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Geological Production And Microbial Consumption of Hydrogen During Low-Temperature Water-Rock Reactions

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GEOLOGICAL PRODUCTION AND MICROBIAL CONSUMPTION OF HYDROGEN DURING LOW TEMPERATURE WATER-ROCK REACTIONS

By

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A thesis submitted to the Faculty of the Graduate School of the University of Colorado
in partial fulfillment of the requirement for the degree of

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The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.
ABSTRACT

Geological Production And Microbial Consumption of Hydrogen During Low-Temperature Water-Rock Reactions

Lisa Ellen Mayhew (Ph.D., Geological Sciences)
Thesis directed by Assistant Professor Alexis S. Templeton

Geologically derived hydrogen gas supports hydrothermal vent ecosystems on Earth today and may have been an abundant energy source available during the origin and evolution of early life on Earth. The existence of deep subsurface microbial ecosystems is dependent on the ability of the water-rock environment to provide a suitable habitat for life, which has not been well quantified. This dissertation provides insights into the potential for low temperature water-rock reaction systems to support H₂-utilizing microbial life and, in turn, for microbial activity to directly affect the water-rock reaction pathways and processes.

The partitioning of Fe into secondary minerals is an important control on the generation of H₂. However, characterization of these phases is hampered by the small amount of reaction products generated in laboratory scale experiments. A synchrotron x-ray based method integrating micro-spectroscopy and micro-XRF data collection and processing was developed to characterize the speciation of Fe in the rare, microscale solid phase reaction products. In H₂-generating experiments conducted at 100°C, Fe(III)-oxides were detected on the surface of spinel particles, while Fe(II)-brucites and talcs were associated with dissolving olivine and pyroxene surfaces. The spinels may be required to mediate electron transfer between Fe(II) and water. Thus, H₂-generation is likely a surface controlled process catalyzed by spinels and accommodated by the formation of Fe(III)-oxides. In such a system, microbial colonization of reactive surfaces may be strongly controlled by the heterogeneous distribution of H₂ production.
To determine if an Archaeal methanogen present in-situ would affect the solid and aqueous geochemistry of water-rock reactions in distinct ways, a water-basalt system amended with Fe\(^0\) was inoculated with a methanogen. The reaction products in the abiotic experiment were dominated by Fe-phyllosilicates in contrast to the culture experiment in which an Fe-bearing pyroxene was detected and Fe-phyllosilicates were absent. The unique secondary mineral assemblage in the presence of an active methanogen suggests that H\(_2\)-utilizing microorganisms do influence the reaction pathways. Therefore, the work presented in this dissertation has helped to advance our understanding of low-temperature water-rock reactions and the potential for microbial activity to survive in and affect these systems.
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CHAPTER 1

Introduction
1. H$_2$ fueled life in the subsurface of Earth

Life on Earth today is thought to reflect the geologic environments in which its ancestor arose and evolved (e.g. Nisbet and Sleep, 2001; Saito et al., 2003; Dupont, et al., 2006). Chemolithoautotrophs, dependent on chemical sources of energy and capable of fixing carbon from CO$_2$ into biomass, are thought to be the most closely related extant life to that which originated and existed early in Earth’s history. H$_2$ gas is one of the most important electron donors for extant chemolithotrophs and it has been postulated that the last common ancestor was an H$_2$-utilizing microorganism (Nisbet and Sleep, 2001). Thus, it seems likely that life originated in an environment where H$_2$ was plentiful. Today, subsurface water-rock reactions (e.g. serpentinization) are an important geologic source of H$_2$ gas in both terrestrial and submarine environments on Earth. Terrestrial H$_2$-rich environments include ophiolite complexes (Neal and Stanger, 1983; Abrajano et al., 1988; Fritz et al., 1992; Schulte et al., 2006; Johnson et al., 2006), hot springs (Chapelle et al., 2002; Reysenbach et al., 2000a; Spear et al., 2005), and subsurface aquifers (Olson et al., 1981; Stevens and McKinley, 1995). Submarine H$_2$-rich environments include shallow (e.g. Vulcano Island, Italy (Amend et al., 2003)) or deep, ridge (e.g. Rainbow (Charlou et al., 2002)) and off-axis hydrothermal vent systems (e.g. Lost City (Kelley et al., 2001)) and other unique geologic settings such as the mid-Cayman Rise, a slow-spreading system that is isolated from the global ridge system (German et al., 2010; Connelly et al., 2012).

It has been postulated that H$_2$-generating water-rock reactions were even more prevalent on early Earth, due to the higher heat budget soon after planetary accretion and the exposure of more ultramafic rocks (e.g. Nisbet and Sleep, 2001; Hellevang et al., 2011). Perhaps H$_2$ was one of the earliest sources of energy on Earth (e.g. Sleep et al., 2004; Russell et al., 2010) as H$_2$-utilizing Bacteria and Archaea occur almost exclusively on many of the deepest branches of the
tree of life (Woese et al., 1990; Pace, 1997) also suggesting the antiquity of H2-based metabolisms (Pace, 1991). The production and conservation of energy from the conversion of hydrogen and carbon dioxide to methane by microorganisms, methanogenesis, is considered to be one of the earliest metabolic processes on Earth; organisms that possess this capability are also predominately found at the base of the tree of life (e.g. Woese, 1990; Pace, 1997; Reysenbach and Shock, 2002; Martin and Russell, 2007). Since the discovery of hydrothermal vent systems, these unique geologic systems have been postulated to be the site of the origin and earliest habitats of life on Earth (e.g. Pace 1991; Russell et al., 1989, 1994; Nisbet and Sleep, 2001; Sleep et al., 2004; Fruh-Green et al., 2004; Holm et al., 2006; Martin and Russell, 2007; Martin et al., 2008).

In the case of submarine hydrothermal vent chimneys, dissolved or gaseous H2 derived from high temperature (>150°C) water-rock reactions supports growth of microbial ecosystems when transported to low(er) temperature sites colonized by H2-utilizing microorganisms (e.g. Kelley et al., 2002; Takai et al., 2004; Martin et al., 2008; Schrenk et al., 2010; Edwards et al., 2011). Lost City and Rainbow hydrothermal vent systems are two of the best and most well studied examples of hydrothermal activity driven by water-rock reactions (Kelley et al, 2001; Charlou et al., 2002; Fruh-Green et al., 2003; Allen and Seyfried, 2004; Kelly et al., 2005). At Lost City, water-rock reactions occurring at up to 200°C (Ludwig et al., 2005) feed vents with fluid temperatures of 40-90°C and elevated concentrations of H2 (Kelley et al., 2005). The fluids support extensive biofilms in which the predominant metabolic processes oxidize H2 (e.g. methanogens and sulfate reducers) (Schrenk et al., 2004; Brazelton et al., 2006; Brazelton et al., 2011). The Rainbow hydrothermal vent system is an on-axis system driven by serpentinization of harzburgites but influenced by the proximity of a magma chamber (Lowell and Rona, 2002;
Allen and Seyfried, 2004). Rainbow vent fluids are extremely enriched in H₂ (up to 16 mmol/kg) (Charlou et al., 2002), the composition of which is thought to be controlled by reaction temperatures up to 400°C (Allen and Seyfried, 2003). Functional gene analyses of chimney structures with temperatures of 40-50°C and hydrothermal sediments at Rainbow show a predominance of H₂-oxidizing metabolisms, including methanogenesis and sulfate-reduction (Nercessian et al., 2005a). Pyrosequencing analyses of 16S rRNA vent deposits at Rainbow reveal the dominance of Archaeal methanogens as well as the presence of hydrogen oxidizing sulfate reducers (Flores et al., 2011). Thus, the importance of rock-derived hydrogen in supporting microbial ecosystems at submarine hydrothermal vents is relatively well defined.

However, focused hydrothermal vents are rare when considering the entire extent of the ocean crust. The majority of potential microbial habitats supplied with rock-derived H₂ gas are likely to be within the crustal rocks themselves. However, it has generally been thought that the rate and extent of production of H₂ from water-rock reactions at temperatures within the range suitable for life (<~130°C) will be limited (e.g. Anderson et al., 1998; 2001; McCollom and Bach, 2009). Direct evidence for the existence and potential distribution of H₂-based microbial communities within the igneous ocean crust is limited (e.g. Cowen et al., 2003; Fisk et al., 2003; Mason et al., 2010). Thus, while the interior of rocks has the potential to house a huge amount of microbial life, there is still little known about the extent of colonization and the specific metabolic strategies employed by potential subsurface communities

2. The subsurface ocean crust as a microbial habitat

The possibility of extensive microbial life existing in Earth’s crust was first proposed by Gold (1992) though published observations of subsurface microbes date to the 1920s (Boston et
Gold (1992) hypothesized that in the absence of photosynthesis, subsurface life would depend on chemical sources of energy provided by fluids flowing up from even greater depths in the Earth. He also suggested that perhaps ‘microbial life exists in all the locations where microbes can survive’ given that a location has a chemical energy supply and a temperature below the maximum temperature limit of life. A hyperthermophilic methanogen can survive to 122°C at 20MPa, the current known temperature limit of life (Takai et al., 2008). However, it may be possible for life to survive at temperatures up to 130-150°C (e.g. Daniel et al., 2004). At high temperatures life is limited by the breakdown of key biomolecules including nucleic acids and enzymes yet the stability of many of these molecules at high temperatures is only loosely constrained (Daniel et al., 2004). Considering the temperature limits of life, Gold (1992) calculated the total pore space within crust down to 5 km, the depth at which temperature would become limiting, and estimates that 2 x 10^{14} tons of bacterial biomass could potentially be housed in this environment if adequate chemical energy sources were available. Gold (1992) thus suggested that subsurface biomass may be equal to the biomass on the surface of the Earth. Calculations conducted by Whitman et al. (1998) (including sediment volumes) estimated prokaryotes in the subsurface to total 3.8-6 x 10^{30} cells; approximately 90-94% of all prokaryotes on Earth (Baross et al., 2004).

It has been suggested that a huge amount of biomass may call the subsurface their home, perhaps even a greater number than those living at the surface. A more recent study uses the correlation of specific surface area measurements of ocean crust basalt with porosity to estimate the total surface area of the 500 m thick shallow basaltic ocean crust (Layer 2A) (Nielsen and Fisk, 2010). This value (10^{34} m^2) along with assumptions of the amount of pore space occupied per cell and an average cell volume are then used to estimate the biomass in Layer 2A to be 3-20
x 10^{29} \text{ cells} - \text{a density of } 0.3-2 \times 10^{6} \text{ cells/m}^2 \text{ (Nielsen and Fisk, 2010). Thus, the potential for low temperature water-rock reactions to take place in these geologic environments and produce H}_2 \text{ is an important aspect to understanding these systems as potential microbial habitats.}

It is apparent that water-rock reaction processes play an important role in the ability for life to exist and persist in the interior of subseafloor igneous rocks. Though little is known specifically about the microbial communities that inhabit the igneous ocean crust due to access and sampling technology limitations (e.g. Edwards et al., 2011; Orcutt et al., 2011), H}_2 \text{ may play an important role in supporting these communities. Cowen et al. (2003) were the first to access and investigate fluids circulating through basaltic ocean crust. Using 16S rRNA molecular techniques they identified a number of organisms, many of who are most closely related to nitrate and sulfate reducing organisms. The authors propose that H}_2 \text{ gas, produced from water-basalt reactions, may act as the electron source for these organisms. This work was evidence for the existence of a subsurface biosphere away from the localized hydrothermal vent systems of the mid-ocean ridges (Cowen et al., 2003). DNA extracted from drill core samples of Hawaii basalts revealed the prevalence of Archaea similar to Crenarchaeota that were previously identified from hydrothermal, mid-ocean ridge, and deep sediment habitats (Fisk et al., 2003). More recently, the first investigation of life in the interior of gabbroic rocks was published (Mason et al., 2010). A suite of molecular techniques detected 16S rRNA genes representative of proteobacteria and functional genes corresponding to nitrate, sulfate, and metal reduction, methane cycling, hydrocarbon degradation, as well as carbon and nitrogen fixation. The wide diversity of possible microorganisms and metabolisms is correlated to high percentages of rock alteration, perhaps due to increased permeability and oxidation state gradients in the more highly altered rock (Mason et al., 2010).}
3. Composition and structure of the ocean crust

The ocean crust covers ~65% of the Earth’s surface and is often described as the largest aquifer on Earth and the entire volume of the ocean can circulate through the $2.3 \times 10^{18}$ m$^3$ of rock in approximately $10^5$-$10^6$ years (e.g. Johnson and Pruis, 2003; Schrenk et al., 2010; Orcutt et al., 2011). Knowledge of the structure and composition of the ocean crust is derived from shipboard seismic surveys, ophiolites (ocean crust that has been abducted onto continental crust), and drill hole and dredge samples of the crust itself (Alt, 1995 and references therein). The ocean crust can be described as being comprised of four distinct layers, from shallow to deep these include 1) sediments (0-1 km), 2) pillow basalts and sheeted dikes (1-3 km), 3) gabbros (4-5 km), and 4) peridotites which are part of the underlying lithospheric mantle (e.g. Alt, 1995). A number of recent reviews have described in detail the different components of the ocean crust and their potential to provide microbial habitats (e.g. Edwards et al., 2005; Schrenk et al., 2010; Edwards et al., 2011; Orcutt et al., 2011). In the context of this thesis, only those components relevant to igneous rock-hosted environments will be discussed. Deep sediment environments, which are known to be an important habitat for subsurface life (e.g. Whitman et al., 1998), will be knowingly omitted from the following discussion.

Subsurface igneous rock habitats can generally be broken down into 4 distinct locales 1) mid-ocean ridge associated, 2) ridge flank, 3) seamounts, and 4) trenches (e.g. Schrenk et al., 2010; Edwards et al., 2011). The majority of fluid circulation is thought to occur in the upper most basaltic layer because of its higher porosity and permeability (Alt, 1995 and references therein; Fisher and Becker, 2000; Alt and Bach, 2003). However, because of the dependency on porosity and the variability of this parameter throughout the ocean crust the depth of circulation is not well defined and may extend to much greater depths (Alt, 1995 and references therein;
Edwards et al., 2005). The temperature of seawater circulation varies, with higher temperatures in the vicinity of mid-ocean ridges (>250°C) and lower temperatures in the ridge flanks (<200°C) (Alt, 1995 and references therein). Recharge zones where seawater penetrates ocean crust are locations of strong temperature gradients and alteration of the crust can occur at low temperatures (Alt, 1995 and references therein).

The generalized description of potential ocean crust habitats does not include unique geologic environments such as at oceanic core complexes, the setting of the Lost City hydrothermal vent field (Kelly et al., 2001, 2005, 2007). This fault-controlled geology exposes typically deep-seated gabbros and peridotites to pervasive seawater circulation thus creating an environment with great H₂ generation potential. Oceanic core complexes are also observed in association with the Mid-Cayman Spreading Center. In this system, gabbros and peridotites are exhumed along shear zones and uplifted by a combination of high angle and detachment faults putting the ultramafic rocks in contact with mafic basalts (Hayman et al., 2011). Diabase intrusions are present and have experienced varying amounts of hydrothermal alteration. Multiple types of hydrothermal vent systems are present at the Mid-Cayman Spreading Center indicative of seawater circulation though the variety of rock types that are present. High temperature black smoker type systems thought to be associated with basalts have been identified within the volcanic field (German et al., 2010; Hayman et al., 2011). Lower temperature systems have been detected off-axis and are most likely driven by serpentinization of peridotites exposed in the oceanic core complexes (German et al., 2010; Hayman et al., 2011). Active hydrothermal vent systems have not been specifically located in conjunction with the diabase intrusions however, alteration of these rocks is evidence that hydrothermal activity occurred in association with the intrusions. In locations where oxygen has been effectively
removed from the circulating seawater, low temperature H₂-producing water-rock reactions are likely to occur. Thus, zones of seawater circulation with temperatures within the limits of life are likely to exist and provide energy for life.

4. Sources of H₂ gas

There are a number of different methods by which H₂ gas may be generated from geologic processes. Hydrogen production may result from gas phase equilibrium between C-H-O-S in magmas according to reactions 1 - 3 (e.g. Apps and van de Kamp, 1994). This hydrogen is released during magmatic degassing (e.g. Aiuppa et al., 2011).

1) \(2\text{H}_2\text{S} = \text{S}_2 + 2\text{H}_2\)

2) \(\text{S}_2 + 4\text{H}_2\text{O} = 2\text{SO}_2 + 4\text{H}_2\)

3) \(\text{SO}_2 + 2\text{H}_2\text{O} = \text{H}_2\text{SO}_4 + \text{H}_2\)

In hydrothermal systems and often in association with magma bodies, H₂ may be produced through reaction of pyrrhotite or amorphous iron sulfide minerals with hydrogen sulfide gas as depicted in Reaction 4 (Drobner et al., 1990).

4) \(\text{FeS} + \text{H}_2\text{S} \rightarrow \text{FeS}_2 + \text{H}_2\)
In similar conditions, H₂ may also be generated from the breakdown of methane gas. At temperatures greater than 600°C, methane gas will decompose to graphite and hydrogen according to Reaction 5 (Apps and van de Kamp, 1994).

\[ 5) \text{CH}_4 = \text{C} + 2\text{H}_2 \]

Hydrogen can also be generated from methane according to reaction 6 which is favored at temperatures \( \geq 1200^\circ\text{C} \) (Apps and van de Kamp, 1994).

\[ 6) \text{CH}_4 + 2\text{H}_2\text{O} \rightarrow \text{CO}_2 + 4\text{H}_2 \]

There are multiple ways in which water can be hydrolyzed resulting in the production of H₂. Radiolysis of water occurs when alpha or gamma particles emitted from radionuclides splits water into free radicals which react to form O₂ and H₂ (e.g. Savary and Pagel, 1997; Apps and van de Kamp, 1994). Hydrogen generated through this process is thought to support microbial ecosystems (e.g. Pederson et al., 1997; Lin et al., 2005). Hydrolysis of water and production of H₂ can also be driven by brittle fracturing of silicate minerals within rocks (e.g. Wakita et al., 1980; Kita et al., 1982; Apps and van de Kamp, 1994; Hirose et al., 2011). Reactive sites on mineral surfaces generated by fracturing can bind water and promote hydrolysis as demonstrated in laboratory experiments (Kita et al, 1982). This process can occur as a result of tectonic events but the amount of H₂ produced is difficult to quantify (Wakita et al, 1980; Hirose et al., 2011). Hydrogen gas may also be derived thermal decomposition of alkanes, alkenes and organic acids (Seewald, 2001) or fermentation by bacteria (e.g. Jackson and McInerney, 2002).
However, hydrolysis of water by ferrous minerals in mafic and ultramafic rocks is thought to be the most important geologic source of H₂ (Apps and van de Kamp, 1994) and will be the process focused on throughout the remainder of this dissertation.

5. Water-rock reactions in the subsurface

5.1 Water-basalt reactions

As stated in section 2, the majority of seawater circulation is likely to occur in basalts and as such, they are likely to house a large proportion of the subsurface biosphere. Whether this life can be sustained by H₂ production from low temperature water-basalt reactions is debated (see section 7) and complicated by the higher activity of SiO₂ in basalts relative to peridotites (see section 5.2.3). However, it is important to consider this potential microbial habitat and the geochemical reactions that might fuel it. There exists an extensive body of literature concerned with basalt-seawater interactions from the perspective of understanding the mechanisms of hydrothermal alteration and its effects on seawater composition. While this work does not include measurements of H₂ production, it provides useful insights into the secondary mineral assemblages associated with water-basalt reactions.

Investigations into both experimental and natural basalt alteration reveal phyllosilicates, including smectite and chlorite, and Fe oxyhydroxides, as the predominant secondary minerals, +/- talc and carbonates in more reducing and/or higher temperature conditions. Experimental investigations have covered a range of temperatures (25-500°C), pressures (400-1000 bars), and water-rock ratios (0.5-125). Tremolite, actinolite, and talc have been reported at high temperatures (400-500°C) (Mottl and Holland, 1978). Smectite, anhydrite, and chlorite +/- Fe...
oxyhydroxides are common across a wide range of temperatures (150-400°C) (Mottl and Holland, 1978; Seyfried and Bischoff, 1979, 1981; Seyfried and Mottl, 1982; Seewald and Seyfried, 1990). Carbonate and phyllosilicates, including serpentine phases, have been reported at 50 and 90°C (Thomassin and Touray, 1982; Crovisier et al., 1983; Thomassin et al., 1989). Secondary mineral phases produced experimentally are similar to those that occur naturally. Talc is generally limited to high temperature alteration zones (200-350°C) (Shau and Peacor, 1992) but has been reported from alteration at <150°C (Hunter et al., 1999). Phyllosilicates and carbonates (ankerite and siderite) are reported at temperatures ~ <70°C (Shau and Peacor, 1992; Laverne, 1993; Alt and Teagle, 2003). Many of these minerals are Fe(III)-bearing and thus suggest that Fe redox processes occur during basalt-water reactions. With the oxidation and incorporation of Fe into secondary mineral phases, it seems likely that in anoxic conditions these processes could generate hydrogen. As such, transformations of Fe associated with basalt alteration could potentially support H₂-based microbial ecosystems. However, data concerning H₂ generation from basalt-water reactions is limited (see sections 6 and 7). Most of what is know of H₂ generation from water-rock reactions is instead based in our understanding of the process of serpentinization.

5.2 Serpentinization

The most commonly discussed and well-studied water-rock reaction is serpentinization; defined as the hydration of ultramafic rocks that results in the formation of serpentine group minerals. Through hydration and alteration of primary igneous rocks serpentinization greatly affects the mineralogy and density of the ocean crust. As this process occurs extensively throughout the oceanic lithosphere it has great implications for the rheology, chemistry, and
microbial habitability of the ocean crust (e.g. Klein et al., 2009 and references therein). For these reasons, and because of the unique geochemical conditions represented by serpentinizing systems, many in-depth petrologic and experimental studies of the serpentinization process have been conducted (Frost, 1985 and references therein). The serpentinization reaction is generally represented as written in reaction 7 (e.g. McCollom and Bach, 2009).

(reaction 7)

\[
\text{Mg}_{1.8}\text{Fe}_{0.2}\text{SiO}_4 + 1.37\text{H}_2\text{O} \rightarrow 0.5\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4 + 0.3\text{Mg}(\text{OH})_2 + 0.067\text{Fe}_3\text{O}_4 + 0.067\text{H}_2
\]

Olivine (Fo90)  serpentine  brucite  magnetite

There is evidence for pH values up to ~ 12, values that result from the fluid being in equilibrium with the mineral assemblage and the production of H\textsubscript{2} maintains reducing conditions that are often indicated by the presence of native iron and iron alloys (e.g. Moody 1976b and references therein; Bach et al., 2006; Seyfried et al., 2007; McCollom and Bach, 2009; Klein et al., 2009). The production of H\textsubscript{2} results from oxidation of Fe, from Fe(II) in primary minerals to Fe(III) in secondary minerals, a process that hydrolyzes water to produce H\textsubscript{2} gas Thus, the oxidation of Fe(II) and the incorporation of Fe(III) into secondary mineral phases is key to H\textsubscript{2} generation from water-rock reactions.

5.2.1 Experimental investigations of H\textsubscript{2} and secondary mineral production during serpentinization
The results of experimental investigations suggest that serpentinization is not as straightforward as written in Reaction 1. A variety of secondary mineral phases can be produced dependent on the starting phases and the reaction conditions. Laboratory investigations of H₂ generation and secondary mineral characterization in peridotite-, olivine-, and pyroxene-water experiments have been conducted at 200-400°C (e.g. Berndt et al., 1996; Allen and Seyfried, 2003; Seyfried et al., 2007; Jones et al., 2010). At 400°C, olivine is relatively unreactive while orthopyroxene, orthopyroxene + clinopyroxene, and olivine + orthopyroxene + clinopyroxene produce ~5 mmolal H₂ and magnetite, serpentine, talc, and tremolite as secondary mineral phases (Allen and Seyfried, 2003). At 300°C, the alteration of olivine to serpentine + brucite + magnetite produced ~160 mmolal H₂ (Berndt et al., 1996). At 200°C, olivine altered to chrysotile and magnetite and produced 9-13 mmolal H₂ in carbonate-undersaturated conditions while in carbonate-oversaturated conditions less magnetite and H₂ were produced as Fe(II) was partitioned into carbonate phases (Jones et al., 2010). In peridotite-water reactions conducted at 200°C, serpentine, anhydrite, and brucite were produced as was 77 mmolal H₂ in the absence of detectable magnetite precipitation (Seyfried et al., 2007). The production of H₂ in the absence of magnetite precipitation suggests that the complexity of the serpentinization process is not fully represented as written in Reaction 7.

5.2.2. Partitioning of Fe during serpentinization

The partitioning of Fe into secondary mineral phases has important implications for the amount of H₂ produced during water-rock reactions. In reaction 7, all of the Fe is incorporated into magnetite and the maximum H₂ is generated. However, experimental results and geochemical models of peridotite-water reactions show Fe (II and III) substitution into secondary
serpentine and brucite phases (Whittacker and Wicks, 1970; Moody 1976a; Berndt et al., 1996; Seyfried et al., 2007; McCollom and Bach, 2009; Klein et al., 2009). Analyses of naturally serpentinized rocks also reveal Fe substitution into secondary minerals (e.g. Moody 1976a, Bach et al., 2006; Klein et al., 2009). In serpentinization experiments conducted at ~300-360°C, 0 to 18 mol% Fe(OH)₂ was measured in precipitated brucites and higher Fe(OH)₂ contents were correlated with the production of less magnetite and lower reaction temperatures (Moody 1976a). Modeling of peridotite-water reactions also predict enhanced Fe(II) substitution into brucite at lower reaction temperatures (Sleep et al., 2004; McCollom and Bach, 2009; Klein et al., 2009). The instability of Fe-brucite at high temperatures causes Fe to be preferentially partitioned into magnetite because the assemblage Mg-brucite + magnetite is more stable than Fe-brucite at high temperatures while at lower temperatures Fe-brucite is more stable (Moody 1976a). Thus, at lower reaction temperatures, more Fe(II) is incorporated into brucite, limiting the oxidation of Fe(II), formation of magnetite, and amount of H₂ generation (Moody, 1976a, 1976b; Bach et al., 2006; Seyfried et al., 2007; McCollom and Bach, 2009; Klein et al., 2009) and potentially the ability of these low temperature reactions to support life.

Secondary minerals other than magnetite can accommodate Fe(III) in their structures and allow for H₂ generation. In both modeling and experimental investigations H₂ production is documented in the absence of magnetite formation (Seyfried et al., 2007; Klein et al., 2009). Experiments at 200°C yielded Fe-brucites (~ 30 mol % Fe) as well as Fe-bearing serpentes (~ 7 mol % Fe) (Seyfried et al., 2007). Mossbauer analyses indicated that both ferrous (58%) and ferric (42%) iron was incorporated into serpentine. Due to the absence of significant quantities of magnetite, it was inferred that the observed H₂ production was due to the oxidation and incorporation of Fe(III) into chrysotile (reaction 8) (Seyfried et al., 2007).
reaction 8 – (Seyfried et al., 2007)

\[ 4.31(Mg_{0.90}Fe_{0.10})_2SiO_4 + 2.64(Mg_{0.87}Fe_{0.13})SiO_3 + 7.99H_2O \rightarrow \]

Olivine (Fo90) orthopyroxene

\[ 3.48(Mg_{0.915}Fe_{0.04}Fe_{0.03})_3Si_2O_5(OH)_4 + 0.83(Mg_{0.70}Fe_{0.30})(OH)_2 + 0.21H_2 \]

Fe-serpentine Fe-brucite

In geochemical models, when Fe(III) is allowed to partition into serpentine, the stability of magnetite is limited to temperatures \( \geq \sim 200^\circ C \) (Klein et al., 2009). The lack of magnetite precipitation in 200°C water-peridotite experiments (Seyfried et al., 2007) may be related to the decreased magnetite stability at \( \sim 200^\circ C \). Serpentine minerals in naturally occurring serpentinites possess significant amounts of Fe(III) (up to 70% Fe(III)) (e.g. O’Hanley and Dyar, 1994; Evans et al., 2009) as do serpentines formed in laboratory reactions at 300°C (up to 37% Fe(III)) (Marcaillou et al., 2011). Klein et al. (2009) detect 30-50 mol % Fe(III) in abyssal peridotites and conclude that significant amounts of H\(_2\) generation can be attributed to Fe(III) incorporation into serpentine. The partitioning of Fe(III) into serpentine is accompanied by the partitioning of Fe(II) into brucite at low temperatures (150-250°C) and water-rock ratios. Evans et al. (2009) conclude that H\(_2\) production due to incorporation of Fe(III) into serpentine, in the absence of magnetite, will be approximately one-third per mole of H\(_2\)O as compared to magnetite producing reactions as illustrated in Reaction 9.
Fe$_2$SiO$_4$ + 3H$_2$O $\rightarrow$ (Fe$^{2+}$Fe$^{3+}$)(Fe$^{3+}$Si)O$_5$(OH)$_4$ + SiO$_2$$_{aq}$ + H$_2$$_{aq}$  \\
(Reaction 9)

olivine (Fa)  Fe(III)-serpentine

(Evans et al., 2009)

Thus, the partitioning of Fe(II) and the potential for Fe(III) to be incorporated into secondary phases other than magnetite, further complicates our understanding of the mechanisms of H$_2$ generation during serpentinization reactions.

5.2.3 The role of SiO$_2$$_{aq}$ in serpentinization

It is clear from natural examples and experimental investigations of serpentinization that the activity of SiO$_2$ can influence the types and amounts of reaction products. Hostetler et al. (1966) noted the absence of brucite in serpentinites containing >40% pyroxene, which are more Si-rich than olivine. Similarly, brucite is absent from the secondary mineralogy of water-pyroxene reactions and correlated with the relatively high concentrations of SiO$_2$ observed in these systems (Allen and Seyfried, 2003). In natural serpentinites, Klein et al. (2009) observe zoning of secondary minerals where brucite is present at the olivine interface and the mineral assemblage transitions to serpentine + brucite +/- magnetite at intermediate locations and serpentine + magnetite furthest from the olivine grain boundaries. As brucite is not stable at log aSiO$_2$ > $\sim$10$^{-3.5}$, this zonation is attributed to gradients of Si that form as olivines (relatively Si-poor) and pyroxenes (relatively Si-rich) simultaneously undergo alteration in a peridotite (Klein et al., 2009). Similarly, the occurrence and association of talc, which is stable at relatively high concentrations of SiO$_2$ (log aSiO$_2$ > 10$^{-5}$), with pyroxene grains also suggests the existence of Si gradients within serpentinites (Viti et al., 2005). Frost and Beard (2007) suggest that the absence of magnetite from some serpentinites can be explained by the activity of SiO$_2$. Serpentinization
occurring at specific SiO₂ activities, within which brucite is stable but above the range of magnetite stability, would result in the absence of magnetite, and the incorporation of Fe into brucite and serpentine (Frost and Beard, 2007). Higher SiO₂ favors increased incorporation of Fe into serpentine and brucite, the incorporation of Fe into these minerals results in less H₂ production relative to Fe incorporation into magnetite (Evans, 2009) therefore, the activity of SiO₂ is an important control on the amount of H₂ production.

