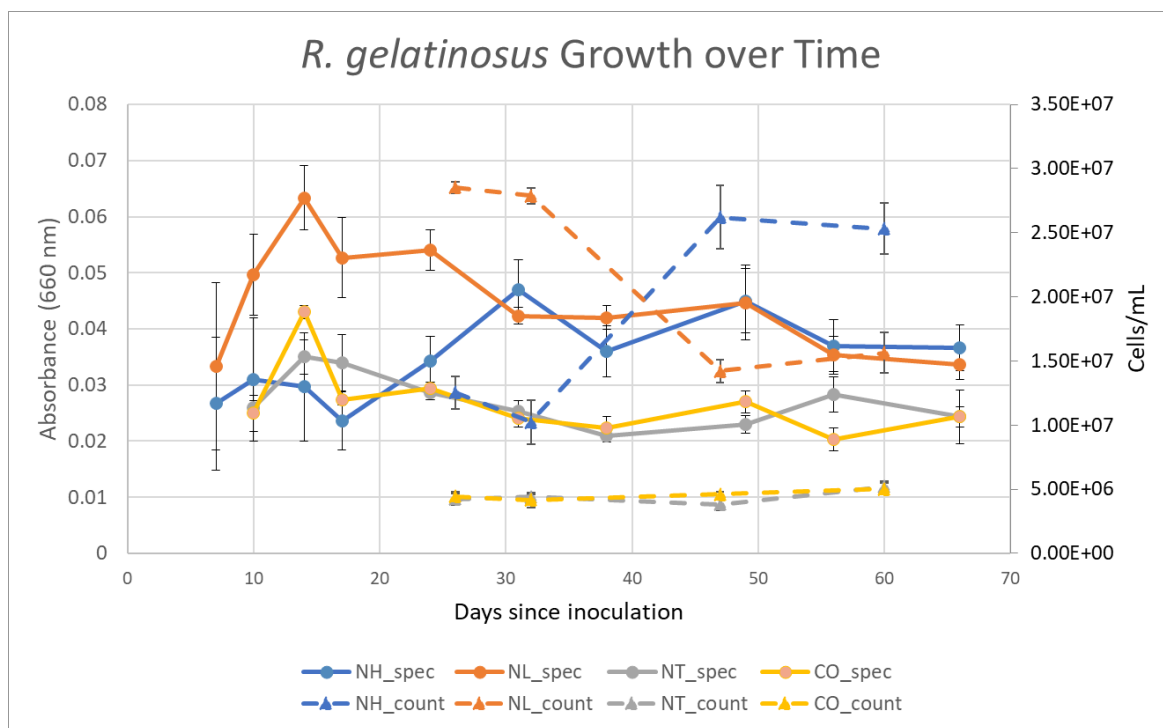


Figure 5.9. Absorbance measurements and cell counts of *R. gelatinosus* grown in light with modified medium 27 with variable reducing agent, cysteinium chloride. Each point represents the average of measurements from three bottles. Error bars represent one standard error. Gas headspaces were either nitrogen or CO at half a bar over pressure. NH=nitrogen headspace, 100% nominal cysteinium chloride; NL= nitrogen headspace, 20% nominal cysteinium chloride; NT= nitrogen headspace, 1% nominal cysteinium chloride; CO= CO headspace, 1% nominal cysteinium chloride. Solid lines represent measurements with the spectrophotometer (left axis) and dashed lines represent cell counts converted to cells/mL media (right axis).

Discussion

The results of this work largely suggest that while CO remains a promising energy source for microbes, its practical use on Mars in the past or present may be limited. The thermodynamics of CO use for both photosynthetic and non-photosynthetic reactions suggest that CO oxidation is favorable under a variety of environmental conditions. Experiments looking at the use of CO by *A. ehrlichii* MLHE-1 showed mixed results. While CO consumption appeared to occur in low pressure environments with some water present, the experiments with desiccated conditions with *A. ehrlichii* MLHE-1 maintained a similar headspace composition as the comparable control. These results indicate that

water activity can be a critical factor limiting microbial use of CO. Given that Mars today is extremely dry (<10 precipitable μm as opposed to cm on Earth (Harri et al. 2014)), a microbe like *A. ehrlichii* MLHE-1 would probably not be able to survive on a CO-based metabolism even if it were able to withstand or be protected from the other environmental stressors, such as UV radiation, which is a major biocidal environmental factor (Khodadad et al. 2017). Furthermore, experiments with acetate indicated that *A. ehrlichii* MLHE-1 could rapidly grow even with limited amounts of acetate. However, little to no growth was observed when acetate was removed entirely, leaving CO as the only possible electron donor. These results indicate that *A. ehrlichii* MLHE-1 would not be able to proliferate with CO as its only electron source even when water was available as a brine. Small amounts of growth may have occurred over several months of incubation in both CO-containing and air-only bottles (Figure 5.4). One explanation for this increase in biomass is that the microbes grew on residual acetate from the transfer from the 2.5 mM acetate bottles. This would explain the growth in both headspaces. However, the trend of CO-containing headspace microbes lagging behind the air-only headspaces did not continue for these extended growth measurements. Rather, the CO-containing bottles showed the same or perhaps slightly increased growth compared to the air controls. It is possible that this difference in growth could be due the presence of CO – biomass may have been from direct CO oxidation or through the use of dissolved CO in the form of formate. Additional long-term growth experiments would need to be conducted to confirm the use of CO for growth. In such a case, a more hospitable ancient Martian environment may have been able to support CO-based microbial growth.

CO-based photosynthesis, on the other hand, may be even more difficult in a Martian environment based on the CO-oxidizing, anoxygenic photosynthetic microbe *R. gelatinosus*. Growth experiments found that CO tended to substantially diminish growth of the microbe in otherwise ideal conditions with a suite of carbon and electron sources in the light compared to cultures grown in nitrogen. No growth was observed in the dark, in contrast to similar experiments reported previously where *R. gelatinosus* grew on CO (Uffen 1983). In more minimal medium experiments, it was found that *R. gelatinosus* did not grow without yeast extract regardless of headspace composition. While the type strain of *R. gelatinosus* was unsuccessful at growing photosynthetically with CO as an electron donor, perhaps other strains or similar species would be able to conduct this type of metabolism. Based on genomic studies, the strain *R. gelatinosus* CBS may be able to transfer the electrons from CO to form H₂ to then be shuttled to photosynthetic electron carriers (Wawrousek et al. 2014). However, the direct use of CO as a source of reducing power to drive photosynthetic production of reduced carbon does not appear to be present in the microbes tested here.

Conclusion

Carbon monoxide remains an interesting gas in astrobiology because of various Earth microbes' known abilities to metabolize CO. The presence of CO in planetary atmospheres allows for its potential use in those environments by known microbial processes. The thermodynamics of CO oxidation largely show that these types of reactions are thermodynamically favorable, which means they could be readily coupled to microbe's energy requirements. When tested in the laboratory, however, extremely dry conditions appear to present a barrier to CO oxidation by *A. ehrlichii* MLHE-1, even if it can survive

other environmental stressors. Further, the direct use of CO as a source of reducing power in anoxygenic photosynthesis by *R. gelatinosus* presents a separate challenge as an alternative CO-based metabolism in the lab. Taken together, the results presented here suggest that entirely CO-based survival and growth present challenges to microbes under even select Mars-like conditions. Especially under the multiple extreme environmental conditions on present-day Mars (freezing temperatures, desiccated, hypobaric, irradiated), it is unlikely that CO could realistically support microbial growth or survival in spite of its thermodynamic promise.

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Chapter 6: Dissertation Summary and Future Directions

This dissertation investigated the presence, distribution, and viability of potential electron donors on Mars that could support a putative microbiology in the past or present. Chapter 2 established a novel analysis of EGA data from SAM to identify samples on Mars that are likely to contain reduced sulfur. This chapter used quadratic discriminant analysis to statistically compare a suite of evolved gases during SAM analysis to laboratory analogue mixtures. It was found that reduced sulfur was likely present in several samples analyzed throughout Gale crater, suggestive of a long, variable history of reducing conditions that could have supported a sulfide-based microbial metabolism if life existed.

Chapter 3 expanded upon the work of Chapter 2 and combined multiple analyses of SAM EGA data to identify samples in the ‘clay-bearing’ Glen Torridon region of Gale crater. Using evolution temperatures of SO₂ peaks, quadratic discriminant analysis, and sulfur isotopes from EGA data, it was found that there was general agreement across these complementary methods in suggesting presence of reduced sulfur or not. These analyses indicated reduced sulfur in two samples in the Glen Torridon region and nearby rocks. The results of this chapter continue the findings that reduced sulfur has been available in the past at Gale crater and may be derived from alteration processes.

Chapter 4 investigated methane and chloromethane that are observed during the thermal decomposition of Martian samples. Possible reactions resulting in the formation and equilibration of these gases were investigated. Additionally, it was found that methane and chloromethane can have carbon isotope depletions relative to a precursor organic carbon from reactions during pyrolysis. These reactions and their isotopic effects should

be considered if the carbon isotopes are measured on Mars to understand the nature of the precursor carbon.

Chapter 5 looked at the possible use of carbon monoxide as an electron donor on Mars. Thermodynamic calculations indicated that CO-based metabolisms should be favorable in Martian conditions. However, laboratory work did not support this finding. *Alkalilimnicola ehrlichii* MLHE-1, which has previously been reported to be able to oxidize CO under Mars-relevant conditions, did not oxidize CO in hypobaric conditions without water. Similarly, no CO-based growth was observed. Further, *Rubrivivax gelatinosus*, a known CO-oxidizer and anoxygenic photosynthetic microbe, did not use CO as an external electron donor in photosynthesis. Taken together, while CO-based metabolisms on Mars are thermodynamically favorable and could, in principle, occur in a putative microbe, these two promising Earth microbes would likely not be able to use the abundant electron donor on Mars.

Overall, this dissertation has found electron donors in the form of reduced sulfur on Mars, supported the future interpretation of reduced carbon from Martian solid samples, and assessed the potential viability of CO as a chemical energy source on Mars. This work will aid the continued assessment of Martian habitability. The Curiosity rover is continuing its ascent of Mt. Sharp to determine the habitability of Gale crater through time and major ancient environmental changes. The MSL mission will also continue its monitoring of the modern environment. In this exciting time of Martian exploration, the work performed for this dissertation will improve future interpretations of habitability on Mars in the past or present with the availability of native sulfide and/or reduced carbon as sources of chemical energy.

Appendix A: The Mars Science Laboratory (MSL) Goals, Instruments, and Landing Site

The Mars Science Laboratory (MSL) Goals and Instruments

The Mars Science Laboratory (MSL) builds upon previous explorations of Mars that have ‘followed the water.’ The main goal of the MSL mission is to assess the environments of Mars for habitability in the past and present. Habitability is defined here as an environment that contained liquid water, a source of carbon, a source of energy, and the elemental building blocks of life (CHNOPS). Additionally, MSL aims to study the geology of its landing area, understand Martian processes affecting habitability, and record the levels of radiation in preparation of human exploration (Grotzinger et al. 2012). In support of these varied goals, MSL carried a wide array of instruments on board the rover, Curiosity. The function of the rover and its instruments are summarized below with information from Grotzinger et al. (2012).

The Curiosity rover was designed to support MSL goals in exploring Mars as a robotic geologist. To that end, the rover is able to drive to targets of interest through instructions provided from Earth through either direct communication from the rover’s high gain antenna or through relays with the Mars Odyssey and Mars Reconnaissance Orbiters. The rover’s mobility system is capable of driving up to 4 cm/s and can drive up to 200 m in a single sol, though most drives are much shorter. The rover is equipped with several cameras to help with navigation and hazard avoidance. On its mast is a camera, Mastcam, which provides a ‘geologist’s’ view of the landscape. The rover also contains an arm equipped with the Sample Acquisition, Processing, and Handling (SA/SPaH) system, which allows for the rover to interact with Martian materials. Included in the SA/SPaH system are a drill used to sample rocks (drill holes are 1.6 cm in diameter and up to 5 cm

deep), a dust removal tool (DRT) to clear Martian bedrock of dust, and a scoop to sample sand. The SA/SPaH system can deliver these solid samples to the analytical instruments inside the rover (discussed below) for further characterization. All rover and instrument processes are powered by a Multi-Mission Radioisotope Thermoelectric Generator (MMRTG). Such an energy source negates any reliance on solar panels (as was the case for previous rovers), though the mission will inevitably be limited by the radioactive decay of the MMRTG's plutonium.

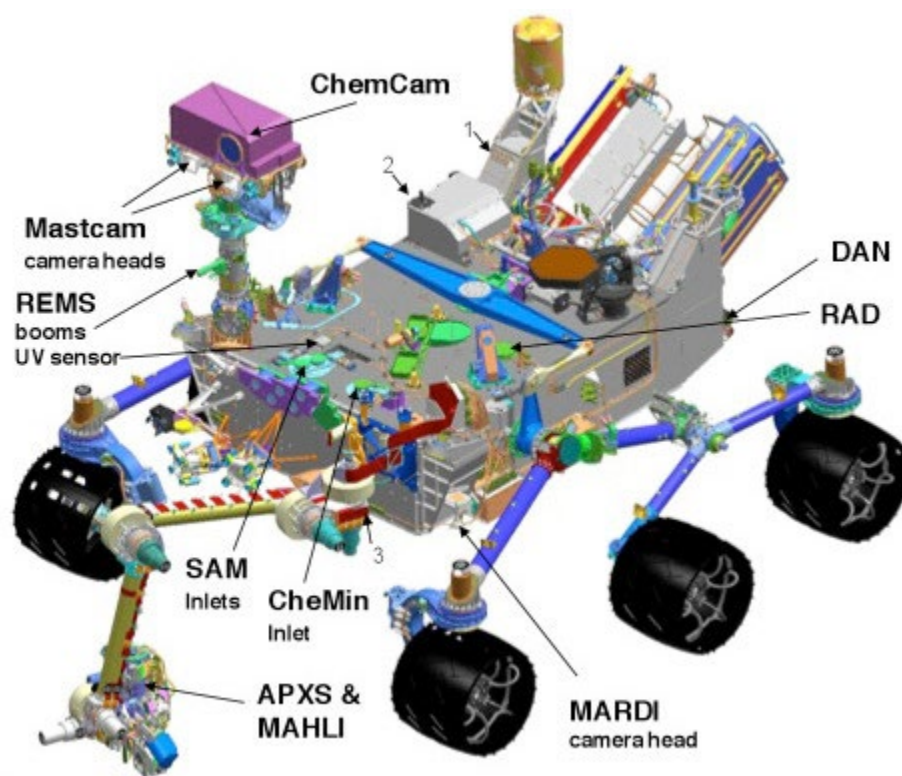


Figure A.1. Diagram showing the placement of MSL instruments on the Curiosity rover. SAM and CheMin are internally located in the body of the rover. Courtesy NASA/JPL-Caltech.

MSL's scientific payload is comprised of ten different instruments that can be classified in four categories: remote, environmental, contact, and analytical (Figure A.1). The remote science instruments are located at the top of the rover's mast and include Mastcam and ChemCam. Mastcam, standing at 1.97 m above the bottom of the wheels,

includes two high resolution cameras that can produce stereoscopic images to provide 3D geologist's point-of-view images. ChemCam uses laser-induced breakdown spectroscopy (LIBS) to determine the elemental composition of small areas around the rover. ChemCam also includes the Remote Micro-Imager (RMI), which is a telescopic lens that can provide geological context for the LIBS spots.

There are four instruments included in the environmental group: the Dynamic Albedo of Neutrons (DAN), the Radiation Assessment Detector (RAD), the Rover Environmental Monitoring System (REMS) and the Mars Descent Imager (MARDI). These instruments largely support the MSL goal of preparing for human exploration. DAN measures H- and OH-bearing (suggesting water) materials up to ~1 m beneath the surface. RAD measures radiation particles from galactic cosmic radiation, solar energetic particles, and secondary neutrons in the atmosphere and regolith. REMS keeps measurements of Martian weather with records of wind speed/direction, air/ground temperature, UV radiation, relative humidity, and atmospheric pressure. Finally, MARDI is a fixed camera whose primary purpose was to record the rover's descent from orbit to the ground and, since landing, regularly records images of the ground directly beneath the rover.

The two contact science instruments, the Alpha Particle X-ray Spectrometer (APXS) and the Mars Hand Lens Imager (MAHLI), are found at the end of the rover's arm. APXS is a heritage instrument technique that also found use on the Mars Exploration Rovers, Spirit and Opportunity. This instrument provides elemental composition with high sensitivity and low detection limits, especially for salt-forming elements. MAHLI is a high-resolution camera that provides close-up, detailed images of samples of interest. MAHLI can be used determine grain size of sedimentary rocks down to sandstones and provide

useful local geological context for other measurements. Together, MAHLI and APXS provide detailed geological and elemental information for samples sent to the analytical instruments.

The analytical instruments on board the Curiosity rover are Chemistry and Mineralogy (CheMin) and the Sample Analysis at Mars (SAM) (Blake et al. 2012; Mahaffy et al. 2012). Both instruments are located within the body of the rover and are delivered solid sample from the SA/SPaH system. CheMin is an x-ray diffractometer that is designed to assess the crystalline mineralogy of Martian solid samples. CheMin is able to detect crystalline minerals in abundance above ~1 wt.% of the delivered sample. It is unable to detect composition of amorphous or trace species, so other instruments, such as CheCam, APXS, and SAM are used to further infer sample composition. SAM is an instrument suite that is dedicated to assessing sample habitability. SAM is comprised of a pyrolysis oven, mass spectrometer, gas chromatograph, and tunable laser spectrometer. Together, these instruments can detect trace volatiles (such as organic molecules or those derived from the decomposition of solid samples) to assess samples for habitability. Isotopes of certain elements (C, O, H, S, Cl) can also be determined using SAM's instruments. Much of this dissertation focuses on work performed with the Sample Analysis at Mars. Details on SAM's capabilities are therefore discussed below.

The SAM instrument suite is capable of three types of analysis: evolved gas analysis-mass spectrometry (EGA-MS or EGA), gas chromatography-mass spectrometer (GCMS), and tunable laser spectrometry (TLS). Together, these techniques can inform us about both solid sample and atmospheric composition, isotope ratios, and possible biosignatures.

EGA is the ‘bread and butter’ of SAM, with most solid samples on Mars being analyzed with this method (Mahaffy et al. 2012; Leshin et al. 2013; Ming et al. 2014; Sutter et al. 2017; Stern et al. 2018; McAdam et al. 2020). The principle of EGA is simple: a solid sample is heated in a pyrolysis oven and sample components decompose at characteristic temperatures with volatiles being carried to the mass spectrometer for identification. On SAM, solid samples are heated to approximately 850°C at ~35°C/min under 30 mbar He flow. Compounds in the sample will thermally decompose at characteristic temperatures and release volatiles (e.g. calcium carbonate, CaCO_3 , will release CO_2 around 700°C or melanterite, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, will release SO_2 around 600°C under these conditions). The released gases are carried to SAM’s quadrupole mass spectrometer (QMS) by a constant He flow during EGA. The QMS scans for mass-to-charge (m/z) ratios from m/z 2 to m/z 535 for each sample. From the QMS data and modeling of the temperature programs, pyrograms of intensity versus temperature can be generated for each detected m/z . Importantly, complex sample mixtures and oven reactions can complicate the interpretation of EGA data. For example, decomposition temperatures can change with different mixtures of minerals or different volatiles can react with each other or the sample to form unexpected products. Therefore, extensive laboratory studies are required to provide more complete interpretations of EGA data. Furthermore, isotope ratios of certain elements can be calculated during EGA. For example, m/z 64 and m/z 66 represent $^{32}\text{SO}_2$ and $^{34}\text{SO}_2$, respectively. Based on these evolved masses in a single peak at a given temperature, a sulfur isotope ratio can be calculated for a specific compound, which can inform about the redox state and geological history of a sample (Franz et al. 2017; Franz et al. 2020).

GCMS is another analytical capability of SAM whose main purpose is to identify organic molecules. GCMS is among the most sensitive techniques used in chemical identification and characterization in planetary science and was originally used on Mars by the Viking landers. Like EGA, the principle of GCMS is straightforward: volatile compounds are sent through a chromatography column that separates them based on their chemical characteristics and they are sent to a mass spectrometer. Molecules are then identified based on both their mass spectra (m/z of their fragments after ionization from the mass spectrometer) and retention time (time it took for the molecule to go through the chromatography column). On SAM, gases evolved during EGA can be trapped during specific temperature ‘cuts’ of interest based on an initial EGA run. Trapped volatiles are then released for separation and identification by GCMS. Like EGA, compounds can react during heating and complicate data interpretations, so laboratory analogue studies are performed for improved data comparisons.

Additionally, two sets of experiments were loaded onto SAM to aid in the identification of organic molecules during GCMS (Mahaffy et al. 2012). These are referred to as the SAM ‘wet chemistry’ experiments and focus on exposing solid samples to one of two derivatization agents – either *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide in dimethyl formamide (MTBSTFA-DMF) or tetramethylammonium hydroxide (TMAH). Both derivatization agents react with organic compounds to make them more volatile to allow for greater chance of detection during GCMS. MTBSTFA reacts with hydroxyl groups to form more volatile silylated compounds. TMAH reacts especially well with fatty acids to increase their volatility and likelihood of detection. Unfortunately, at some point prior to the MSL landing, one of the cups containing MTBSTFA leaked into the SAM

Sample Manipulation System (SMS). Due to this leak, all samples are exposed to small amounts of MTBSTFA, which further complicates analysis of both EGA and GCMS (Glavin et al. 2013). The amount of MTBSTFA varies for each sample and is a function of the amount of sample on which the MTBSTFA can adsorb and length of time between sample collection and analysis. Byproducts of reactions between MTBSTFA and the sample and fragments from MTBSTFA must be taken into account for all EGA and GCMS analyses.

Finally, the TLS allows for high precision measurements of specific gases and isotopes from atmospheric sampling or from gases released during EGA. The TLS uses specific, narrow infrared laser frequencies to allow highly sensitive detection of specific gases, chiefly CO₂, CH₄, and H₂O from either the atmosphere or gases from temperature cuts during EGA (Mahaffy et al. 2012). The isotopes of C, H, and O can also be detected with high precision from these gases, provided the gases are abundant enough for confident isotope detection. The TLS has been used for determining isotope values of atmospheric CO₂ through direct sampling, with results pointing toward atmospheric loss (Webster et al. 2013). The TLS has also been widely reported for its detections of trace amounts of methane in the atmosphere that varies with seasonality (Webster et al. 2015; Webster et al. 2018). Additional measurements are often made during EGA runs of solid samples, during which evolved gases are sent to the TLS Herriott cell for analysis over specified temperatures (Franz et al. 2020). Such gases and isotope ratios can inform about the nature of carbon (e.g. organic vs. inorganic, redox state) and/or environmental conditions under which phyllosilicates formed. In both atmospheric and EGA runs, the TLS data can be compared to results derived from QMS data.

MSL Landing Site: Gale Crater, Mars

Site selection for MSL focused on four primary criteria to best serve the mission's objectives: diversity, context, habitability, and preservation. The mission necessitated diverse geology to explore with the rove. That is, a site had to have diverse geomorphology, mineralogy, and stratigraphy based on what could be observed from orbit. Such as site also needed to be able to be placed into a regional context in which local results could be extrapolated regionally or even globally. In terms of habitability, orbital studies of the selected site needed to demonstrate evidence of past liquid water. Finally, the site needed to show a history of preservation as is the case for long sequences of sedimentary rocks, which can preserve environmental history stratigraphically during deposition.

Gale crater was ultimately chosen for exploration by MSL based on the above criteria. Gale crater is ~155 km diameter impact crater of Hesperian age near the Martian crustal dichotomy (Milliken, Grotzinger, and Thomson 2010). In the middle of Gale crater is a mountain, informally known as Mt. Sharp, that consists of ~ 5 km of sedimentary strata recording the environmental and geologic history of Mars (Wray 2013). From orbit, several potential scientific targets were identified. Among these targets were high thermal inertia rocks (consistent with well-cemented sedimentary rocks), geomorphology consistent with past liquid water (such as alluvial fans), and a ridge with spectrally-identified elevated hematite (suggestive of a redox boundary) (Fraeman et al. 2016; Grotzinger et al. 2012). To the south of the hematite ridge is a region with elevated levels of clay minerals, which could provide enhanced preservation of organic molecules (Keil and Mayer 2013). Just stratigraphically above the clay-bearing unit is a sulfate-bearing unit. The transition from clays to sulfates is thought to represent a major environmental change in Martian geological

history from the more neutral/alkaline waters a more acidic time period (Grotzinger et al. 2012). Some studies have also suggested that sulfates can provide enhanced preservation of organics, though thermal decomposition of sulfates and organics together can complicate interpretations (Lewis et al. 2015; Francois et al. 2016). Stratigraphically above the sulfate unit is an unconformity that represents younger strata of anhydrous iron oxides. Strata above the sulfate unit are presently beyond the mission concept. At the time of writing (Fall 2020), the Curiosity rover is finishing its exploration of the clay-bearing unit and will soon begin heading toward the sulfate unit. The rover's traverse and drill holes through July 2020 are shown in Figure A.2 (courtesy NASA/JPL-Caltech/MSSS).

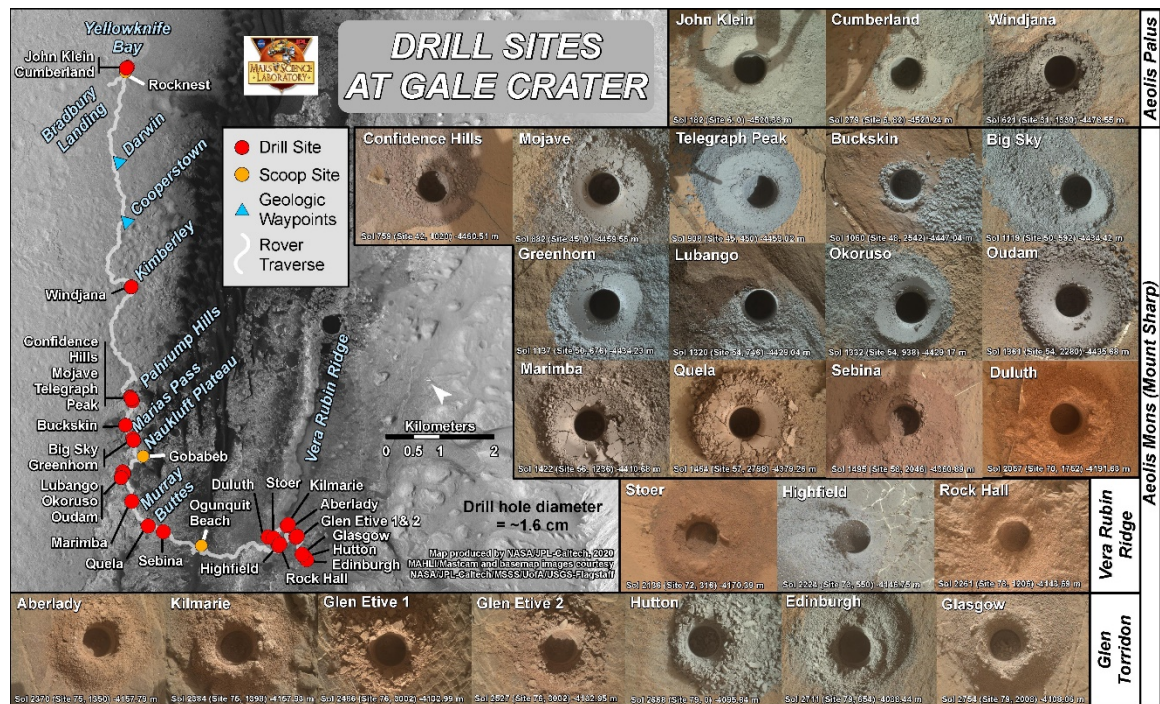


Figure A.2. Compilation of drill holes along Curiosity's traverse in Gale crater, Mars. Total drive distance as of sol 2829=23.06 km. Each drill hole has a diameter of ~1.6 cm. Courtesy NASA/JPL-Caltech/MSSS.

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Appendix B: Supplementary Information for Chapter 2

This appendix for Chapter 2 is reproduced and reformatted from the Supporting Information for the Wong et al. (2020) article “Detection of reduced sulfur on Vera Rubin ridge by quadratic discriminant analysis of volatiles observed during evolved gas analysis” published in the Journal of Geophysical Research: Planets. Reproduced with permission from John Wiley and Sons.

Introduction

The figures in the supporting information below provide additional and more detailed information about the main text. Figure B.1 is a general stratigraphic column of the area explore at Gale crater with labeled drill holes. Figure B.2a-g provides an alternative view of the information in Figure 2.1 of the main text, where the five EGA volatiles in each sample are directly compared to each other (i.e. Figure 2.1f). Figure B.3 shows additional example laboratory EGA profiles. Figure B.4 presents an alternative test (GCMS) that supports the conclusion drawn from EGA in Figure 2.2 of the main text that H₂S is not necessarily a good discriminator of sulfur redox. Figure B.5 shows the integrated counts of CO₂, COS, and CS₂ compared to the moles of carbon added to different sulfur compounds. Figure B.6 shows the results of data validation runs for the quadratic discriminant analysis. Figure B.7 shows a more detailed view of Figure 2.3 in the main text with a focus on the relevant Mars samples. Figure B.8a-j shows the same data presented in main text Figure 2.3, but in 2D, pairwise scatter plots for a better understanding of the Spearman correlation trends and details of particular samples.

Table B.1 shows the integrated counts of different m/z ratios for main EGA peaks of different sulfur compounds that support m/z 64 being due to SO₂.

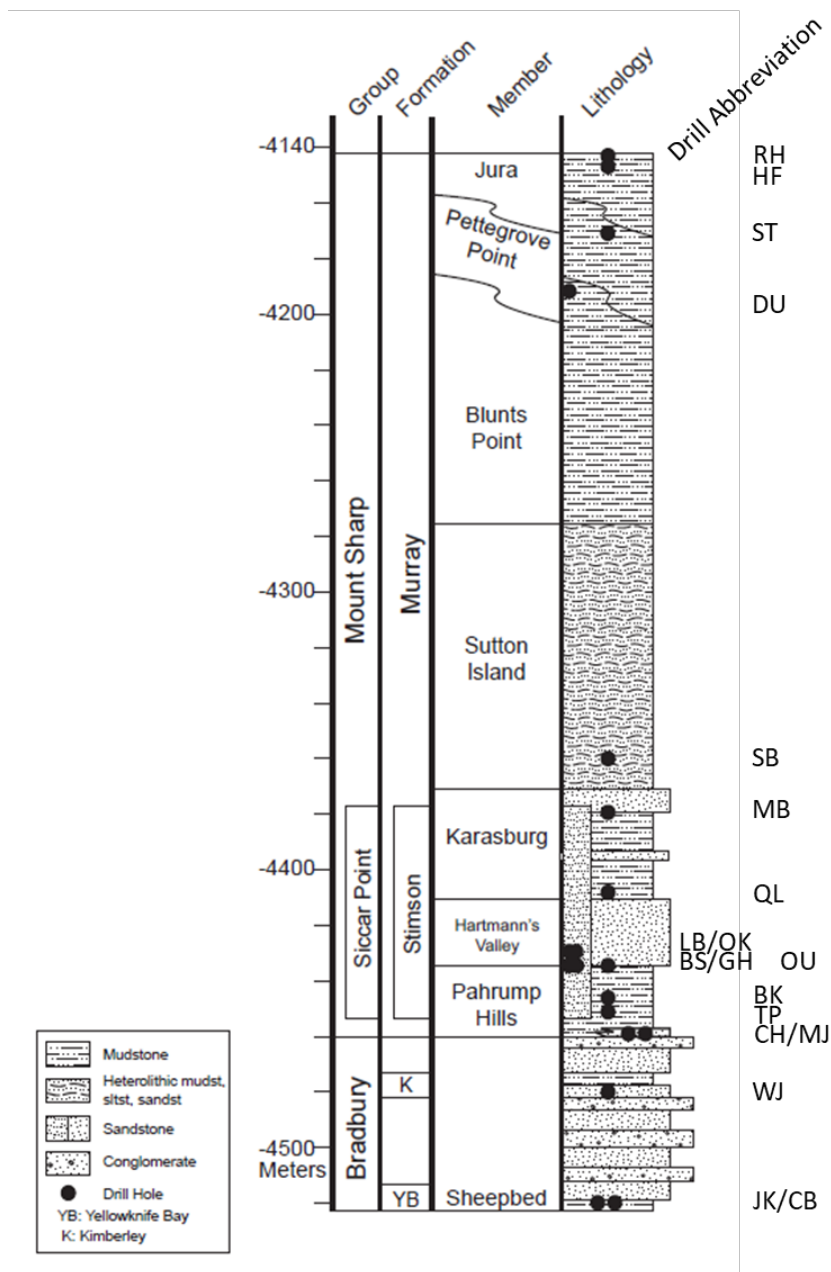


Figure B.1. This is a general stratigraphic column based on Curiosity's exploration of Gale crater through the VRR campaign. Drill holes are marked with black circles and each hole's sample name abbreviation is to the right. Each drill hole was analyzed by SAM and CheMin with the exceptions of LB, OK, and SB, which were only analyzed by CheMin. JK: John Klein. CB: Cumberland. WJ: Windjana. CH: Confidence Hills. MJ: Mojave. TP: Telegraph Peak. BK: Buckskin. BS: Big Sky. GH: Greenhorn. OU: Oudam. LB: Lubango. OK: Okoruso. QL: Quela. MB: Marimba. SB: Sebina. DU: Duluth. ST: Stoer. HF: Highfield. RH: Rock Hall. Not shown are scooped samples: RN (Rocknest), GB (Gobabeb), and OG (Ogunquit Beach). (Credit: MSL Sed-Strat Working Group).