5.2.4 Serpentinization as a surface controlled process

Reaction mechanisms and rates are also important controls on the progress of serpentinization and thus H₂ production. For instance, the rate of dissolution of olivine has been shown to be directly dependent on proton activity at pH values < 9 (Pokrovsky and Schott, 2000; Rosso and Rimstidt, 2000; Oelkers, 2001). However, at a range of temperatures and pressures, the rate determining step in the serpentinization process has been suggested to be the nucleation and growth of serpentine on mineral surfaces (e.g. Martin and Fyfe, 1970). However, the diffusion of water, through any secondary mineral layers, to the olivine surfaces is also an important control on the reaction progress (e.g. Martin and Fyfe, 1970). For silicate minerals, the hydration of metal-oxygen bonds at the mineral surface is a key component controlling dissolution (e.g. Schott and Berner, 1983; Schott et al., 2009 and references therein). At low temperatures, numerous researchers have demonstrated the passivation of olivine surfaces by formation of secondary mineral phases (e.g. Bearat et al., 2006; Andreani et al., 2009; King et al., 2010; Daval et al., 2011). Formation of Si-rich layers occurs due to incongruent dissolution of olivine that preferentially releases Fe and Mg to solution (e.g. Schott and Berner, 1983; Schott et al., 2009; Giammar et al., 2005; Bearat et al., 2006; Andreani et al., 2009; King et al., 2010;
Daval et al., 2011). Eventually, these layers become thick enough to limit diffusion of water to the mineral surface, hydration of the Fe-O bonds, and release of Fe(II) from the mineral structure. This, in turn, limits the amount of Fe available to be oxidized in conjunction with hydrolysis and H₂ production.

6. Microbe-Fe-silicate interactions

It has been well established in the literature that microbes can affect both the dissolution and precipitation of minerals. Mineral precipitation can be either biologically induced (BIM) or biologically controlled (BCM) (e.g. Frankel and Bazylinski, 2003 and references therein). In biologically controlled mineralization, precipitation is likely directly controlled by the metabolic and genetic processes associated with the organisms and minerals are generally precipitated in direct contact with the cells (e.g. Frankel and Bazylinski, 2003 and references therein). The close association between cells and minerals and in this process leads to well-defined mineral structures and sizes. Cellular metabolic processes that induce changes in the chemical environment leading to nucleation and growth of minerals are termed biologically induced mineralization (e.g. Frankel and Bazylinski, 2003). These phases generally cannot be distinguished from abiotically precipitated phases on the basis of size and structure. This process is more prevalent in anaerobic, sub surface environments; habitats where respiration of metal ions is dominant (e.g. Frankel and Bazylinski, 2003). Biologically induced mineralization can result from the creation of microenvironments on mineral surfaces. Microorganisms that can limit the diffusion of ions (e.g. Fe, Mg, Si) away from the mineral surface, create a localized supersaturated environment that favors the precipitation of minerals (e.g. Ullman et al., 1996; Benzerera et al., 2004). Cell surfaces may also act as a reactive surface for nucleation and
initiation of mineral precipitation (e.g. Urrutia and Beveridge, 1994; Fortin et al., 1997 and references therein; Fortin et al., 1998; Frankel and Bazylnski, 2003 and references therein).

Microbes can also affect silicate mineral dissolution (e.g. Ehrlich, 1996). Increased release of Si, Fe, and Mg from mineral structures may be accomplished through microbial production of ligands that act to solubilize minerals (e.g. Ullman et al., 1996; Barker et al., 1997; Barker et al., 1998; Liermann et al., 2000; Kalinowski et al., 2000; Welch and Banfield, 2002; Daughney et al., 2004). Organic acids produced by organisms can cause enhanced mineral dissolution by acting as ligands that promote dissolution (e.g. Ullman et al., 1996; Welch and Ullman, 1999; Liermann et al., 2000) or by lowering the pH of the aqueous phase (e.g. Barker et al., 1997; Wu et al., 2007). Similarly, pH values at mineral surfaces in contact with cells can also be very low (e.g. Barker et al., 1998). Organic materials produced by cells can also inhibit mineral dissolution by binding to and passivating mineral surfaces (e.g. Ullman et al., 1996 and references therein; Benzerara et al., 2004). Byproducts of microbial metabolism can also inhibit dissolution. Santelli et al. (2001) observed decreased dissolution of fayalite in the presence of Acidithiobacillus ferrooxidans, an Fe oxidizing bacteria, and attributed it to production of Fe(III), which then adsorbed to the fayalite surfaces. Thus, there are a variety of ways in which mineral assemblages can be, at least partially, controlled by microbial activity.

The alteration of Fe-silicate minerals has been investigated in the presence of microorganisms in a variety of experimental contexts (e.g. Kalinowski et al., 2000; Liermann et al., 2000; Santelli et al., 2001; Benzerara et al., 2004; Daughney et al., 2004; Wu et al., 2007). In the presence of microbial consortia or microorganisms that produce siderophores (i.e., Fe chelating organic compounds), aqueous Fe concentrations are higher than in abiotic control experiments (Kalinowski et al., 2000; Liermann et al., 2000; Daughney et al., 2004; Wu et al.,
2007). In the presence of aerobic, non-siderophore producing organisms, dissolution of orthopyroxene and fayalite decreased due to passivation of mineral surfaces either by adsorption of Fe(III) or precipitation of an amorphous layer on mineral surfaces (Santelli et al., 2001; Benzerara et al., 2004). Fe-bearing silicate minerals have also been discovered in close association with bacterial surfaces, suggestive of microbially induced precipitation, in a variety of natural environments (Fortin et al., 1997 and references therein; Fortin and Beveridge, 1997; Fortin et al., 1998).

In experiments investigating microbe-Fe-silicate interactions in anaerobic conditions, *Shewanella putrefaciens* was found to couple reduction of Fe(III) in smectite (clay) to energy generation (Kostka, et al., 1996). A number of other pure bacterial strains, as well as bacterial consortia enriched from a variety of soils, can also reduce Fe(III) in smectite suggesting that this is not an uncommon metabolic strategy (e.g. Kostka et al., 1999). Thus, while it has only recently been demonstrated, it is not surprising that methanogens were found to be capable of reducing Fe(III) in nontronite (Fe(III)-bearing clay) (Liu et al., 2011). As such, methanogens are capable of promoting dissolution of the clay and release of Fe(II) to solution (Liu et al., 2011). However, it may also be possible for methanogens to encourage mineral precipitation. Orange et al. (2011) demonstrated the sorption of Fe(III) and Ca$^{2+}$ to the S-layer and plasma membrane of *Methanocaldococcus jannaschii*. Increased silicification of *M. jannaschii* was observed following sorption of Fe(III) to the cell wall (Orange et al., 2011).

7. Potential ecosystems in low temperature H$_2$-generating water-rock systems

In order for H$_2$-metabolizing microorganisms to be able to influence water-rock reactions these reactions must take place with the temperature limits of life. However, adequate
production of H₂ from low temperature water-rock reactions to support life is debated. Stevens and McKinley (1995) were the first to propose the existence of a subsurface lithoautotrophic microbial ecosystem (SLiME) supported by H₂ production from low temperature water-basalt reactions within the Columbia River Basalt aquifer. Measured H₂ concentrations of 60 micromolar in CRB ground waters were attributed to water-basalt reactions and stable isotope and microcosm experiments suggested methanogens were present and active in the ground waters (Stevens and McKinley, 1995). However, this proposal was challenged because of questions concerning the amount and duration of H₂ generation from low temperature water-rock reactions as will be discussed below (e.g. Anderson et al., 1998).

Other potential SLiMEs have been suggested including a methanogen-dominated community at Lidy Hot Springs (Chapelle et al., 2002) and a H₂ driven HyperSLiME at the Kairei hydrothermal system (Takai et al., 2004). Vent fluids have temperatures >250°C and contain 2.5 mM H₂. Despite the high temperatures, the presence of hyperthermophilic Archaeal methanogens in the vent fluids was detected by both molecular and cultivation methods (Takai et al., 2004). Microhabitats within temperature gradients, defined by proximity to the heat source and interactions with circulating seawater, are proposed to provide reasonable habitats within the temperature limit of life despite the high vent fluid temperatures at the Kairei hydrothermal system (Takai et al., 2004). It is clear from the discussion of the proposed SLiME localities that a consensus concerning the existence of microbial communities within rocks undergoing alteration and H₂ production has yet to be reached. In the absence of a well-defined natural SLiME environment, laboratory investigations can provide important insights into the potential for low temperature H₂ production to support microbial communities.
8. Can low temperature water-rock reactions support H₂ based ecosystems? Predictions from low temperature water-rock experiments

To further investigate the potential for low temperature water-basalt reactions to support subsurface microbial ecosystems, Stevens and McKinley (1995) tested abiotic H₂ production from a variety of basalts incubated with anaerobic water (pH 6) at room temperature. Over the course of five days, rapid H₂ production was documented (Stevens and McKinley, 1995). The results of these experiments stimulated further investigations by other researchers in the field. Anderson et al. (1998) attempted to replicate these results and, at pH 6, observed H₂ production to concentrations 6-fold higher than that documented by Stevens and McKinley (1995). However, H₂ production ceased after ~200 hours of incubation and was not reinitiated upon flushing of the headspace. H₂ production did not occur under pH 8; conditions argued to be more environmentally relevant and indicative of the natural environment. To explain H₂ production at pH 6, Anderson et al. (1998) proposed that the creation of reactive surface sites due to crushing of the rocks produces silica radicals that react with water to make H₂. However, they did not explain how reactive sites could be responsible for H₂ production at pH 6 but not at pH 8. The authors conclude that microbial ecosystems in basalt aquifers are not supported by H₂ production from water-basalt reactions. Instead, they suggest that oxidation of organic matter coupled with sulfate reduction supports a portion of the subsurface community while fermentation of organic matter produces H₂ for methanogenesis (Anderson et al., 1998).

These results inspired further work to test the effect of rock composition, pH, temperature, sterilization method, reducing agents, and product removal on H₂ production (Stevens and McKinley, 2000). Basalt and specific mineral separates (forsterite, fayalite, augite, labradorite) were tested in an attempt to further define the components responsible for H₂
generation. H₂ production was observed from numerous basalt samples at 30 and 60°C. The wide variation in H₂ concentrations was attributed to differences in mineral composition especially with respect to ferrous iron content. Low(er) pH and high(er) temperature were also found to enhance H₂ production. Furthermore, continued H₂ production was inhibited by accumulation of reaction products (i.e. H₂) and stimulated by removal of these products. The authors conclude that Fe(II)-bearing olivine and pyroxene minerals present in basalt react with water at moderate temperatures to produce the H₂ present in the Columbia River Basalt aquifer, a process that they suggest is analogous to serpentinization (Stevens and McKinley, 2000).

In more recent experiments, olivine (Fo₀₉₁)-water reactions at 30, 50, and 70°C were found to produce only very small amounts of H₂ after 9 months of incubation (Neubeck et al., 2011). However, the results were not discussed in the context of previous reported results (i.e. Stevens and McKinley, 2000) and thus explanations for the discrepancies in H₂ production were not provided. The likely precipitation of Fe-bearing secondary minerals is proposed to explain low aqueous Fe concentrations in the 50 and 70°C experiments though the composition of such phases was not investigated (Neubeck et al., 2011). As discussed above, H₂ generation from serpentinization reactions is attributed to the oxidation of Fe(II) in primary minerals to Fe(III) in secondary phases. Thus, the oxidation state of Fe and speciation of Fe-bearing mineral phases is key to understanding H₂ production from water-rock reactions at any temperature. However, characterization of secondary mineral products has not been included in any published study of H₂ production from low temperature water-rock reactions.

This gap in our understanding of low temperature water-rock reactions is filled, in part, by geochemical models that provide important insights into H₂ generation and secondary mineral formation at low temperatures (Palandri and Reed, 2004; McCollom and Bach, 2009; Klein et
The formation of brucite, serpentine, clinopyroxene, chlorite, and minor magnetite are predicted to accompany H₂ production (though in concentrations that are small relative to peak production at ~315°C) at temperatures <150°C (Palandri and Reed, 2004; McCollom and Bach, 2009; Klein et al., 2009). Investigations of H₂ production from a wider variety of rock and mineral substrates and characterization of the corresponding secondary mineral assemblage are necessary to truly understand the potential for low temperature water-rock reactions to support microbial ecosystems.

9. X-ray spectroscopic techniques provide insights into low temperature H₂ production

As discussed in section 5.2.2, understanding the behavior and partitioning of Fe is key to unraveling the reactions and mechanisms responsible for H₂ generation at low temperatures. However, determining the speciation and distribution of Fe on the microscale in complex geologic materials is challenging. Mossbauer spectroscopy is an extremely valuable tool for determining the oxidation state of Fe in geological materials but generally requires large amounts of powdered materials (e.g. Dyar et al., 2006 and references therein). As the amount of reaction products generated during laboratory scale experiments is often quite small, application of the Mossbauer technique is not always realistic. Other techniques more suited for microanalyses pose their own problems. For instance, electron microprobe requires the phases of interest to be exposed at the surface and highly polished, which can be difficult to achieve with phyllosilicates that often occur as surface layers on primary mineral phases. Additionally, electron microprobe analyses do not yield reliable information concerning the speciation of redox active elements in silicate minerals (e.g. Dyar et al., 1989; Sobolev et al., 1999). Similarly, electron dispersive spectroscopy produces only qualitative elemental weight percents and gives no indication of the
speciation of redox active elements. More recently, researchers have been turning to synchrotron x-ray based analyses to interrogate Fe speciation on the microscale. At synchrotron light sources, electro magnets are used to accelerate electrons to close to the speed of light and the full spectrum of light is emitted from these electrons. Monochromators are tuned to select for x-rays within a narrowly defined energy range specifically selected to excite core level electrons from a particular element of interest (Sham and Rivers, 2002; Newville, 2004). The x-ray beam can be focused to obtain spatial resolutions of 10s of nanometers to millimeters enabling interrogation of microscale phases (e.g. Manceau et al., 2002; Templeton and Knowles, 2009). X-ray adsorption near edge spectroscopic (XANES) techniques collect x-ray absorption and fluorescence data as a sample is exposed to the x-ray beam at small energy increments (e.g. 0.3 – 1 eV steps over a few hundred eV range) (Stohr, 1992). At the Fe K-edge, the edge and peak positions of spectra vary on the order of a few electron volts dependent on the valence state of Fe. Analyses of the pre-edge features of the spectra can be applied to pure materials to more quantitatively determine the oxidation of Fe (e.g. Delaney et al., 1998; Wilke et al., 2001; Berry et al., 2003). Recently, this technique has been implemented to investigate the speciation of Fe in bulk samples of serpentine minerals produced in laboratory experiments at 300°C (Marcaillou et al., 2011). X-ray fluorescence (XRF) mapping using precisely tuned x-rays has enabled production of oxidation state maps. Energies for mapping are chosen to maximize the signal for Fe(II) and Fe(III) respectively. Ratios of Fe(II) to Fe(III) can then be determined for each pixel within the mapped area thus the oxidation state of Fe present in diverse and complex samples can be determined on the microscale (e.g. Buss et al., 2008; Templeton and Knowles, 2009; Templeton et al. 2009; Mayhew et al, 2011). Thus, synchrotron-based x-ray spectroscopic techniques are well suited to interrogating the speciation of Fe on the microscale and as such can
provide important insights into understanding H₂ production from water-rock reactions.

10. Dissertation goals and format

Low temperature (≤150°C) water-rock reactions that generate H₂ gas have the potential to fuel an extensive subsurface biosphere. Much is known about the process of serpentinization at high temperatures (200-400°C) and it is clear from this work that the secondary mineral assemblage, Fe partitioning, silica activity, and surface reactivity are important controls on H₂ production from water-rock reactions. Little is known about the geochemistry of water-rock reactions that take place within the temperature limits of life despite the potential role of for these reactions in supporting H₂-based subsurface microbial ecosystems. In the event that these systems do support rock-hosted life, this life may be able to influence the geochemistry of the water-rock reactions through the possible creation of microenvironments. For example, the creation of localized areas of low pH, perhaps associated with cell surfaces, may enhance mineral dissolution. On the other hand, cells adhered to mineral surfaces may inhibit dissolution by limiting the diffusion of ions away from the mineral surfaces. This may lead to locally higher concentrations of ions in solution and thus supersaturation and precipitation of mineral phases. Additionally, reactive sites on cell surfaces may act as sites for the nucleation and growth of minerals. Microorganisms may also influence reaction progress by consuming H₂, a reaction product. However, the effect of the presence of anaerobic, H₂-utilizing microorganisms on the geochemistry of water-rock reactions has not been extensively studied. There are clearly gaps in our understanding of H₂ production from low temperature water-rock reactions, the ability of these reactions to sustain long-term biological activity, and, in turn, the potential influence of
biological activity on the progress and product of these reactions. To address these open
questions, the objectives of this dissertation are threefold:

1) to further our understanding of the mechanism of H₂ generation from low temperature water-
rock reactions, particularly the behavior and partitioning of Fe into secondary mineral phases that
occurs during this process

2) to assess the potential for these reactions to support microbial methanogenesis, an example of
a H₂-based metabolism likely to be active in the subsurface biosphere

3) to determine if rock-hosted microbial life that metabolizes H₂ gas as it is produced can
influence the geochemistry of the reactions and reaction products

Three subsequent chapters comprise the core of this dissertation, each of which is
presented in the form of a stand-alone manuscript that will ultimately be refined for journal
publication. Chapter 2 describes the Fe-bearing secondary mineral assemblage produced in an
abiotic, highly reducing, anaerobic, water-Fe⁰-basalt reaction system at 55°C after short (48
hours) and long (10 months) reaction times. A synchrotron radiation based microscale technique
is developed to locate and identify numerous unique Fe-bearing phases within complex samples.
The integration of x-ray fluorescence (XRF) and x-ray adsorption near edge spectroscopy
(XANES) data collection and processing allows for determination of Fe speciation and
distribution at the microscale. Use of this method allowed for detection and identification of
incipient Fe-bearing reaction products associated with phenocrysts of olivine after only 48 hours
of reaction at 55°C. Pervasive precipitation of Fe-bearing phyllosilicates was detected after 10 months of reaction.

In Chapter 3, a methanogen, *Methanothermobacter thermoflexus*, is introduced to the water-Fe\(^0\)-basalt reaction to test the effect of H\(_2\) metabolism on the geochemistry of the system. Time series measurements of key aqueous and gaseous constituents are used to characterize the geochemical system and track changes in the geochemistry of the culture relative to the abiotic experiments. Growth of *M. thermoflexus* is supported by the water-Fe\(^0\)-basalt system over 1+ year as evidenced by methane production and visualization of cells. Using the method described in Chapter 2, a unique Fe-bearing mineral assemblage, characterized by the presence of an Fe-bearing pyroxene and the absence of Fe-bearing phyllosilicates, is detected and identified in the culture relative to the abiotic experiment. Potential explanations for the different secondary mineralogies are explored.

Chapter 4 then sets out to experimentally determine the extent of H\(_2\) generation from several compositions of olivine, pyroxene, and peridotite as well as magnetite at 55 and 100°C. The synchrotron-based method described in Chapter 2 is used to determine that Fe(III)-oxides formed both as precipitates and surface coatings on spinel particles that were present in the starting materials. The formation of Fe(III)-oxides likely accommodated H\(_2\) generation. The spinels appear to catalyze electron transfer between Fe(II), present in solution from the dissolution of olivine and pyroxene minerals, and water. Fe(II)-bearing phyllosilicate coatings detected on pyroxene particles may inhibit dissolution of these phases and contribute to decreased H\(_2\) production over longer time scales. Another important goal of Chapter 4 is to identify more environmentally relevant geologic materials (in comparison to water-Fe\(^0\)-basalt)
that can be used as substrates for long-term H₂ production in future in-situ microbial growth and bioalteration experiments.

Chapter 5 assesses how the findings of Chapters 2 through 4 advance our understanding of the potential for low temperature water-rock reaction systems to be suitable habitats for H₂-utilizing microbial life and, in turn, for this life to affect the reaction pathways. I also address the challenges and limitations of my experimental approaches and provide brief summaries of my scientific pursuits that are relevant to, but not included in, this dissertation. The implications of this work are discussed and questions to be addressed in future work are posed.

Appendices A-C follow Chapter 5. Appendix A is supplementary information for Chapter 2 as published online (Mayhew et al., 2011). Appendix B is supplementary information for Chapter 3 and will be submitted as such for publication to accompany the main manuscript. Appendix C is a collection of other H₂-generating abiotic water-rock experiments that have been conducted over the course of writing this dissertation. This data is not included in Chapter 4 but is representative of work that has been conducted during the course of this dissertation that adds to the current understanding of H₂ production from low temperature water-rock reactions.

Appendix D includes data collected from experiments designed to grow M. thermoflexus in a water-olivine reaction system. While there is much that still needs to be understood about this data, it represents a significant experimental effort conducted as part of my PhD work and still holds promise for eventual publication.
CHAPTER 2

Microscale imaging and identification of Fe speciation and distribution during fluid-mineral reactions under highly reducing conditions

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Abstract

The oxidation state, speciation, and distribution of Fe are critical determinants of Fe reactivity in natural and engineered environments. However, it is challenging to follow dynamic changes in Fe speciation in environmental systems during progressive fluid-mineral interactions. Two common geological and aquifer materials – basalt and Fe(III) oxides – were incubated with saline fluids at 55°C under highly reducing conditions maintained by the presence of Fe°. We tracked changes in Fe speciation after 48 hours (incipient water-rock reaction) and 10 months (extensive water-rock interaction) using synchrotron-radiation μXRF maps collected at multiple energies within the Fe K-edge. Immediate PCA analysis of the ME maps was used to optimize μXANES analyses; in turn, refitting the ME maps with end-member XANES spectra enabled us to detect and spatially resolve the entire variety of Fe-phases present in the system. After 48 hours, we successfully identified and mapped the major Fe-bearing components of our samples (Fe(III) oxides, basalt and rare olivine), as well as small quantities of incipient brucite associated with olivine. After 10 months, the Fe(III)-oxides remained stable in the presence of Fe°, whereas significant alteration of basalt to minnesotaite and chlinochlore had occurred, providing new insights into heterogeneous Fe speciation in complex geological media under highly reducing conditions.
1. Introduction

Iron is the most abundant transition metal in Earth surface environments and is dynamically cycled between oxidized and reduced forms in numerous biogeochemical processes (O’Day et al., 2004). Fe minerals often dominate the reactivity of soils, sediments, rock matrices and engineered environments such as permeable reactive barriers (PRB). Any environmental or geological sample will likely contain iron in one or more of its three valence states (Fe$^0$, Fe(II), and Fe(III)) and in numerous chemical forms. One of the greatest challenges is to identify the most reactive iron phases and secondary precipitates that may be present, even in low abundance. It is also difficult to measure dynamic changes in Fe speciation in environmental systems during progressive fluid-mineral interactions. Thus, there is a need to be able to rapidly profile not only the bulk average oxidation state and speciation of Fe, but to identify the distribution and abundance of iron phases at the micro-scale as a function of time.

One of our major goals is to determine the abiotic mineralogical transformations of common Fe-bearing geological substrates, basalt and Fe(III)-oxides, under highly reducing conditions. Moderate temperature (<100°C) hydration and alteration of Fe-bearing silicate minerals (e.g. olivines, pyroxenes, amphiboles, micas) present in peridotites (Moody, 1976; Fruh-Green et al., 2004) and basalts (Stevens and McKinley, 2000) has not been extensively studied under anoxic conditions. Once the initial Fe-bearing reaction products are determined, we intend to explore how the activity of H$_2$ consuming organisms can change the water-rock reaction pathways and rates of H$_2$-generation in deep subsurface basalt aquifers (e.g. Stevens and McKinley, 2000). Identifying initial reaction products will also be valuable to other environmental studies, such as predicting coupled Fe$^0$-glass-clay alteration reactions at elevated temperatures relevant to geological disposal of radioactive waste (e.g. de Combarieu et al., 2011),
or modeling how incipient basalt alteration products formed in saline fluids may control the release of Fe, Mg and Ca required for mineral trapping of CO₂ injected into basalt aquifers (e.g. Goldberg et al., 2008). In near surface environments, there is significant interest in defining the heterogeneous Fe mineralogy that forms within zero-valent-iron (ZVI)-PRB designed to degrade or immobilize contaminants (e.g. chlorinated organics, heavy metals, radionuclides) in groundwater (e.g Gu et al., 1999; Furukawa et al., 2002; Phillips et al., 2000). Similarly, it is critical to determine how strongly reducing conditions can induce the reductive dissolution and re-precipitation of Fe-(hydr)oxides, which exert a critical control on the retention and release of metals and metalloids into the environment, leading to widespread environmental problems, such as high arsenic concentrations in groundwater (Hansel et al., 2005; Dixit and Hering, 2003; Tufano and Fendorf, 2008).

In these environmental applications, Fe speciation is expected to be temporally and spatially variable. Increasingly, synchrotron-based x-ray fluorescence (SR-μXRF) mapping is coupled with micro-x-ray absorption near edge spectroscopy (μXANES) to identify the oxidation state, speciation, and distribution of discrete Fe phases within a sample (Marcus, 2010 and references therein). However, μXANES spot locations are often chosen because they represent hotspots of a particular element (Lombi and Susini, 2009) but this does not guarantee sampling the entire variety of phases present nor provide a full spatial determination of phase distribution. We present a method wherein SR-μXRF maps collected at multiple energies within the Fe K-edge undergo Principal Components Analysis (PCA) and spots defined as unique are chosen for μXANES analyses. Then “end-member” (EM) XANES spectra representative of the unique components (both in Fe oxidation state and speciation) can be identified through PCA of the μXANES data sets and independently fit using an extensive Fe model compound library.
Ultimately the EM XANES spectra are used to fit the multiple energy maps and thereby fully define the distribution of each unique Fe component within a complex sample.

In our experimental system, basalt glass, Fe(III) oxides, and Fe$^0$ were combined and anaerobically incubated with artificial seawater at 55°C. One experiment was incubated for 48 hours to mimic an incipient water-rock reaction and a second was incubated for ~ 10 months to mimic a more extensive water-rock reaction environment. ZVI (which can occur in ultramafic rocks or PRB) is reactive in contact with water and was used to drive down the redox potential of the fluids and produce fluxes of Fe(II) and H$_2$. The objective of these experiments was twofold. First, using the method described above, we tested whether a variety of distinct Fe species within complex geologic materials would be detectable, identifiable, and map-able with micron-scale resolution. Second, we followed the moderate temperature alteration of common geological materials – basalt glass and Fe(III)-oxides – revealing the preservation of Fe(III)-oxides coupled with pervasive precipitation of Fe-bearing phyllosilicates that will significantly differ in terms of their reactivity during subsequent fluid-mineral interactions.

2. Methods

2.1 Water-rock reaction samples

The geologic components in each experiment included volcanic glass, Fe$^0$, and Fe(III) oxides synthesized through base titration of ferric chloride (Ryden et al., 1977). Glassy Hawaiian basalt was ground and sieved to 53-425µm. The basalt and all other geologic materials were sterilized by heating at 100°C in an oven for >24 hours. All experimental apparatus and liquid media were autoclaved and kept sterile prior to assembly. 1.25g basalt powder, 0.1g Fe$^0$ chips (1-
2mm), 0.25g basalt chips (2-5mm), 35ml of anaerobic artificial seawater media (Templeton et al., 2005), 0.1ml of 1M Fe(III) oxides, and 150mM sulfate were combined in a serum vial with an 80% N₂, 20% CO₂ headspace. The vial was capped with an airtight rubber stopper and incubated at 55°C. After 48 hours and 10 months, 1ml of mineral bearing media was removed using a 23g needle, resulting in the preferential selection of particles <100µm (due to size, Fe⁰ particles were not selected). Sample preparation was conducted in an anaerobic chamber to avoid exposure to oxygen. The sample was diluted in anaerobic MilliQ water to remove salts and obtain a reasonable particle density, 200µl of diluted sample was applied directly to a pure quartz microscope slide (ESI Co.) and dried overnight. After drying, epoxy (Buehler) was applied to the sample and it was removed from the anaerobic chamber and allowed to harden at 55°C. To reduce the chance of oxidation of the sample, epoxy above the grain surfaces was removed with a diamond wheel. The grains were exposed during dry grinding on silica carbide sandpaper, polished using 0.25µm diamond in ethanol and cleaned with acetone; never coming in to contact with water (see SI).

2.2 Synchrotron microprobe μXRF mapping and μXANES

Synchrotron-based hard x-ray microprobe measurements of element distributions were conducted at beam line 2-3 at the Stanford Synchrotron Radiation Lightsource (SSRL). The incident energy was selected using a Si (111) double crystal monochromator detuned 15% at 7keV with the SPEAR accelerator ring containing ~150 to 200 mA at 3.0 GeV. The spot size was focused to 2.5 x 2.5µm using Pt-coated Kirkpatrick-Baez mirrors (Xradia Inc.) that also provided harmonic rejection of energies greater than 22keV. The sample was rastered across the micro-focused x-ray beam at a 45 degree incident angle, using a pixel step-size of 2.5µm, with a
dwell time of 100 milliseconds per pixel. Element maps were generated in a continuous raster scanning mode using a single-element Si drift Vortex detector (SII NanoTechnology USA Inc). Windowed counts for each element extracted from the full X-ray fluorescence spectra were normalized to the intensity of the incident X-ray beam (I₀). Regions of the map area of particular interest for chemical speciation mapping at the Fe K-edge were identified from an 11keV map. Fe K-edge mapping was conducted at 5 discrete energies (7123, 7126, 7128, 7130, and 7133eV) to determine changes in the Fe oxidation state and mineralogy across the sample. The 5 energies were specifically chosen because examination of preliminary data revealed that differences in normalized intensity between μXANES analyses was maximized. If preliminary data is not available, comparisons of model spectra can be used for the initial choice of energies. Background fluorescence does not contribute significantly to our data. However, mapping at a background energy should be considered for samples where the target element does not dominate the total fluorescence yield (e.g. Marcus, 2010).