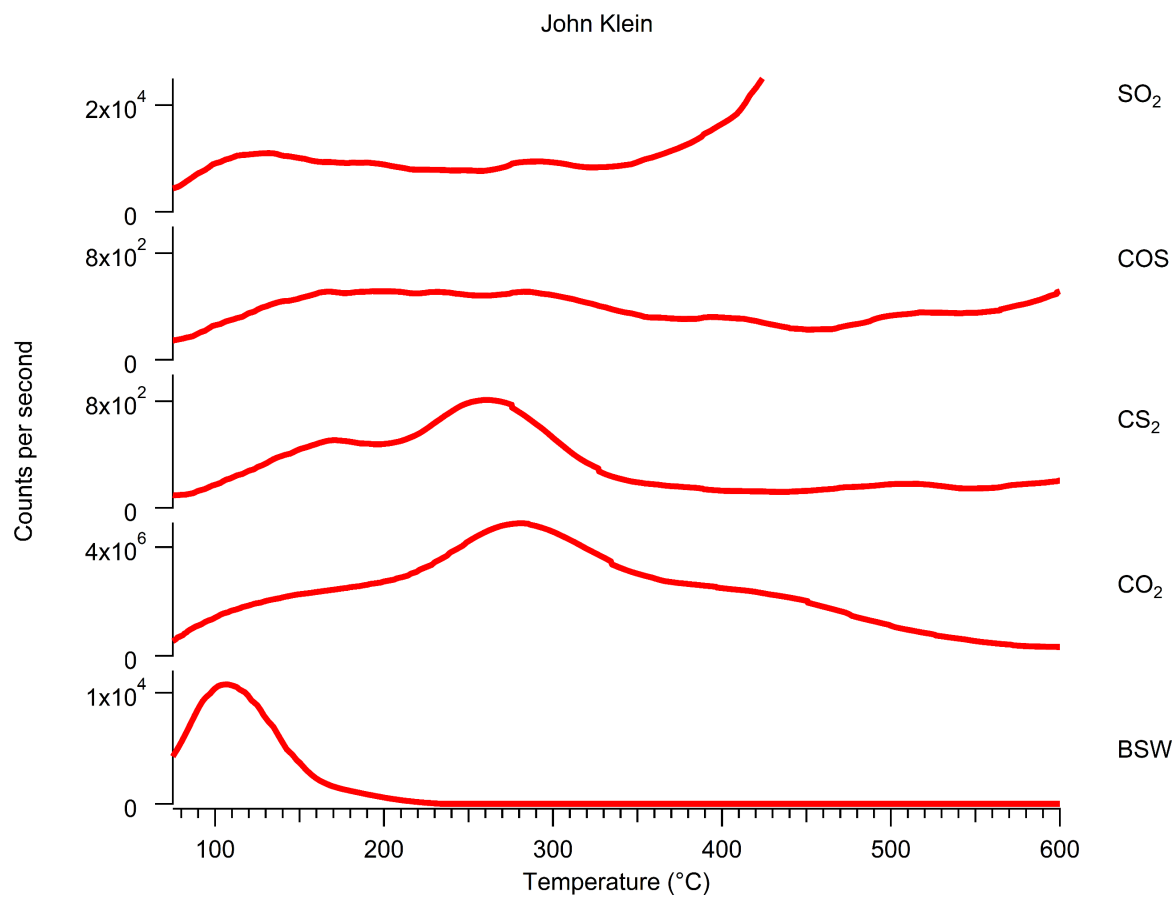


Figure B.2a. John Klein. The plots in Figure B.2a-g show the same data as Figure 2.2a-e in the main text, but consolidated so that volatiles can be compared to each other within each sample. Vertical axis limits have been adjusted to show the volatiles' evolution structures.

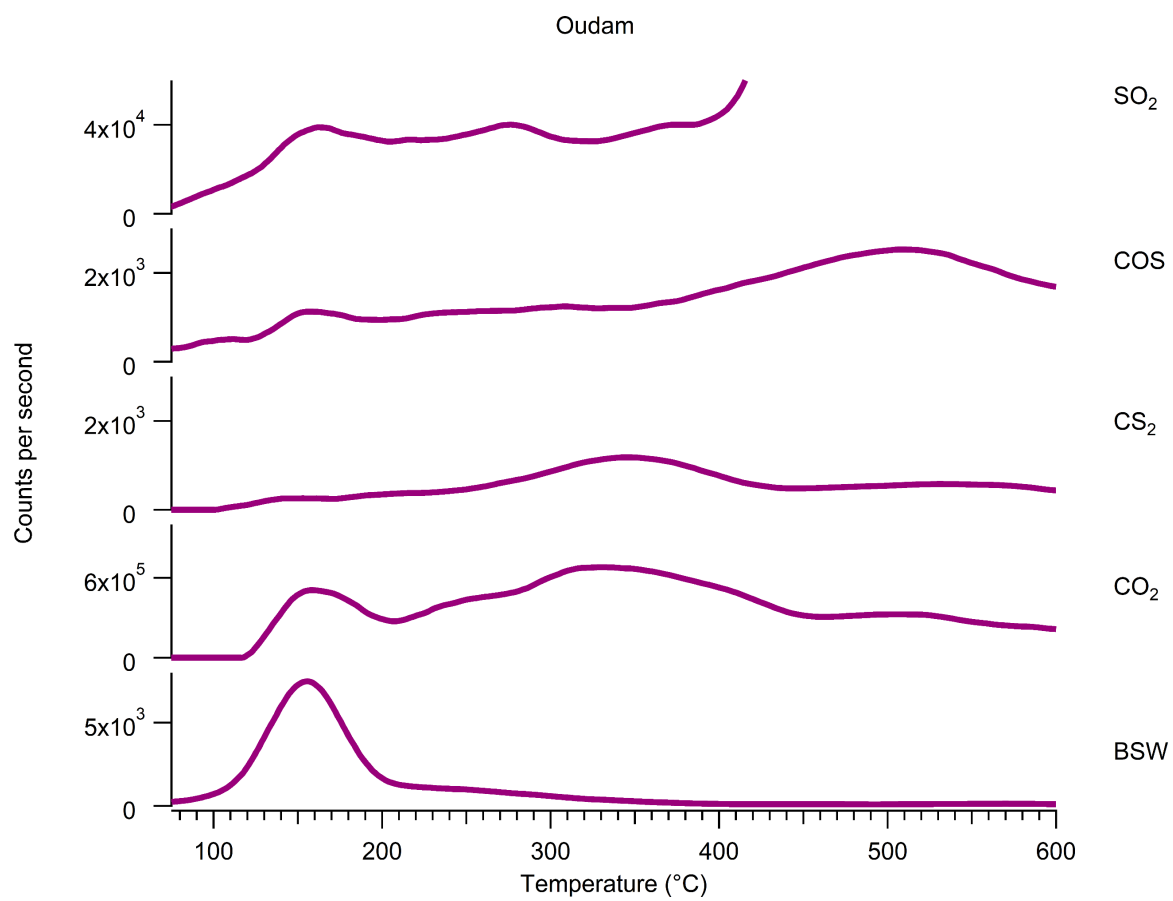


Figure B.2b. Oudam.

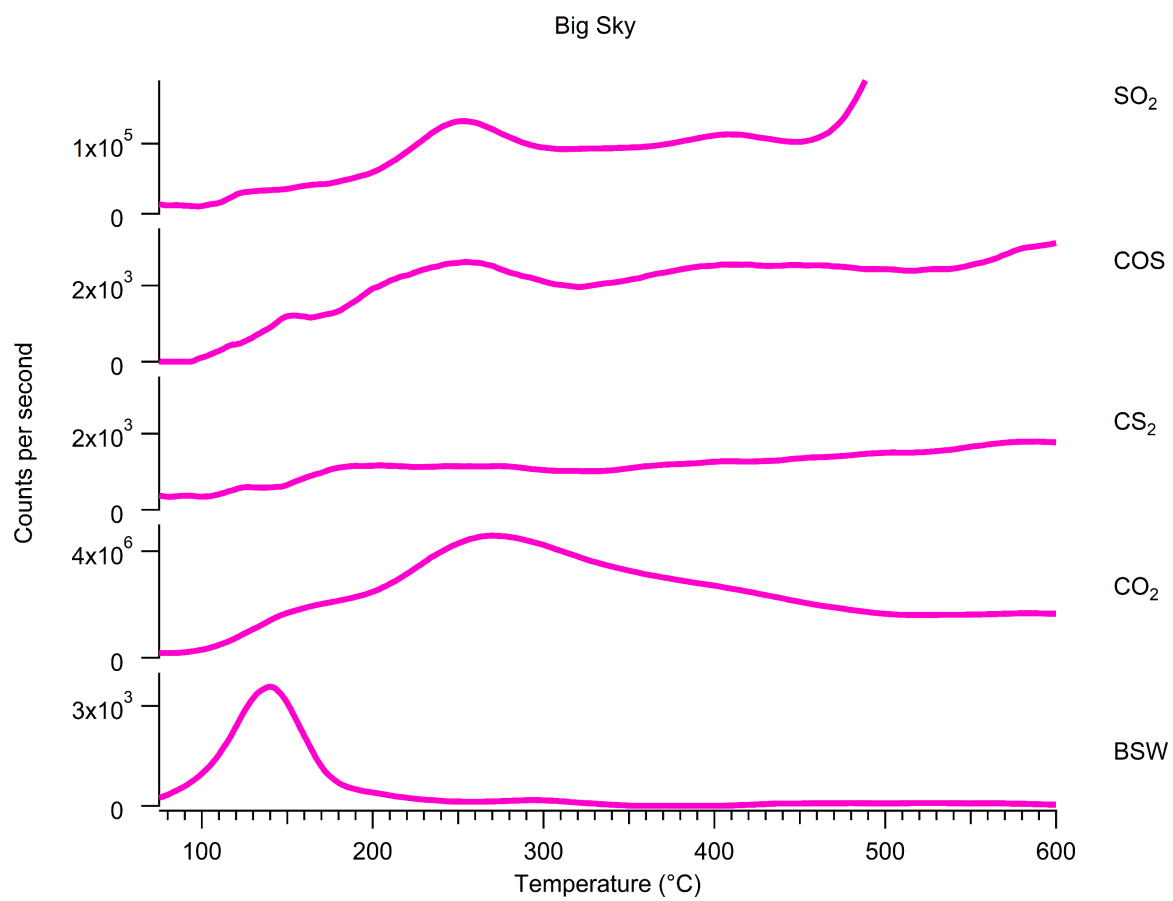


Figure B.2c. Big Sky.

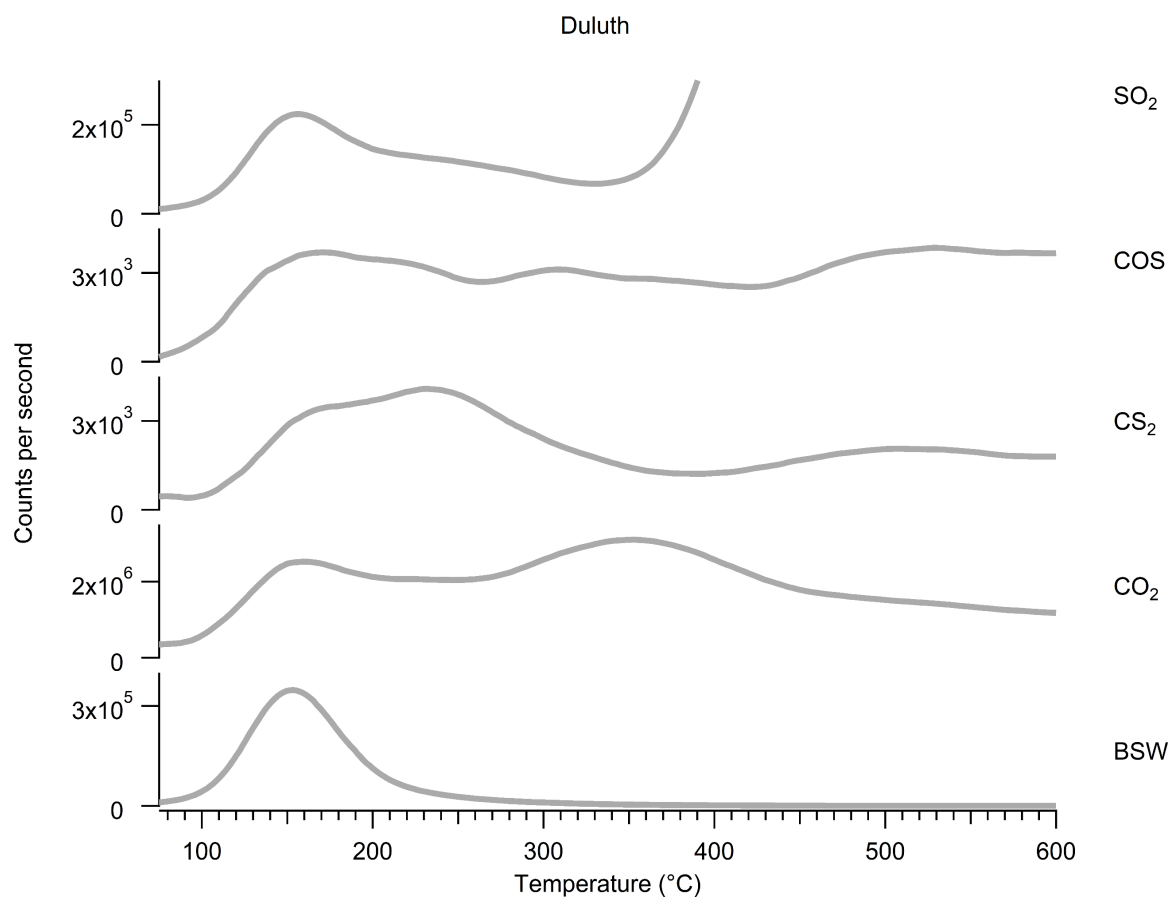


Figure B.2d. Duluth.

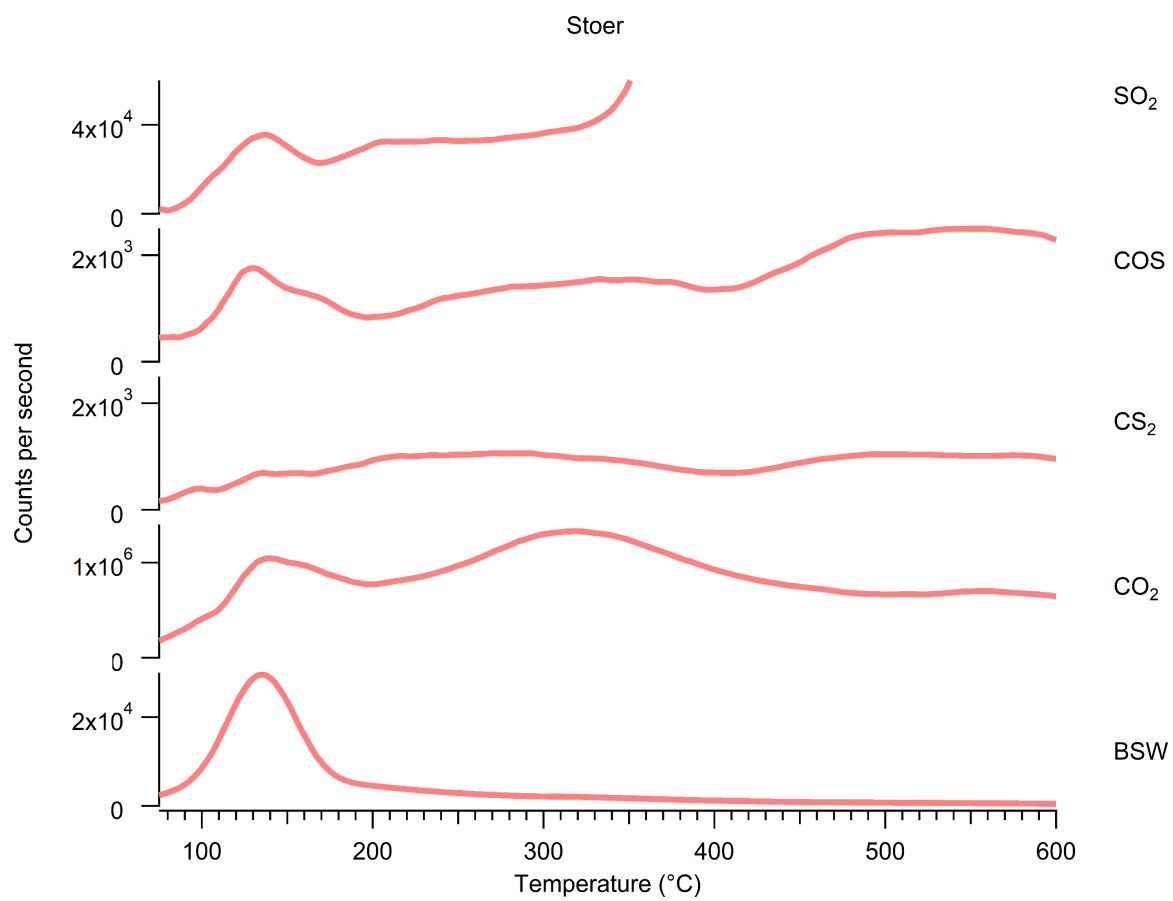


Figure B.2e. Stoer.

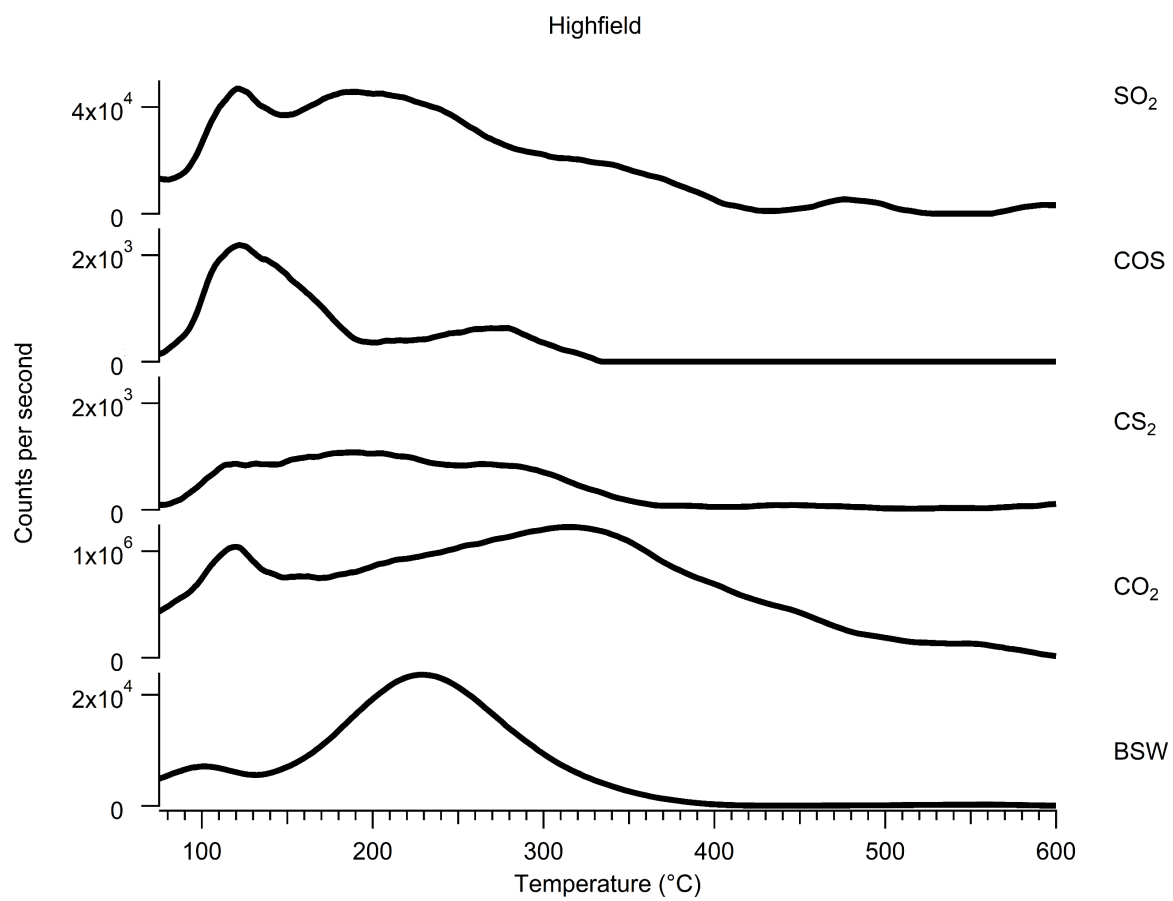


Figure B.2f. Highfield.

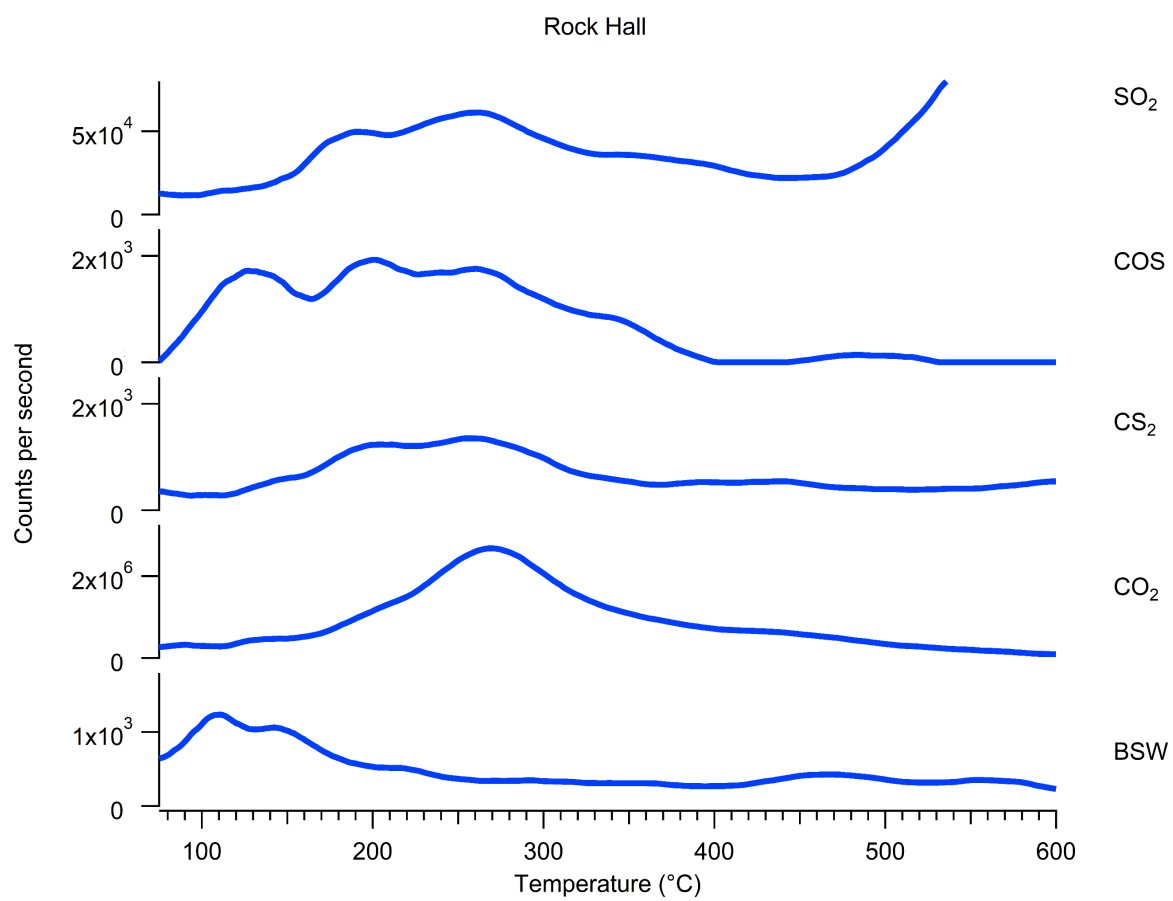


Figure B.2g. Rock Hall.

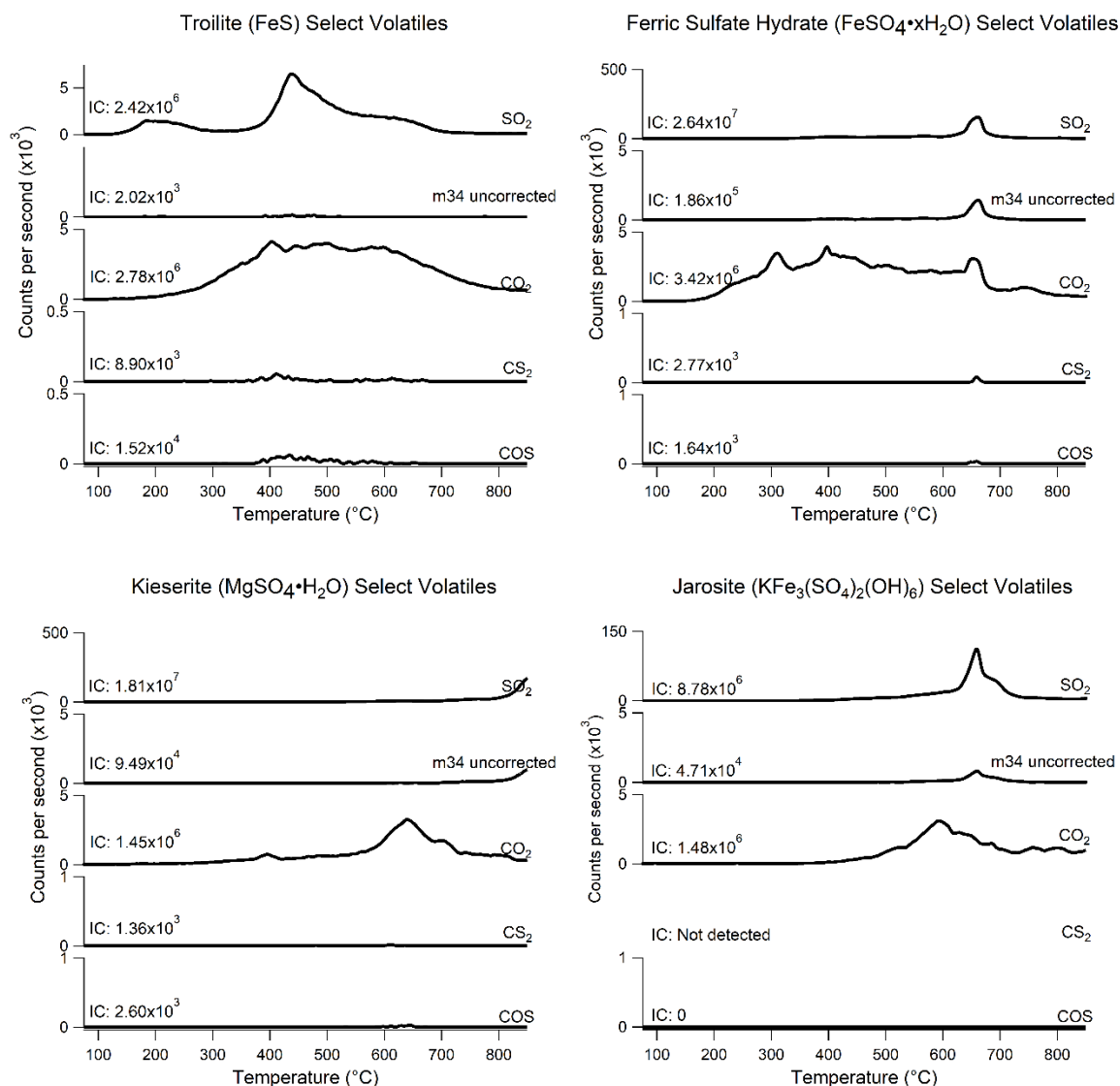


Figure B.3. Additional example laboratory EGA profiles for select carbon and sulfur volatiles for different sulfur minerals.

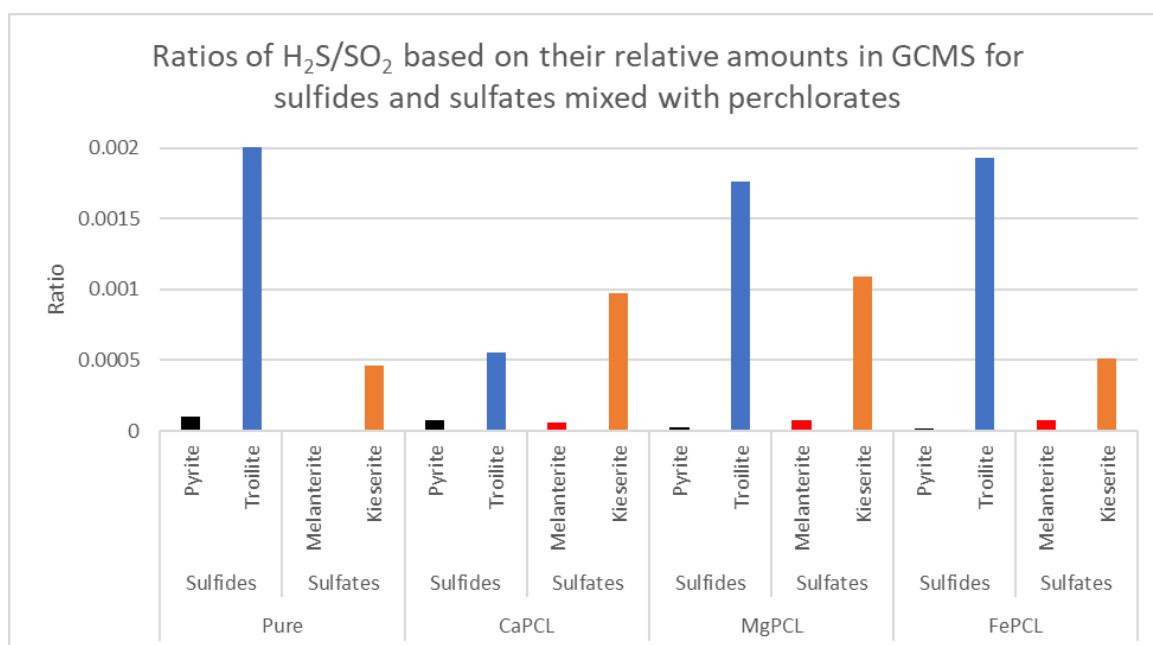


Figure B.4. This Figure shows the ratios of H₂S to SO₂ observed during pyrolysis GCMS analyses of sulfides and sulfates mixed with different perchlorates. Samples were prepared by the same methods described in the main text. They were then heated to 850°C and the evolved gases were eluted through a SAM-like GC column. Peaks of SO₂ were identified and integrated from the total ion counts. Evolved H₂S was much less abundant and peak areas of m/z 34 counts were integrated for these values. While troilite, a sulfide, typically had a higher ratio of H₂S/SO₂ than any of the other samples in most mixtures, the sulfides did not reliably produce a large relative amount of H₂S that could be used to identify them compared to the sulfates. Pure=sulfur minerals in fused silica, CaPCL=calcium perchlorate, MgPCL=magnesium perchlorate, FePCL=iron(III) perchlorate additions.

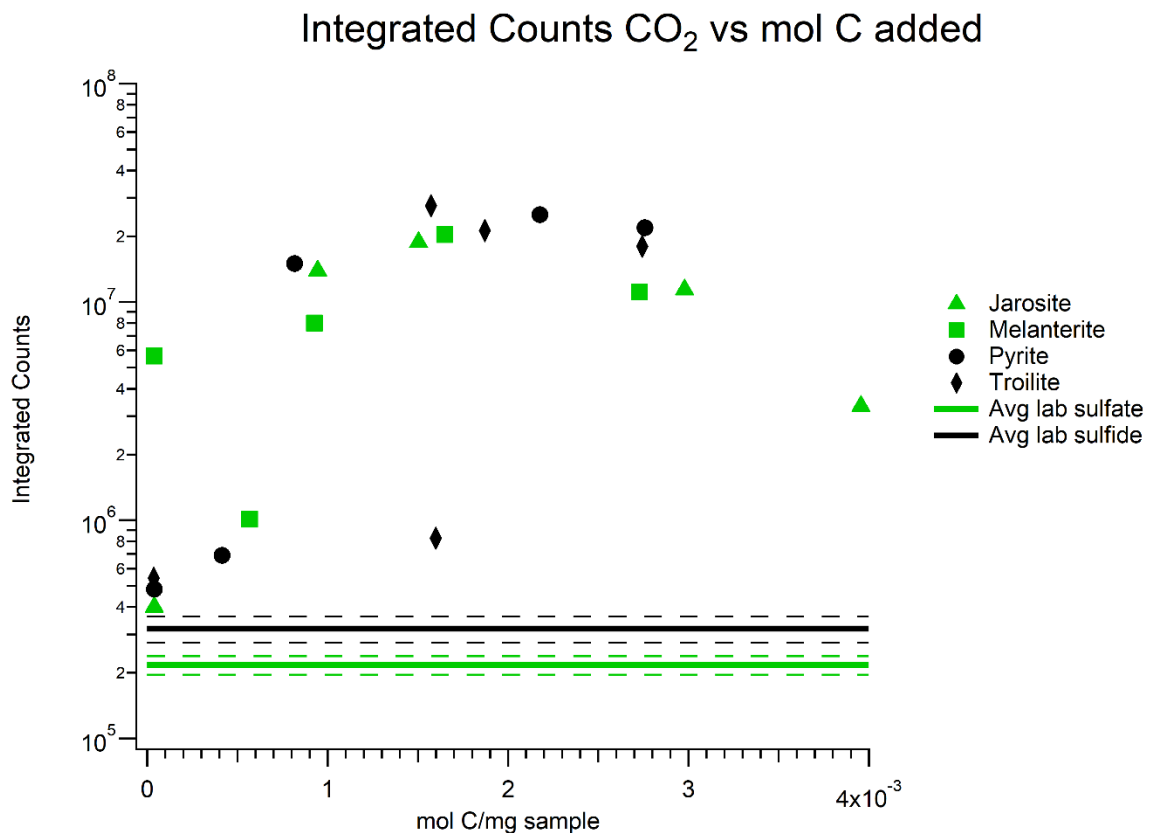


Figure B.5a-c. These plots show the integrated counts of (a) CO₂, (b) COS, and (c) CS₂ per mg sample compared to the moles of carbon compounds per mg sample added to different sulfur compounds. Carbon compounds added include MTBSTFA-DMF, siderite, Mg acetate, Fe (III) oxalate, and myristic acid. Solid lines represent the average integrated counts for all laboratory data for sulfides and sulfates. The dashed lines represent the averages plus/minus one standard error about the means.