Multiple energy (ME) maps were achieved by raster mapping a single line at each incident monochromator energy and repeating this process at each successive line. Prior to collection of μXANES data, the maps were dead time corrected and underwent PCA using the MicroAnalysis Toolkit (http://smak.sams-xrays.com/). Maps of unique components were used to guide the selection of spots for μXANES investigations, ensuring that the μXANES collected would represent the variety of Fe-phases present in the samples. Each μXANES spectrum was collected from ~ 240eV below the Fe K-edge to 300eV above the edge. All μXANES data were dead time corrected, background subtracted, and normalized to unit step edge using standard methods using SIXPACK software package (Webb, 2005) before further processing. Maps and spectra were calibrated using the first inflection of a Fe⁰ foil at 7112 eV. The short dwell times
for $\mu$XRF mapping (50-100 $\mu$s) and $\mu$XANES analyses (8 mins) minimized potential radiation
damage to the sample.

2.3 Model compounds

Iron-bearing minerals representing a variety of different mineral groups were obtained for
bulk Fe K-edge XANES (Table A1). Materials were ground to a fine powder using porcelain and
agate mortar and pestles, mixed with boron nitride (typically 1:10) to achieve adequate beam
transmission, loaded into mylar sample holders and sealed with kapton tape. Independent
identification of mineral phases was verified by X-ray powder diffraction.

2.4 XAS Measurements

Bulk XANES measurements were performed at SSRL on beam lines 11-2 and 4-1. Fe K-
edge XANES spectra were collected from 7,000 to ~8,000 eV using a Si(220) phi=0
monochromator crystal set and a slit size of ~1x10mm. The monochromator was detuned 30-
50% for harmonic rejection. Two to four scans of each sample were collected either in
transmission mode using an ion chamber or fluorescence mode using a Lytle detector and
XANES data reduction was conducted as described above.

3. Data Analysis

3.1 PCA of multiple energy Fe K-edge $\mu$-XRF maps

For each sample, the dead time corrected Fe channel of each individual energy map were
collected into a single data file and PCA was performed on the series of maps (Figure A1).
Application of PCA to image data is a common technique for processing soft x-ray transmission data (e.g. Lerotic et al., 2004) and but has not been extensively applied to hard x-ray μ-XRF data.

3.2 Identification of end-member XANES spectra

PCA is commonly applied to XANES data sets to determine the components necessary to explain the variation within the dataset (Fay et al., 1992; Ressler et al., 2000; Wasserman, 1997). We conducted PCA on the XANES spectra collected from each sample using SIXPACK software (Webb, 2005). Following previously published procedures, we identified the number of principal components required to explain our dataset (Lerotic et al., 2004; Malinowski, 1977; Manceau et al., 2002; Manceau and Matynia, 2010). We then used the number of components to identify end-members of the sample spectra. EMs are those sample spectra that are the most different from one another and, when combined, describe all of the variation of the data set. The component loadings tabulated in the PCA matrix were used to identify end-members by plotting the loadings for all spectra for 1 principal component versus the loadings for all spectra for a second principal component. The spectra identified as the most unique on these plots were chosen as the EMs (see SI). This method is equivalent to that outlined in Bateson and Curtiss, (1996). Spectra identified as EMs were over-plotted to visually verify differences between the spectra (Figure A2).

3.3 Least squares fitting (LSF) of XANES spectra

XANES spectra identified as end-members were fit from 7110-7150eV with linear combinations of Fe model compounds. All fits were done using the ‘Cycle Fit’ function in SIXPACK (Webb, 2005) and a model compound library that represented as many possible
constituents of the system without including nearly identical XANES spectra (see Appendix A; Table A1). All other XANES spectra were fit internally with the EM spectra to verify that they could be accurately described by the end-members (Figure A6).

3.4 Fitting maps with Fe(II)/Fe(III) model XANES and end-member XANES spectra

XANES fitting of the ME maps allows for calculation of oxidation state maps or spatial maps of the EM spectra; as discussed by Marcus (2010). Using SMAK, we fit the Fe K-edge ME maps in a non-negative linear least-squares sense with normalized fluorescence values for standards and model spectra corresponding to the 5 different map energies. Our basalt glass XANES spectra was used as a model for Fe(II) (~90% Fe(II)) and ferrihydrite was used as a model for Fe(III) (Figure A3). The normalized fluorescence values for basalt, ferrihydrite, and EM spectra were determined at each of the mapping energies: 7123, 7126, 7128, 7130, and 7133 eV (Tables A2 and A3). These values were used for the XANES fitting routine to produce oxidation state or speciation maps. After fitting EM XANES spectra with XANES model compounds, these maps can be interpreted as maps of particular mineral assemblages.

4. Results and Discussion

4.1 Multiple Fe K-edge energy maps

Synchrotron-based hard x-ray fluorescence is commonly used to generate elemental maps of geological samples (Manceau et al., 2002 and references therein). Mapping at 10s of discrete energies around the edge of one particular element has been extensively applied to x-ray absorption spectroscopy collected in transmission mode (e.g. Lerotic et al., 2004). However, multiple energy mapping is less commonly used with hard x-rays, though it has been applied to
environmental samples for determination of the oxidation state and/or speciation of iron and other metals (e.g. Marcus, 2010; Sutton et al., 2009; Templeton et al., 2009). We XANES fit the 48-hour ME map data using an Fe(II) and an Fe(III) standard (Figure 1b), which easily resolves the oxidation state of the particles due to the large difference in the Fe K-edge spectra for basalt and ferrihydrite (Figure A3). Fe(II)/Fe(III) XANES fitting reveals that most particles in the sample are enriched in Fe(II). Dark orange particles in the optical view (Figure 1a) correspond to Fe(III)-rich areas within the map, and a few smaller Fe(III)-rich particles also become apparent (Figure 1c). To map the distribution of specific Fe phases, PCA of the 5 ME maps of each sample were used to determine the number and distribution of unique components (Vekemans et al., 1997). In the 48-hour sample, PCA suggests the existence of three components containing reasonable spatial data (Figure 2) while in the 10-month sample, four components are suggested (Figure A4).

4.2 PCA identification and least-squares fitting of μXANES spectra

Collecting μXAS (XANES/EXAFS) data on the same sample as μXRF maps is a common technique (e.g. Marcus, 2010 and references therein; Fay et al., 1992). Lombi and Susini (2009) note that the choice of μXANES locations may be biased by the experimenter’s preference for hotspots of particular elements. Our protocol differs from previous work because we immediately conduct PCA on the ME maps and use the component maps (Figures 2 and A4) to guide XANES spot selection to pixels where Fe speciation clearly must differ. Then we collect spectra from several spots associated with each unique component. Therefore, we have statistically defined unique Fe-bearing areas within the sample prior to μXANES analyses instead of relying solely on the experimenter’s qualitative impression of the data.
Figure 1. a) Plane polarized light photomicrograph of the sample area of interest (10x mag). b) SR-uXRF total Fe intensity mapped at 11KeV. c) Distribution of FeII and FeIII obtained from XANES fitting the 5 energy maps. Numbers correspond to location of microXANES analyses. Scale bars = 40μm.
Figure 2. a-c) Distribution of distinct components (1-3 respectively) determined from PCA of the 5 energy Fe K-edge maps. PCA results in pixels with both positive and negative values, represented by red and blue respectively. Numbers correspond to location of microXANES analyses. Scale bars = 40 um.
To stringently identify unique Fe K-edge XANES EM spectra, PCA was conducted on the full set of XANES spectra collected from each sample and revealed the existence of four unique components in each sample. Plotting the loadings of component 2 versus 3 and 3 versus 4 resulted in the identification of four EM spectra for each sample (Figure 3; also see SI methods). These EM XANES spectra represent the entire diversity of the data sets from each sample, and non-negative linear-combinations of all of the EM spectra can be used to fit all of the other XANES data. The EM XANES are also independently fit using linear combinations of model compounds to determine Fe speciation within samples. Although it is difficult to use Fe K-edge XANES to distinguish between members of one mineral group due to spectral similarity, it is possible to distinguish between classes of minerals such as Fe(II)- and Fe(III)-bearing silicates, oxides, oxyhydroxides, clays, carbonates and sulfides (O’Day et al., 2004).

At 48-hours, EM XANES 7 (48/7) is fit with a large fraction of basalt, the dominant geological material, with minor Fe-sulfide, and EM XANES 9 (48/9) represents the Fe(III)-rich component of the starting materials. Several Fe(III)-model compounds are invoked in the final fit for 48/9, such as ferrihydrite, hematite, and Fe(III)-clay (Table A4); however, µEXAFS analyses would be required to accurately quantify these species (Hansel et al., 2003). Olivine is present as physically and chemically distinct crystallites in the initial basaltic glass and was directly detected in the fits of EM XANES spectra 3 and 2 (48/3 and 48/2). The San Carlos olivine model spectrum dominates the fit of 48/3 (Table A4) and represents an olivine with a composition approximately equal to Fo90 (Mg1.8Fe0.2SiO4) (McCollom and Bach, 2009). The Sapat Valley olivine spectrum occurs as a minor component and may represent an olivine with submicron intergrowths of pyroxene. 48/2 is fit with both olivine species but additionally Fe-substituted brucite (MgxFe1-x(OH)2) is included in the fit (Table A4).
Figure 3. a-d and f-i) The occurrence of end-member XANES in the 48-hour and the 10-month sample respectively. Bold numbers on maps correspond to the EM XANES spectrum used to fit the map (plotted in 3e and 3j) and indicate the location of the analysis. e and j) Stackplots of the end-member XANES spectra in the 48-hour and the 10-month sample respectively (spectra are offset by 1 for clarity). Lines indicate energies at which uXRF maps were collected. Scale bars = 40 um for a-d and 50um for f-i.
In the 10-month sample, EM XANES 14 (10/14) is fit with a large fraction of basalt associated with minor sulfate-green-rust, while EM XANES 7 (10/7) represents the oxidized iron component with nearly the same proportions of Fe(III)-model compounds required for the fit of 48/9 (Table A4). EM XANES 15 (10/15) is fit by a combination of San Carlos olivine and Fe-substituted brucite, again similar to 48/2. In contrast, EM XANES 5 (10/5) is fit entirely with Fe-phyllosilicates, species not previously detected. Minnesotaite, an Fe(II)-bearing talc \((\text{Fe,Mg})_3\text{Si}_4\text{O}_{10}(\text{OH})_2\)), and low-Fe clinochlore, an Fe(II)-bearing chlorite \((\text{Fe,Mg})_4\text{Si}_3\text{Al}_2\text{O}_{10}(\text{OH})_8\)), compose the fit to this spectrum (Table A4).

Clearly PCA of the ME maps enabled us to identify particles representative of the starting materials (both abundant and rare) as well as secondary phases produced during the reaction progress. In both the 48-hour and 10-month samples, Fe-bearing brucite is only detected in association with olivine. Brucite is a predicted alteration product for olivine under low silica activity, and thermodynamic predictions suggest that up to 30 mol% Fe can substitute for Mg at temperatures below 150°C (McCollom and Bach, 2009). However, it is surprising to detect brucite in experiments where the aqueous concentration of SiO_2(aq) is 450-550 µM (Table A5), since talc should (and does) precipitate. This suggests the existence of microenvironments within the heterogeneous sample, or large gradients in Si activity near dissolving olivine interfaces as proposed by Klein et al. (Klein et al., 2009).

After 10-months of reaction, new phases were detected where the Fe-speciation is fit by Fe-talc (e.g. minnesotaite) and low-Fe chlorite (e.g. clinochlore). The occurrence of minnesotaite has previously been observed in experiments of Fe\(^{0}\)-basalt glass-clay interactions at 90°C (de Combarieu et al., 2011). In natural settings, minnesotatite is often used as an indicator mineral of very low-grade metamorphism (>100°C) of banded iron formations (Klein, 2005).
The detection of minnesotaite at 55°C may be the lowest temperature occurrence that has been documented. Low-Fe clinochlore is often associated with hydrothermal environments and serpentinization processes (Iyer et al., 2008; Seyfried et al., 2011); however, low Fe-clinochlore is stable across a wide range of [Si(aq)] at both 25 and 75°C (Aja and Small, 1999). The detection of minnesotaite and clinochlore in the 10-month sample reflect significant changes in both aqueous and solid phase chemistry as the water-rock reaction progressed.

4.3 Fit of ME maps with end-member XANES

Identification of end-member XANES and subsequent least squares fitting with Fe model compounds enables an understanding of the variety of Fe species present at 48 hours and 10 months of reaction progress. Using EM spectra to fit non-EM spectra produces fits with excellent R² values (e.g. Figure A6), supporting our choice of EM XANES and the validity of characterizing the entire sample with the smaller subset of XANES data. However, fitting the XANES spectra only informs us of the Fe-speciation at the points where the XANES spectra were collected. Further data processing is required to understand the spatial distribution of the Fe phases represented by the EM spectra.

Ultimately, our goal was to determine the specific distribution and abundance of the Fe phases present in our samples. To accomplish this, we conducted LSF of the multiple energy µXRF maps collected from each sample. With hard x-ray data, the most common procedure is to fit ME maps to represent the distribution of discrete valence states (Marcus, 2010 and references therein) (e.g. Figure 1c). In this work, we collected five µXRF maps across the Fe K-edge, and because we identified only four EM XANES it is possible to fit the µXRF maps with the EM XANES. The images generated represent the specific distribution of the four EM XANES.
spectra in each sample (Figure 3; Tables S2 and S3) and therefore correspond to the distribution of unique Fe-bearing phases.

In the 48-hour sample, EM XANES maps make it clear that most of the particles possess some amount of basalt glass (48/7 map, Figure 3a), the exception being the Fe(III)-bearing particles (48/9 map, Figure 3b). Compared to basalt, the other Fe phases (48/9, 48/2, and 48/3) are much more limited in occurrence (Figure 3a-d). In this relatively simple experimental system it is possible to observe straightforward correspondences between PCA maps and maps of EM XANES. When compared to the component maps (Figure 2), there is a strong correspondence between map component 1 which approximates total Fe distribution (Figure 2a) and basalt (48/7, Figure 3a); this is not surprising since basalt is the primary material reacted with artificial seawater in this experiment. We also observe a strong similarity in the distribution of map component 2 (Figure 2b), which distinguishes phases on the basis of oxidation state, and the Fe(III)-bearing phases (48/9, Figure 3b). In order to fully describe the speciation of component 3 from PCA of the ME maps, both 48/2 and 48/3 are necessary, suggesting that map component 3 represents a more complex assemblage of Fe-bearing phases that are distinct from the basalt and Fe(III)-phases. As discussed above, both 48/2 and 48/3 represent a minor component of the experimental system, olivine, +/- brucite, an incipient alteration product. Thus, map component 3 appears to be particularly sensitive to localization of rarer phases and secondary products compared to the dominant basalt and Fe(III)-oxides represented by map components 1 and 2 (Figure 2). However, in order to visualize the distributions of the different Fe-bearing mineral assemblages within map component 3 it was necessary to fit the µXRF maps with the EM XANES (Figure 3c,d).
EM XANES maps of the 10-month sample indicate that there is significantly less basalt glass (10/14 map, Figure 3f) relative to the 48-hour sample (see Figures 1a and S4f for further evidence). In contrast, the 10/5 map reveals that the secondary Fe-silicates, minnesotaite and clinochlore, are pervasive and widely distributed across the mapped area (Figure 3i). However, it is important to note that the EM maps are not scaled to directly represent mineral abundance; for example, although the minnesotaite/clinochlore areas in the 10/5 map appear to have significant intensity (Figure 3i), upon examination of the total Fe map (Figure A4f) it is possible to see that these are areas of relatively low Fe abundance. Altogether, our interpretation is that the dissolution of basalt is directly coupled with the precipitation of minnesotaite and clinochlore as the reaction progressed.

Surprisingly, the Fe(III)-bearing phases (10/7 map, Figure 3g) have not significantly changed by 10 months of reaction. Although the particles are smaller in size and more widely distributed than those in the 48-hour sample (Figure 3b), the XANES spectra for the Fe(III) particles are nearly identical over time, resulting in similar fits for 48/9 and 10/7 (Table A4). Although the Fe-mineralogy associated with ZVI-PRB has been shown to be highly heterogeneous over small spatial scales (Phillips et al., 2000), and ferrihydrite is a common reaction product that can even coat Fe⁰ surfaces (Furukawa et al., 2002), typically oxic fluids must be reacting with the barrier to stabilize Fe(III)-oxides. Instead, we expected to observe reductive dissolution and conversion of the Fe(III)-oxides to magnetite due to large fluxes of Fe(II) produced during anoxic corrosion of the Fe⁰. However, ferrihydrite will be stabilized in the presence of dissolved ligands such as the high concentrations of calcium, sulfate, and dissolved silica in our experiments (e.g. Hansel et al., 2005; Voegelin et al., 2010) (Table A4). Moreover, the conversion of ferrihydrite to magnetite only occurs above a threshold of ~300 µM.
Fe(II) (Hansel et al., 2005; Hansel et al., 2003), whereas the Fe(II) concentration remained at a steady-state value of ~100 µM throughout these experiments (Table A5). Therefore we have determined that the persistent precipitation of minnesotaite and chlinochlore buffered [Fe(II)_{aq}] to low values, resulting in a high Si/Fe ratio (>4) and long-term passivation of the Fe(III)-oxides.

5. Assessment of Integrated Method and Broad Applicability

In this work we have clearly defined an integrated approach to µXRF and µXANES data collection and processing that optimizes for identification of numerous Fe-bearing species in complex samples. PCA of the ME maps identified unique Fe-bearing components and this information guided our µXANES spectra collection and thereby enabled analysis of the variety of phases represented by the components. Using PCA of XANES spectra and LSF of end-member XANES we were then able to specifically identify the diverse Fe-bearing species within each component (to the mineral group level).

Using only spectral analysis, we independently corroborated the presence of the initial bulk reaction materials. By conducting PCA of the ME maps prior to µXANES spectroscopy, incipient reaction products such as brucite can be detected in the 48-hour sample, even though they represent a very minor proportion of the total Fe-speciation within the sample. The detection of new secondary minerals, minnesotaite and chlinochlore, in the 10 month sample illustrates that our integrated approach to data collection and processing enables us to track changes in microscale Fe-speciation during anaerobic water-basalt reactions over time. Notably, a number of the particles chosen for µXANES analyses were relatively small and non-descript in terms of Fe fluorescence intensity; for example, the spots containing minnesotaite/chlinochlore or olivine/brucite do not necessarily correspond to either the most intense areas of Fe fluorescence.
(Figure A4e) or well-defined particles (Figure A4f). Thus without conducting PCA of the ME maps we may not have chosen them for \(\mu\)XANES and would have missed sampling unique Fe species within the sample area.

Subsequent XANES fitting of the ME maps allows the ME maps to be quantitatively reassessed to reveal the spatial distribution and abundance of each EM XANES spectrum. It was even possible to visualize highly localized incipient reaction products (brucite) that comprise only a small fraction of the total Fe in the sample (but must occur in proportions of \(\geq 10\%\) in a 2.5x2.5\(\mu\)m pixel). For the 10-month sample, the loss of basalt and diffuse growth of minnesotaite/chlinochlore regions was clearly visualized (Figure 3).

Accurate detection and quantification of fluid-mineral reaction products is crucial to understanding changes in Fe speciation and reactivity during secondary mineralization processes. This SR-based integrated approach to data collection and processing can be applied to complex geologic samples (e.g. soils, sediments, rock matrices and mineral alteration rims) to detect micron-scale geochemical transformations of a target element. This technique is not limited to Fe-bearing species but rather can be used to detect and interrogate the distribution and speciation of any redox active element (e.g. Mn, U, Cr, As, S) where specific chemical forms can be resolved using XANES analyses, giving rise to species-dependent spatial variability in multiple-energy maps. In addition, since PCA can rapidly be conducted on the ME maps and \(\mu\)XANES data immediately after collection, additional information can be gleaned by targeted \(\mu\)XRD or \(\mu\)EXAFS analyses to better constrain the mineralogy within spots of interest. Ultimately, increasing availability of brighter synchrotron radiation sources and development of new detector technologies (e.g. Ryan et al., 2007) will enable acquisition of hard x-ray fluorescence data at more energies more quickly, producing image stacks similar to those that are commonly
generated in transmission mode for low-Z elements (Lerotic et al., 2004), and thereby enabling full-spectral analysis of element speciation in every pixel. In the meantime, the method employed here can be easily and effectively applied to environmental samples to be analyzed at almost any existing microspectroscopy beamline.

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Supporting Information is Available

Supporting information includes further explanation of SR-based data collection and processing and additional figures and tables that enhance these descriptions.
 Brief – Integrated PCA of multiple energy μXRF maps and μXANES spectra identifies unique Fe speciation in heterogeneous media, revealing precipitation of Fe(III)-phyllosilicates and passivation of Fe(III)-oxides during Fe0-basalt-water reaction
References


CHAPTER 3

The effect of methanogenesis on the geochemistry of low temperature water-basalt-Fe$^0$
reactions

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Abstract

The potential for hydrogen (H$_2$) gas production from low temperature (<100°C) water-rock reactions to support microbial life has long been debated in the literature. Geochemical signatures of the presence of life in these systems are of great interest as we search for life in extreme environments on Earth and other planets, and attempt to understand the signs of life that are preserved in Earth’s geologic record. We designed a laboratory experiment to investigate the ability of H$_2$-utilizing microorganisms to survive and thrive during long-term H$_2$ production and to determine the effect of in-situ H$_2$ consumption on an anaerobic, reducing water-rock reaction system. Seawater and basalt were incubated at 55°C and a subset of experiments were inoculated with *Methanothermobacter thermoflexus*, a thermophilic, alkaliphilic methanogen. Fe$^0$ was added to produce measurable quantities of H$_2$ to support long-term (1+ year) growth of *M. thermoflexus*. We detect distinct differences in the geochemistry of abiotic versus culture experiments. Hydrogen concentration increased with time in the abiotic experiment, whereas in the culture experiment H$_2$ was consumed and converted to CH$_4$. However, less methane was generated in the culture experiment than was expected based on H$_2$ levels in the abiotic experiment. Additionally, aqueous Fe and Si concentrations were higher in the culture than in the abiotic experiment. Fe-bearing talc and clinochlore-like secondary Fe-bearing minerals, predicted to be in equilibrium with the aqueous geochemistry measured in both experiments, dominated the Fe-bearing secondary mineralogy of the abiotic reactions, but were absent from the culture experiment after one year of reaction. Instead a unique pyroxene-like phase was detected. We propose that the presence and activity of *M. thermoflexus* in the culture experiment is responsible for the nucleation and extensive precipitation of the pyroxene-like phase. In addition, we propose that *M. thermoflexus* is capable of H$_2$-dependent Fe(III)-oxide reduction.
and that this process explains the discrepancy between predicted and actual CH₄ production, and contributes to the higher aqueous Fe(II) concentration in the culture experiment.
1. INTRODUCTION

The potential for subsurface chemolithoautotrophic life to survive and thrive solely on rock-derived H₂ gas has great implications for our understanding of subsurface life on Earth today, the origin and evolution of life on early Earth, and the potential existence of life on other planets. In extant subsurface environments, dissolved or gaseous H₂ derived from high temperature (>150°C) water-rock reactions may support microbial ecosystems when transported to low(ER) temperature sites colonized by H₂-utilizing microorganisms, such as at submarine hydrothermal vent chimneys (e.g. Kelley et al., 2002; Martin et al., 2008; Schrenk et al., 2010). Alternatively, H₂ gas may be produced from low temperature (≤100°C) water-rock reactions and consumed in-situ by H₂-utilizing microorganisms. The existence of such in-situ microbial ecosystems is dependent on the hydrogen generation potential of the host rock at temperatures ≤100°C. Proving the existence of such in-situ ecosystems in hinges upon the ability of microorganisms to influence the aqueous and solid-phase chemistry of the system in detectable ways. Of particular interest is the precipitation of biogenic minerals that have the potential to be preserved in the geologic record and to provide a signature of life long after that life has gone. Thus, laboratory investigations of rock and mineral alteration at low temperatures in the presence and absence of H₂-utilizing microorganisms is crucial to understanding the potential for these microbial ecosystems to exist and influence the water-rock reaction pathways and processes.

The redox transformation of iron under anaerobic conditions from reduced Fe(II) in primary minerals (e.g. silicates) to oxidized Fe(III) in secondary mineral phases (e.g. oxides and layer silicates such as magnetite and clays, respectively) is primarily responsible for the production of H₂ gas from water-rock reactions. Understanding the rates and mechanisms of Fe-silicate mineral transformations under anoxic conditions in the presence of microorganisms is
relevant to a variety of scientific and environmental questions. It has been proposed that \( \text{H}_2 \) generation and microbial consumption occurs within deep basaltic aquifers (e.g. Stevens and McKinley, 1995) although that proposal has been challenged on the basis of the small amounts of \( \text{H}_2 \) generated in experiments (e.g. Anderson et al., 1998). Nevertheless, it has spurred a broad discussion of potential subsurface lithoautotrophic microbial ecosystems (SLiMEs) (e.g. Chapelle et al., 2002; Takai et al., 2004; Nealson et al., 2005). Nealson et al. (2005) defines a SLiME as a hydrogen-driven ecosystem that uses geochemically supplied electron acceptors. Examples include the methanogen-dominated subsurface SLiME and HyperSLiME communities at Lidy Hot Springs (Chapelle et al., 2002) and at the Kairei hydrothermal vent system (Takai et al., 2004), respectively.

Previous studies of the alteration of Fe-silicate minerals in the presence of microbial consortia or microorganisms that are known to produce siderophores (Fe chelating organic compounds) have found higher concentrations of Fe in solution than in abiotic control experiments (Kalinowski et al., 2000; Liermann et al., 2000; Daughney et al., 2004; Wu et al., 2007). However, it is not known if these increased Fe concentrations were accompanied by Fe-bearing secondary mineral precipitation or if there were any notable differences in the secondary mineralogy between cultures and abiotic controls. In the presence of an aerobic, non-siderophore producing organism, \textit{Ramlibacter tataouinensis}, a decrease in the release of Fe to solution from the dissolution of orthopyroxene was attributed to enhanced precipitation of an amorphous mineral layer that inhibited the exchange of ions between the mineral and the solution (e.g. Benzerara et al., 2004). In the presence of an acidophilic, iron oxidizing bacteria, \textit{Acidothiobacillus ferroxidans}, enhanced Fe(III) production inhibited fayalite dissolution by passivating the mineral surfaces (Santelli et al., 2001). However, virtually no microbial Fe-
silicate alteration experiments have been conducted in anoxic conditions despite the influence of secondary Fe-bearing phases on the reactivity of these water-rock systems.

Numerous investigations of basalt alteration conducted at temperatures > 100°C have characterized the secondary mineral assemblages that were formed, but the elevated temperatures of those studies precludes investigation of the role of microorganisms (e.g. Seyfried and Bischoff, 1979; Crovisier et al., 1983; Thomassin et al., 1989; James et al., 2003). Recently there has been an intensified focus on laboratory studies of H₂-production from water-silicate reactions at temperatures within the range conducive to life. Neubeck et al. (2011) measured nanomolar concentrations of H₂ and release of Fe and Si to solution from abiotic water-olivine reactions at 30-70°C. Less Fe was measured in solution at 70°C than at lower temperatures and one possible explanation was the precipitation of secondary Fe-silicates, although the mineralogy of the precipitates was not reported (Neubeck et al., 2011). In a study of the effect of microorganisms on low temperature H₂ production, the presence of microbial consortia was found to correlate with elevated H₂ production (at µmolar levels) from water-basalt-sediment slurries relative to sterile controls (Parkes et al., 2011). In this case, increased H₂ production was attributed to enhanced mechanochemistry in the presence of microorganisms. These investigations present intriguing results, yet the H₂ producing reactions and the associated mineral products still remain to be understood.

The objectives of this study were twofold: (1) to determine if microbial activity could be maintained for year-long time scales during progressive H₂-generation due to low temperature water-rock interaction and (2) to determine if in-situ consumption of H₂ by microorganisms affects the chemistry of low temperature, anoxic water-rock reaction systems and the secondary mineralization pathways. Here we explore a set of conditions (low temperature, reducing, and
anoxic) not previously investigated in the literature and make comparisons between biotic and abiotic systems. Moreover, we conduct a comprehensive long-term investigation of not only the aqueous chemistry but also the gaseous and solid phase chemistry thus tracking changes to the water-rock system over time. We also investigate the influence of *Methanothermobacter thermoflexus* on the extent of H₂-generation and changes in Fe mineralogy.