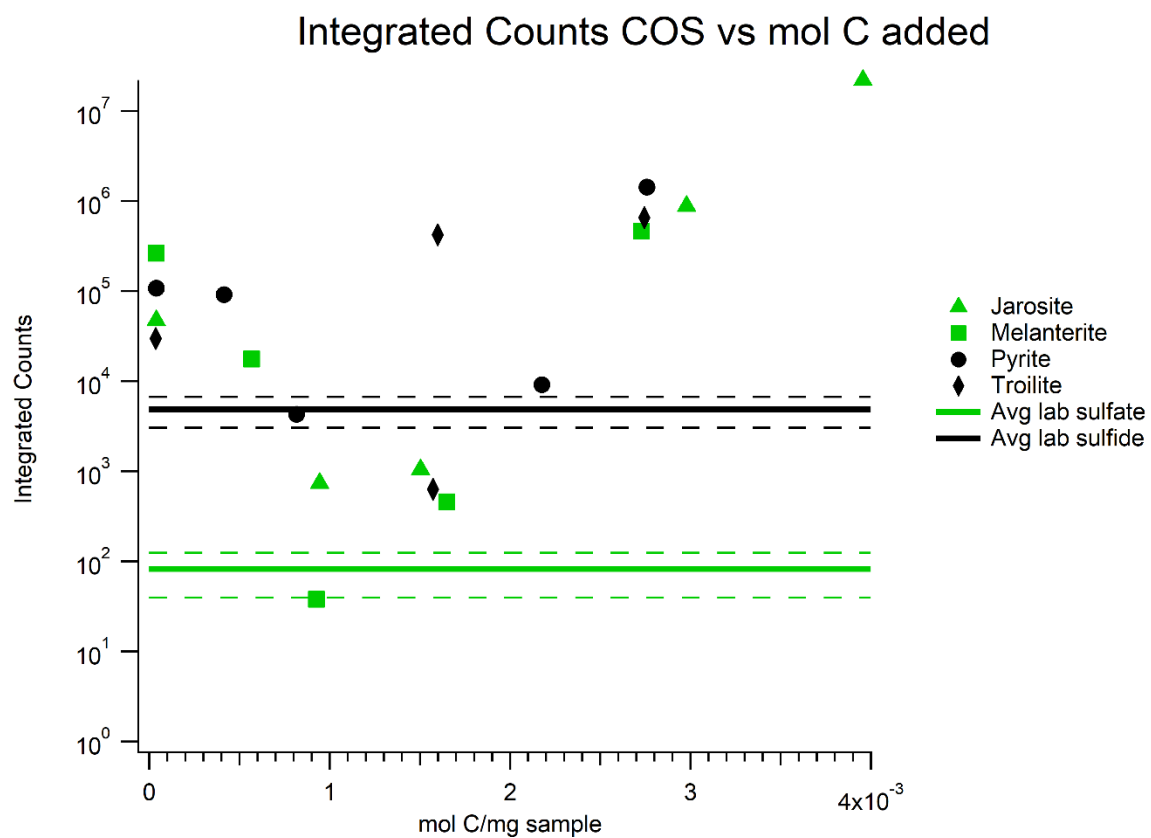


Figure B.5b. Counts of COS vs mol C per mg total sample.

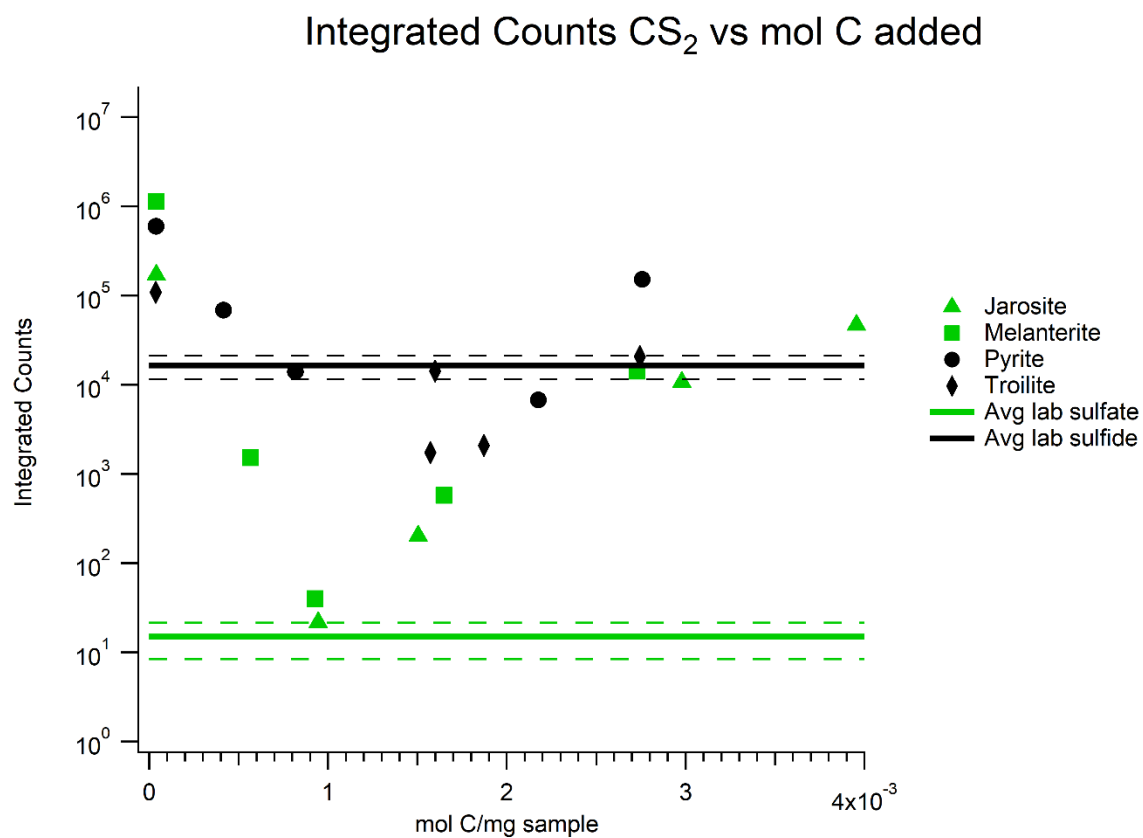


Figure B.5c. Integrated counts of CS₂ vs mol C added per mg total sample.

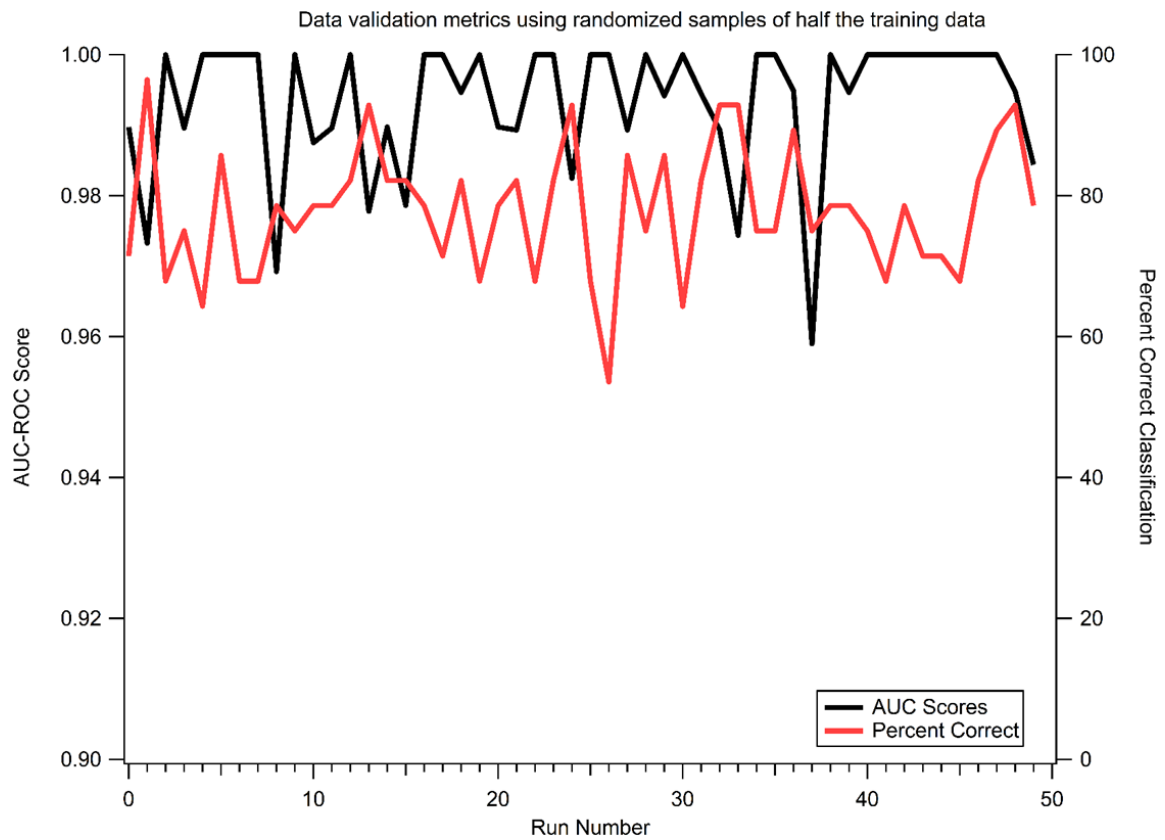


Figure B.6. This Figure shows the results of 50 QDA training data validations runs. AUC-ROC scores are plotted with the left axis in black. Percent of training data samples correctly clustered is on the right and plotted in red. Half ($N=28$) of the total training data used in the analysis was randomly selected as a training dataset. QDA was applied to these data and predictions for the other half of the data were made based on the results of the QDA. This process was repeated 50 times.

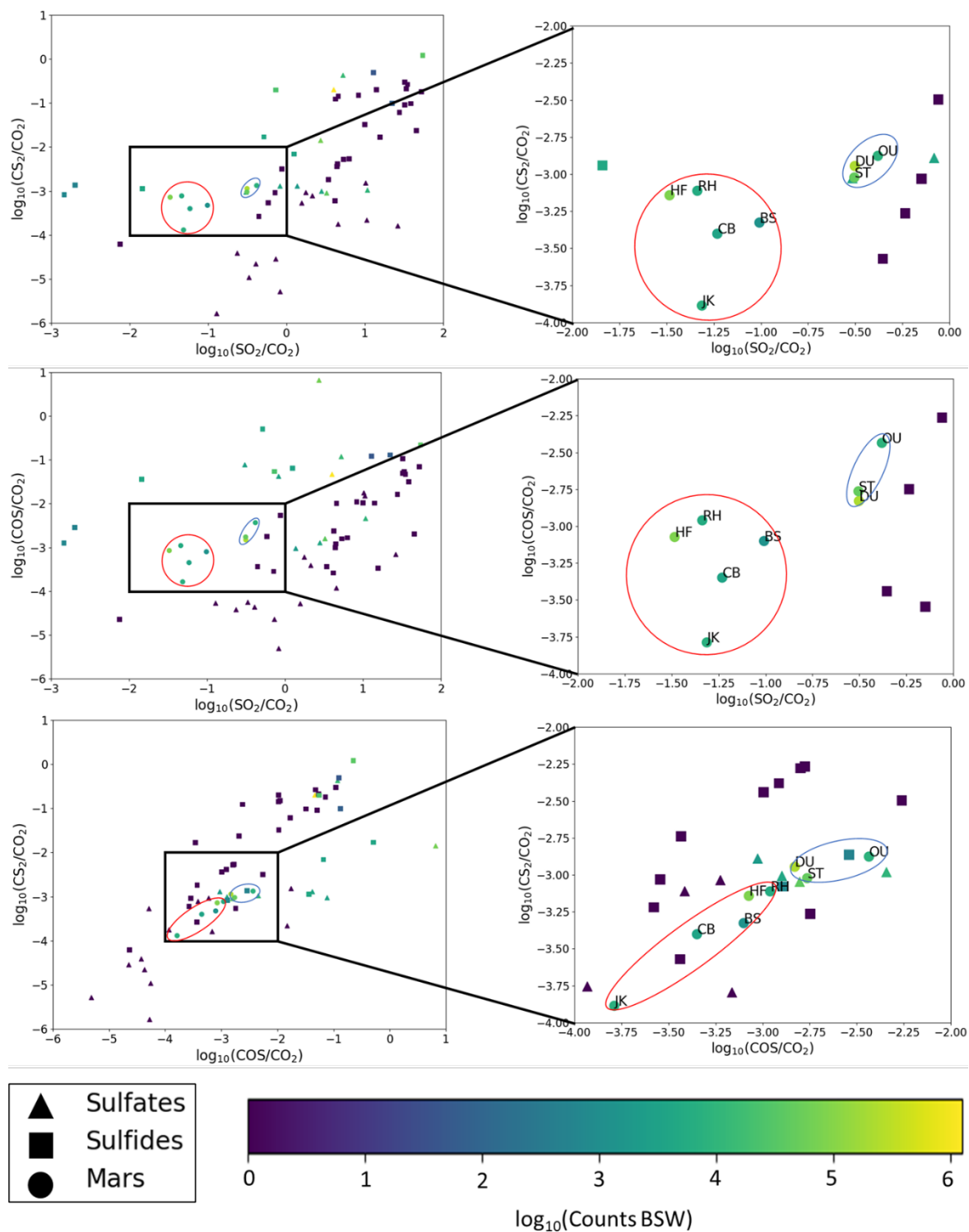
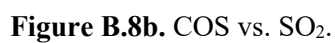


Figure B.7. These plots show zoomed-in areas for Figure 2.3 in the main text. The plots on the left are from the main text. The plots on the right focus on the separation between Mars samples that cluster with sulfides (circled in red) and those that do not (circled in blue).



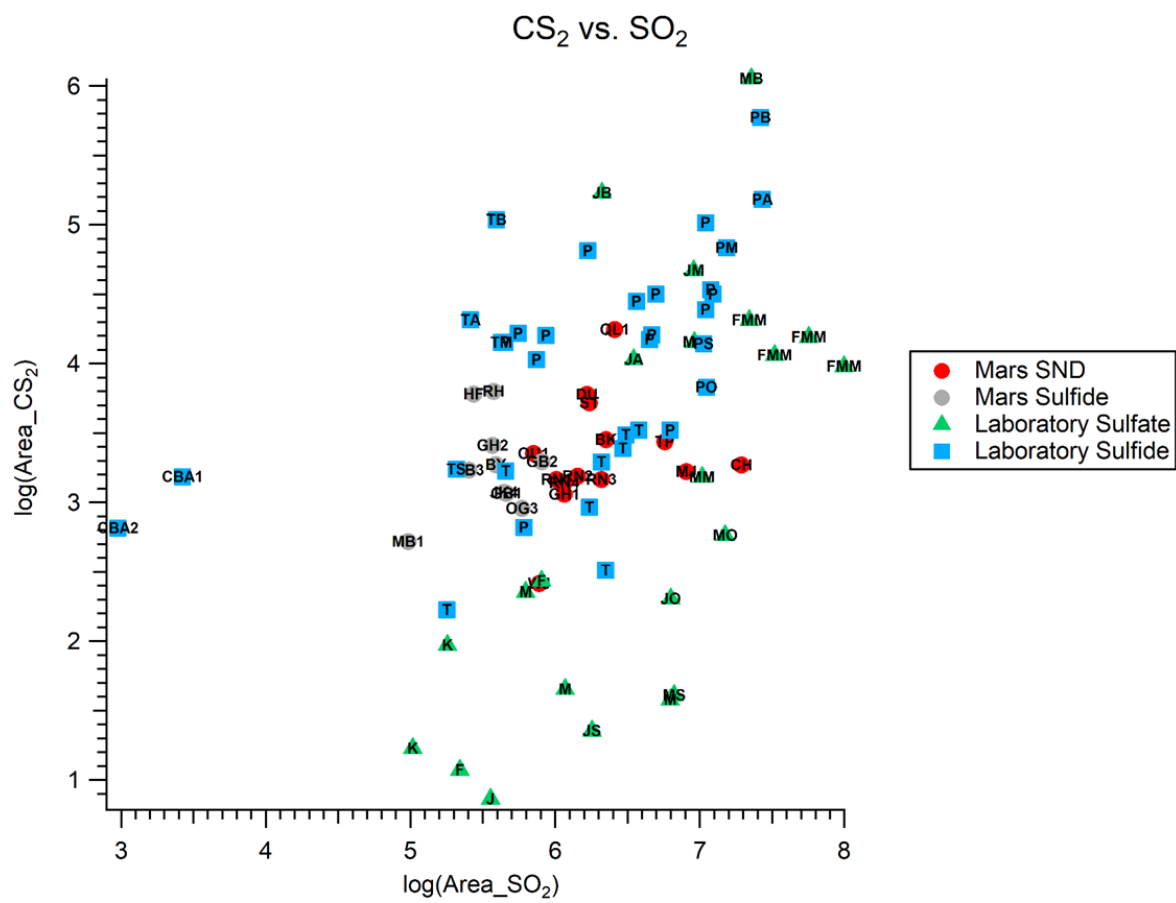


Figure B.8c. CS_2 vs. SO_2 .

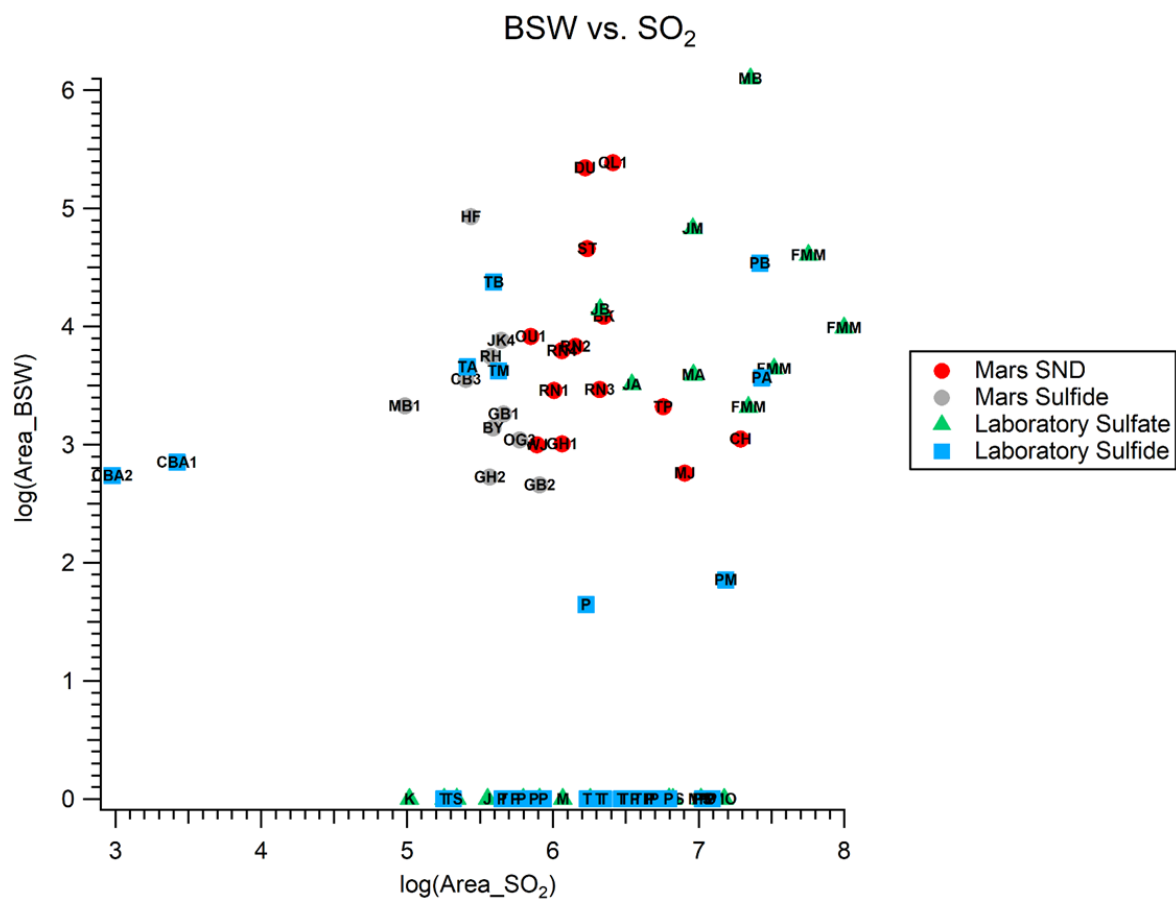


Figure B.8d. BSW vs. SO₂.

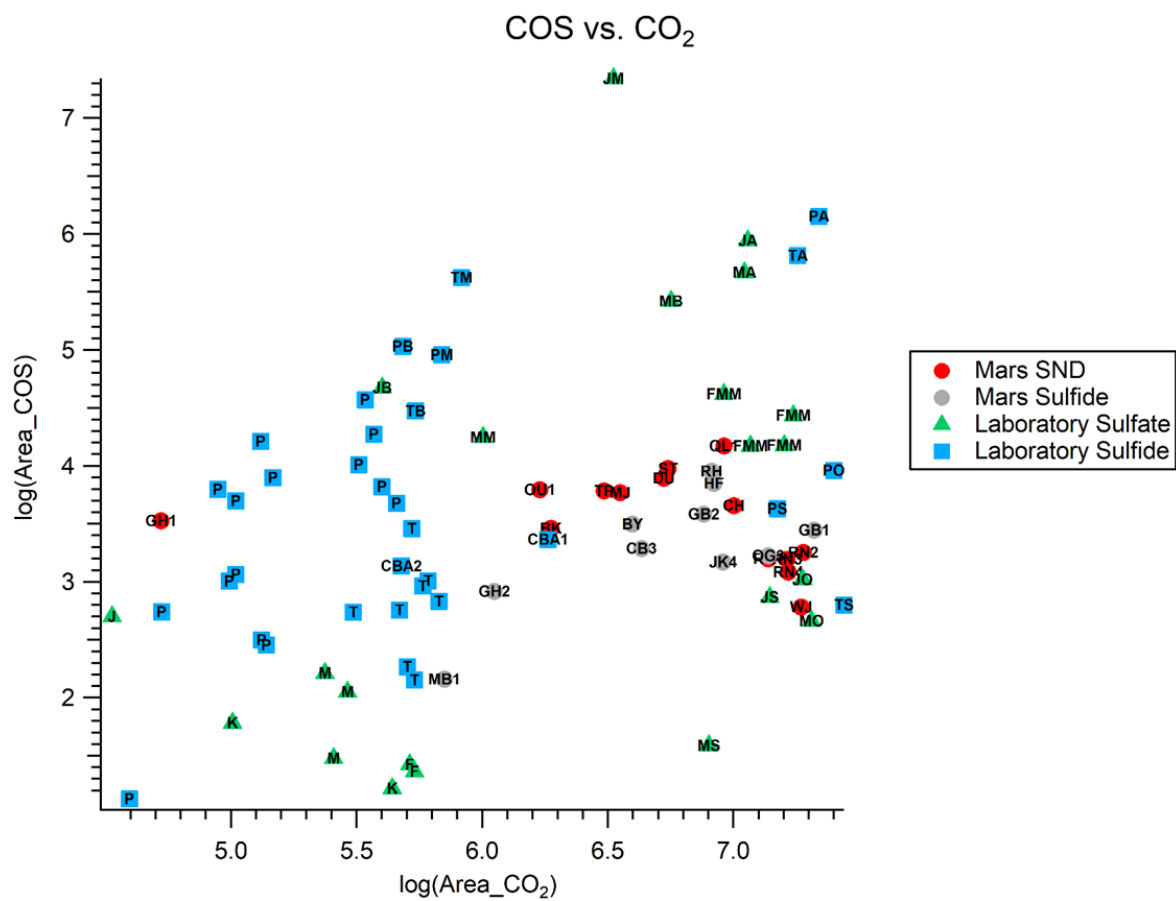
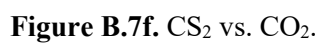


Figure B.8e. COS vs. CO₂.



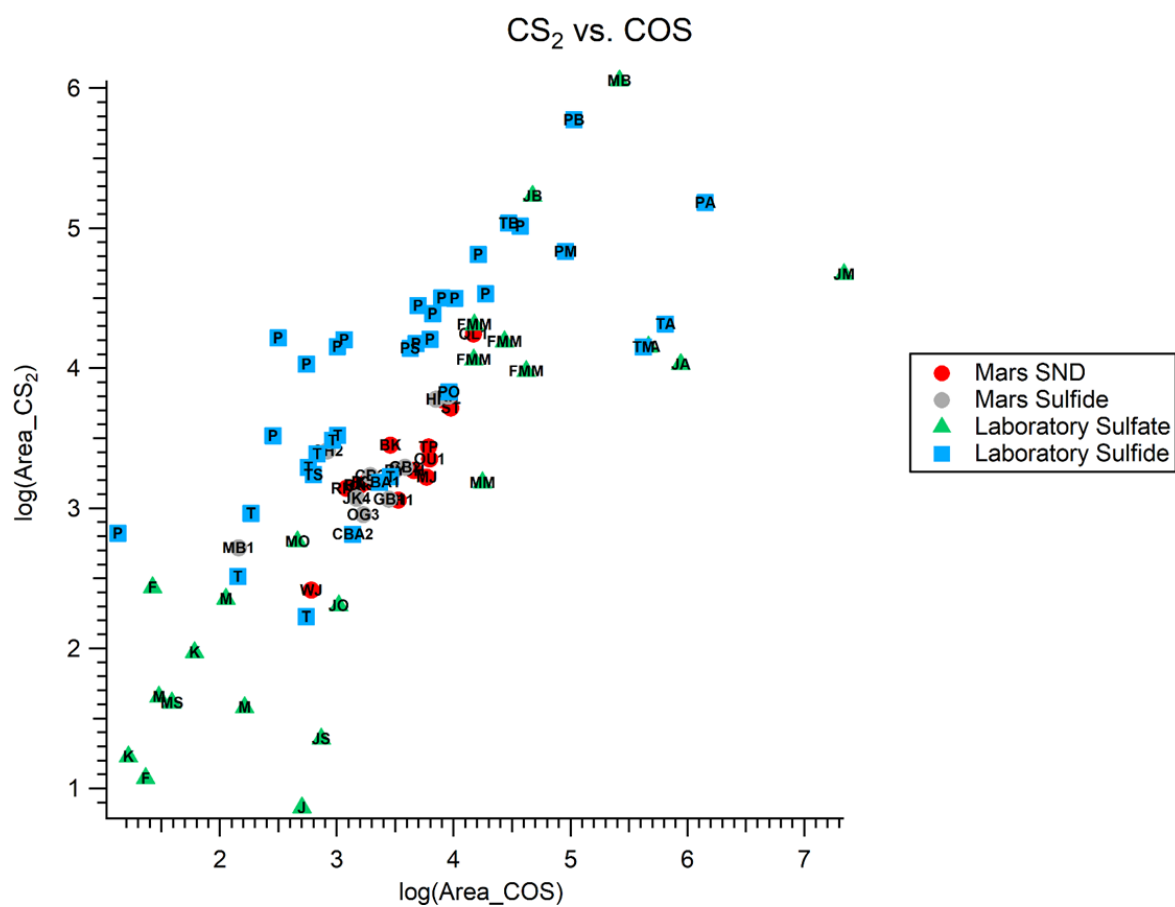


Figure B.8h. CS_2 vs. COS.

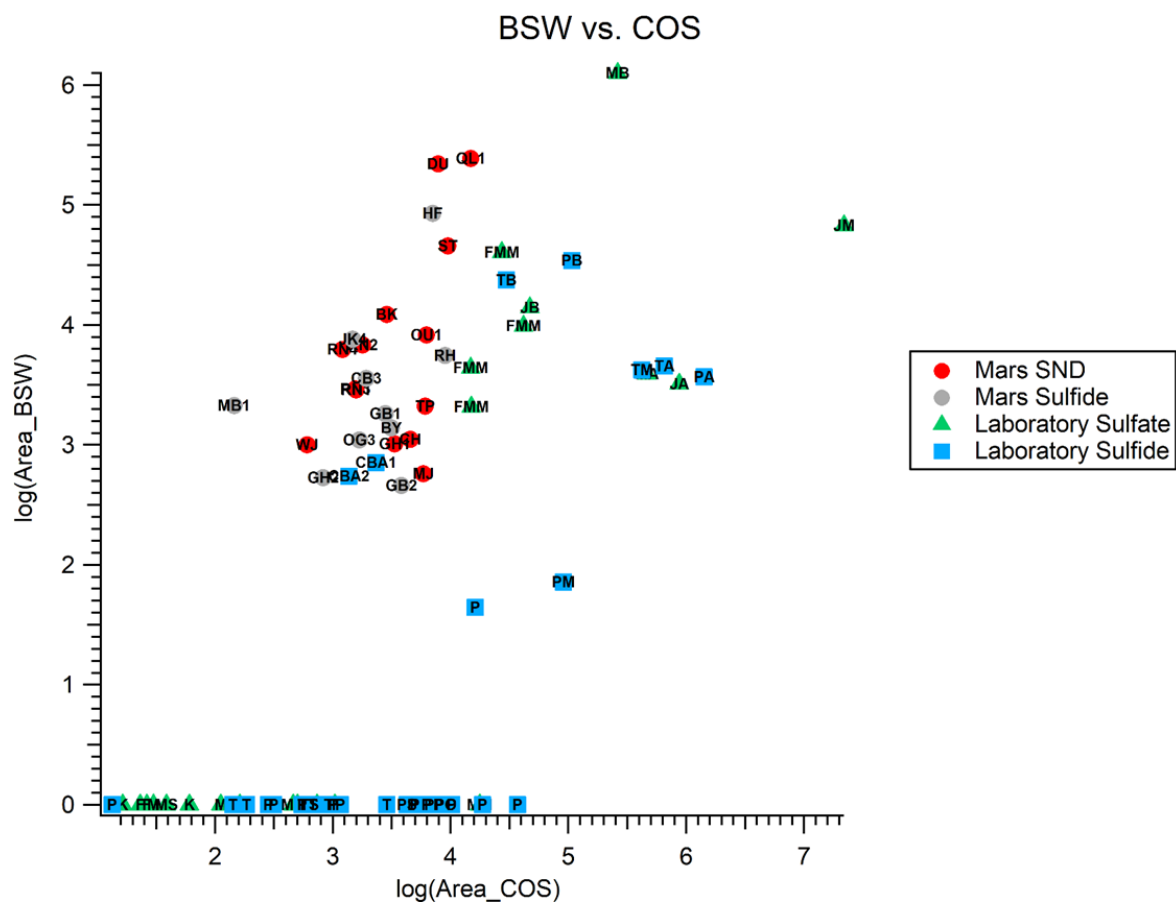


Figure B.8i. BSW vs. COS.

<i>m/z</i>	Possible ions	Pyrite	Troilite	Melanterite	Ferric sulfate hydrate	Jarosite	Kieserite
32	O ₂ ⁺ , S ⁺	5.83E+06	3.47E+05	8.87E+06	3.97E+06	4.21E+06	2.07E+07
48	SO ⁺	2.69E+07	8.64E+05	4.09E+07	1.17E+07	1.05E+07	5.51E+07
64	SO ₂ ⁺ , S ₂ ⁺	5.14E+07	1.60E+06	7.72E+07	1.62E+07	1.63E+07	1.05E+08
96	S ₃ ⁺	ND	ND	0.00E+00	9.93E+02	ND	ND
128	S ₄ ⁺	ND	ND	ND	ND	0.00E+00	ND
160	S ₅ ⁺	ND	ND	ND	ND	ND	ND
192	S ₆ ⁺	ND	ND	ND	ND	0.00E+00	ND
Temp. range (°C)		460-602	372-539	474-609	592-697	619-728	781-1031

Table B.1. This table shows the integrated counts of different *m/z* ratios for the sulfur minerals tested on their own in the lab. The temperature range indicates the range of the main peak of *m/z* 64, which was used to calculate integrated counts. The column of ‘Possible ions’ is a non-exhaustive list of potential ions with the associated *m/z* ratios with a focus on sulfur-related ions. SO⁺ (*m/z* 48) is a fragment of SO₂ and unlikely to be observed if elemental or molecular sulfur is fragmented in the mass spectrometer. ND=not detected during entire EGA run. Molecular sulfur ions were not commonly detected during pyrolysis of sulfides or sulfates. Together, these data suggest that *m/z* 64 is SO₂.