We investigate the effect of an Archaeal methanogen on the chemistry of a water-Fe0-basalt reaction at low temperatures. Methanogens convert hydrogen and carbon dioxide to methane. This metabolic process is considered to be one of the earliest on Earth and is predominately found in organisms that populate the base of the tree of life (e.g. Woese et al., 1990; Pace, 1997; Reysenbach and Shock, 2002). It is likely that these earliest methanogens inhabited, and perhaps originated in, rock-hosted environments such as hydrothermal vent systems (e.g. Pace, 1991; Martin et al., 2008). It has been postulated that geochemical H₂ production on other planets and moons, including Mars and Europa, may support extraterrestrial microbial methanogenesis (e.g. Boston et al., 1992; McCollom, 1999). Some methanogens are also capable of reducing Fe(III), a function that is thought to date back to the Archean (4-2.5 Gya) when the supply of terminal electron acceptors was limited, and Fe(III) could be produced photochemically (Vargas et al., 1998). *Methanothermobacter thermoflexus* (Kotelnikova et al., 1993; Wasserfallen et al., 2000), the organism used in this study, is one of only a few known and isolated thermophilic Archaea that grow optimally at high pH (Wagner and Wiegel, 2008). Thus, methanogens in general and *M. thermoflexus* specifically are particularly suited for investigations into the effect of in-situ H₂ consumption on the chemistry of low temperature, anoxic water-rock reaction systems. This long-term study provides insights into the interrelationships of water-rock reaction systems and the microbial life they may support.
2. MATERIALS AND METHODS

2.1 Water-rock reaction environment

Laboratory culturing environments were designed to mimic the production and slow release of H₂ gas from water-rock reactions that are more relevant to subsurface environments than typical culture experiments with high concentrations of headspace H₂ gas. Volcanic glass with a basaltic composition was chosen to represent ocean crust environments. Further, basaltic glass provided all of the elemental components of basalt without the variety of mineral phases thus simplifying and facilitating the detection of any secondary mineral phases. Electron microprobe analyses of the glassy Hawaiian basalt reveal a composition of (in weight percent oxide) 51.87% SiO₂, 13.57% Al₂O₃, 11.43% CaO, 10.90% FeO, 6.65% MgO, 2.59% TiO₂, 2.37% Na₂O, 0.37% K₂O, and 0.06% Cr₂O₃. Olivine phenocrysts (Fo₈₁) constitute approximately 9% of the material. Plagioclase and spinel occur as minor (≤1%) phases. Fe⁰ was added to maintain anaerobic conditions and produce measurable quantities of H₂ gas over long (1+ year) time periods. Fe(III) oxides synthesized through base titration of ferric chloride (Ryden et al., 1977) were added to create a system in which Fe was far from equilibrium in order to observe if, and how, the Fe would equilibrate in the system in the presence and absence of *M. thermoflexus*. Glassy Hawaiian basalt was ground and sieved to 53-425µm. Basalt powder (1.25g) and Fe⁰ (0.1g of 1-2mm grains), were combined in glass serum vials with 35ml of anaerobic artificial seawater media (Templeton et al., 2005), 0.1ml of 1M Fe(III) oxides for a final concentration of 3mM, and 150mM sulfate. The headspace was 80% N₂, 20% CO₂ and the vials were capped with an airtight butyl rubber stopper and incubated at 55°C. All experimental components were sterilized either by heating at 100°C in an oven for >24 hour or autoclaving and were kept sterile prior to assembly.
2.2 Inoculum

The water-rock systems were inoculated with a pure culture of *Methanothermobacter thermoflexus* (DSM 7268), a methanogenic Archaea (Kotelnikova et al., 1993; Wasserfallen et al., 2000). *M. thermoflexus*, was chosen as the inoculum because it: 1) utilizes H$_2$(g) for growth producing CH$_4$(g), the concentration of which can be tracked over time, 2) tolerates pH>8 and seawater salinity, and 3) exists as long rods and chains of rods that are visible by light and fluorescence microscopy even when embedded among mineral particles.

2.3 Fluorescence and Field Emission Scanning Electron Microscopy

*Methanothermobacter thermoflexus* cultures and abiotic controls were frequently visualized using a Zeiss Axio Imager Z.1 fluorescence microscope and a field emission scanning electron microscope (FESEM) (JEOL JSM-7401F) to verify the continued existence of *M. thermoflexus* in the cultures (Supplementary Information). Aliquots of media and mineral particles were aseptically sampled using sterile syringe needles (21g) and syringes. For fluorescence microscopy, aliquots were deposited directly onto microscope slides and cells were detected using the autofluorescence of methanogenic F420 with excitation of 390-420 nm and emission of 450 nm. For FESEM, aliquots were stored in 2.5% gluteraldehyde at 4°C. Samples were pipetted onto 0.2µm polycarbonate filters, dehydrated using an ethanol dehydration protocol where filtering replaces centrifugation, and critical point dried. The filters were mounted onto EM stubs using carbon tape and gold coated to a thickness of ~7nm to reduce charging.
2.4 Cell Enumeration

The culture experiment was inoculated with \(\sim 4.75 \times 10^6\) cells/ml grown on 20% H\(_2\), 80% CO\(_2\) overpressured to 2 atm in DSMZ media #141 at a dilution of 1:100 for a starting cellular concentration of \(4.75 \times 10^4\) cells/ml. Fluorescence microscopy verified the persistence of living cells over time but accurate direct cell counts could not be conducted because of the close cell-mineral associations. Removal of cells from mineral particles for direct cell counting was not attempted due to the common problems of incomplete separation and cell loss that result in inaccurate assessment of cell numbers (maximum yield \(\sim 10\%\)) (e.g. Riis et al., 1998; Gough and Stahl, 2003).

2.5 Concentrations of gases

Analysis of H\(_2\), CH\(_4\), and CO\(_2\) concentrations in the headspace was performed by gas chromatography (GC). Headspace gases were aseptically extracted using sterile needles and a gas-tight syringe. For detection of H\(_2\), the sample was injected into an SRI 310C Gas Chromatograph equipped with an Alltech Molecular Sieve (5A 80/100) 6' x 0.085” ID column and a thermal conductivity detector (TCD) with N\(_2\) as the carrier gas. For detection of CO\(_2\) and CH\(_4\), the sample was injected into an 8610C SRI Gas Chromatograph equipped with a PORAPAK Q 6' x 0.085" I.D. column with He as the carrier gas. A flame ionization detector (FID) was used for measurements of CH\(_4\) and a TCD was used to detect CO\(_2\). Headspace concentrations are converted to aqueous concentrations using the Ideal Gas Law and Henry’s Law using coefficients from the Sander Compilation (Sander, 1999).
2.6 Concentrations of Aqueous Species

Dissolved concentrations of aqueous Fe, Si, Mn, Mg, and Ca were measured using an Inductively Coupled Optical Emission Spectrometer (IC-OES). Media was aseptically removed from the experiments and filtered through a 0.2µm sterile Millipore Millex filter, acidified with trace metal grade nitric acid (67-70% as HNO₃, Fisher Scientific), and diluted 10x to reduce interference in the measurements caused by the high concentration of total dissolved solids (TDS) in the seawater-based media. Spiked matrix blanks were measured to further diminish error associated with the TDS of the media. To maintain a constant media volume and to avoid pulling a vacuum in the system, the media removed was replaced with an equal volume of sterile, anaerobic media.

2.7 Synchrotron microprobe µXRF mapping and µXANES

2.7.1 Sample preparation

Reacted mineral particles from culture and abiotic experiments were prepared for optical imaging and spatially resolved synchrotron radiation x-ray fluorescence mapping and spectroscopy. For each synchrotron analysis, 1ml of mineral bearing media was removed aseptically using a 23g needle with preferential selection of particles <100µm (due to size, the mm-sized Fe⁰ particles were not selected). Preparation was conducted in an anaerobic chamber to avoid exposure to oxygen and the sample was diluted in anaerobic MilliQ water to remove salts and obtain a reasonable particle density. Diluted sample was applied directly to a pure quartz microscope slide (ESI Co.) and dried at room temperature overnight. Epoxy (Buehler) was applied to the dried sample and it was removed from the anaerobic chamber and allowed to harden at 55°C. To reduce the chance of oxidation of the sample, epoxy above the grain surfaces
was removed with a diamond wheel and grains were exposed during dry grinding on silica carbide sandpaper, polished using 0.25µm diamond in ethanol and cleaned with acetone, never coming in to contact with water.

2.7.2 Multiple energy x-ray fluorescence mapping (µXRF) and spot x-ray absorption near edge spectroscopy (µXANES) data collection

Synchrotron-based hard-x-ray microprobe measurements of major element distributions and µXANES were conducted at BL 2-3 at the Stanford Synchrotron Radiation Lightsource (SSRL). Solid-phase samples were collected after short (48 hours for the abiotic control and 2 months for the culture experiment) and longer reaction times (~1 year for both experiments) to track changes in Fe speciation with time and to test the effect of microbial methanogenesis on the Fe speciation. A detailed explanation of the integrated approach to synchrotron data collection and processing developed to identify unique Fe-bearing components within our samples is given in Mayhew et al. (2011). Any deviations from the beamline configuration and data collection protocol are stated here. Data collection occurred with the SPEAR accelerator ring containing ~150 to 300 mA at 3.0 GeV. The focused spot size was either 2x2 or 2.5 x 2.5 µm with a pixel step-size of 2 or 2.5µm, respectively. Dwell times were either 50 or 100 milliseconds dependent on x-ray flux. Fe K-edge maps were generated in a continuous raster scanning mode using a single-element vortex detector, and all windowed counts for each element extracted from the full X-ray fluorescence spectra were normalized to the intensity of the incident X-ray beam (I₀). Fe K-edge mapping was conducted at 5 or 7 discrete energies chosen to maximize the differences in normalized intensity between representative µXANES. The abiotic experiment was mapped at 7123, 7126, 7128, 7130, 7133 +/- 7127 and 7129 eV. The culture experiment was mapped at 7123, 7127, 7128, 7130, 7133 +/- 7126 and 7129 eV. Approximately 10-30 µXANES were
collected from each map area in order to sample the variety of Fe-phases present. Each \( \mu \)XANES spectrum was collected from \( \sim 240 \text{eV} \) below the Fe K-edge to \( 300 \text{eV} \) above the edge. All \( \mu \)XANES data were dead time corrected, background subtracted, and normalized to unit step edge using standard methods using SIXPACK software package (Webb, 2005) before further processing. Maps and spectra were calibrated using the first inflection of a Fe\(^0\) foil at 7112 eV. The short dwell times for \( \mu \)XRF mapping (50-100 ms) and \( \mu \)XANES analyses (8 minutes) minimized potential radiation damage to the sample.

2.7.3 Multiple energy \( \mu \)XRF maps and \( \mu \)XANES data analysis

A brief description of the data analysis is given here; see Mayhew et al., 2011 for more detail. Prior to collection of \( \mu \)XANES data, the XRF maps were dead time corrected and underwent Principal Components Analysis (PCA) using the MicroAnalysis Toolkit (http://smak.sams-xrays.com/) to produce maps of the spatial distribution of unique components. These were used to guide the selection of \( \mu \)XANES locations, to ensure that the \( \mu \)XANES would represent the diversity of Fe-bearing phases as suggested by the unique components. PCA was then conducted on deadtime corrected, normalized XANES spectra using SIXPACK software (Webb, 2005) in order to identify the end-member spectra representative of the entire data set. The multiple energy Fe K-edge maps were fit with end-member spectra to produce maps of the spatial distribution of each end-member throughout the map area. To understand the geochemical significance of this data, the end-member XANES (EM) spectra were fit with Fe model compounds to identify the speciation of Fe represented by each EM spectra.
2.7.4 XAS measurements of iron model compounds for use in least squares fitting of unknown spectra

Bulk Fe K-edge XANES were obtained for iron-bearing minerals representing a variety of different mineral groups. Materials were ground to a fine powder using porcelain and agate mortar and pestles, mixed with boron nitride (typically 1:10) to achieve adequate beam transmission, loaded into mylar sample holders and sealed with Kapton tape. Bulk XANES measurements were performed at SSRL on beam lines 11-2 and 4-1. Fe K-edge XANES spectra were collected from 7,000 to ~8,000 eV using a Si(220) phi=0 monochromator crystal set and a slit size of ~1x10mm. The monochromator was detuned 30-50% for harmonic rejection. Two to four scans of each sample were collected either in transmission mode using an ion chamber or fluorescence mode using a Lytle detector and XANES data reduction was conducted as described above. Independent identification of mineral phases was verified by X-ray powder diffraction.

XANES spectra identified as end-member (EM) XANES spectra for any given mapped sample were fit from 7110-7150eV with linear combinations of Fe model compounds that represented as many possible constituents of the system without including nearly identical XANES spectra (Table A1). All fits were done using the ‘Cycle Fit’ function in SIXPACK (Webb, 2005). All models were fit individually as 1 component fits and then the fit cycle was repeated for 2 component fits using the best 1 component model compound paired with the remaining model compounds. This process is repeated until adding an additional component no longer decreases the $R^2$ value by 10% or greater. A plot of the model spectra used in fits of end-member XANES is available in the Supplementary Information. All other XANES spectra were
fit internally with the EM spectra to verify that they could be accurately described by the end-members.

2.8 Geochemical modeling

The Geochemist’s Workbench (Version 8, RockWare, Inc., Golden, CO.) was used to create mineral stability diagrams on the basis of the bulk aqueous and gaseous chemistry at the time of sampling for synchrotron analyses. The thermo.dat thermodynamic database, modified to include solid solutions of select Fe-Mg end-member minerals, was used for all models. Equilibrium constants of solid solutions were calculated according to (Nordstrom and Munoz, 2006).

3. RESULTS

3.1 Gas chemistry

Hydrogen production from Fe\textsuperscript{0} occurs according to reaction 1.

\[
\text{Reaction 1)} \quad 2\text{Fe}^0 + 2\text{H}^+ \rightarrow 2\text{Fe}^{2+} + \text{H}_2
\]

Measurements of H\textsubscript{2} and CH\textsubscript{4} gas concentrations were conducted over the course of the experiments to investigate the effect of \textit{M. thermoflexus} on the extent of H\textsubscript{2} generation, and the concomitant conversion of H\textsubscript{2} to CH\textsubscript{4} (Reaction 2), during progressive low-temperature Fe\textsuperscript{0}-basalt-water reaction.
Reaction 2) \[ CO_2 + 4 \text{H}_2 \rightarrow \text{CH}_4 + 2 \text{H}_2\text{O} \]

3.1.1 \textit{H}_2 \textit{production and behavior in the abiotic experiment}

The \textit{H}_2 \textit{concentration in the abiotic experiment increased with increasing incubation time} (Figure 1a). The concentration of \textit{H}_2\textit{aq} reaches a peak of \(\sim 180 \mu\text{M} \) at 4700 hours. To reset the system and to determine if steady \textit{H}_2 \textit{production could be reinitiated, the headspace was flushed with N\textsubscript{2}/CO\textsubscript{2} just prior to 6000 hours (Figure 1a). The \textit{H}_2 \textit{concentration was drawn down to \(\sim 3 \mu\text{M} \) during flushing. Subsequently, steady \textit{H}_2 \textit{production was renewed and maintained for the remainder of the experiment. The CH}_4 \textit{concentration throughout the duration of the experiment was maintained at essentially 0 \mu\text{M} (Figure 1a).}

3.1.2 Consumption of \textit{H}_2 and production of \textit{CH}_4 by \textit{M. thermoflexus}

In the culture experiment the metabolic activity of \textit{M. thermoflexus} consumes \textit{H}_2 as it is produced, maintaining the aqueous \textit{H}_2 \textit{concentration at \(\sim 0.25 \mu\text{M}, \text{approximately 700-fold less than in the abiotic experiment. CH}_4 \textit{gas is produced and \mu\text{molar quantities are dissolved in the aqueous phase (Figure 1b). We observe an offset in the timing of the decrease of \textit{H}_2 and the onset of \textit{CH}_4 production. We documented a decrease in the concentration of \textit{H}_2 as soon as we began measuring \textit{H}_2 \textit{concentration at 264 hours (~8 days after inoculation). However, \textit{CH}_4 production did not begin in earnest until after 792 hours (~30 days after inoculation) (Figure 1b). The concentration of \textit{H}_2\textit{aq reached its relatively steady background concentration (~0.25 \mu\text{M}) as \textit{CH}_4 production was just beginning (Figure 1b). The concentration of \textit{CH}_4\textit{aq reaches a relatively steady concentration of ~40 \mu\text{M} at ~2200 hours and is maintained for ~3000 hours.}

Similar to the abiotic experiment, the headspace was flushed just prior to 6000 hours to determine if steady \textit{H}_2 and \textit{CH}_4 production could be reinitiated. This decreased [\textit{H}_2]\textit{aq by an
Figure 1. Aqueous hydrogen and methane concentrations in A.) the abiotic experiment and B.) the culture experiment. The legend is the same for both plots.
order of magnitude (0.03 µM). The concentration of CH₄aq was reduced to 0.9 µM during flushing. Unlike after the initial inoculation, there was no delay in CH₄ production after flushing the headspace. Steady CH₄ production persisted for the duration of the experiment and the concentration of CH₄aq approached ~40 µM when measurements were stopped at ~10,000 hours. After flushing the headspace, the concentration of [H₂]aq remained low at ~0.2 µM essentially the same amount as prior to flushing.

3.2 Aqueous Fe concentration

The total concentration of dissolved Fe (Feₐq) was tracked to observe its behavior over time and with changing gas concentrations. The total Feₐq is considered to be approximately equal to the total dissolved Fe(II)ₐq under the experimental geochemical conditions. In the abiotic experiment, Feₐq increases to its peak concentration of ~900 µM during the first six weeks of the experiment (Figure 2a). The concentration of Feₐq then steadily decreases over the course of ~2600 hours to ~50 µM, the average background concentration that persists until the headspace is flushed. Flushing the headspace initiated a rise in Feₐq to a peak concentration of ~480 µM over the course of ~300 hours. Again, this is followed by a steady decrease in the concentration of Feₐq over the course of ~2600 hours to ~100 µM, the average background concentration maintained after flushing. Throughout the experiment, H₂ production persists as the concentration of Feₐq fluctuates.

Due to our sampling schedule, we did not document the initial rise of the Feₐq concentration in the culture experiment. The highest concentration measured (~775 µM) was the first measurement at 336 hours (Figure 2b). This is followed by a decrease in the concentration of Feₐq over the course of ~4000 hours to a steady background concentration of ~67 µM.
Figure 2. Aqueous Fe concentration plotted with the dominant gas phase in A.) the abiotic control and B.) the culture experiment. The legend is the same for both plots.
Analysis of Variance (ANOVA) analyses conducted in R (a Language and Environment for Statistical Computing, (R Development Core Team, 2011) reveal that the difference in the concentration of Fe$_{aq}$ between the abiotic and culture experiments is significant (P<0.01). Flushing the headspace initiated a rise in the concentration of Fe$_{aq}$ to ~375 µM (Figure 2b). The following decrease in the concentration of Fe$_{aq}$ was gradual and a consistently low concentration was not obtained during the remainder of the culture experiment. The concentration of Fe$_{aq}$ is significantly higher in the culture versus the abiotic experiment (P<5.3e$^{-9}$) after flushing. Throughout the experiment, CH$_4$ production persists as the concentration of Fe$_{aq}$ decreases.

### 3.3 Solid phase Fe chemistry

#### 3.3.1 Fe speciation and distribution after short reaction times

The speciation of Fe in the solid phase was investigated in the abiotic and culture experiments at three distinct time points. The abiotic experiment was investigated after 48 hours of reaction to determine if any measurable alteration occurred to the solid phase materials after a very short reaction time. Four unique end-member spectra were identified in the sample and the Fe speciation was defined by fitting with spectra of Fe model compounds (Table 1; Figure B1) (Mayhew et al., 2011). Two of the end-member XANES spectra represent the initial experimental starting materials, basalt and Fe(III)-oxides (Table 1; Figure 3a). A third spectrum represents olivine which occurs as distinct phenocrysts within the basalt. The fourth end-member spectrum (EM 2) also represents olivine but is unique because of its association with Fe-substituted brucite (Mg$_x$Fe$_{1-x}$(OH)$_2$), an incipient reaction product (Mayhew et al., 2011).

The culture experiment was first investigated after 2 months of reaction time and five end-member spectra were identified. As in the abiotic experiment, three of the end-member
Figure 3. A.) Representative end-member spectra of the starting materials that are detectable at all time points (basalt, San Carlos olivine + brucite, and Fe(III) oxides). The end-member spectra of the unique secondary phases in the culture and abiotic experiments at 1 year of reaction (plotted in the dashed grey and black respectively) are included to illustrate the distinct difference in Fe-speciation represented by these end-member spectra. B.) Least squares fit to the unique end-member spectrum from the abiotic experiment at 1 year of reaction. C.) Least squares fit to the unique end-member spectrum from the culture experiment at 1 year of reaction. Axes are the same units on all plots.
spectra represent the initial experimental starting materials (Table 1). The other two end-member spectra represent mixtures of different proportions of olivine and Fe-substituted brucite, the same secondary phase identified in the abiotic experiment (Table 1). The definite identification of the other phases used in the fits to these end-members is not certain as they occur in proportions of ~5-10% of the Fe absorbance, what is considered to be the detection limit of the technique determined from empirical mixtures (O’Day et al., 2004; Ostergren et al., 1999).

XANES fitting of the multiple energy maps with the end-member spectra reveals that the speciation and distribution of Fe looks similar in the abiotic experiment at 48 hours of reaction and the culture experiment at two months of reaction (Figure 4). In both experiments the majority of material within the mapped area is basalt. There are a few isolated particles of Fe(III)-oxides and olivine with little to no overlap between the distinct phases (Figure 4).

3.3.2 Fe speciation after 1 year of reaction

After approximately one year of reaction time, four end-member spectra were identified in the abiotic experiment. As in the 48-hour sample, three of the end-member spectra represent the starting materials (basalt, Fe(III)-oxides and olivine). The fourth end-member spectrum (EM 5) is fit entirely by spectra of Fe-phyllosilicates, including an Fe-bearing talc $((\text{Fe,Mg})_2\text{Si}_2\text{O}_6)$ and low-Fe clinohlore, an Fe(II)-bearing chlorite $((\text{Fe,Mg})_4\text{Si}_3\text{Al}_2\text{O}_{10}(\text{OH})_8)$ (Table 1; Figure 3b).

Four end-member spectra were also identified in the culture experiment after 1 year of reaction. Again, three end-member XANES spectra represent the starting materials. The fit to the fourth end-member XANES spectrum (EM 9) is dominated by an Fe-bearing pyroxene $((\text{Fe,Mg})_2\text{Si}_2\text{O}_6)$, (Table 1; Figure 3c). None of the end-member spectra in the culture experiment
Figure 4. Maps of the distribution of end-member XANES in A.) the abiotic experiment after 48 hours of reaction and B.) the culture experiment after 2 months of reaction. Green represents the basalt-bearing end-member, red represents the Fe(III)-oxide bearing end-member, and blue represents the olivine-bearing end-member. Scale bars = 40μm.
were fit with the Fe-bearing talc and clinochlore phases used to fit EM 5 in the abiotic experiment.

The distribution of Fe-species at one year of reaction looks distinctly different than after the short reaction times (Figure 5). Distinct particles of all three starting materials (basalt, Fe(III)-oxides, and olivines) are still identifiable though less of the map area is represented by basalt particles than after 48 hours of reaction (5% versus 30%) (Figure 5a). The secondary Fe-phyllosilicates are the dominant phases, covering approximately 25% of the map area, within the abiotic experiment. Similarly, in the culture experiment, less of the map area is represented by basalt particles than after two months of reaction (14% versus 34%) (Figure 5b). However, the basalt particles are riddled with the pyroxene-like phase creating a very different appearance than the basalt particles in the abiotic experiment. Only ~5% of the map area contains the pyroxene-like spectral signature though it is widely distributed (Figure 5b).

3.3.3 Fe speciation and distribution after 2 years of reaction

The solid phase Fe chemistry was also examined after two years of reaction time. The aqueous and gaseous chemistry at the time of sampling is reported in Table 2, since continuous sampling was not carried out between year one and two of the reaction. As at both the short and one-year reaction times, three end-member spectra represent the starting materials in the abiotic experiment (Table 1). The fourth end-member (EM 26) represents the secondary phases and the fit is dominated by the Fe-bearing talc. Similarly, three end-member spectra represent the starting materials in the culture experiment (Table 1). However, the secondary Fe-speciation in the culture experiment after two years of reaction is distinct from earlier time points. The fourth end-member (EM 28) represents the secondary phases and the fit is dominated by the Fe-bearing talc, although this phase was not present after one year of reaction. Additionally, the pyroxene-
Figure 5. Maps of the distribution of end-member XANES after 1 year of reaction in A.) the abiotic experiment (scale bar = 50um) and B.) the culture experiment (scale bar = 30um). In both experiments green represents the basalt-bearing end-member and red represents the Fe(III)-oxide bearing end-member. Blue represents the Fe-phyllosilicate bearing end-member in the abiotic experiment and the pyroxene-bearing end-member in the culture experiment.
like phase detected after one year of reaction was not detected after two years of reaction.

For the abiotic experiment, the map of Fe speciation and distribution after two years of reaction is very similar to the map after one year of reaction (Figures 5a and 6a). Distinct particles of basalt and Fe(III)-oxides remain apparent. The Fe-bearing talc is again pervasive and widely distributed. The Fe speciation and distribution in the culture experiment at two years of reaction is distinctly different from that at one year. There are still distinct basalt particles, though they are no longer preferentially associated with the pyroxene-like phase. Fe(III)-oxides also remain distinct. Although the Fe-bearing talc and clinochlore-like phases were absent at one year of reaction, at two years the Fe-bearing talc end-member spectrum is pervasive occurring as large, aerially extensive sheets that cover 20% of the map area (Figure 6b). The occurrence of the Fe-bearing talc end-member at two years is similar to that of the abiotic experiment after one and two years of reaction (Figure 5a and 6a).
Figure 6. Maps of the distribution of end-member XANES after 2 years of reaction time in A.) the abiotic experiment and B.) the culture experiment. Green represents the basalt-bearing end-member, red represents the Fe(III)-oxide bearing end-member, and blue represents the Fe-phyllosilicates bearing end-member. Scale bars = 60 um.
Table 1. Non-negative least square fits of Fe model compounds to end-member XANES spectra.

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<td>CH$_4$(aq)</td>
<td>CO$_2$(aq)*</td>
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* this is solely H$_2$CO$_3$(aq), not total inorganic carbon
4. Discussion

4.1 Growth of Methanothermobacter thermoflexus

A first test in this study was whether a H₂-dependent microorganism such as *M. thermoflexus*, a thermophilic, alkalophilic, methanogenic *Archaea* (Kotelnikova et al., 1993), could be sustained over long time scales by slow rates of hydrogen generation during water-Fe⁰ interaction. We document the persistent presence and sustained metabolic activity of *M. thermoflexus* in the culture experiment over the course of 10,000 hours (~13 months), which is consistent with the previously observed short-term growth of other methanogens using Fe⁰ as an energy source (e.g. Daniels et al., 1987; Belay and Daniels, 1990; Lorowitz et al., 1992; Karri et al., 2005). Coupled observations of cells (Figure B2) and the persistent consumption of H₂ and production of CH₄ demonstrate cellular survival and microbial methanogenesis. Quantitative cell counts were not obtained due to the low yield of cell-mineral separation techniques, so cell specific activity cannot be calculated. However, microscopic examination of fluid samples does allow us to make some first-order estimates of cell abundance. Fluorescence microscopy revealed an initial increase in cell density during the first ~4 days after inoculation after which time the cell density appeared to remain constant to 10,000 hours. While these cells may not have been multiplying, the reinitiation of CH₄ production after the headspace was flushed at ~6000 hours and the continued production of CH₄ throughout the duration of the experiment (>10,000 hours) is evidence for the persistent metabolic activity of *M. thermoflexus*.

4.2 Actual versus predicted CH₄ production in methanogenesis experiments

As stated in section 3.1, H₂ production from Fe⁰ occurs according to reaction 1 thus, we hypothesized that microbial H₂ consumption, which removes the products of this reaction, would
drive the reaction to the right, stimulating increased H₂ production. Therefore we would expect the culture experiment, in the presence of *M. thermoflexus*, to have a higher total H₂ production than predicted from the abiotic experiment. This would be similar to observations by Lorowitz et al. (1992) who demonstrated enhanced Fe⁰ oxidation and H₂ production in the presence of a methanogen relative to abiotic experiments.

To compare reducing equivalents generated in the abiotic and biotic experiments, we calculated the maximum amount of CH₄ that could be produced if all of the H₂ in the abiotic experiment was converted to CH₄ by microbial methanogenesis according to reaction 1. This was compared to the total moles of CH₄ measured in the culture experiment (Figure 7). The average amount of CH₄ in the culture experiment is ~25% less than the total CH₄ predicted from the abiotic experiment (70 vs. 90 µmoles, respectively). After flushing the headspace, the actual amount of CH₄ at each time point in the culture experiment is consistently 40-60% of the predicted amount of CH₄ at each corresponding time point in the abiotic experiment. Thus, there appears to be less CH₄ produced in the culture than predicted from the abiotic experiment. Even taking into account the background amount of H₂ (maximum ~0.5 µmoles) maintained in the culture experiment does not make up the difference between what is predicted and what is observed. This is in contrast to the results of Lorowitz et al. (1992). While it is possible that less H₂ was produced from the water-rock reaction in the culture experiment, reproducibility of H₂ production is generally 5-10% as determined from experimental replicates of the abiotic system (data not shown) and is therefore likely not the (sole) reason for the discrepancy between the actual and predicted amount of CH₄. However, our approach only accounts for the conversion of H₂ to CH₄ and does not consider the potential consumption of H₂ or reducing equivalents in other
Figure 7. Comparison of the measured methane concentration in the culture experiment to the predicted methane concentration (calculated from the actual hydrogen concentration) in the abiotic experiment.
geochemical reactions. In these experiments, one other possible sink for H₂ is the microbially-mediated reduction of Fe(III) oxides according to reaction 3.

\[
\text{Reaction 3: } \quad \text{H}_2 + 2\text{Fe(OH)}_3 \rightleftharpoons 2\text{Fe}^{2+} + 6\text{OH}^- + 2\text{H}^+
\]

Twenty-four hours after inoculation with \textit{M. thermoflexus} a distinct color change from red to green was observed in the culture experiment. This may explain the earlier rise in the concentration of Fe\textsubscript{aq} in the culture versus the abiotic experiment. The following decrease in the concentration of Fe\textsubscript{aq} is slower in the culture experiment and the steady background concentration is higher than in the abiotic experiment both before and after flushing the headspace (Figure 2).

Thus our observation of less CH\textsubscript{4} measured then predicted can be explained by microbial Fe(III) reduction. Methanogen-catalyzed Fe(III)-oxide reduction is known to result in decreased methane concentrations relative to Fe(III)-free experiments (Bond and Lovley, 2002; van Bodegom et al., 2004; Liu et al., 2011). We also observe a decrease in the concentration of H₂ from 264 hours, long before vigorous CH\textsubscript{4} production begins at ~800 hours, that supports the hypothesis that H₂ is being used in a process other than CH\textsubscript{4} production. All of these observations can be explained by the existence and persistence of microbial H₂-dependent Fe(III) reduction, acting as a second source of Fe(II) to solution and a second sink of H₂, in the culture experiment that is not present in the abiotic experiment. Fe(III) oxidation by \textit{M. thermoflexus} was unexpected as it has not yet been demonstrated in the literature. However, this process explains many of the geochemical trends in the culture experiment. Physiological studies of \textit{M. thermoflexus} are currently underway to verify this conclusion.
4.3 **Geochemical parameters define two fields of data defined by the presence/absence of an Fe-bearing talc**

There is a strong correlation between the aqueous H$_2$ concentration and aqueous Fe and Si concentrations at the time of sampling for each synchrotron investigation and the detection of secondary minerals, particularly the Fe-bearing talc. In order to see the relationships between these variables, ternary diagrams were constructed. In these plots the concentrations of the three variables are ratioed to one another so that the variables sum to 100 (Figure 8). The position of points on the plot indicate the relative percentage of each variable at that time. Two unique fields are defined by the data and characterized by the presence or absence of the Fe-bearing talc and clinochlore-like phases (Figure 8a). One field is characterized by low H$_2$ concentrations and the absence of the Fe-bearing talc. This includes the abiotic experiment after 48 hours of reaction, before large amounts of H$_2$ accumulated, and the culture experiment at early time points when low H$_2$ concentrations are maintained by the metabolic activity of *M. thermoflexus*.