Appendix C: Supplementary Information for Chapter 3

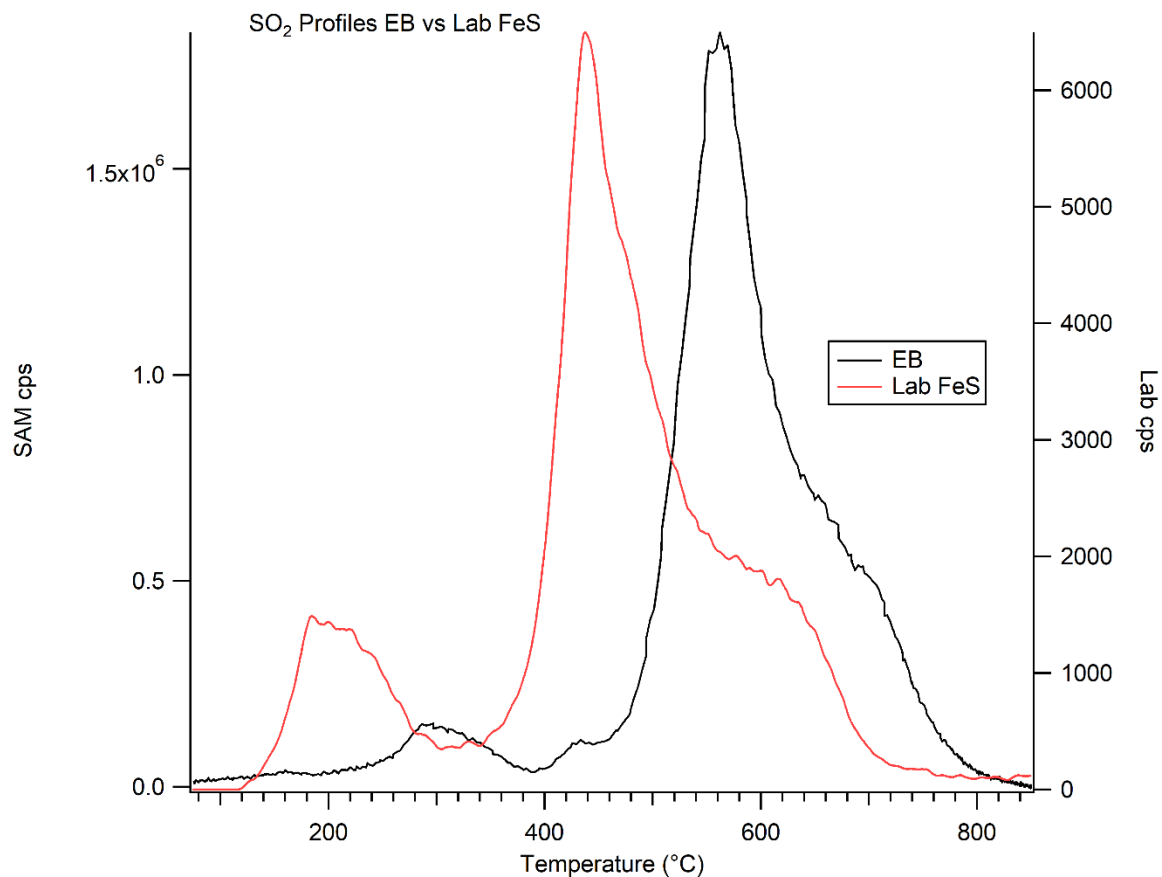


Figure C.1. EGA comparison of SO₂ evolved from the EB sample on Mars with SAM (black, left axis) and a laboratory EGA run of FeS mixed in an inert fused silica matrix (red, right axis). Profiles of SO₂ release are similar, but the laboratory SO₂ is shifted ~100°C lower than the SAM data. The temperature shift could be due to differences in oven conditions, flow rate, or sample mixtures.

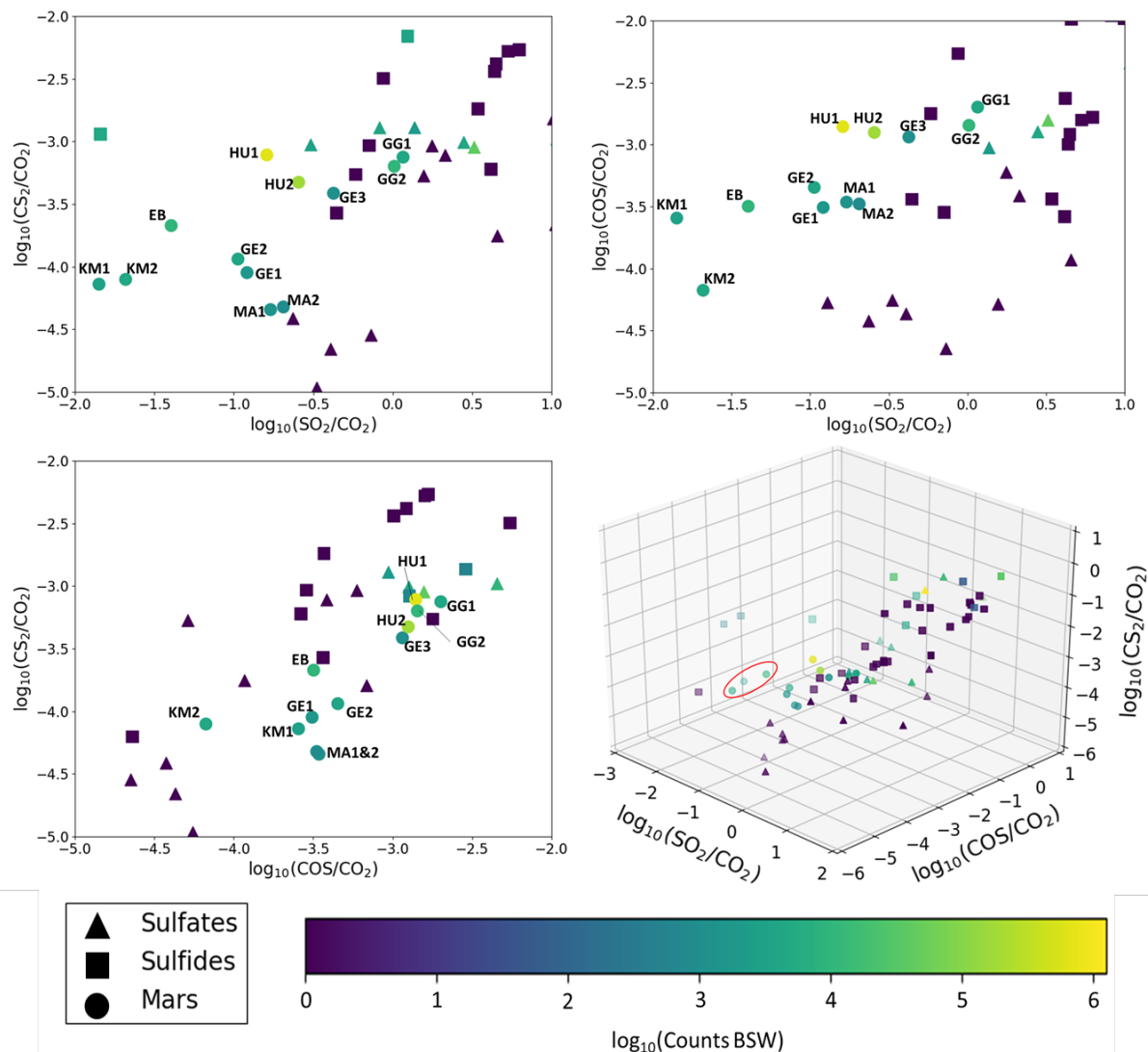


Figure C.2. This figure shows the relationships between evolved QDA volatiles in 2-D and 3-D space for the Glen Torridon samples and laboratory samples described in Wong et al. (2020) and tabulated in Wong (2020). SO_2 , COS, and CS_2 have been normalized by CO_2 . Color bar represents the evolved BSW in each sample. KM1, KM2, and EB clustered with laboratory sulfides (>50% posterior probability) in QDA calculations. Red ellipse in the 3-D plot is illustrative only to show the separation of KM1, KM2, and EB compared to the rest of the GT samples in variable space.

Table C.1. This table shows the log-normalized integrated counts per mg sample for SO₂, CO₂, COS, CS₂, and BSW for the samples discussed in Chapter 3. These data were used in the QDA calculations of posterior probabilities (Table 3.1) and for placement of “Mars data” in Figure C.1.

SAMPLE	AREA SO ₂	AREA CO ₂	AREA COS	AREA CS ₂	AREA BSW
EB	5.703285	7.097292	3.599682	3.425511	3.996162
HU1	6.925654	7.717867	4.86311	4.610186	5.818607
HU2	6.523512	7.115393	4.213529	3.789452	5.229732
GG1	6.454498	6.389141	3.692157	3.263892	3.642642
GG2	6.440712	6.4309	3.586626	3.232244	4.026651
MA1	6.299431	7.068412	3.604777	2.725564	3.132632
MA2	6.358023	7.046069	3.567698	2.724469	2.95328
GE1	6.10051	7.017538	3.510538	2.96985	3.260652
GE2	6.081972	7.05582	3.710436	3.116728	3.598947
GE3	6.264177	6.636403	3.697915	3.222701	3.040528
KM1	5.364374	7.212865	3.620243	3.072963	3.475179
KM2	5.473038	7.153941	2.978375	3.052152	3.656498

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Appendix D: Supplementary Information for Chapter 4

This section has a table of carbon isotope ranges for different materials and additional plots showing K_{eq} vs. temperature for the reactions listed in Table 4.2 that were not shown in the main Chapter 4 text. Figures D.1 and D.2 support the extremely favorable reactions involving CH_3 under all SAM oven temperature conditions. Figures D.3 and D.4 show the equilibrium constants for the reactions that make up the net reaction $CH_4 + Cl_2 \leftrightarrow CH_3Cl + H_2$ discussed in the main text of Chapter 4.

Table D.1. Range of carbon isotopic compositions for select carbon sources. Ranges are from Leshin et al. (2013) except where otherwise noted.

Carbon source	Approximate range of $\delta^{13}C$ (‰, V-PDB)
Martian meteorite carbonate	+10 to +65
Martian refractory carbon	-28 to -5
Martian magmatic carbon	-30 to -20
Atmospheric CO₂ from SAM	+42 to +50
Bulk carbonaceous chondrites	-25 to 0
Terrestrial reduced carbon	-80 to -15
CO₂ evolved from solid samples^a	-25±20 to +56±11

^aValues from Franz et al. (2020)

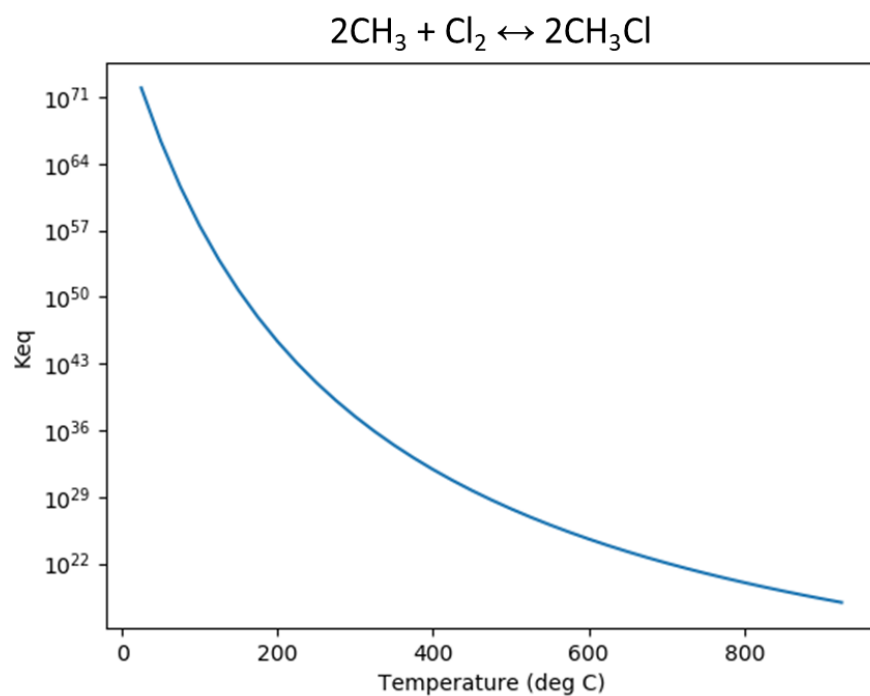


Figure D.1. K_{eq} vs. temperature for Reaction 1 in Table 4.2, showing that the reaction of methyl with chlorine strongly favors the formation of chloromethane at all temperatures in the SAM oven range.

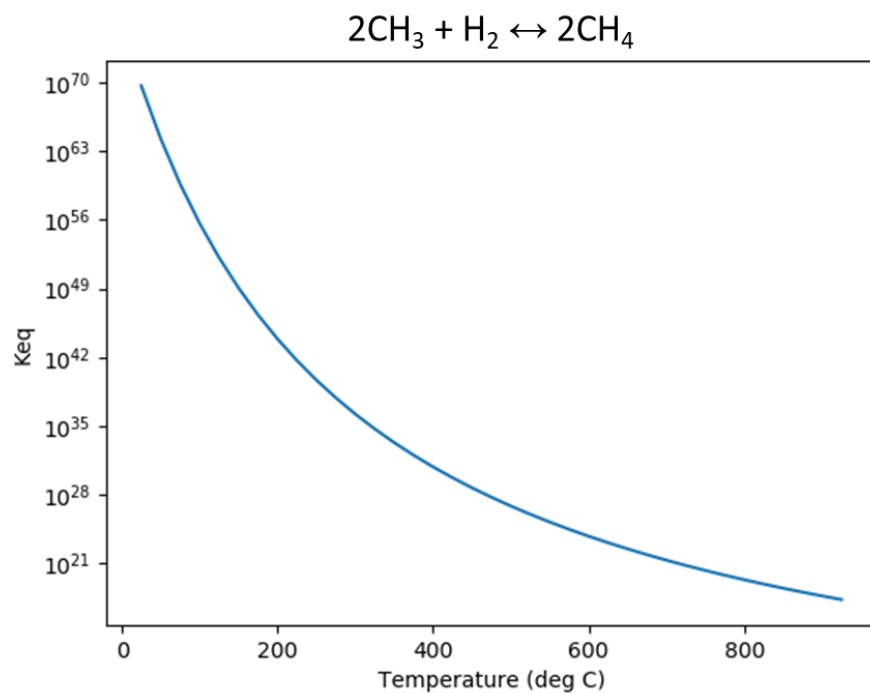


Figure D.2. K_{eq} vs. temperature for Reaction 2 in Table 4.2, showing that the reaction of methyl with molecular hydrogen strongly favors the formation of methane at all temperatures in the SAM oven range.

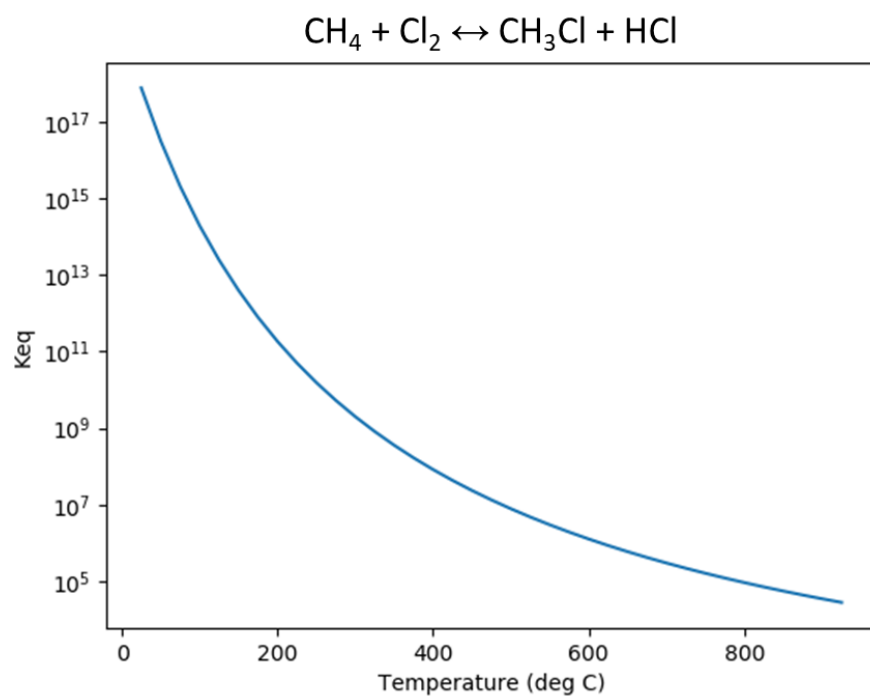


Figure D.3. K_{eq} vs. temperature for Reaction 4 in Table 4.2, showing that the reaction of methane and chlorine favors the formation of chloromethane and HCl in the SAM temperature range.