The second field is characterized by high H$_2$ concentrations and the presence of the Fe-bearing talc (Figure 8a). The Fe-bearing talc was detected in the abiotic experiment at one and two years of reaction and the culture at two years of reaction. The higher concentration of H$_2$ in the culture experiment is indicative of a decrease in the metabolic activity of *M. thermoflexus* at this time. Talc generally forms from basalt alteration at reducing and/or higher temperature conditions such as in experimental investigations at 400-500°C (Mottl and Holland, 1978). In naturally altered basalts, talc is generally limited to high temperature alteration zones (200-350°C) (Shau and Peacor, 1992) but has been reported from alteration at <150°C (Hunter et al., 1999). Minneostaite, the Fe-end-member of the talc solid solution series, is a common alteration product of Fe-silicates in contact with reduced, high Si fluids at temperatures down to ~150°C.
Figure 8. Ternary diagrams of A.) the relative percentages of aqueous Fe and Si and H₂ gas and B.) the relative percentages of aqueous Fe and H₂ and CO₂ gas, for each experiment at the time of sampling for synchrotron investigations. Fe and Si are scaled to the maximum concentration of H₂ in (8A.) and Fe is scaled to the maximum concentration of CO₂ in (8B.).
(Miyano, 1978; Rasmussen et al., 1998). While we do not detect the pure Fe-end-member of talc, these geochemical characteristics are similar to those in our experiments and further support our detection of a Fe-bearing talc.

We hypothesized that the consumption of H$_2$ by *M. thermoflexus* would exert some control over the secondary mineralogy in our water-Fe$^0$-basalt system. The concentration of H$_2$ dictates the redox state of the experimental system by defining the oxygen fugacity of the system (e.g. Frost, 1991). Active microbial methanogenesis is an important control on the concentration of H$_2$ in our experimental system. Geochemical models of peridotite-water reactions illustrate the influence of the concentration of H$_2$ on the nature of the secondary mineral phases. For example, when H$_2$ is removed from the system, Fe-brucite is predicted to convert to magnetite and produce H$_2$ to maintain equilibrium (McCollom and Bach, 2009). In this scenario, brucite remains in the mineral assemblage, but the Fe content of the brucite decreases. Hydrogen metabolizing microorganisms, such as methanogens, can be responsible for a decrease in H$_2$ concentration in water-rock reaction systems. It therefore seemed likely that in an experimental water-rock reaction system, through consumption of H$_2$, a methanogen could effect the partitioning of Fe between secondary mineral phases. However, using the bulk aqueous chemistry and the concentration of H$_2$ to model both the abiotic and culture systems, we find a Fe-bearing talc to be stable across the range of H$_2$ concentrations (Figure 9). We further investigated the stability of talc by using a range of values of Fe content (Mg$\# = 1, 0.9, 0.5, 0.1, \text{ and } 0$) and found all variations to be stable across the range of H$_2$ concentrations (data not shown). Thus, the difference in H$_2$ concentration does not directly explain the absence of Fe-bearing talc and clinochlore-like phases in the low H$_2$ field. Similarly, despite the statistically significant higher concentrations of Fe$_{aq}$ and Si$_{aq}$ in the culture experiment at one year of reaction, Fe-bearing talc
Figure 9. Mineral stability diagram showing the Fe-bearing talc and pyroxene stable across the range of log fH2 (-0.49 - -3.21) represented by the culture and abiotic experiments. The model parameters are [Fe^{2+}]=10^{-4} M, [Ca^{2+}]=10^{-3} M, [Mg^{2+}]=10^{-1.5} M, [Si^{4+}]=10^{-3.3} M, log fCO_{2} = 10^{-1.2}. 
is predicted to be stable across the entire range of Fe$_{aq}$ and Si$_{aq}$ values. Figure 9 was constructed with [Fe]$_{aq} = 10^{-4}$, however if Fe$=10^{-3.6}$ M, equal to the concentration in the culture experiment, the stability fields do not change significantly. The talc ((Mg$_{2.70}$Fe$_{0.30}$)Si$_4$O$_{10}$(OH)$_2$) stability field remains essentially the same while a more Fe-rich talc ((Mg$_{0.30}$Fe$_{2.70}$)Si$_4$O$_{10}$(OH)$_2$) is stabilized across a narrow pH range (~6 – 6.25) (data not shown). Figure 10 shows the stability of talc across the range of Si concentrations. The presence or absence of a Fe-bearing talc does correlate with the concentration of Fe$_{aq}$ and Si$_{aq}$ in the experiments. After two years of reaction, when Fe-bearing talc is detected in the culture experiment, the concentrations of Fe$_{aq}$ and Si$_{aq}$ in the culture experiment have dropped to levels much more similar to those in the abiotic experiment. This suggests that the relatively lower concentrations of Fe$_{aq}$ and Si$_{aq}$ are being buffered by the Fe-bearing talc.

The results also show a strong correlation between the aqueous CO$_2$ concentration and the presence or absence of Fe-bearing talc and clinochlore-like phases (Figure 8b). Again, two unique fields are defined, a high CO$_2$ field characterized by the absence of Fe-bearing talc and a low CO$_2$ field characterized by the presence of Fe-bearing talc (Figure 8b). As in Figure 8a, the abiotic at 48 hours, culture at 2 months and 1 year fall are characterized by the absence of a Fe-bearing talc while the abiotic at 1 and 2 years and the culture at 2 years are characterized by the presence of a Fe-bearing talc. Throughout the first year of the experiment, the concentration of CO$_2$ in the culture is ~1 mM greater than the concentration in the abiotic experiment. Again, geochemical models indicate that a Fe-bearing talc is stable across the range of CO$_2$ concentrations represented by the abiotic and culture systems. Thus, the difference in CO$_2$ concentration also does not directly explain the absence of Fe-bearing talc and clinochlore-like phases in the high CO$_2$ field.
Figure 10. Mineral stability diagram showing the thermodynamically stable Fe-bearing talc, pyroxene, and carbonate under the bulk aqueous and gaseous chemical conditions of the experimental system. Dashed red lines show how the stability field of talc changes with increasing Fe content. The black dashed line shows how the stability field of pyroxene changes with increasing Fe content. The culture and abiotic experiment are plotted on the diagram to show in which stability field the bulk aqueous chemistry fell at one year of reaction. The length of the symbol along the x-axis represents the uncertainty in pH measurements. The model parameters were $[\text{Fe}^{2+}]=10^{-4}$ M, $[\text{Ca}^{2+}]=10^{-5}$ M, $[\text{Mg}^{2+}]=10^{-1.5}$, $\log H_{\text{aq}} = 10^{-3.92}$ M, $\log \text{CO}_2_{\text{aq}} = 10^{-2.95}$ M.
The low CO$_{2\text{aq}}$ in the abiotic experiment correlates with a higher pH (7.5) while the higher CO$_{2\text{aq}}$ in the culture experiment correlates with a lower pH (7). The higher pH in the abiotic experiment enhances deprotonation of H$_2$CO$_{3\text{aq}}$ (aka. CO$_{2\text{aq}}$) and causes an increase in the concentration of HCO$_3^-$ and CO$_3^{2-}$. The higher concentration of CO$_3^{2-}$ may cause carbonate phases to precipitate. In fact, the bulk aqueous solutions of both the abiotic and culture experiments, after short reaction times, are supersaturated with respect to dolomite (CaMg(CO$_3$)$_2$) and siderite (FeCO$_3$). It is possible that these phases precipitated in the abiotic experiment whereas in the culture experiment *M. thermoflexus* may have disrupted the nucleation and precipitation of carbonates by holding the initial concentration of CO$_2$ at levels too low to reach supersaturation. Kinetic hindering of the nucleation and precipitation of secondary mineral phases under supersaturated solution conditions is commonly observed in water-rock systems (e.g. Giammar et al., 2005; King et al., 2010). If carbonate phases did precipitate in the abiotic experiment we might expect to detect any Fe-bearing carbonates using our synchrotron methods. However, Fe(II) in a carbonate-like coordination is not detected in significant amounts in either the abiotic or culture experiment. It may be that dolomite is the dominate carbonate phase. However, we are unable to detect dolomite because of the Fe specificity of our synchrotron technique and due to limitations of sample size conventional XRD techniques are also not a viable approach.

Regardless of carbonate phase precipitation and despite the differences in CO$_2$, H$_2$, Fe and Si concentrations between the abiotic and culture experiments after one year of reaction, Fe-bearing talc is predicted to be thermodynamically stable in the bulk geochemical conditions of both systems. Therefore the absence of Fe-bearing talc in the active methanogenesis experiments is intriguing and unexpected.
4.4 Detection of a pyroxene-like Fe XANES spectral signature

After one year of reaction, a clearly unique pyroxene-like Fe XANES spectral signature was detected and distributed throughout the culture experiment (Figures 4, 6). This unique spectrum was not detected in the abiotic experiment or primary materials, nor could we identify phenocrysts of a primary pyroxene phase in unreacted samples using electron microprobe. Therefore we infer that a secondary phase structurally-similar to pyroxene has formed in the culture experiment. Surprisingly, models of the bulk geochemistry of the abiotic and culture experiments do not predict the formation of smectite, a common products of basalt alteration at a range of temperatures (e.g. Mottl and Holland, 1978; W. Seyfried Jr and Bischoff, 1979; Seyfried and Bischoff, 1981; Seyfried and Mottl, 1982; Seewald and Seyfried, 1990), despite the inclusion of a variety of clays (e.g. smectites, illite) in the database. Instead, the models predict the stability of a Fe-bearing pyroxene in the system. Hedenbergite (CaFeSi$_2$O$_6$), the Fe-endmember of the Fe clinopyroxene solid solution series, requires a pH of 9-10 to be stable. However, solid solutions of hedenbergite and diopside (CaMgSi$_2$O$_6$), the Mg-end-member of the clinopyroxene group, are stable to lower pH values (Figure 10). As the Mg content of the pyroxene is increased, the bulk chemistry of the abiotic experiment (within the error of the pH measurements) is pushed into the pyroxene stability field (Figure 10). As the Fe content of the Fe-bearing talc is increased the stability field of pyroxene moves to lower pH values, within the error of that measured in the culture experiment. Therefore it may be possible to form a secondary Mg,Fe pyroxene in our experiments if a pyroxene of with an approximate composition of CaMg$_{0.9}$Fe$_{0.1}$Si$_2$O$_6$ can be nucleated. As with the Fe-bearing talc, we do not detect the Fe-end-member of the pyroxene however, the geochemical conditions under which the Fe-end-member occurs naturally are similar to the conditions in our experimental system. Hedenbergite is found
in highly reducing conditions, often in the presence of native metals and across a range of temperatures (Burton et al., 1982; Krot et al., 2000; Hu et al., 2011 and references therein). In meteorites hedenbergite may precipitate directly from high Fe(II), Ca, and Si bearing fluids at relatively low temperatures (~150°C) (Krot et al., 2000). While the formation of a Fe-bearing pyroxene is consistent with the geochemical conditions in our experiments, these conditions do not represent those of normal low-temperature basalt alteration.

What is significant about our detection of a Fe-bearing pyroxene as a secondary phase in the culture experiment is not its relevance to basalt alteration but its absence from the abiotic experiment. The Fe-bearing pyroxene is predicted to be thermodynamically stable under the geochemical conditions of both the culture and the abiotic experiments but it is only detected in the culture experiment. The intriguing question is if the presence of this phase can be explained by the presence of *M. thermoflexus*. Microorganisms can create microenvironments on mineral surfaces by limiting the diffusion of ions (e.g. Fe, Mg, Si) away from the mineral surface (e.g. Ullman et al., 1996; Benzerara et al., 2004). Additionally, microbes can facilitate increased release of Si, Fe, and Mg from mineral structures by producing ligands that act to solubilize minerals or lowering pH (e.g. Ullman et al., 1996; Barker et al., 1998; Liermann et al., 2000; Kalinowski et al., 2000; Santelli et al., 2001; Welch and Banfield, 2002; Daughney et al., 2004; Wu et al., 2007). The presence of *M. thermoflexus* on a mineral surface may increase the release of Si from mineral surfaces (though we did not investigate ligand production or localized cell-mineral pH values in our experiments) and/or limit the diffusion of Si to solution. In both cases, creating a locally higher concentration of Si that could result in the localized precipitation of the pyroxene (Figure 10).
Cell surfaces may also act as a reactive surface for nucleation and initiation of mineral precipitation (e.g. Urrutia and Beveridge, 1994; Fortin et al., 1997 and references therein; Fortin et al., 1998). Fe- and Mg-bearing silicate minerals have been discovered in close association with bacterial surfaces in a variety of natural environments (Fortin et al., 1997 and references therein; Fortin and Beveridge, 1997; Fortin et al., 1998) and are often smaller than abiotic minerals (e.g. Urrutia and Beveridge, 1994). Following the mechanism proposed by Urrutia and Beveridge (1994), Fe ions may have sorbed to negatively charged reactive sites on the cell wall of *M. thermoflexus*. The sorption of Mg to negatively charged carboxylic sites on polysaccharide walls associated with cyanobacteria and diatoms has also been documented (Defarge et al., 1994; Tesson et al., 2008). The possibility of metal sorption to the cell walls of Archaeal methanogens has been explored by (Orange et al. (2011) who demonstrated the sorption of Fe(III) and Ca\(^{2+}\) to the S-layer and plasma membrane of *Methanocaldococcus jannaschii*. Silicate anions could then be deposited at the site through complexation with the metal ions and these clusters would then act as a nucleation site for the growth of Fe-, Mg-silicates (e.g. Urrutia and Beveridge, 1994). In fact, Orange et al. (2011) observe increased silicification of *M. jannaschii* after sorption of Fe(III) to the cell wall, indicative of the deposition of silica anions at these sites. Thus, it is not unlikely that the presence of *M. thermoflexus* similarly facilitates the nucleation of Fe-silicates.

The size and spatial distribution of the pyroxene-like phase may also suggest that *M. thermoflexus* participates in the precipitation of this phase. The pyroxene-like phase predominantly occurs as one to four pixels thus (~ 2.5x2.5 – 10x10 µmicrons) (Figure 5). This is much smaller and spatially localized than the Fe-bearing talc precipitates in the abiotic experiment that appear to form sheets up to 50 µmicrons across. Furthermore, the pyroxene-like phase is almost always associated with and distributed across basalt particles, suggestive of
precipitation in association with cells that are attached to the basalt surface (Figure 5). That the presence and activity of *M. thermoflexus* may play a role in the precipitation of the Fe-bearing pyroxene is further supported by the absence of the pyroxene-like phase in the culture experiment after two years of reaction when *M. thermoflexus* is no longer active as evidenced by the accumulation of H₂ to concentrations similar to those in the abiotic experiment.

The mechanism of low-temperature pyroxene formation requires further mechanistic investigation to explore the effects of metabolic activity and cell surface properties on the nucleation and stabilization of these types of phases under the bulk and interfacial conditions., However, the detection of this phase allowed for greater insight into the complexity of the biogeochemical processes at work in our experimental system. Due to limited sample sizes, conventional XRD methods were not an option, although even if they had been it may not have been possible to detect this small and relatively rare phase among the predominant primary materials. SEM investigations revealed surface coatings (presumably not Fe-bearing) that made it difficult to see smaller mineral phases on the basalt surfaces (data not shown). Thus, the application of synchrotron-based spectroscopic techniques facilitated detection of this secondary phase that is unique to the culture experiment.

Secondary minerals, such as those detected in our abiotic and biotic systems, have the potential to be biosignatures because the unique secondary mineral assemblage in the culture appears to be indicative of the presence and activity of *M. thermoflexus*. Mineral biosignatures, including Fe-bearing minerals (e.g. Fortin and Langley, 2005 and references therein), can be more robust than carbon-based materials that are fragile in the face of common geologic process (e.g. weathering, hydrothermal alteration, and metamorphism). Recently, Izawa et al. (2010) proposed that titanite in-fillings in putative biogenic tubules in basalt preserve microbial trace
fossils over geologic time scales. Furthermore, silicification of microorganisms can lead to their preservation as microfossils in a variety of natural environments (e.g. Fortin et al., 1997 and references therein). Metal ions associated with the cell wall often encourage precipitation of silicates and preservation of the microorganism (e.g. Ferris et al., 1988; Orange et al., 2009). While the potential for silicate minerals to be biosignatures is high, the pyroxene-like phase in this study appears to be transient and further work is required to understand its potential for preservation in the geologic record.

5. Conclusions

*M. thermoflexus*, a methanogenic Archaea, is sustained by H2 generated from water-Fe0 reactions for long periods of time (1+ years) but produces less methane than predicted from the H2 concentration in the abiotic experiment. We propose that *M. thermoflexus* directs some H2 to the reduction of Fe(III)-oxides, diverting this H2 away from methane production. Fe(III) reduction also acts as a second source of Fe(II) to solution thereby maintaining higher aqueous Fe(II) in the culture relative to the abiotic experiment despite continued precipitation of secondary Fe-bearing phases. Fe(III) reduction by methanogens has important implications for understanding Fe redox processes and methane production in anoxic geologic environments (e.g. van Bodegom et al., 2004; Liu et al., 2011). This process can also influence the effectiveness and longevity of engineered systems such as permeable reactive barriers (e.g. Gu et al., 1999; Furukawa et al., 2002). Fe(III) oxide reduction by *M. thermoflexus* has not been previously demonstrated in the literature and further work is currently being conducted to verify this observation.
The secondary mineral assemblage in the culture is distinct from that in the abiotic experiment despite the prediction from geochemical models that the same phases should be stable in both systems. We propose that the active presence of *M. thermoflexus* destabilizes a Fe-bearing talc phase in favor of a Fe-bearing pyroxene phase in the culture experiment at one year of reaction. This could be accomplished through the creation of geochemical microenvironments at the cell-mineral interface that enhance the supersaturation of a Mg-rich pyroxene coupled with the cells providing reactive surfaces for the nucleation and precipitation of the pyroxene-like phase. When *M. thermoflexus* is no longer actively metabolizing, the pyroxene-like phase is absent and the Fe-bearing talc phase is present, further suggesting that the activity of *M. thermoflexus* is integral to the formation of the pyroxene-like phase.

The demonstration of a microbial effect on the secondary mineral assemblage in this experimental system has important implications for understanding the effect of microbial activity on natural water-rock reaction systems. This work suggests that H₂-utilizing microorganisms can affect the geochemistry of secondary mineral assemblages associated with natural, environmentally relevant systems in detectable and identifiable ways. With this knowledge and in combination with our study of abiotic peridotite-, olivine-, and pyroxene-water reactions at low temperatures (Chapter 4), we are poised to shift future experimental work to investigate the effects of microbial H₂-utilization on more natural/relevant water-rock reaction systems. In these systems, understanding the potential for these minerals to persist in the environment over both short and long time scales (e.g. preservation in the rock record) will have important implications for the potential of these minerals to act as biosignatures, indicators of the presence and activity of life, in water-rock reaction systems.
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Supporting Information is Available

Supporting information includes SEM images of cells in culture experiments and a plot of Fe model compounds used to fit unknown XANES spectra.

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CHAPTER 4

Hydrogen production from abiotic water-rock reactions at low temperatures
Abstract

The production of H$_2$ gas from the hydration of (ultra)mafic rocks is a primary source of energy for life in extreme environments. However, focused hydrothermal venting is relatively rare compared to large-scale, low temperature hydrothermal circulation through the ocean crust. Thus there is the potential for low temperature water-rock reactions to support extensive subsurface life sustained by in-situ water-rock reactions. However, little is known about the amount, rate, and mechanisms of H$_2$ generation from low temperature water-rock reactions. We investigated H$_2$ production from natural peridotite, olivine (Fo$_{90}$, Fa$_{88}$), and clinopyroxene at 55 and 100°C. To unravel the mechanisms of H$_2$ production, the speciation of Fe in the primary and secondary mineral assemblage was determined using synchrotron-based micro x-ray fluorescence multiple energy mapping (µXRF) and micro x-ray adsorption near edge spectroscopy (µXANES) and correlated with changes in $p$H$_2$, $p$CO$_2$, and aqueous Fe and Si activity. Five of six substrates tested, including petedunnite, a pyroxene, San Carlos peridotite, fayalite and associated magnetite, and San Carlos olivine, produced significant H$_2$ gas (~50 – 200 nmoles H$_2$/gram mineral). Hedenbergite, also a pyroxene, did not produce H$_2$ above background levels. All of the water-rock reactions are thermodynamically predicted to result in H$_2$ generation; however, it is notable that the experiments that did produce H$_2$ were strongly correlated with the presence of spinel phases (e.g. magnetite, chromite and gahnite) associated with the primary substrates. The dominant change in Fe speciation is the formation of Fe(III)-oxide layers on the spinel surfaces and the precipitation of Fe(II)-bearing talcs intimately associated with the pyroxene phases. We propose that H$_2$ generation is likely promoted by heterogeneous surface-mediated electron transfer reactions with water molecules adsorbed to the surface of spinels possibly driven by Fe(II) sorption. This continuous flux of Fe(II) is sustained.
by the dissolution of the olivine and pyroxene components of the starting materials. We infer that the cessation in H$_2$ production is due to passivation of the spinel surfaces by the formation of oxidized surface layers and the significant adsorption of aqueous silica. Furthermore, the formation of secondary silicate surface layers (e.g. talc) may limit further dissolution of pyroxene phases and the continued release of Fe(II) to solution. This work provides insights into the mechanism of H$_2$ generation from low temperature water-rock reactions and suggests that low temperature H$_2$-producing reactions cannot be ruled out as a potentially important and significant source of energy for microbial life in the subsurface.
1. INTRODUCTION

The production of H₂ gas from the hydration of (ultra)mafic rocks is a primary source of energy for life in extreme environments. Geochemically generated hydrogen gas is released to the seafloor at hydrothermal vents which teem with microbial life. These systems are thought to be the oldest inhabited systems on Earth (e.g. Reysenbach and Shock, 2002) and perhaps even the site of the origin of life (e.g. Martin et al., 2008). Serpentinization, the process of hydration and alteration of ultramafic rocks, is the most well studied example of a H₂ producing water-rock reaction (e.g. Whittaker and Wicks, 1970; Moody, 1976b; Moody, 1976a; Berndt et al., 1996; Allen and Seyfried, 2003; Bach et al., 2006; Seyfried et al., 2007; McCollom and Bach, 2009; Klein et al., 2009). The accumulation and release of the products of serpentinization at hydrothermal vents are known to fuel H₂-based microbial ecosystems such as at Lost City (Kelley et al., 2001; Schrenk et al., 2004; Kelley et al., 2005; Brazelton et al., 2006; Brazelton et al., 2011) and Rainbow (Charlou et al., 2002; Nercessian et al., 2005; Flores et al., 2011). However, little is known about the potential for life to survive in the subsurface below such systems. Lost City is an example of an environment where serpentinization is thought to occur at >110°C (Proskurowski et al., 2006); the lower estimate being below the known temperature limit of life (~120°C (Kashefi and Lovley, 2003; Takai et al., 2008). However, little is known about the potential for water-rock reactions at low temperatures (~<150°C) to produce ample H₂ to support rock-hosted microbial ecosystems.

Focused hydrothermal venting is relatively rare and large-scale diffuse hydrothermal circulation may be equally or more important in supporting a H₂ driven subsurface biosphere (e.g. Hellevang, 2008). The entire volume of the ocean can circulate through the 2.3×10¹⁸ m³ of rock in approximately 10⁵-10⁶ years (e.g. Johnson and Pruis, 2003; Schrenk et al., 2010; Orcutt
et al., 2011). The temperature of seawater circulating through ridge flanks is thought to be
<200°C (Alt, 1995 and references therein). At recharge zones, where seawater penetrates the
ocean crust, strong temperature gradients exist, thus there are multiple locales where alteration of
the crust can occur at low temperatures (Alt, 1995 and references therein). In anoxic conditions,
H₂ production from low temperature hydration of the host rock may occur. The subsurface of
Earth’s crust has been postulated to house a large amount of microbial biomass on the basis of
pore space volume available within the crust (e.g. 2 x 10¹⁴ tons of bacterial biomass (Gold,
1992); 7-9 x 10²⁹ cells - a density of 0.3-2 x 10⁶ cells/m² - in the shallow subsurface ocean crust
(Nielsen and Fisk, 2010). H₂ generation from low temperature water-rock reactions associated
with diffuse hydrothermal circulation is likely to play an important role in supporting this
microbial life.

For life to survive in rocks undergoing alteration necessitates temperatures at or below
the known temperature limit of life ~120°C (Kashefi and Lovley, 2003; Takai et al., 2008).
Additionally, the production of H₂ at these temperatures must be in sufficient quantities and rates
to support H₂-utilizing life (e.g. Hoehler, 2004). In laboratory experiments, autotrophic growth
of methanogens has been maintained on H₂ concentrations as low as a few to tens of nmolar
(Kral et al., 1998). However, the ability of low temperature water-rock reactions to generate
enough H₂ at rates sufficient to support microbial life in natural environments has been
questioned (e.g. Anderson et al., 1998; McCollom and Bach, 2009).

Geochemical models (e.g. Sleep et al., 2004; Palandri and Reed, 2004; McCollom and
Bach, 2009; Klein et al., 2009) and laboratory experiments (Stevens and McKinley, 1995;
Stevens and McKinley, 2000; Stevens and McKinley, 2001; Anderson et al., 1998; Anderson et
al., 2001; Neubeck et al., 2011; Parkes et al., 2011) provide important insights into H₂ production
from water-rock reactions. Most of our understanding of H\textsubscript{2} production from water-rock reactions stems from studies of serpentinization (Reaction 1), a process characterized by the formation of serpentine group minerals during the alteration of olivine and pyroxene rich rocks/minerals etc. (e.g. Whittacker and Wicks, 1970; Moody, 1976a, 1976b; Berndt et al., 1996; Allen and Seyfried, 2003; Bach et al., 2006; Seyfried et al., 2007; Evans, 2008; Evans et al., 2009; McCollom and Bach, 2009; Klein et al., 2009).

(Reaction 1)
\[\text{Mg}_{1.8}\text{Fe}_{0.2}\text{SiO}_{4} + 1.37\text{H}_2\text{O} \rightarrow 0.5\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4 + 0.3\text{Mg(OH)}_2 + 0.067\text{Fe}_3\text{O}_4 + 0.067\text{H}_2\]
olivine (Fo90)  serpentine  brucite  magnetite

In high temperature conditions and at low oxygen fugacity, the reaction responsible for H\textsubscript{2} generation is the oxidation of Fe, from Fe(II) in primary minerals to Fe(III) in secondary minerals. Fe oxidation hydrolyzes water and reduces protons to produce H\textsubscript{2} gas.

Laboratory investigations of H\textsubscript{2} generation in peridotite-, olivine-, and pyroxene-water experiments have focused on high temperatures (~200-400°C) (e.g. Berndt et al., 1996; Allen and Seyfried, 2003; Seyfried et al., 2007; Jones et al., 2010). These experiments and geochemical models (e.g. Palandri and Reed, 2004; McCollom and Bach, 2009; Klein et al., 2009) have demonstrated that the temperature of reaction exerts a strong control on Fe partitioning and thus H\textsubscript{2} production. At temperatures > ~350°C, little H\textsubscript{2} is generated from olivine because it is essentially unreactive (Allen and Seyfried, 2003; Klein et al., 2009). Peak H\textsubscript{2} production occurs at ~315-340°C and coincides with the formation of significant amounts of magnetite (Moody, 1976a; McCollom and Bach, 2009; Klein et al., 2009). Below ~315°C, H\textsubscript{2} production decreases
with decreasing temperature and less magnetite is formed (McCollom and Bach, 2009; Klein et al., 2009). In reaction 1, all of the Fe is incorporated into magnetite and the maximum H\textsubscript{2} is generated. However, analyses of natural serpentinites, results from laboratory experiments, and modeling of peridotite-water reactions show Fe (II and III) substitution into secondary serpentine and brucite phases (Whittacker and Wicks, 1970; Moody 1976a; O’Hanley and Dyar, 1998; Berndt et al., 1996; Seyfried et al., 2007; McCollom and Bach, 2009; Klein et al., 2009; Evans et al., 2009; Marcaillou et al., 2011). Geochemical models indicate that lower temperatures of reaction favor the incorporation of Fe(II) into secondary minerals (e.g. Palandri and Reed, 2004; McCollom and Bach, 2009; Klein et al., 2009). The extent of Fe(II) substitution into secondary minerals limits the oxidation of Fe(II) and therefore affects the amount of H\textsubscript{2} generation (Moody, 1976; Bach et al., 2006; Seyfried et al., 2007; McCollom and Bach, 2009; Klein et al., 2009). Fe(III) can also substitute into brucite and serpentine (O’Hanley and Dyar, 1994; Seyfried et al., 2007; Klein et al., 2009; Evans et al., 2009; Marcaillou et al., 2011). At 200°C, hydrogen production has been observed in the absence of significant quantities of magnetite and attributed to the oxidation and incorporation of Fe(III) into chrysotile (Seyfried et al., 2007). However, H\textsubscript{2} production accommodated by incorporation of Fe(III) into serpentine produces only one mole of H\textsubscript{2} per mole of fayalite reacted versus two moles of H\textsubscript{2} per mole of fayalite reacted when magnetite is produced as illustrated in Reactions 2 and 3 (Evans et al., 2009).

\[
\text{Fe}_2\text{SiO}_4 + 2\text{H}_2\text{O} \rightarrow 2\text{Fe}_3\text{O}_4 + 3\text{SiO}_2\text{aq} + 2\text{H}_2\text{aq} \quad \text{(Reaction 2)}
\]

olivine (Fa) \hspace{1cm} \text{magnetite}

\[
\text{Fe}_2\text{SiO}_4 + 3\text{H}_2\text{O} \rightarrow (\text{Fe}_2^{2+}\text{Fe}^{3+})(\text{Fe}^{3+}\text{Si})\text{O}_5(\text{OH})_4 + \text{SiO}_2\text{aq} + \text{H}_2\text{aq} \quad \text{(Reaction 3)}
\]

olivine (Fa) \hspace{1cm} \text{Fe(III)-serpentine}
The activity of SiO$_2$aq is also thought to exert control on H$_2$ production. Frost and Beard (2007) suggest that the absence of magnetite from some serpentinites can be explained by the activity of SiO$_2$. Serpentinization occurring within the range of SiO$_2$ activities, in which brucite is stable but that are above the range of magnetite stability, would result in the absence of magnetite, and the incorporation of Fe into brucite and serpentine (Frost and Beard, 2007; Evans, 2009). As mentioned, Fe partitioning into serpentine and brucite results in the generation of less H$_2$ than partitioning into magnetite (Evans, 2009). Thus, temperature and silica activity affect Fe partitioning during water-rock reactions thereby influencing H$_2$ production and the ability of these systems to support microbial ecosystems.

Geochemical models have been used to predict the secondary mineral assemblage associated with these reactions at lower temperatures (25-150°C) (e.g. Palandri and Reed, 2004; McCollom and Bach, 2009; Klein et al., 2009) but experimental data are limited and variable and the associated secondary mineralogy has not been characterized. Stevens and McKinley (1995) proposed that basalt-water reactions at 22°C could produce ample H$_2$ (nanomolar headspace concentrations) to support microbial growth in the subsurface of the Columbia River Basalts. They conducted abiotic basalt-, olivine-, and pyroxene-water reactions at 30°C and 60°C to verify this conclusion (Stevens and McKinley, 2000; 2001). This work was challenged on the basis that the H$_2$ production was not sustained over time scales necessary to support ecosystems and that the pH of the experiments (6) was not environmentally relevant (Anderson et al., 1998; Anderson et al., 2001). However, subsequent to this work there have been only limited investigations into the potential for low temperature water-rock reactions to produce H$_2$. Recently, abiotic olivine-water reactions at 30-70°C have been shown to produce sub-nanomolar amounts of H$_2$ and CH$_4$ (Neubeck et al., 2011). In other work, H$_2$ production from
mechanochemical processes in sterile water-basalt-sediment slurries at ~0-100°C was found to be negligible although in the presence of a microbial consortia H₂ production was enhanced (Parkes et al., 2011). These studies present intriguing and widely variable results and despite the influential role of Fe partitioning on H₂ production, characterization of Fe-bearing secondary mineral assemblages was not included in these low temperature experiments. There is still much to understand about mechanisms of H₂ generation from natural geologic substrates at low temperatures (<~150°C).

This study was designed to address the gap in our understanding of H₂ production from low temperature water-rock reactions. The goal of this work is twofold, 1) to investigate H₂ production from peridotite, olivine (Fo₉₀, Fa₈₈), and clinopyroxene at 55 and 100°C and 2) to characterize the secondary mineral products and the behavior of Fe and Si to better constrain the H₂ producing reactions and understand the variability in H₂ production at lower temperatures. We used natural mineral samples and therefore the composition and modal abundances of the starting material were thoroughly investigated. To detect and identify the limited amount of secondary material that formed during reaction at the microscale, synchrotron-based x-ray fluorescence (µXRF), Fe K-edge x-ray adsorption near edge spectroscopy (µXANES) and capillary XRD were employed. Integration of the gaseous, aqueous, and solid phase geochemistry is used to propose the key processes involved in low-temperature H₂ generation. This work provides insights into the specific reactions responsible for H₂ generation from water-rock reactions and the potential for such low temperature systems to support in-situ microbial ecosystems.
2. MATERIALS AND METHODS

2.1 Rock and mineral substrates

Peridotite (San Carlos, AZ), olivine (San Carlos, AZ), fayalite and magnetite (Forsythe Iron Mine, Canada) were purchased from Ward’s Scientific (Rochester, NY). Two clinopyroxenes, an Fe-rich hedenbergite (South Mountain, Owyhee Co., Idaho) and a Zn-rich petedunnite (Barton Mine, North River, Warren Co., NY) were purchased from Excalibur Minerals (Peeskill, NY). All rocks and minerals were ground by hand in a porcelain mortar and pestle and sieved to 53-212µm. No metal tools were used in the crushing and grinding process. Ground materials were washed with deionized water to remove smaller grains adhered to the larger particles. SEM imaging was used to verify removal of small grains. The materials were allowed to dry at room temperature to minimize aerobic water-rock reactions during the preparation process. Mineral separates were further purified using a hand magnet and a Frantz Magnetic Separator. The fayalite and magnetite are two different fractions separated from the Forsythe Mine material. Nitrogen adsorption isotherms were collected for each geologic substrate to calculate the specific surface area by the BET method.

Semi-quantitative evaluation of mineral abundance by QEMSCAN® scanning electron microscopy was conducted to identify the components of the starting reaction materials and modal abundance of each component. QEMSCAN® simultaneously collects energy dispersive x-ray spectrometer (EDS) spectra and calibrated backscatter electron (BSE) intensity information (Hoal et al., 2009). A BSE signal and an EDS spectrum are collected from each pixel (20x20µm) to make a phase identification based on prescribed BSE values and elemental intensities. Instrument operating conditions include 25 kV accelerating voltage and a 5 nA beam current.
Quantitative weight percent oxides of the major mineral components in each starting material were acquired by electron microprobe (JEOL JXA 8600).

2.2 Water-rock reactions

Five grams of each powder (except for 1.5 grams of fayalite) was combined with 35ml of artificial seawater media (27.5 g NaCl, 5.38g MgCl₂, 0.73g KCl, 0.2g NaHCO₃, 1.4g CaCl₂, 1g NH₄Cl, and 0.05g K₂HPO₄ in 967 ml of MilliQ water) in glass serum vials. Sulfate was not included in the seawater media to avoid complications of Fe-S interactions. The media and vials were purged with 80% N₂, 20% CO₂ and the vials were capped with an airtight butyl rubber stopper. The rubber stoppers were boiled 3x to release extractable hydrocarbons and hydrogen. Media only experiments were also conducted. All experiments were conducted in triplicate. Three replicates of each experiment were incubated at 55 and 100°C. All geologic substrates were sterilized by heating at 100°C in an oven for >24 hour. The glass vials, rubber stoppers, and media were autoclaved. All components were kept sterile prior to assembly in a biohazard hood. Sterilization was conducted prior to assembly to avoid initiating the reaction during autoclaving. Glass vials were heated to 550°C for at least 3 hours to remove any combustible carbon species.

2.3 Concentrations of gases

Analysis of H₂, CH₄, and CO₂ concentrations in the headspace was performed by gas chromatography (GC). Headspace gases were aseptically extracted using sterile needles and a gas-tight syringe. For detection of H₂, the sample was injected into an SRI 310C Gas Chromatograph equipped with an Alltech Molecular Sieve (5A 80/100) 6’ x 0.085” ID column and a thermal conductivity detector (TCD) with N₂ as the carrier gas. For detection of CO₂ and
CH₄, the sample was injected into an 8610C SRI Gas Chromatograph equipped with a PORAPAK Q 6' x 0.085" I.D. column with He as the carrier gas. A flame ionization detector (FID) was used for measurements of CH₄ and a TCD was used to detect CO₂. Headspace concentrations are converted to aqueous concentrations using the Ideal Gas Law and Henry’s Law using coefficients from the Sander Compilation (Sander, 1999).

### 2.4 Concentrations of Aqueous Species

Dissolved concentrations of aqueous Fe, Si, Mn, Mg, and Ca were measured using an Inductively Coupled Optical Emission Spectrometer (IC-OES). Aqueous Ni concentrations were measured using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Media was aseptically removed from the experiments and filtered through a 0.2µm sterile Millipore Millex filter, acidified with trace metal grade nitric acid (67-70% as HNO₃, Fisher Scientific), and diluted 10x to reduce interference in the measurements caused by the high concentration of total dissolved solids (TDS) in the seawater-based media. Spiked matrix blanks were measured to further diminish error associated with the TDS of the media. To maintain a constant media volume and to avoid pulling a vacuum in the system, the media removed was replaced with an equal volume of the sterile, anaerobic media from the same batch of media that was used in the initial experiment setup.

### 2.5 Synchrotron microprobe µXRF mapping and µXANES

#### 2.5.1 Sample preparation

Reacted mineral particles were prepared for optical imaging and spatially resolved synchrotron radiation x-ray fluorescence mapping and spectroscopy. Fayalite, San Carlos
olivine, San Carlos peridotite, and petedunnite were sampled after seven weeks of reaction. Hedenbergite was sampled after 14 weeks of reaction. Mineral bearing media was removed aseptically using a 23g needle with preferential selection of particles ≤ ~150µm. Preparation was conducted in an anaerobic chamber to avoid exposure to oxygen. The samples were washed in anaerobic MilliQ water to remove salts, applied directly to a pure quartz microscope slide (ESI Co.) and dried at room temperature overnight. Epoxy (Buehler) was applied to the dried sample and it was removed from the anaerobic chamber and allowed to harden at 55°C. To reduce the chance of oxidation of the sample, epoxy above the grain surfaces was removed with a diamond wheel and grains were exposed during dry grinding on silica carbide sandpaper, polished using 0.25µm diamond in ethanol and cleaned with acetone, never coming in to contact with water.

2.5.2 Multiple energy x-ray fluorescence mapping (µXRF) and spot x-ray absorption near edge spectroscopy (µXANES) data collection

Synchrotron-based hard-x-ray microprobe measurements of major element distributions and µXANES were conducted at BL 2-3 at the Stanford Synchrotron Radiation Lightsource (SSRL). A detailed explanation of the integrated approach to synchrotron data collection and processing developed to identify unique Fe-bearing components within our samples is given in Mayhew et al. (2011), which comprises Chapter 2 of this thesis. Data collection occurred with the SPEAR accelerator ring containing ~ 350 mA at 3.0 GeV. The focused beam size was approximately 2x2 µm with a pixel step-size of 2 or 4 µm, depending on the sample, and a 25 millisecond dwell time. Fe K-edge maps were generated in a continuous raster scanning mode using a single-element vortex detector, and all windowed counts for each element extracted from the full X-ray fluorescence spectra were normalized to the intensity of the incident X-ray beam (I₀). Fe K-edge mapping was conducted at 5 (7123, 7127, 7128, 7130, 7133 eV) or 6 (+ 7129 eV)
or 7 (+ 7129, 7131) or 8 (+ 7126, 7129, 7131 eV) discrete energies, chosen to maximize the
differences in normalized intensity between representative µXANES. Approximately 10-30
µXANES spectra were collected from each map area in order to sample the variety of Fe-phases
present. Each µXANES spectrum was collected from ~ 240eV below the Fe K-edge to 300eV
above the edge. All µXANES data were dead time corrected, background subtracted, and
normalized to unit step edge following standard methods using the SIXPACK software package
(Webb, 2005) before further processing. Maps and spectra were calibrated using the first
inflection of a Fe⁰ foil at 7112 eV. The short dwell times for µXRF mapping and µXANES
analyses (8 minutes/scan) minimized potential radiation damage to the sample. A single scan was
conducted at each µXANES location.

2.5.3 Multiple energy µXRF maps and µXANES data analysis

A brief description of the data analysis is given here; see Mayhew et al., 2011 for more
detail. Prior to collection of µXANES data, the multiple energy XRF maps were dead time
corrected and run through a Principal Components Analysis (PCA) using the MicroAnalysis
Toolkit (http://smak.sams-xrays.com/) to produce maps of the spatial distribution of unique
components. These were used to guide the selection of µXANES locations, to ensure that the
µXANES would represent the diversity of Fe-bearing phases as suggested by the unique
components. PCA was then conducted on deadtime corrected, normalized XANES spectra using
the SIXPACK software (Webb, 2005) in order to identify the end-member spectra representative
of the entire data set. The multiple energy Fe K-edge maps were then fit with end-member
spectra to produce maps of the spatial distribution of each end-member throughout the map area.
To understand the geochemical significance of this data, the end-member XANES (EM) spectra
were fit using linear combinations of Fe model compounds to identify the speciation of Fe represented by each EM spectra.

2.5.4 XAS measurements of iron model compounds for use in least squares fitting of unknown spectra

Bulk Fe K-edge XANES were obtained for iron-bearing minerals representing a variety of different mineral groups (Mayhew et al., 2011). Briefly, materials were powdered and, if necessary, mixed with boron nitride to achieve adequate beam transmission. Measurements were performed at SSRL on beam lines 11-2 and 4-1. Fe K-edge XANES spectra were collected from 7,000 to ~8,000 eV using a Si(220) phi=0 monochromator crystal set and a slit size of ~1x10mm. The monochromator was detuned 30-50% for harmonic rejection. Two to four scans of each sample were collected either in transmission mode using an ion chamber or fluorescence mode using a Lytle detector and XANES data reduction was conducted as described above. Independent identification of mineral phases was verified by X-ray powder diffraction or electron microprobe analysis.

XANES spectra identified as end-members (EM) of each sample were fit from 7110-7150 eV with linear combinations of Fe model compounds (see Table C1). All fits were done using the ‘Cycle Fit’ function in SIXPACK (Webb, 2005). All models were fit individually as 1 component fits and then the fit cycle was repeated for 2 component fits using the best 1 component model compound paired with the remaining model compounds. This process is repeated until adding an additional component no longer decreases the $R^2$ value by 10% or greater. A plot of the model spectra used in fits of end-member XANES is available in the supplementary information. All other XANES spectra were fit internally with the EM spectra to verify that they could be accurately described by the end-members.
2.6 X-ray diffraction

X-ray diffraction analyses of the bulk starting material and secondary mineral phases were conducted to complement the Fe specific, spatially resolved synchrotron analyses. Conventional powder x-ray diffraction (XRD; PANalytical X Pert PRO MRD, Almelo, The Netherlands) with Cu Kα radiation, an XCelerator solid-state linear array detector, and Bragg-Brentano focusing optics was used to obtain diffraction patterns from the un-reacted starting materials. The starting materials were ground in isopropyl alcohol in an agate mortar and pestle and mounted as acetone smears onto glass petrographic slides. Each sample was scanned from 5–120 degrees using a 0.0063-degree step size, with a 100 second per point count time. These patterns were used for comparison to diffraction patterns of reacted samples to aid in identification of secondary phases that formed during incubation at 100°C.

Secondary phases from each experiment were sampled and concentrated by shaking the vials to suspend the fine phases. Ten milliliters of media and suspended material were removed from each of the three vials from each individual experiment and then combined. The mineral phases were pelleted by centrifugation and the supernatant removed. The minerals were then suspended in MilliQ water, pelleted, and the supernatant removed. This process was repeated 5x to remove soluble salts which may have precipitated out of the seawater media. Due to limited sample size, none of the conventional powder mounting techniques could be used to analyze the reacted materials. Instead, powders were packed in 0.7-mm OD boron-rich glass capillaries (Charles Supper Company, Natick, MA). These samples were run using the MRD with Cu tube and XCelerator detector; however, a multilayer focusing optic was used to generate a parallel, high-intensity beam for capillary measurements. Samples were scanned between 9.5–76.5 degrees using a 0.0063-degree step size and a 1000 second per point count. Capillaries were

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spun at 20 rpm during the analysis to maximize crystallite orientation sampling. The irradiated length for each sample was determined by a 20-mm mask. When necessary to obtain sufficient volume and ensure homogeneous illumination, samples were diluted with amorphous silica gel (Grace Davidson). This material increased measured background to some extent, but contributed no peaks to the diffraction pattern as opposed to the boron-rich glass of capillary tubes. The capillaries were not entirely amorphous and contributed two peaks to the diffraction patterns. These were accounted for during analysis of the diffraction data.

2.7 Geochemical modeling

The Geochemist’s Workbench (Version 8, RockWare, Inc., Golden, CO.) and thermo.dat database were used to create mineral stability diagrams on the basis of the bulk aqueous and gaseous chemistry of each individual experiment at the time of sampling for synchrotron analyses (Table 3). Model parameters are given in the appropriate figure captions.

3. RESULTS

3.1 Production of H₂ from water-rock reactions

San Carlos peridotite, San Carlos olivine, the fayalite and magnetite fractions of Forsythe fayalite, and petedunnite-water reactions produced H₂ above background levels during reaction at 55 and 100°C as shown in Figure 1. At 55°C, the magnetite-fraction produced the most H₂ followed by petedunnite. H₂ production occurred during the first 96 and 336 hours respectively, with rates of production of 1.86 and 0.38 nmoles/g/hour. These experiments reached concentrations 2.5-3x greater than the fayalite fraction and 5-6x greater than San Carlos peridotite and San Carlos olivine. Hydrogen production from the fayalite fraction, San Carlos
Figure 1a. Hydrogen gas produced in abiotic water-rock reactions at 55°C. All experiments were conducted in triplicate and the results from individual experiments are plotted. Values reported are calculated from the gas phase only. Error bars represent the analytical uncertainty.
peridotite, and San Carlos olivine was longer lived but the rates of generation were much slower
e.g. 0.02-0.06 nmoles/g/hour). Hedenbergite did not produce H₂ at this temperature.

At 100°C, petedunnite produced the most H₂ while the San Carlos peridotite, San Carlos
olivine, and fayalite and magnetite fraction experiments also produced significant hydrogen.
Hydrogen production from magnetite was short-lived, lasting only 48 hours while being
produced at a rate of 2.71 nmoles/g/hour. Steady H₂ production was maintained for 336 hours in
the petedunnite, San Carlos peridotite, and fayalite fraction experiments at rates of 0.63, 0.42,
and 0.21 nmoles/g/hour respectively. After initial H₂ generation, the concentration of H₂
generally plateaued. In contrast, San Carlos olivine produced H₂ above background levels for the
first 1200 hours of incubation at a relatively constant rate (0.05 nmoles/g/day) though the
concentration was significantly less than that for other minerals. Little H₂ was generated from
hedenbergite; although the average H₂ concentration in the hedenbergite experiment is above that
of the media-only controls, they are not statistically different. All substrates produced 1.5-5.6x
more H₂ at 100 than at 55°C, with the exception of the magnetite fraction which produced only
0.7x of the amount produced at 55°C. The rate of H₂ generation increased for all substrates at
100°C, ranging from 1.5-8.4x as fast as at 55°C.

3.2 Mineral alteration

The starting geologic materials were characterized using XRD and electron microprobe.
Quantitative measurements of the weight percent oxides of each major mineral phase were
acquired from electron microprobe analyses and mineral formulas were calculated from these
values (Table 1). QEMSCAN was used to further characterize the components of each substrate
and obtain the modal abundance of each phase (Table 2). Surface areas ranged from 0.2506-
Figure 1b. Hydrogen gas produced in abiotic water-rock reactions at 100°C. All experiments were conducted in triplicate and the results from individual experiments are plotted. Values reported are calculated from the gas phase only. Error bars represent the analytical uncertainty.
0.5738 m²/g for all substrates with the exception of hedenbergite, which had a much higher surface area (6.9959 m²/g) (Table 1). Additionally, Fe K-edge synchrotron µXRF and µXANES analyses were conducted on the starting materials to enable accurate comparison to analyses of reacted materials. The high resolution synchrotron µXRF and µXANES method is limited to a small map area (~0.0025 - 0.01 cm²) thus it was important to obtain a more representative picture of the starting phases using QEMSCAN and XRD.

Table 1. Major mineral constituents of the starting geologic substrates as identified by XRD and/or electron microprobe analyses. Chemical formulas were calculated from average normalized weight percent oxides of each phase as measured by electron microprobe.

<table>
<thead>
<tr>
<th>Starting material/ phases</th>
<th>Calculated mineral formula</th>
<th>SSA m²/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forsythe mine fayalite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fayalite fraction</td>
<td>Fe₁.₈₄Mg₀.₁₄SiO₄ - Fa₈₈</td>
<td>0.2506 ± 0.0072</td>
</tr>
<tr>
<td>fayalite</td>
<td>Fe₃O₄</td>
<td></td>
</tr>
<tr>
<td>magnetite</td>
<td>Fe₁.₈₄Mg₀.₁₄SiO₄</td>
<td>0.5738 ± 0.0063</td>
</tr>
<tr>
<td>clinopyroxene</td>
<td>(Ca₀.₉₂Na₀.₀₄)(Mg₀.₂₄Fe₀.₇₈Al₀.₀₄)(Si₁.₉₄Al₀.₀₆)O₆</td>
<td></td>
</tr>
<tr>
<td>magnetite fraction</td>
<td>Fe₃O₄</td>
<td></td>
</tr>
<tr>
<td>fayalite</td>
<td>Fe₁.₈₄Mg₀.₁₄SiO₄</td>
<td></td>
</tr>
<tr>
<td>clinopyroxene</td>
<td>(Ca₀.₉₂Na₀.₀₄)(Mg₀.₂₄Fe₀.₇₈Al₀.₀₄)(Si₁.₉₄Al₀.₀₆)O₆</td>
<td></td>
</tr>
<tr>
<td>San Carlos olivine</td>
<td>Mg₁.₈Fe₀.₂SiO₄ - F₀₉₀</td>
<td>0.2728 ± 0.0102</td>
</tr>
<tr>
<td>Hedenbergite</td>
<td>CaFe₀.₇Mn₀.₁Mg₀.₁Si₂O₆</td>
<td>6.9959 ± 0.0702</td>
</tr>
<tr>
<td>Petedunnite</td>
<td>Ca₀.₉Zn₀.₃Mn₀.₃Fe₀.₃Mg₀.₂Si₂O₆</td>
<td>0.2806 ± 0.0078</td>
</tr>
<tr>
<td>gahnite</td>
<td>NA*</td>
<td></td>
</tr>
<tr>
<td>San Carlos peridotite</td>
<td>Mg₁.₈Fe₀.₂SiO₄ - F₀₉₀</td>
<td>0.3442 ± 0.0052</td>
</tr>
<tr>
<td>olivine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>clinopyroxene</td>
<td>(Ca₀.₈Na₀.₁)(Mg₀.₈Fe₀.₁Al₀.₂)(Si₁.₉Al₀.₁)O₆</td>
<td></td>
</tr>
<tr>
<td>orthopyroxene</td>
<td>Ca₀.₀₂Mg₀.₈₅Fe₀.₀₈Si₀.₉₇Al₀.₀₇O₃</td>
<td></td>
</tr>
</tbody>
</table>

* phase was not analyzed by microprobe – theoretical formula: ZnAl₂O₄

The majority of the starting geologic materials were found to be heterogeneous prior to incubation at 100°C. The fayalite and magnetite fractions were obtained from the Forsythe Mine fayalite deposit but we were unable to completely purify the separates. Thus, the fayalite fraction is ~87 volume % fayalite but also contains magnetite, pyroxene, amphibole, and chlorite.
Similarly, the magnetite fraction is ~44 volume % magnetite but also contains a considerable amount of fayalite (~40 volume %) with lesser amounts of pyroxene, amphibole, siderite, and chlorite. Olivine (Fo₉₀) is the predominant phase (~85 volume %) in the San Carlos peridotite with lesser amounts of orthopyroxene, clinopyroxene, siderite, and spinels as well as some alteration phases including chlorite, talc, and serpentine. San Carlos olivine (~91 volume %) is separated from the San Carlos peridotite and also contains minor amounts of orthopyroxene, siderite, and spinels as well as chlorite and serpentine. Petedunnite (~85 volume %) contains minor amounts of olivine, carbonates, and gahnite (a Zn-bearing spinel). Hedenbergite is predominantly clinopyroxene (~91 volume %) with minor amounts of olivine, amphibole, and carbonates.

Table 2. Comprehensive characterization of phases present in the starting geologic materials and their modal abundance in volume percent from QEMSCAN analyses.

<table>
<thead>
<tr>
<th>Mineral phase</th>
<th>Fayalite Fraction</th>
<th>Magnetite Fraction</th>
<th>San Carlos olivine</th>
<th>Hedenbergite</th>
<th>Petedunnite</th>
<th>San Carlos peridotite</th>
</tr>
</thead>
<tbody>
<tr>
<td>olivine</td>
<td>86.9</td>
<td>40.0</td>
<td>90.8</td>
<td>0.2</td>
<td>0.6</td>
<td>85.3</td>
</tr>
<tr>
<td>orthopyroxene</td>
<td>0.6</td>
<td>1.0</td>
<td>3.5</td>
<td>0</td>
<td>0</td>
<td>2.4</td>
</tr>
<tr>
<td>clinopyroxene</td>
<td>6.2</td>
<td>1.8</td>
<td>0</td>
<td>80.4</td>
<td>1.1</td>
<td>3.8</td>
</tr>
<tr>
<td>petedunnite</td>
<td>0</td>
<td>0</td>
<td>9.8</td>
<td>85.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>amphibole</td>
<td>2.4</td>
<td>1.6</td>
<td>0</td>
<td>0.9</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Fe(III)-bearing oxides*</td>
<td>0.4</td>
<td>43.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>gahnite</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>chromite</td>
<td>0</td>
<td>0</td>
<td>0.05</td>
<td>0</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>other spinels</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>siderite</td>
<td>1</td>
<td>3.2</td>
<td>0.7</td>
<td>5.6</td>
<td>0.7</td>
<td>1.9</td>
</tr>
<tr>
<td>other carbonates</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>3.6</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>chlorite</td>
<td>0.2</td>
<td>0.2</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>talc</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>serpentine</td>
<td>0</td>
<td>0</td>
<td>1.4</td>
<td>0</td>
<td>0</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* category includes magnetite, hematite, and goethite due to difficulties distinguishing between them due to their similar EDS spectra and BSE signals
Reacted substrates were investigated by synchrotron µXRF and µXANES analyses which focus on the Fe-bearing mineral phases in close association with the primary mineral phases in the water-rock reactions. Examples of end-member XANES spectra are shown in Figure C1. Separate XRD analyses of concentrated reaction products provides insight into the bulk secondary mineral assemblage. These two analytical approaches to secondary mineral characterization provide a complementary characterization of the solid phases present after reaction at 100°C. The following presentation of results and discussion focuses on the 100°C experiments as solid phase data was not collected for the 55°C experiments.

3.2.1 Olivine-water reactions

The complex starting mineralogy of the fayalite fraction was corroborated by identification of end-member XANES spectra representative of pyroxene and magnetite as well as fayalite (Table C2). The end-member spectrum representative of magnetite also required an additional Fe(III) component in the fit, suggestive of some oxidation of the magnetite. Figure 2a illustrates the relative proportions of these phases within the mapped area. After reaction at 100°C, we were able to detect the growth of alteration phases (Figure 2c). An Fe-bearing talc is the predominant alteration phase and was not detected prior to reaction. This coating is preferentially associated with the clinopyroxene particles (see optical image in comparison to green particles in map, Figure 2c and 2d). We also observe the growth of an oxidized Fe phase. An Fe(III)-oxide appears to coat all of the magnetite particles (see optical image in comparison to blue particles in map, Figure 2c and 2d). Using XANES analyses we are unable to distinguish the distinct type of Fe(III) oxide because of the similar peak positions and structures of spectra from a variety of Fe(III) oxides including ferrihydrite, hematite, and goethite. Oxidation of the iron in the fayalite fraction experiment is corroborated by the observation of an Fe(III)-bearing
Figure 2a-b. A.) Distribution of unique Fe-bearing phases in the starting fayalite fraction (red = fayalite, green = pyroxene, blue = magnetite). B.) Optical image of the mapped area.

Figure 2c-d. C.) Distribution of unique Fe-bearing phases in the fayalite fraction reacted at 100°C (red = fayalite, green = Fe-bearing talc + the bulk fayalite fraction, blue = Fe(III)-oxides). D.) Optical image of the mapped area.
precipitate upon visual examination of the fayalite reaction vials. Brucite and serpentine were not
detected in the reacted minerals from the fayalite fraction experiment. It was not possible to
determine if any new magnetite formed in the fayalite fraction experiment. Capillary XRD data
is not currently available for the fayalite experiment.

The starting San Carlos olivine was more pure than the fayalite although partially altered
prior to reaction at 100°C. This was corroborated by identification of end-member XANES
spectra in the unreacted material that are representative of olivine + brucite indicating partial
alteration of the olivine (see Figure C1 for an example of end-member spectra). A number of the
particles within the µXRF map area are olivine coated to some degree with brucite (blue in
Figure 3a). End-member spectra identified from the reacted material possess a greater proportion
of Fe-bearing brucite and a greater number of particles are completely coated by the brucite
(Figure 3b). We also detect a new oxidized Fe phase that is present in limited abundance but
which was not detected at all in the unreacted material (Figure 3b). A Mg-Ca carbonate not
detected in the XRD of the primary material was also detected in the concentrated reaction
material using capillary XRD methods. Magnetite and serpentine were not detected through
diffraction or spectroscopic measurements.

3.2.2 Pyroxene-water reactions

Electron microprobe and X-ray diffraction analyses revealed that one of the starting
pyroxenes originally purchased as “hypersthene” is a petedunnite, a Zn-bearing pyroxene
(Ca_{0.9}Zn_{0.3}Mn_{0.3}Fe_{0.3}Mg_{0.2}Si_{2}O_{6}). XRD also identified gahnite, a Zn-bearing spinel (ZnAl_{2}O_{4}) in
the mineral assemblage. Fe K-edge XANES and XRF revealed that the starting material included
alteration phases primarily localized around the gahnite inclusions (Figure 4a). The majority of
Figure 3A.) Distribution of unique Fe-bearing phases in the starting San Carlos olivine (red = San Carlos olivine, green = San Carlos olivine > brucite, blue = brucite > San Carlos olivine.

Figure 3b-c. B.) Distribution of unique Fe-bearing phases in the San Carlos olivine reacted at 100°C (red = San Carlos olivine, green = Fe(III)-oxide, blue = brucite > San Carlos olivine). C.) Optical image of the mapped area.
the map area is fit with petedunnite (green particles in Figure 4a). There also appears to be an oxidized coating on some pyroxene particles (red particles in Figure 4a). The distribution of a unique end-member spectrum that is not fit well by our existing model compound library corresponds with Zn hotspots (the best fit is dominated by Fe(III)-serpentine). These Zn hotspots are likely associated with the Zn-bearing spinel (Figure 4a and 4e). We also detect an altered petedunnnite spectrum in which Fe-bearing talc is a component of the fit but it is limited to only a few scattered pixels (data not shown).