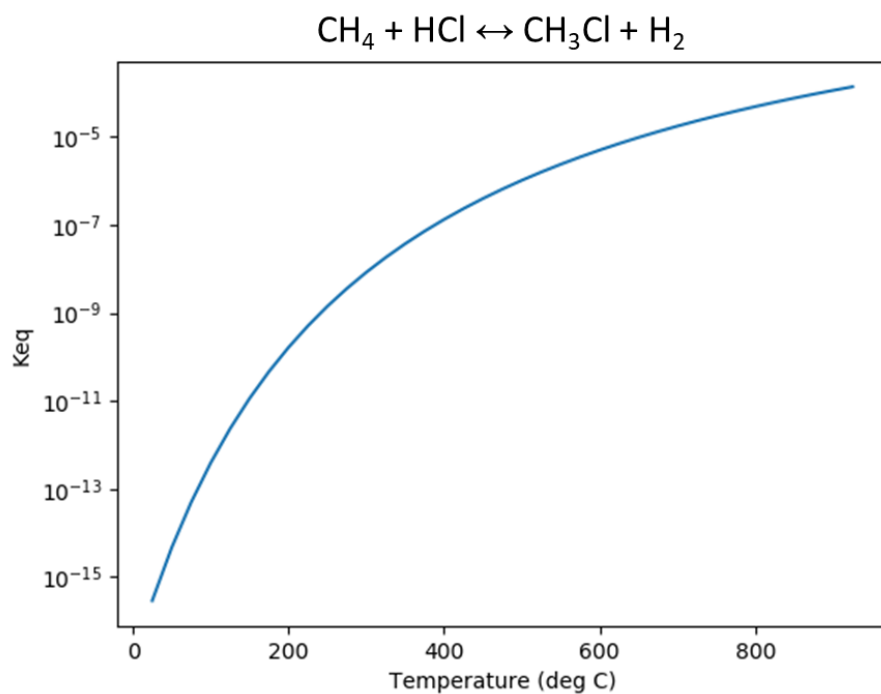


Figure D.4. K_{eq} vs. temperature for Reaction 5 in Table 4.2, showing that the reaction of methane and HCl does not favor the formation of chloromethane and H_2 in the SAM temperature range.

References

- Franz, H B, P R Mahaffy, C R Webster, G J Flesch, E Raaen, C Freissinet, S K Atreya, et al. 2020. "Indigenous and Exogenous Organics and Surface-atmosphere Cycling Inferred from Carbon and Oxygen Isotopes at Gale Crater." *Nature Astronomy* 4 (5): 526–32. doi:10.1038/s41550-019-0990-x.
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Appendix E: Supplementary Information for Chapter 5

Below are additional plots showing the gas chromatography results from the experiment testing long-term use of CO by *Alkalilimnicola ehrlichii* MLHE-1 described in Chapter 5, Table 5.1. Also included is a plot of additional the long-term spectrophotometer measurements of *A. ehrlichii* MLHE-1.

Figures E.1 and E.2 show the average CO₂ % observed in the bottle headspaces for the acetate experiments. No CO was added to these bottles and none was observed during GC analysis. Figure E.1 shows the CO₂ for bottles kept at atmospheric pressure for uninoculated bottles that had been rehydrated (“Control Wet”) and inoculated bottles that had been rehydrated (“Microbe Wet”) or that remained desiccated (“Microbe Dry”). Likewise, Figure E.2 shows the same control vs. inoculated bottles for bottles that had been brought to low pressure (<10 mbar). If microbes were actively metabolizing, it would be expected that there would be an increase in CO₂ compared to the control bottles. No significant increases in CO₂ were observed ($p>0.05$) in any of these cases.

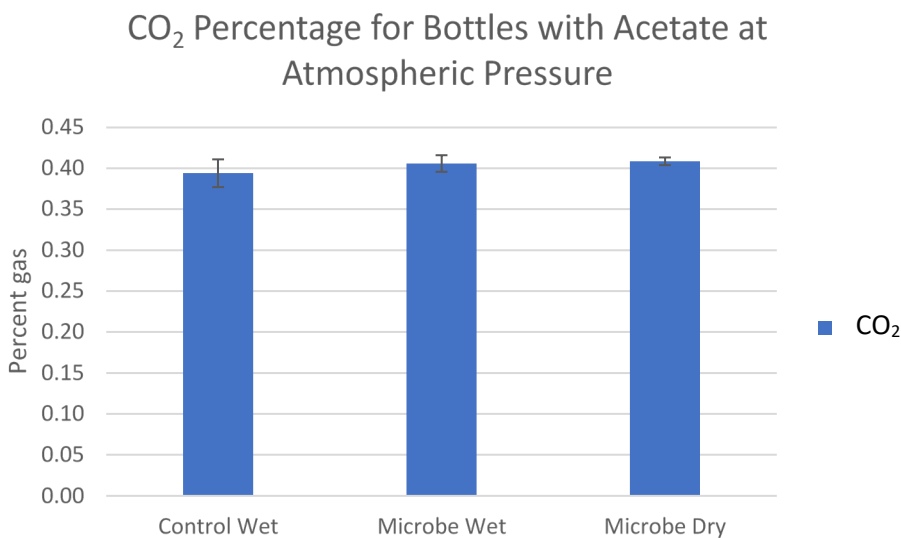


Figure E.1. Observed percent of CO₂ in the headspace of bottles kept at atmospheric pressure without the addition of CO. Error bars represent the mean of three replicates. Error bars are the standard error of the replicates.

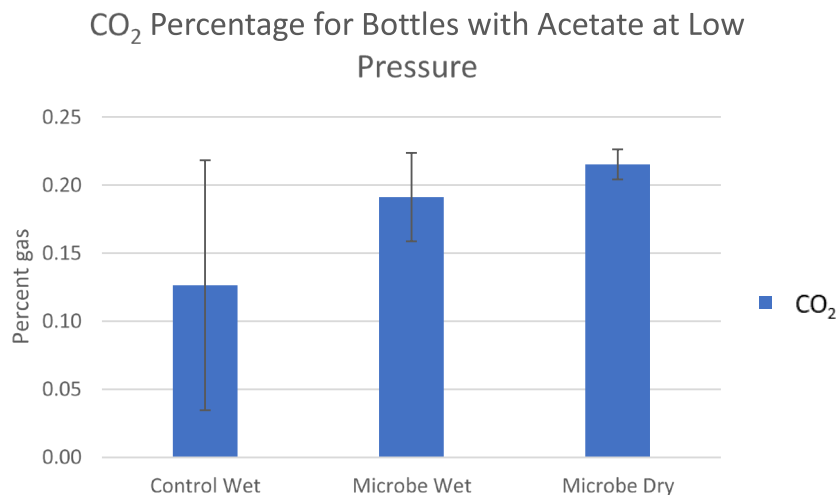


Figure E.2. Observed percent of CO₂ in the headspace of bottles kept at low pressure (<10 mbar) without the addition of CO. Error bars represent the mean of three replicates. Error bars are the standard error of the replicates.

Figure E.3 shows the CO and CO₂ headspace percentage comparisons of inoculated bottles that were kept at atmospheric pressure with the addition of CO for desiccated (“Microbe Dry”) and rehydrated (“Microbe Wet”) conditions. The top plot focuses largely on the percentage of CO in the headspace. The lower plot zooms in to focus on the CO₂. There was no significant difference in the average CO₂ measurements. The measured CO in the dry experiment, however, was significantly different from the wet experiment. While it is tempting to view this difference as microbial use of CO in a dry environment the lack of CO increase suggests that CO was not oxidized by microbial metabolism, especially given the abundance of available CO. Rather, the differences in CO percentages may be due to differences in initial CO additions to the bottles.

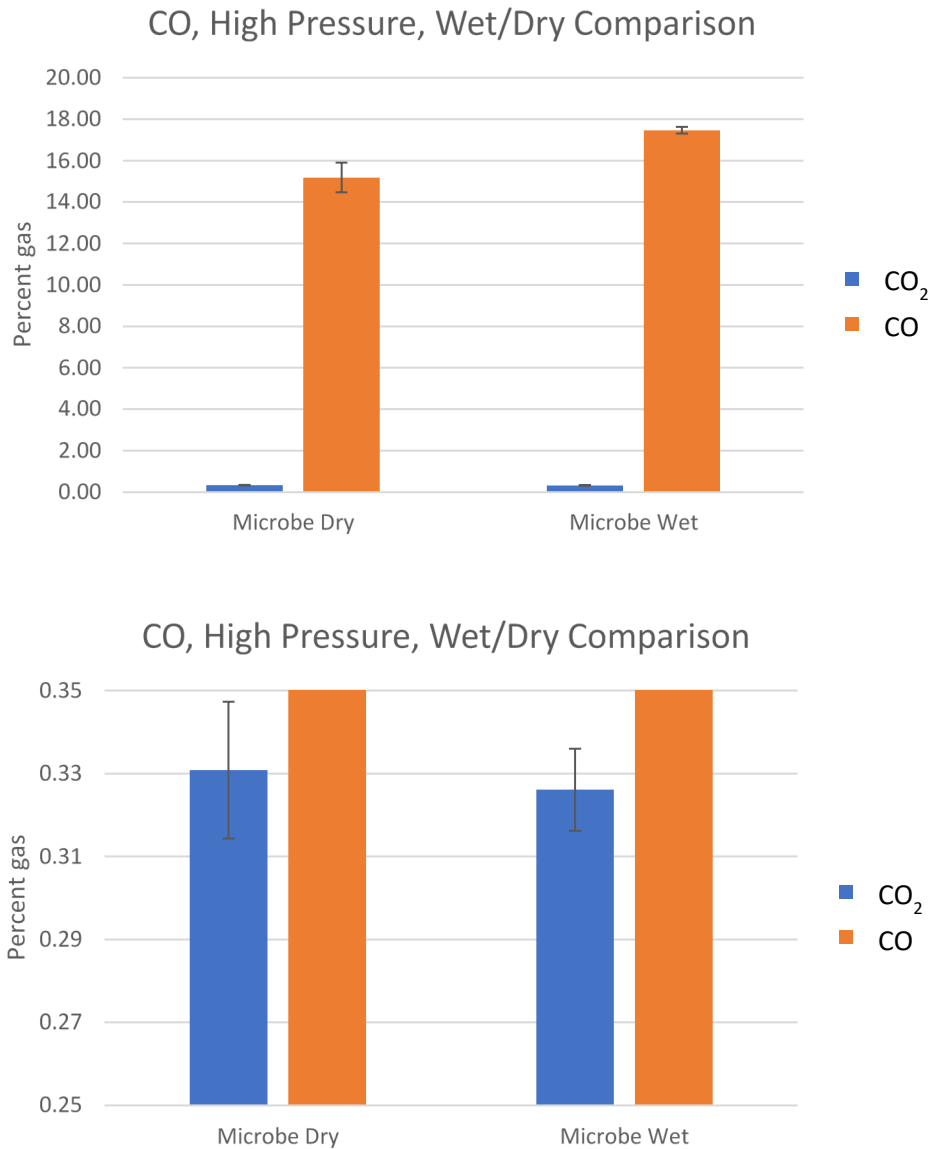


Figure E.3. Observed percent of CO (orange) and CO_2 (blue) in the headspaces of bottles kept at atmospheric pressure with the addition of CO. Error bars represent the mean of three replicates. Error bars are the standard error of the replicates. Top plot shows the full range of percentages observed. Bottom plot focuses on the measured CO_2 (note y-axis range from 0.25-0.35%).

A. ehrlichii MLHE-1 growth with or without CO (absorbance over time, no shaking)

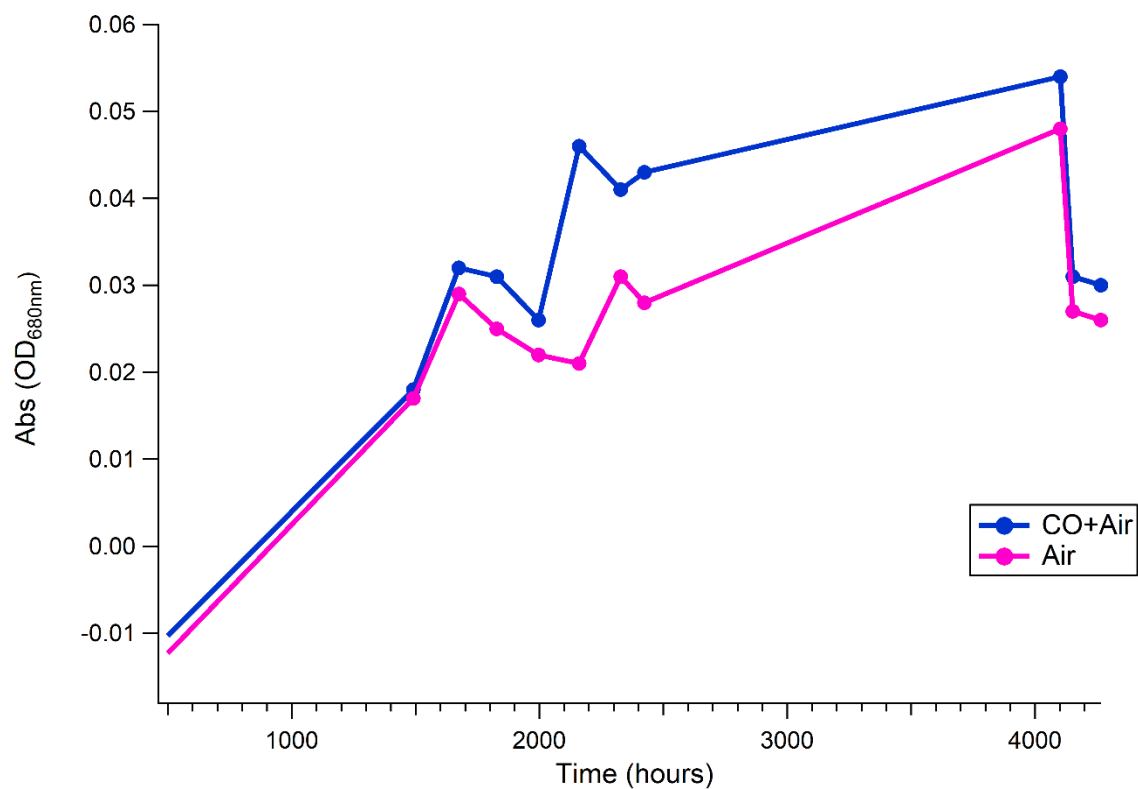


Figure E.4. Extended spectrophotometer measurements of *A. ehrlichii* MLHE-1 incubated at room temperature without shaking in media without acetate. Headspace consisted of air only or air supplemented with CO. Only a single bottle of each condition was analyzed.

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EDUCATION

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- 2015 **B.A. Molecular Biology & Biochemistry; Biology with Honors**
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- 2017-2020 **NASA Earth and Space Science Fellow**, Penn State University
- 2015 **Biologist I**, Sierra Lobo, Inc./Kennedy Space Center
- 2012-2015 **Undergraduate Student Researcher**, Wesleyan University
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PUBLICATIONS

- Wong, G. M.** +9 others (2020) Detection of reduced sulfur on Vera Rubin ridge by quadratic discriminant analysis of volatiles observed during evolved gas analysis. *JGR: Planets*, 125, e2019JE006304.
- Fraeman A. A. +42 others (incl. **G. M. Wong**). (*In press*) Evidence for a Diagenetic Origin of Vera Rubin Ridge, Gale Crater, Mars: Summary and Synthesis of Curiosity's Exploration Campaign. *JGR: Planets*. DOI:10.1029/2020JE006527
- McAdam, A., B. Sutter, P. Archer Jr., H. Franz, **G. Wong**, +18 others. (*In press*) Constraints on the Mineralogy and Geochemistry of the Vera Rubin ride, Gale crater, Mars, from Mars Science Laboratory Sample Analysis at Mars Evolved Gas Analyses. *JGR: Planets*. DOI:10.1029/2019JE006309
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- 2015-2016 **Teaching Assistant:** Natural Disasters (SP 2016); The Earth System (FA 2015)

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- 2019 **1st Place Poster in Geosciences Graduate Student Colloquium**
- 2017 **EMS Centennial Graduate Research Travel Award**