Upon comparison of the unreacted and reacted material, it is clear that growth of alteration phases occurred during reaction at 100°C. A few unaltered pyroxene grains remain (green particles in Figure 4c) but the Fe-bearing talc coating is present in much greater abundance (blue particles in Figure 4c). Petedunnite with Fe(III)-oxide that appears to be unique from the oxidized coating detected in the unreacted material is detected and correlated with the Zn-hotspots (Figure 4c and 4f). We no longer detect the oxidized Fe coating that was seen in the unreacted material.

Synchrotron investigations of the starting hedenbergite corroborate the XRD, electron microprobe, and QEMSCAN results. The majority of the map area is composed of pyroxene particles (green particles Figure 5a). An alteration phase appears to partially coat some of the pyroxene grains (blue pixels in Figure 5a) though we have been unable to adequately identify the alteration phase. The synchrotron results also reveal the presence of an oxidized Fe component that is primarily represented by a single particle (red in Figure 5a). Analyses of the reacted material reveal growth of an oxidized Fe phase during reaction at 100°C. The oxidized component is now present in a much greater proportion of the map area (red particles in Figure 5c) and are also evident in the optical image (Figure 5d). We also observe the precipitation of an
Figure 4a-b. A.) Distribution of unique Fe-bearing phases in the starting petedunnite material (red = Fe(III)-oxides, green = pyroxene, blue = gahnite. B.) Optical image of the mapped area.

Figure 4c-d. C.) Distribution of unique Fe-bearing phases in the petedunnite reacted at 100°C (red = Fe(III)-oxides, green = pyroxene, blue = Fe-bearing talc). D.) Optical image of the mapped area.
Figure 4e-f. Distribution of Zn in e) the unreacted petedunnite and f) the petedunnite reacted at 100°C. Locations of Zn hotspots correspond to the location of the oxidized Fe component in Figure 4c.
Figure 5a-b. A.) Distribution of unique Fe-bearing phases in the starting hedenbergite material (red = Fe(III)-oxides, green = pyroxene, blue = altered pyroxene).  B.) Optical image of the mapped area.

Figure 5c-d. C.) Distribution of unique Fe-bearing phases in the hedenbergite reacted at 100°C (red = Fe(III)-oxides, green = pyroxene, blue = Fe-bearing talc).  D.) Optical image of the mapped area.
Fe-bearing talc phase on several pyroxene grains (blue particles in Figure 5c) which was not present in the unreacted material.

3.2.3 Peridotite-water reactions

Olivine and pyroxene were detected in synchrotron analyses of the starting San Carlos peridotite (green and red particles respectively in Figure 6a). Brucite was also found to be present on some of the olivine and pyroxene particles (blue in Figure 6a). Chromite, which we know is present in the starting material from electron microprobe and QEMSCAN analyses, was not detected in the synchrotron analyses but this is due to the fact that the chosen map area did not include any chromite particles. After reaction at 100°C, we were able to detect multiple alteration phases in the San Carlos peridotite. Unaltered olivine is still detectable (green particles in Figure 6b) but Fe-bearing brucite is now a major component of one of the end-member spectra and appears to be a coating on approximately one half of the olivine particles (blue particles in Figure 6b). An oxidized Fe component, not detected in the starting material, is detected after reaction but is limited in its distribution to only three particles (Figure 6b). The distribution of this phase correlates to the chromite particles as evidenced by both the optical image (Figure 6c) and the map of Cr distribution throughout the mapped area (Figure 6d).

3.2 Geochemical characterization of water-rock reaction experiments

3.2.1 Concentration of aqueous ions

The concentrations of aqueous species in the water-rock reaction experiments at the time of sampling for synchrotron and XRD analyses are summarized in Table 3. The concentration of aqueous Si varies between substrates; San Carlos peridotite and olivine have only slightly elevated concentrations versus the media-only control (~1570 vs. 940 µM, respectively) while
Figure 6A.) Distribution of end-member XANES spectra in the starting San Carlos peridotite (red = pyroxene, green = olivine, blue = olivine + brucite).

Figure 6b-c. B.) Distribution of end-member XANES spectra in the San Carlos peridotite reacted at 100°C. (red = oxidized Fe, green = olivine, blue = olivine + brucite, grey = pyroxene). C.) Optical image of the mapped area.
Figure 6d. Distribution of chromium in the San Carlos peridotite reacted at 100°C. Locations of Cr enrichments correspond with chromite particles in the optical image (see Figure 6c) and Fe(III)-oxide coatings in the Figure 6b.
hedenbergite, petedunnite, and the magnetite fraction have intermediate Si concentrations (~2500 µM) and the fayalite fraction has the highest concentration (~4280 µM). All experiments have Mn concentrations above the media-only controls. The concentration of Fe_{aq} is below detection limit (0.1 µM) in all samples at all times with the exception of fayalite and magnetite fraction experiments, which reach values as high as 150 and 240 µM, respectively. The composition of the starting media, designed to mimic seawater, makes it difficult to detect small variations in Mg and Ca concentration due to dissolution or precipitation of Mg- and Ca-bearing minerals in the experiments. Only after 600 hours do Mg concentrations in the experiments deviate from the media-only control (data not shown). San Carlos peridotite, San Carlos olivine, hypersthene, and hedenbergite all have concentrations approximately 1 mM less than the media only control (33 mM). Similarly, the concentration of Ca for all samples is within ~1 mM of the media only control (18 mM). The sole exception is hedenbergite that has a Ca concentration approximately 3 mM greater than all other experiments.

Table 3. Geochemical parameters at the time* of sampling for synchrotron and XRD analyses. Values are averages of replicate vials.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>pH</th>
<th>log f/H₂</th>
<th>TIC mM</th>
<th>CO₂(aq) µM</th>
<th>log f/CO₂</th>
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<td></td>
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<td></td>
<td></td>
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<td>4280</td>
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<td>-1.11</td>
<td>2870</td>
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<tr>
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<td>5.81</td>
<td>1180</td>
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</tr>
<tr>
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<td>5.36</td>
<td>960</td>
<td>-1.23</td>
<td>2620</td>
</tr>
<tr>
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<tr>
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<td>3.61</td>
<td>1100</td>
<td>-1.17</td>
<td>940</td>
</tr>
</tbody>
</table>

* San Carlos peridotite, fayalite, San Carlos olivine, and petedunnite were sampled at ~1200 hours. Hedenbergite was sampled at ~2300 hours. BDL – below detection limit
3.2.2 CO$_2$, TIC, and pH

CO$_2$(aq) was calculated from measurements of CO$_2$(g) in the headspace according to Henry’s Law. There is variation in the initial concentration of CO$_2$(aq) between experiments (550 – 850 µM) due to variations in the amount of time individual vials were bubbled with N$_2$/CO$_2$ during the experimental setup. After a short reaction time (~336 hours) the CO$_2$ concentration in all water-rock reactions is below that in the media-only control. After further reaction, CO$_2$ concentration increases in all water-rock experiments while remaining constant in the media-only control. By the end of the experiment, only hedenbergite and petedunnite have concentrations that are lower than that in the media-only control (Table 3).

The starting seawater media had a pH of 5.5. After 24 hours of incubation, the media-only control increased to 6 and remained at this value for the duration of the experiment. The pH of the water-rock experiments was more variable. Final pH values varied from 6 to 7 (Table 3).

The total inorganic carbon (TIC) of the experiments was calculated from the concentration of CO$_2$(aq) and starting concentration of bicarbonate in the media and pH. Assessment of the saturation of the bulk aqueous media with respect to carbonate phases, was made by comparing the ion activity product (IAP) to the Ksp of individual carbonate phases. All experiments were super-saturated with respect to dolomite, calcite, and magnesite but below saturation for siderite after 24 hours of reaction and at the end of the reaction time.

4. DISCUSSION

4.1 H$_2$ production from water-rock reactions at 100°C

Hydrogen gas was generated from water-rock reactions at 100°C over the course of 2300 hours at concentrations greater than those observed in media-only controls (Figure 1). Five of six
tested substrates, petedunnite (a pyroxene), San Carlos peridotite, the magnetite and fayalite fractions of the Forsythe fayalite, and San Carlos olivine produced significant H$_2$ gas. Hedenbergite, also a pyroxene, did not produce H$_2$ above background levels. There are large variations in H$_2$ production and secondary phase formation between substrates, even those belonging to the same mineral group, suggesting that the processes and pathways of reaction differ between assemblages.

Equilibrium H$_2$ production values extrapolated from models of dunite (100% olivine Fo$_{90}$)-water reactions at 100°C and water:rock = 1 and 150°C and water:rock = 7 are approximately 70-280 µmol H$_2$/gram dunite (Klein et al., 2009). The fayalite fraction produced 90 nmol H$_2$/gram, San Carlos olivine produced 62 nmol H$_2$/gram mineral, and San Carlos peridotite produced ~155 nmol H$_2$/gram, values that are three orders of magnitude less than those predicted by equilibrium models. The lesser amount of H$_2$ generated in the experiments is consistent with the non-equilibrium conditions and the small amount of alteration that occurred. The design of the experimental system essentially encourages disequilibrium because of the large headspace volume (35 ml) that acts to remove H$_2$ from the aqueous phase thereby not allowing equilibrium between H$_2$ and the mineral assemblage. Furthermore, the bulk aqueous chemistry will not reach equilibrium with olivine and pyroxene, the majority of the starting minerals, because of the precipitation of talc which holds the fluid chemistry in the talc stability field. Talc precipitation also causes surface passivation that discourages further reaction. Due to the fact that only a small fraction of the primary minerals have reacted during the time period of these experiments, substantially less H$_2$ should be generated than that predicted from the equilibrium models.
4.2 Formation of Fe(III) oxides

The only Fe(III)-bearing phase frequently detected in the experiments, and likely related to H₂ generation, is Fe(III) oxides. Fe(III) oxides are formed in all of the reactions though to varying degrees. In the fayalite fraction, petedunnite, and San Carlos peridotite experiments Fe(III) oxide coatings are detected on the spinel phases (Figures 2c, 4c, and 6b). A smaller amount of oxidized Fe is detected in the San Carlos olivine experiment (Figure 3b) but this may be because there were no spinel phases included in the map area. The precipitation of Fe-oxides is visually apparent in the magnetite fraction experiments and is confirmed by Fe K-edge XANES analysis of the precipitates. It was not possible to obtain data from the magnetite surfaces. Thus far, we have been unable to mineralogically identify the specific oxidized Fe component because of the similar white line positions and structures of XANES spectra from a variety of Fe(III) oxides including ferrihydrite, hematite, and goethite, for example. The signal from these phases is not adequate enough to collect high quality Fe K-edge EXAFS data which can be used to differentiate between Fe(III)-oxides (Hansel et al., 2003). X-ray diffraction analyses of the concentrated Fe(III)-oxide products in the magnetite and fayalite fraction experiments are complicated by the high Fe contents of these phases due to Fe fluorescence when excited by the Cu-sourced X-rays. Attempts are being made to address this issue and obtain XRD data for these samples.

Regardless of the specific oxidized Fe phase, it is clear that Fe oxidation, largely associated with spinel surfaces, has occurred in the water-rock reaction experiments, and that the Fe(III) product is an isolated oxide phase rather than being substantially incorporated into silicates such as clays, serpentine or talc which are spectrally distinct by XANES. Other researchers have reported the formation of Fe(III)-oxides in olivine-water reactions conducted at
120°C and attributed the formation of these phases to oxidation of Fe(II) coupled to reduction of water to H₂ (Olsson et al., 2012). Similarly, hematite was observed after short reaction times in olivine-water reactions at 200°C (King et al., 2010). Hematite is predicted to be present at large water-rock ratios (> ~3100) in geochemical models of peridotite-freshwater reactions at 25°C due to the oxidizing nature of the system (Palandri and Reed, 2004). However, the precipitation of Fe(III)-oxides is not generally predicted from geochemical models of olivine-water reactions at low temperatures.

### 4.3 Formation of brucite, talc, and carbonates

Geochemical models (Klein et al., 2009) and laboratory experiments of olivine-water reactions conducted at 200°C (Seyfried et al., 2007), attribute H₂ production to the formation of Fe(III)-bearing serpentine and brucite in the absence of magnetite. Numerous modeling (e.g. Palandri and Reed, 2004; McCollom and Bach, 2009; Klein et al., 2009) and experimental (e.g. Moody 1976a; Seyfried and Dibble, 1980; Janecky and Seyfried, 1986; Allen and Seyfried, 2003; Seyfried et al., 2007) investigations into peridotite-water reactions at 200 – 400°C have been conducted. At <200°C, the secondary mineral assemblage in both geochemical models and experiments consistently include serpentine, magnetite, clinopyroxene, +/- brucite and chlorite. At these temperatures, H₂ production is attributed to the incorporation of Fe(III) into magnetite (e.g. McCollom and Bach, 2009; Klein et al., 2009). At temperatures ≤ 200°C, magnetite has either been absent or present in only very limited quantities (Seyfried et al., 2007; Klein et al., 2009; McCollom and Bach, 2009). Jones et al. (2010) reported abundant magnetite precipitation at 200°C in CO₂ undersaturated conditions and less magnetite in CO₂ supersaturated conditions.
As in the case of the olivine-water reactions, H₂ production at these temperatures is attributed to the incorporation of Fe(III) into serpentine and brucite.

Fe-bearing brucite is detected in association with olivine particles in the San Carlos olivine and San Carlos peridotite experiments (Figures 3b and 6b). The brucite is Fe(II)-rich and therefore acts as an Fe-sink thereby limiting H₂-production (Figure C2). Talc is the other dominant Fe-bearing alteration product. The precipitation of talc is correlated with Si concentration, such that Fe-bearing talc is present in experiments with ~2400 – 4300 µM Si (fayalite and magnetite fractions, petedunnite, and hedenbergite) and absent in experiments with less Si (~1600-1800 µM Si in the San Carlos olivine and peridotite). In the fayalite fraction experiment, growth of an Fe-bearing talc occurs during reaction at 100°C. This phase, which was not present in the starting material, appears to coat the clinopyroxene particles that are present in the fayalite fraction experiment after reaction and is found in 9% of the pixels (green particles in Figure 2c, compare to location of clinopyroxene particles in Figure 2d). In the petedunnite experiment, an Fe-bearing talc is present in 5% of the mapped pixels in the starting material. Growth of the talc phase during reaction is evidenced by the greater abundance (76% of the pixels) of the Fe-bearing talc in the reacted samples (Figure 4c). Fe-bearing talc is also detected in the hedenbergite experiment. No talc was detected in the starting material (Figure 5a), but after reaction 25% of the mapped pixels contain Fe-bearing talc (Figure 5c). Fe-bearing talc likely plays a similar role to brucite in suppressing H₂ generation as it also appears to be Fe(II)-rich (Figure C2).

In addition to the Fe-bearing secondary phases detected in the experiments, a magnesian calcite was detected in the reacted San Carlos olivine and peridotite experiments. These phases were not detected by XRD of the unreacted materials but were detected in the reacted materials.
The experiments were oversaturated with respect to dolomite, calcite, and magnesite thus formation of these phases was not unexpected. Carbonate phases are commonly found in similar experimental studies of olivine-water reactions conducted to investigate the potential for mineral carbonation as a sink for CO₂. In these experiments, precipitation of carbonate phases is observed in carbonate oversaturated conditions at a range of temperatures (95 – 200°C) (e.g. Giammar et al., 2005; Jones et al., 2010; Olsson et al., 2012).

It is exceedingly difficult to characterize the total amount of secondary mineral formation in samples that remain so dominated by the original bulk mineralogy. Similarly, it is not possible to quantitatively assess the partitioning of Fe between the brucite, talc, and Fe(III)-oxide phases because the total amount of each phase cannot be determined from a bulk measurement. The synchrotron analyses employed in this study investigate only a very small amount of the reacted material so that it is not possible to quantitatively extrapolate the results; instead the Fe speciation maps and XANES data are used to detect as many of the different Fe species present as possible. Bulk Fe K-edge XANES analyses would be well-suited to obtaining a more quantitative understanding of the partitioning of Fe but the small proportion of secondary phases are not detectable in bulk analyses because the signal is overwhelmed by the remaining primary material.

We did not detect Fe(II) or Fe(III)-bearing serpentine as a phase greater than 5% of the total Fe in any given secondary mineral assemblage (where 5% is the detection limit for the Fe K-edge XANES fitting); this is likely due to the high pCO₂ in the experiments. In experiments investigating olivine carbonation, serpentine phases have been observed to precipitate under some conditions but because of the high pCO₂ they are not generally the dominant phases (e.g. King et al., 2010; Olsson et al., 2012). Similarly, the formation of new magnetite was not
detectable within the reaction products. This is in line with the absence of magnetite in the secondary mineral assemblage at temperatures $<\sim 200^\circ$C (e.g. Seyfried et al., 2007; Klein et al., 2009; McCollom and Bach, 2009; Jones et al., 2010). However, assessment of the secondary mineralogy in this study is limited to the end time-point at which most of the experimental systems (the exception being San Carlos olivine) were no longer producing $H_2$. It is possible that the phases first formed during $H_2$ generation are different than those captured during sampling; for example, it is possible that early Fe-bearing serpentine was converted to talc due to increasing Si concentration with progressive water-rock reaction. However, for all substrates, hematite is the predicted stable phase calculated from the bulk aqueous chemistry at early time points in the reaction progress while talc is the predicted stable phase as the reaction progresses and the $Si_{aq}$ and $H_2_{aq}$ concentrations increase (Figure 7). In either case, neither serpentine nor brucite are the predicted stable phases, due to the high aqueous Si and CO$_2$ concentrations (e.g. Klein et al., 2009; Klein and Garrido, 2011).

### 4.4 The importance of spinel phases in $H_2$ generation

There is a strong correlation between the abundance of spinel phases (e.g. magnetite, chromite, or gahnite) in the starting reaction material and generation of $H_2$. For example, if we compare the mineral assemblages of the two pyroxene substrates, we find that petedunnite, which produced the most $H_2$ at 100$^\circ$C, contains 1.2 volume % gahnite while there are no spinels present in the hedenbergite, which made no $H_2$ at either 55 or 100$^\circ$C. Similarly, the San Carlos peridotite contains 0.9 volume % spinels, predominately chromite, while the San Carlos olivine contains 4x times less spinels ($\sim 0.2$ volume %). The much greater spinel surface area in the San Carlos peridotite correlates with the production of 10x more $H_2$ than in the San Carlos olivine.
Figure 7. Mineral stability diagram showing the thermodynamically stable Fe-bearing phases in black and Mg-bearing phases in blue under the bulk aqueous and gaseous chemical conditions of the San Carlos olivine system at a) 24 hours and b) 1200 hours of reaction. The bulk aqueous chemistry at the time is plotted on each diagram. The length of the symbol along the x-axis represents the uncertainty in pH measurements. The model parameters were $[\text{Fe}^{2+}]=10^{-7}$ M, $[\text{Ca}^{2+}]=10^{-1.7}$ M, $[\text{Mg}^{2+}]=10^{-1.5}$, $\log H_{\text{aq}} = 10^{-6.6}$ and $10^{-8.2}$ for a) and b) respectively, $\log CO_{\text{aq}} = 10^{-3.1}$ and $10^{-2.9}$ for a) and b) respectively.
experiment during the first 24 hours of reaction. We see a similar pattern with the magnetite and fayalite fractions of the Forsythe fayalite. The magnetite fraction produces ~5x more H₂ than the fayalite fraction during the first 24 hours of reaction and contains 32 volume % magnetite while the fayalite fraction contains only 0.39 volume % magnetite.

We propose that spinel surfaces promote the transfer of electrons from the Fe(II) in solution to H₂O, a process that is thermodynamically favored under the experimental conditions but kinetically hindered, thereby leading to the generation of hydrogen gas (Figure 8). Spinel minerals are known for their catalytic properties including magnetite and chromite as catalysts of organic synthesis reactions at high (e.g. Foustoukos and Seyfried, 2004; Fu et al., 2007) and low (e.g. Nuebeck et al., 2011) temperatures. Spinels are also known to catalyze reduction of metals and thus play a critical role in reducing contaminants in soils (e.g. Watts et al., 1999; Gorski and Scherer, 2010) as well as aqueous species (e.g. White and Peterson, 1996; Shipley et al., 2009). Magnetite, an inverse spinel, is highly conductive (e.g. Hamilton, 1958; Balko and Hoy, 1977; Voogt et al., 1995) due to high electron mobility between Fe(II) and Fe(III) (e.g. Skomurski et al., 2010). Gahnite, a Zn-bearing spinel, is also known for its electron transfer capabilities (Popovic et al., 2011). Similar to contaminant metals, aqueous Fe(II) should strongly interact with the magnetite, chromite, and gahnite in our experiments, as well documented for Fe oxides and spinels (e.g. Tronc et al., 1984; Hansel et al., 2003; Hansel et al., 2005; Williams and Scherer, 2004; Silvester et al., 2005; Larese-Casanova and Scherer, 2007; Tanwar et al., 2008, 2009; Gorski and Scherer, 2010). The sorption of aqueous Fe(II) to the surface of Fe(III)-bearing oxide surfaces has been shown to rapidly result in the oxidation of the aqueous Fe(II) due to the transfer of electrons from aqueous Fe(II) to the mineral phase (e.g. Tronc et al., 1984; Williams and Scherer, 2004; Silvester et al., 2005; Larese-Casanova and Scherer, 2007; Tanwar et al.,
Figure 8. Schematic representation of spinel surface promoted H₂ generation. A.) Primary silicate phases dissolve in water and release Fe(II) to solution however, electrons are not transferred from Fe(II)aq to water in solution. Instead, Fe(II)aq transfers electrons to spinels which then transfer electrons to water adsorbed to the spinel surface. B.) Oxidation of Fe(II)aq at the spinel surface leads to formation of Fe(III) oxides that coat the spinel surface resulting in C.) the passivation of the surface so it is no longer available for electron transfer. Similarly, the primary silicate phases are passivated by the formation of Fe(II)-silicates and no longer undergo dissolution.
Aqueous Fe(II), supplied from dissolution of the primary minerals, is an effective species for transfer of electrons to spinels and is the assumed electron source in these experiments. Magnetite oxidation releases Fe(II) to solution (White et al., 1994). Dissolution of the primary Fe-silicates also releases Fe(II) to solution. This process is pH dependent with increases in dissolution rate corresponding to decreases in pH (e.g. Pokrovsky and Schott, 2000; Haenchen et al., 2006; Schott et al., 2009). The rate of dissolution is also controlled by the hydration of metal-oxygen bonds at the mineral surface (e.g. Schott and Berner, 1983; Schott et al., 2009 and references therein). With this experimental design, it is not possible to measure the moles of mineral dissolved and thus the iron that was released from the starting materials making it difficult to assess the total amount of iron available for reaction. In all experiments other than the magnetite and fayalite fractions the concentration of aqueous Fe(II) is below the detection limit (~10^{-7}M) which is likely due to the precipitation of secondary mineral phases. It is clear that Fe(II) is released to solution from the observation of precipitation of Fe-bearing secondary phases as well as indications of mineral dissolution inferred from the concentrations of other cations in solution.

Applying this hypothesis enables explanation of the pattern in H2 production from the different substrates tested in these experiments. The greater amount of spinel surface area available for electron transfer reactions in the magnetite fraction may be responsible for the
enhanced H₂ production by the magnetite fraction versus the related fayalite fraction. A similar pattern is evident when comparing H₂ production and spinel content from San Carlos peridotite to San Carlos olivine and from petedunnite to hedenbergite. The lack of H₂ production in the hedenbergite sample can then be attributed to the absence of spinel phases in the hedenbergite material. In this study it appears that the presence of spinel phases (e.g. magnetite, chromite, gahnite) in the starting reaction materials is necessary for the production of H₂ above background levels.

The magnetite in these experiments may also act as a reactant as it is not predicted to be thermodynamically stable under the reaction conditions, Magnetite can be oxidized to maghemite through removal of Fe(II) ions from the mineral surface (e.g. Jolivet and Tronc, 1988; White 1990; White et al., 1994) or by oxidation of the surface Fe(II) atoms (e.g. White et al., 1994; Castro et al., 1996; Peterson et al., 1997). Oxidative processes at the surface of magnetite particles have been observed to occur on the order of a few minutes to hours (e.g. White et al., 1994) and decreases in dissolution rates and reactivity with increasing reaction time have been attributed to formation of oxidized surface layers (e.g. White 1990; White et al., 1994; Castro et al., 1996; Peterson et al., 1997).

It is curious that Fe(III) oxides were detected in the hedenbergite after reaction at 100°C, and yet no significant H₂ production occurred in these experiments (nor in experiments at 55°C). The formation of the Fe(III)-oxide phases is extensive and widely distributed (red particles Figure 5c and 5d) but the oxidation of Fe(II) was not coupled with H₂ generation. Furthermore, no spinels were present in the hedenbergite material to catalyze the transfer of electrons to water. Thus another thermodynamically favorable electron acceptor must be present in the hedenbergite experiments. Inadvertent dissolved oxygen was considered as a possibility; however, the
hedenbergite experiments, like all others, were conducted in triplicate at both 55 and 100°C and H₂ was not generated in any of the vials. It seems unlikely that oxygen could have been entrained into all 6 hedenbergite vials and but not into any of the other experiments. Another possibility is the existence of an oxidized manganese component in the starting materials that preferentially undergoes reductive dissolution by reaction with Fe(II). The hedenbergite does contain Mn though the speciation of Mn was not characterized, and high concentrations of Mn were released to solution as the reaction progressed. For now, the mechanism for Fe-oxidation without H₂ production remains an open question.

4.5 Passivation of spinel particles

It is also possible to begin to understand the variation in H₂ production rates and timescales in terms of the reactivity of the spinel phases. The high, but short-lived, rates of H₂ production observed in the first 48 hours of the magnetite fraction experiment suggest that rapid oxidation of the Fe(II) surface atoms occurred. Such rapid oxidation, followed by a decrease in reaction rates, has been observed previously (e.g. White et al., 1994). This hypothesis is supported by observation of an oxidized Fe precipitate present in the magnetite fraction experiment at 48 hours of reaction. The precipitation of an oxidized Fe phase was observed in the fayalite experiment after 96 hours of reaction. This coincides with the timing of a decrease in the rate of H₂ generation in the fayalite fraction experiment, suggesting that oxidation of the spinel surfaces was influencing the rate of H₂ production. After 336 hours at 100°C, a decrease and even cessation of H₂ production occurs in most experiments, San Carlos olivine being the exception. Synchrotron investigations of the surface of spinel particles at the end point of the fayalite fraction and San Carlos peridotite experiments reveal the presence of an oxidized coating
(Figures 2c and 6c). Similarly, an oxidized Fe phase is detected in close association with Zn hotspots in the petedunnite experiment (Figure 4c and 4f). In the high Si conditions of the experiments, it may also be possible for silica ions to adsorb to the surface of the spinel phases and limit further participation in electron transfer reactions (e.g. Hansel et al., 2003, 2005; Pokrovski et al., 2003; Voegelin et al., 2010). The result of either scenario is the partial passivation of the spinels in these experiments as shown in Figure 8.

4.6 Passivation of primary silicate minerals

Passivation of the primary silicate phases (e.g. olivine and pyroxene), also influences the rate and duration of H₂ production by limiting the release of Fe(II) to solution and thus removing the driver of sustained H₂ production. Formation of Si-rich surface layers can occur due to incongruent dissolution of silicates in which preferential release of Fe versus Si, attributed to the location of Fe atoms in less stable metal sites within the mineral structure, occurs (e.g. Schott and Berner, 1983; Schott et al., 2009 and references therein). This process is often observed in experimental systems (e.g. Schott and Berner, 1983; Schott et al., 2009; Giammar et al., 2005; Béarat et al., 2006; Andreani et al., 2009; King et al., 2010; Daval et al., 2011). These Si-rich layers have been found to passivate mineral surfaces and decrease dissolution and release of Fe(II) to solution in olivine carbonation experiments (e.g. Bearat et al., 2006; Andreani et al., 2009; King et al., 2010; Daval et al., 2011). Additionally, Fe-rich clays and talcs have also been reported from naturally serpentinized samples (e.g. Andreani et al., 2009).

We propose that the precipitation of Si-bearing phases is in part responsible for the eventual decrease and even cessation in H₂ production in our experiments. There is ample evidence for the precipitation of Si-rich secondary mineral phases. These phases, such as the Fe-
bearing talcs, occur as coatings on the pyroxene particles in the fayalite fraction and pyroxene experiments, (Figures 2c and 4c). The formation of these phases is correlated to the aqueous Si concentration, such that by the end of the experiment the concentration of Si in fayalite > magnetite > petedunnite > San Carlos peridotite > San Carlos olivine (Table 3). The formation of these phases likely leads to a decrease or cessation in dissolution of the primary phases thus causing a decrease in aqueous Fe(II) available to feed electrons to the spinel surfaces. However, the absence of a surface coating on the fayalite particles in the fayalite fraction experiment may allow for continued dissolution and release of Fe(II) to solution. This could explain the high concentration of aqueous Fe(II) in this experiment as well as sustained production of H₂. In contrast to all other experiments, H₂ production from the San Carlos olivine experiment continues throughout the entire duration of the experiment (Figure 1) suggesting that passivation of the mineral surfaces by precipitation of Si-rich secondary mineral phases does not take place in this experiment. This is supported by the absence of Si-bearing secondary phases in the reacted material (Figure 3b). Thus, it appears that passivation of the mineral surfaces due to formation of Si-rich secondary phases in high Si experiments may in part explain the eventual shut down of H₂ production as shown in Figure 8.

4.7 Comparison of H₂ production in the presence of spinels to previous experimental work

Investigations of H₂ production from low temperature single mineral-water reaction experiments have been conducted at 30, 50 and 70°C (Stevens and McKinley, 2000; Neubeck et al., 2011). Stevens and McKinley (2000) reported hydrogen production from low temperature olivine-water laboratory experiments after four days of reaction at 30°C (100% N₂ headspace, water:rock = 1, pH = 6). After four days of reaction at 100°C, the fayalite fraction had produced
40 nmol H₂/gram fayalite and forsterite had produced 13 nmol H₂/gram forsterite, amounts within the error of the values reported by Stevens and McKinley (2000) (34 nmol H₂/gram fayalite and 14 nmol H₂/gram forsterite). In this study, the experiments were conducted higher temperatures and water-rock ratios, thus greater H₂ production relative to Stevens and McKinley experiments was predicted. The higher pH in the San Carlos olivine experiment (pH = 7) could partially explain the discrepancy between our prediction and results. However, the fayalite fraction experiment had a pH of 5.5 at four days of reaction, a condition that should favor increased H₂ production. The source of the fayalite and forsterite used by Stevens and McKinley (2000) was not stated but if it is assumed that there minerals were natural samples it is likely that spinel phases were present and could have contributed to H₂ generation.

A more recent study of low temperature H₂ production from forsterite-water reaction reported 0.24, 0.19, and 0.20 nmol H₂/gram forsterite at 30, 50, and 70°C respectively after 9 months of reaction (Neubeck et al., 2011). While data from 9 months of reaction is not available for these experiments, there is significantly greater H₂ production in these experiments after 1.5 months of reaction. The lower pH (7 vs. 8.7), higher water:rock (7 vs. 2.4), and significantly smaller grain size (53>212um vs. 0.125-1mm (majority 0.250-0.500mm)) of these experiments versus those of Neubeck et al. (2011) likely all contribute to the significantly higher H₂ production reported in this study. The olivine used by Neubeck et al. (2011) was reported to contain <1% spinel phases as does the San Carlos olivine used in this study (0.21 volume % spinels). In all cases, secondary phases were not reported and therefore cannot be compared to our observations.

Petedunnite studied in this work, with 1.2 volume % gahnite, produces 70x (140 nmol/gram mineral) as much H₂ as pyroxene-water laboratory experiments at 30°C (2 nmol
H$_2$/gram augite) (water:rock = 1, pH = 6) (Stevens and McKinley, 2000). It is unlikely that the large discrepancy in H$_2$ production can be explained solely by the difference in temperature. Models of olivine- and peridotite-water reactions show only an ~ 30 mmol increase in H$_2$ concentration between 30 and 100°C (Klein et al., 2009). Similarly, the difference in the Fe content of the pyroxenes is only 1% which also does not explain the large variation in H$_2$ production. Thus, it seems that the presence of spinels in the petedunnite experiment contributes to the enhanced H$_2$ production in comparison to previous experimental results. The secondary phases were not characterized in these experiments and thus cannot be compared to our experimental data.

5. CONCLUSIONS

In this work we attribute H$_2$ production from low temperature water-rock reactions to the surface-promoted oxidation of Fe(II) and reduction of water. H$_2$ generation does not appear to proceed in the absence of the spinel phases. The spinel phases present in our natural geologic mineral separates (e.g. magnetite, chromite, and gahnite) are known to possess catalytic properties that can enhance electron transfer reactions. Models of interfacial electron transfer processes between Fe(II) in solution and Fe(II/III)-bearing conductive mineral phases provide a framework for understanding the mechanism of H$_2$ production in our mineralogically complex water-rock systems. The dissolution of primary minerals releases Fe(II) to solution essentially generating a source of electrons to be transferred. Precipitation of Fe(III) oxides and adsorption of Si to spinel phases passivates the surfaces causing a decrease in H$_2$ production with time. Similarly, precipitation of secondary silicate minerals on primary olivines and pyroxenes likely
decreases the rate of mineral dissolution and the supply of Fe(II) and electrons. Thus, it appears that H₂ generation is a surface controlled process in these low temperature systems.

In this work, as predicted from geochemical models, Fe(II)-rich talc and brucite precipitate on pyroxene and olivine surfaces respectively, and in taking up Fe(II), the Fe becomes unavailable for oxidation, thus these phases act to suppress H₂ generation. However, in contrast to geochemical models and hydrothermal laboratory experiments (≤200°C) that correlate H₂ generation to the incorporation of Fe(III) into serpentine and brucite secondary phases, we do not detect the Fe(III)-bearing varieties of these minerals. Instead, transfer of electrons from Fe(II) to water to produce H₂ is promoted by spinel surfaces and results in Fe(III) (oxy)hydroxides precipitates and coatings.

The discovery of spinels as catalytic phases for H₂ generation has implications for our understanding of H₂ production in natural systems and the potential for such systems to harbor life. For instance, partially reacted (ultra)mafic rocks often have high spinel contents and thus may be likely to produce significant amounts of H₂ at low temperatures. It is also intriguing to consider the potential for spinel-catalyzed reactions to participate in H₂ production in high temperature experiments. Magnetite, a spinel, is the predominant secondary phase that accommodates H₂ production in high temperature serpentinization experiments. Similarly, H₂ production in natural geologic systems is attributed to magnetite formation. Because magnetite may act as a catalyst for electron transfer and H₂ production, the process of magnetite formation and H₂ generation may become self-catalytic.

The identification of low temperature H₂ generation as a surface-controlled process leads to hypotheses concerning the involvement of microorganisms. Organisms able to position themselves adjacent to spinel particles would maximize their ability to obtain the H₂ being
produced. Appropriate samples of serpentinitized materials (e.g. material retrieved from actively reacting low-temperature geologic environments) could be used for targeted searches for microbial organisms or signatures. Microscopic investigations or molecular approaches such as DNA extractions could be focused to the sites of H₂ generation (i.e. spinel particles).

In addition to the presence of spinel phases, the concentration of aqueous Si is another factor controlling H₂ in these experiments. The concentration of aqueous Si is artificially high due to the experimental design but we propose that in lower Si conditions we would see more sustained H₂ production due to either less poisoning of catalytic surfaces by Si sorption or to the formation of Fe(III)-bearing serpentine and brucite rather than talc. In order to better mimic the conditions examined in geochemical models, laboratory experiments must be designed such that concentrations of aqueous Si are defined only by dissolution of the reacting phases. Similarly, most natural systems are open on geologic time scales with continued input of fluids. Thus, experiments implementing advective flow would also better mimic natural conditions. We suspect that in such a system, H₂ production could be sustained over long time scales due to removal of reaction products. Perhaps disruption of passivating layers, due to both physical and chemical factors, including flow and changes in the chemistry of the bulk aqueous phase, would also facilitate further H₂ production.

This work provides insights into our understanding of the potential mechanisms of H₂ generation from natural geologic substrates in low temperature conditions. Furthermore, it suggests that low temperature H₂-producing reactions cannot be ruled out as a potentially important and significant source of energy for microbial life in the subsurface.

Supporting Information is Available
Supporting information includes figures and tables that enhance the description of the fits of Fe model compounds to unknown XANES spectra. Figures depicting H$_2$ production from geologic materials tested but not included in this chapter are also presented.
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CHAPTER 5

Geology and Biology Intertwined: Geochemically derived H₂ gas and the life it may fuel
1. Low temperature water-rock reactions and life

Geologically derived hydrogen gas is thought to have been an important and abundant energy source available during the origin and evolution of early life on Earth (e.g. Nisbet and Sleep, 2001; Reysenbach and Shock, 2002; Sleep et al., 2004; Russel et al., 2010). On Earth today, circulation of seawater through ocean crust is drives production of H$_2$ gas from water-rock reactions and feeds ecosystems localized at hydrothermal vents (e.g. Kelley et al., 2002; Charlou et al., 2002; Takai et al., 2004; Martin et al., 2008; Schrenk et al., 2010; Edwards et al., 2011). The existence of microbial ecosystems at the site of water-rock reactions occurring within the temperature limits of life is dependent on the ability of the water-rock environment to provide a suitable habitat for life. This has been called into question on the basis of the small amounts of H$_2$ predicted to be generated at low temperatures and kinetic inhibitions on the water-rock reactions leading to limited rates of supply of H$_2$ (McCollom and Bach, 2009).

In geochemical models of peridotite-water reactions McCollom and Bach (2009) predict the formation of Fe-rich brucite at low reaction temperatures (i.e. Mg$\#$ 0.7-0.8 at 50-200°C). The authors further relate the stability of Fe-brucite to the concentration of H$_2$ and predict that the removal of H$_2$ from the reaction system will cause the conversion of the Fe-brucite to magnetite, with accompanied H$_2$ production, to maintain equilibrium. In this scenario, a mechanism for removing hydrogen from the water-rock system will drive further hydrogen production. Hydrogen metabolizing microorganisms, such as methanogens, are one such mechanism. It therefore seems likely that, at the site of the water-rock reactions, through consumption of H$_2$, these organisms could trigger conversion of Fe-brucite to magnetite and the production of H$_2$. This would be of immense value to an organism that survives by harnessing energy from H$_2$ oxidation. In essence, the metabolic activities of such organisms would catalyze further
production of the substrate they require for metabolism, ensuring a source of energy and thus their survival. Sites of low temperature water-rock reactions may then host these beautifully inter-twined biological-geological systems.

This dissertation was designed to provide insight into the potential for low temperature water-rock reaction systems to be suitable habitats for H$_2$-utilizing microbial life. In this habitat, biology and geology are so closely interconnected, it was compelling to also address the potential for life to directly affect the water-rock reaction pathway as speculated on above. Enhanced H$_2$ generation in the presence of an H$_2$-utilizing organism, or other diagnostic geochemical signatures such as unique secondary mineral assemblages, could suggest that microorganisms do influence the reaction pathways. The work presented in this dissertation has helped to advance our understanding of low-temperature water-rock reactions and the potential for microbial activity in these systems.

2. Speciation and localization of rare, microscale solid phase reaction products

The partitioning of Fe into secondary mineral phases during low temperature, anoxic water-rock reactions is an important control on the generation of H$_2$ during low temperature water-rock reactions. To date, laboratory investigations into H$_2$ production during low temperature water-rock reactions have not included characterization of the resultant secondary mineral phases. Characterization of these phases is hampered by the small amount of reaction products generated in laboratory scale experiments. Techniques such as conventional XRD or bulk XANES require large sample sizes in which the phases of interest must be present as 5-10% of the total material to even be detected, let alone quantitatively identified. Electron microprobe requires the phases of interest to be exposed at the surface and highly polished, which can be difficult to achieve
with soft materials often occurring as surface layers on primary mineral phases. Additionally, electron microprobe and SEM-EDS give no indication of the speciation of redox active elements such as Fe.

Given the current limitations, we developed a synchrotron x-ray based method for investigating and characterizing the behavior and speciation of Fe in the rare, microscale solid phase reaction products. This method, described in detail in Chapter 2 (Mayhew et al., 2011), integrates µXANES and µXRF data collection and processing. XRF maps are collected at multiple energies within the Fe K-edge and PCA analyses of the maps reveal the distribution of unique Fe-bearing components. Targeted µXANES analyses enable collection of spectra representative of the entire variety of Fe-bearing components within the complex samples. Subsequent fitting of spectra identified as end-members of the data set, enabled identification of the speciation of a suite of unique Fe-bearing phases. The distribution of these phases at the microscale is determined by fitting of multiple XRF maps with end-member spectra. In abiotic water-basalt-Fe$^0$ systems, incipient Fe-bearing reaction products associated with phenocrysts of olivine were detected after only 48 hours of reaction at 55°C. After 10 months of reaction, precipitation of Fe-bearing phyllosilicates had occurred. Based on fits with model compounds it is likely that the majority of Fe in these phases was Fe(II). Applying this technique to culture experiments, it was determined that the secondary mineral assemblage in the presence of *M. thermoflexus*, characterized by the presence of a Fe-bearing pyroxene and the absence of phyllosilicates, was distinct from that in the abiotic system. The technique was also applied to low temperature abiotic water-rock reactions to determine the secondary phases associated with H$_2$ generation. In olivine-, pyroxene, and peridotite-water reactions at 100°C, it was determined that Fe(III)-oxides formed both as precipitates and surface coatings on spinel particles suggesting
oxidation of Fe associated with these phases. Fe(II)-bearing phyllosilicates were detected as surface coatings on pyroxene particles. Application of this method allowed for identification of a variety of Fe-bearing phases embedded in complex geologic materials containing many different Fe-bearing phases and provided an understanding of the localization of these phases; information that has proved invaluable in understanding the reactions occurring in these experiments.

This method has value and applicability beyond the work presented in this dissertation and can be applied to any system in which the speciation of a redox active element is of interest. In this work, this technique has been exclusively applied to laboratory experiments; however, it will be immensely useful in unraveling the speciation and distribution of any redox active elements in complex natural samples. For example, it could be used to investigate changes in arsenic speciation within water-saturated sediments such as in Bangledesh or transformations of uranium in conjunction with mining and remediation efforts. This technique could be used to glean new information from a number of systems that have already been investigated using synchrotron methods including Se and As speciation in plants (e.g. Pickering et al., 2000, 2006; Amos et al., 2012) or Cr speciation in chromite ore (e.g. Chrysochoou et al., 2009) or soils formed on serpentinites (Oze et al., 2007). One of the most exciting potential applications of this technique would be to identify mineral speciation at cell-mineral interfaces to determine if and how these phases differ from those not associated with cell surfaces. With this knowledge it would be possible to specifically target minerals with a biotic signature for other analyses thus aiding in the characterization of such phases and perhaps providing insight into the mechanisms of their formation. Thus, there is a wide variety of elements and environments that can be interrogated using this technique, making it a valuable contribution to this field.
3. H$_2$ generation potential of geologic substrates and determination of potential H$_2$

generating mechanisms in abiotic water-rock reactions

Given the potential importance of the contribution from low temperature reactions in supporting H$_2$-based ecosystems, it is important to determine the conditions most favorable for H$_2$ production. Chapter 4 describes the production of H$_2$ gas in significant quantities from a variety of natural geologic substrates at both 55 and 100°C, a temperature range within which little experimental work on H$_2$ generation has been published. Hydrogen was generated from olivine-, pyroxene-, peridotite-, and magnetite when electron-transfer catalysts such as spinels were present. Fe(III)-bearing serpentine and brucite secondary phases, predicted from low temperature geochemical models, were not observed. Instead, H$_2$-generation was likely accommodated by the formation of Fe(III)-oxides. Additional hydrogen generation driven by olivine and pyroxene dissolution is likely suppressed by the formation of Fe(II)-bearing talc and brucite phases because the Fe contained in these phases is not available for oxidation and subsequent H$_2$ production. In this work, the importance of spinel mineral phases as catalysts for H$_2$ production was identified. These phases appear to catalyze electron transfer between Fe(II), present in solution from the dissolution of olivine and pyroxene minerals, and water. The precipitation of Fe(III)-oxides both in solution and on the surface of the spinels results from the oxidation of Fe(II) and production of H$_2$ gas. Fe-bearing silicates precipitate on pyroxenes, likely shutting down the further dissolution of these phases and the supply of aqueous Fe(II) able to donate electrons. Thus, it appears that H$_2$ generation in these experiments is a surface controlled process.

The results of this work suggest that the addition of spinel phases (e.g. magnetite) to ‘clean’ geologic materials should result in enhanced H$_2$ generation relative to spinel-absent
experiments. A hypothesis that can be easily tested. Additionally, experiments in which known concentrations of aqueous Fe(II) are added to spinel-absent, spinel-only, and spinel+Fe(II)-silicates geologic substrates would help to elucidate the importance of aqueous Fe(II) as an electron donor for H2 generating reactions at spinel surfaces. Furthermore, if it were possible to stop passivation of both spinel and Fe-silicate surfaces greater and more sustained H2 production would be predicted. There is still much to test and understand about spinel catalyzed H2 production.

This work suggests that geochemical models of H2 generation that do not require catalytic phases or account for surface controlled processes may be missing important components of the reactions responsible for H2 production from low temperature water-rock reactions. The nature of the secondary phases and association with specific primary minerals appears to be especially relevant to understanding reaction mechanisms and it is not something that has been characterized in previous laboratory experiments of H2 generation at low temperatures. High temperature laboratory investigations have characterized the secondary phases but have not considered potential catalysis of H2 generation by spinels. However, the role of spinels may be less important because the reaction between Fe(II) and water is less kinetically hindered at higher temperatures. This can also be tested by high temperature experiments in which the starting material contains known amounts of spinel phases. However, because magnetite is the dominant secondary phase that accommodates H2 production in high temperature serpentinization experiments, the process of magnetite formation and H2 generation may become self-catalytic.

The identification of H2 generation at low temperatures as a surface-controlled process also leads to exciting hypotheses concerning the potential involvement of microorganisms.
Organisms able to position themselves on spinel particles would maximize their ability to obtain the H₂ being produced at the mineral surface. Samples of partially serpentinized materials could be used for targeted searches for microbial organisms. Microscopic investigations or molecular approaches such as DNA extractions could be focused to the sites of H₂ generation (i.e. spinel particles).

4. In-situ microbial life: can it survive and will it affect the geochemistry of low temperature water-rock reactions?

Final H₂ concentrations observed in the abiotic water-rock reactions ranged from $10^{-4.7}$ – $10^{-6.3}$ M at 55°C and $10^{-4.7}$ – $10^{-6.1}$ M at 100°C. The background concentration of H₂ as measured in the control experiments was subtracted from these values. These concentrations are more than enough to generate ~40 kJ/mol of energy from methanogenesis, twice the amount of energy required to sustain growth, and approximately 4x as much energy obtained by methanogens living in some anoxic sediments (Hoehler et al., 1994, 2001, 2004; Sleep and Bird, 2007). Of course, this value will change as the reaction proceeds and the amount of reactants and products changes (e.g. H₂ and CO₂ are consumed and CH₄ is produced). Thus, to first order, the fayalite fraction, magnetite fraction, petedunnite, San Carlos olivine, and San Carlos peridotite generate enough H₂ at both 55 and 100°C to support methanogenesis. However, this exciting result is confounded by the need to sustain methanogenesis over long time scales in order for these low temperature water-rock systems to be suitable habitats for microbial life. With time, microbial life in these systems would encounter a number of issues. Less energy would be generated from methanogenesis as 1) methane is produced and builds up in the system and 2) H₂ and CO₂ concentrations are drawn down. In my current “batch” experimental system both of
these are likely to occur. However, in a modified laboratory system, or in natural systems, in which advective flow occurs, methane accumulation would be avoided and CO₂ could be replenished. A concomitant decrease in H₂ will also occur unless fresh rock/mineral is made available for reaction or Fe oxidation is stimulated. In both experimental and natural systems, passivation of reactive surfaces by precipitation of secondary minerals may inhibit exposure of fresh surface area. Advective flow may be able to cause partial physical disruption of such layers or perhaps flush the system with less rock-reacted fluids thereby enhancing the thermodynamic disequilibrium and causing dissolution of secondary phases. In natural systems, the increase in rock volume associated with hydration should expose fresh surfaces with time allowing the reaction to proceed (e.g. Lowell and Rona, 2002; Kelemen et al., 2011). Evidence from these experiments coupled with evidence for a subsurface biosphere (e.g. Gold 1992, Whitman et al., 1998; Takai et al., 2004b) suggest that it is possible, and perhaps even probable, that life survives at the site of low temperature water-rock reactions.

The work presented in this dissertation takes the first steps towards understanding if, and how, methanogens present at the site of low temperature water-rock reactions would affect the geochemistry of these systems. An Archaeal methanogen was introduced into a water-basalt system amended with Fe⁰. The Fe⁰ was used to produce H₂ in quantities and rates great enough to monitor over long time scales (1+ year). Admittedly, this system deviates from a natural geologic environment in a number of ways; however, it did enable testing of the hypothesis that a H₂-utilizing microorganism present during continuous H₂ producing water-rock reactions would affect the solid phase and aqueous geochemistry of the system in distinct ways. This experiment revealed that the products of the water-rock reaction in the presence of the active methanogen are different than those observed in the abiotic reaction. This is the first time that
this has been demonstrated in an anoxic, low-temperature (55°C) water-rock reaction system. The mechanism that suppressed precipitation of Fe-phyllosilicates and favored precipitation of an Fe-bearing pyroxene in the culture experiment remains a mystery. However, the observation alone is an exciting result because it suggests that organisms in low temperature, anoxic water-rock environments may be able to directly affect the water-rock reaction pathways. If they are indeed capable of this, it seems reasonable that they would do so in such a manner as to maximize the amount of energy made available to them. This then becomes a testable hypothesis and experiments can be specifically designed to interrogate the nature of the cell-water-rock interactions as we advance our understanding of the mechanisms responsible for H₂ generation in these geologic environments and the potential ways in which organisms might exert control over these mechanisms.

In these same culture experiments, Fe(III)-oxides were added to create an environment in which Fe was present in multiple oxidation states and out of equilibrium with the bulk aqueous and gaseous chemistry. Admittedly, this particular aspect of the experimental design complicated the chemistry and interpretations. However, it enabled discovery of the likelihood that *M. thermoflexus*, the Archaeal methanogen used in the experiments, possesses the capability of coupling H₂ oxidation with reduction of Fe(III) oxides. As discussed in Chapter 3, this hypothesis explains many of the observations including the behavior of aqueous Fe(II) and the fewer reducing equivalents present in the gas phase in the culture versus in the abiotic experiment. *M. thermoflexus* has not been previously shown to have this ability. This is another testable hypothesis that has resulted from this work and experiments are currently underway to unequivocally demonstrate that *M. thermoflexus* has this ability. This also leads to interesting hypotheses concerning water-rock systems such as those described in Chapter 4. *M. thermoflexus*
could potentially be an important component of a geochemical H$_2$ and Fe cycle in these
environments. The transfer of electrons from Fe(II)aq thru spinels to water would result in H$_2$
and Fe(III) oxide production. M. thermoflexus could then consume H$_2$ while reducing Fe(III)
oxides resulting in the release of Fe(II) to solution which could then donate electrons to the
spinels to produce more H$_2$. In this way, M. thermoflexus would facilitate continued H$_2$
production by consuming the reaction products (H$_2$ and Fe(III) oxides) and regenerating a
reactant (Fe(II)). Such a system would be another elegant example of the interconnections
between geology and biology.

5. Challenges and limitations

There were many challenges associated with these scientific undertakings, some of which
were described above. In other cases, large efforts were put forth to overcome these challenges
but did not produce results that were described in the core chapters of this dissertation. This
information may be valuable to the scientific community and is thus included in this section of
the dissertation. Also in this section, the limitations of the work are acknowledged and
suggestions provided for how to address these limitations in future work.

As discussed in previous sections, in choosing to do controlled, experimental
investigations I was constrained by both laboratory conditions and time scales, and I was
particularly challenged by the problem of trying to characterize small amounts of reaction
products. Coupling the synchrotron-based $\mu$XANES and $\mu$XRF method with capillary XRD
allowed us to identify both Fe-bearing and non Fe-bearing secondary phases and provided
insight into secondary phases associated with surfaces of primary minerals as well the bulk
materials. There are, however, a couple of overarching limitations of these approaches. The first
is the difficulty in quantitatively determining the iron contents of the secondary phases. Many of
the minerals form solid solutions between Mg- and Fe-end-members, but we are not able to
detect Mg in the x-ray fluorescence spectra collected for each pixel due to significant absorption
of Mg Ka by air between the sample and detector, and because of the type of Si drift vortex
detector used. Now that soft X-ray beamlines will be increasingly common (e.g. Advanced
Photon Source and Stanford Synchrotron Radiation Laboratory), it may be possible in future
work to more quantitatively determine Mg:Fe ratios by careful comparison of Mg K and Fe L
fluorescence peaks. For reasons outlined in section 2, it is difficult to acquire the relevant
information using other techniques, SEM allows for visualization of surface coatings but because
these phases most often occur as coatings, it is difficult to obtain even representative qualitative
proportions from EDS analyses due to interference from the underlying minerals. Depending on
the thickness of these phases, electron microprobe analyses could be similarly compromised
unless they formed discrete secondary minerals as often observed in higher-temperature
experiments. With this experimental design, it is not possible to measure the moles of mineral
dissolved and thus the iron that was released from the starting materials, making it difficult to
assess the total amount of iron available for reaction. Quantitative assessment of the amount of
primary mineral that reacted was not possible because the high concentrations of potential tracer
elements (e.g. Mg, Ca) in the starting seawater media made it impossible to track changes in
concentration due to mineral dissolution. It was not possible to use Si as a tracer element because
of the input of Si from dissolution of the glass vials as well as mineral dissolution. The use of
these elements is also complicated by the precipitation of Mg-, Ca-, and Si-bearing secondary
phases that also cannot be quantitatively assessed. Similarly, it is not possible to quantitatively
assess the partitioning of Fe between the brucite, talc, and Fe(III)-oxide phases because the total
amount of each phase cannot be determined from a bulk measurement. The synchrotron analyses employed in this study investigate only a very small amount of the reacted material so that it is not possible to quantitatively extrapolate the results. Bulk Fe K-edge XANES analyses would be well-suited to obtaining a more quantitative understanding of the partitioning of Fe but the small proportion of secondary phases are not detectable in bulk analyses because the signal is overwhelmed by the remaining primary material. Thus, it is immensely challenging to quantitatively address the fate of iron in my experimental systems.

The second major limitation is the lack of independent Fe(II)/Fe(III) ratios for both the model compounds used to fit unknowns and the secondary phases. Ideally, I would have obtained quantitative Mossbauer analyses of the Fe(II)/Fe(III) ratios in the model compounds however this technique was not available to me. I still hope to accomplish this as I move into my post-doctoral work. This information would help to elucidate the Fe(II)/Fe(III) contents of the starting reaction materials used in Chapter 4. However, because of the lack of spot specificity versus the synchrotron technique, Mossbauer analyses of the reacted materials would be equivalent to a bulk measurement. Inferences of Fe(II)/Fe(III) from fits of model compounds to unknown spectra are certainly not the same as independent analyses of the unknown phases themselves.

I considered conducting analyses of the pre-edge feature of the Fe XANES spectra collected from the unknown phases to independently verify the Fe(II)/Fe(III) content. Analyses of the pre-edge features of the spectra can be applied to pure materials to quantitatively determine the oxidation of Fe (e.g. Delaney et al., 1998; Wilke et al., 2001; Berry et al., 2003). This technique was used to investigate the Fe(III) content in bulk samples of serpentine minerals produced in laboratory experiments at 300°C (Marcaillou et al., 2011). However, this technique
does not provide reliable results when applied to materials in which both the valence state and
coordination site of Fe varies (e.g. Wilke et al., 2001). This is problematic for many of the model
compounds and unknown phases especially in systems such as those described in this work in
which the redox state of Fe is influenced by the bulk chemical environment, surface chemical
reactions, and microbiological activity. The unknown spectra and, in some cases, model
compounds, are also not ideal for these types of pre-edge analyses because they are not often
comprised of a single phase. Most of the spectra are mixtures of different phases that would
further confound attempts to model the pre-edge feature as they are no longer representative of
Fe within a single phase but of Fe occurring in multiple valence states, coordination sites, and
phases. Due to the complications specific to this work, I did not feel that the results of pre-edge
analyses would be reliable and thus were not worth attempting.

In addition to the challenge of identifying reaction products, geologic materials are
complex even before they are subject to laboratory experiments. This complexity can be avoided
by choosing to work with synthetic materials but this is, of course, less representative of the
complexity of the natural world and therefore the results are less applicable to real systems. We
chose to work with natural materials and thus made a significant effort to characterize the
materials prior to using them in our experiments. Though we tried to obtain single mineral
separates in a number of cases, all of the materials were actually mixtures of a number of
different phases. Even the quenched glassy basalt used in Chapter 3 possessed phenocrysts of
olivine and plagioclase. Not only did most of the materials contain more than a single primary
phase, they were often partially reacted, containing alteration phases, prior to being reacted in the
laboratory. For example, a thin section of San Carlos peridotite revealed not only alteration along
grain boundaries but alteration phases such as serpentine and magnetite located along fractures
within many of the olivine grains. We were not able, even through the processes of crushing, grinding, and washing, to completely remove these phases. Therefore, it was extremely important to be as thorough as possible in characterizing the starting substrates to enable direct and accurate comparison to the reacted materials. This is why I chose to interrogate the starting substrates using optical microscopy, electron microprobe, and QEMSCAN in addition to the XRD and synchrotron techniques applied to both the starting and reacted materials. This suite of tools provided a comprehensive view of the starting substrates; enabling me to feel confident about the conclusions I drew from comparisons to the reacted materials.

As evidenced from the work presented in Chapter 4, using our experimental approach, H₂ production from natural substrates at low temperatures does not occur in large amounts and is not sustained over time scales greater than a couple of months. However, prior to learning these things and without altering our experimental approach, I attempted to grow an Archaeal methanogen in olivine-water reaction systems. I found that with such low H₂ concentrations it was difficult to accurately track changes in the concentration with the equipment available. I had similar issues tracking the concentration of methane. However, it was clear from fluorescence microscopy that *M. thermoflexus* was sustained in the culture conditions for over a year. This exciting observation was difficult to back-up without clean H₂ and CH₄ data and hence was not included as a chapter in this dissertation. However, as discussed above, culture experiments conducted in water-basalt-Fe⁰ systems indicated that *M. thermoflexus* is able to influence the reaction geochemistry in detectable ways. Additionally, I learned that in the presence of Fe(III)-oxides, *M. thermoflexus* may use some H₂ to reduce the Fe(III)-oxides. With this knowledge, I can now revisit the olivine-water culture experiments with a better understanding of the chemistry of the system and therefore an improved ability to interpret the data. I suspect that
synchrotron analyses of samples from these experiments will similarly reveal distinct secondary mineral assemblages in the presence of *M. thermoflexus*. Having surveyed a variety of natural geologic substrates for the capability of producing and sustaining abiotic H₂ generation, I feel that I have a much better idea of the substrates and the experimental approach to implement that will allow me to successfully culture microorganisms on natural geologic substrates without amending the experiments with Fe⁰.

Another challenge of this work was finding organisms suitable for use in the culture experiments. My initial goal was to use organisms obtained from related natural geologic systems to make this work as environmentally-relevant as possible. There are no isolates, or even consortia of organisms, reported to be obtained from actively serpentinizing systems and maintained in laboratory cultures. Therefore, I traveled to the Mid-Atlantic Ridge to collect samples of serpentinized rocks and fluids emanating from hydrothermal vents at serpentinizing sites to attempt to enrich organisms from these samples. Unfortunately, these efforts were not successful. I was limited to collecting rocks sitting on the seafloor or exposed at outcrops and able to be shaken loose and carried by the JASON remote-operated vehicle. While my success rate for actually identifying and collecting serpentinized rocks was quite high, I was limited to rocks that were not from actively serpentinizing locations. It is clear that I can support growth of H₂-utilizing microbes in my culture system thus I predict that had I been able to obtain rocks from actively serpentinizing regions I would have had a much better chance at enriching relevant microbial communities. Theoretically, the fluid samples obtained from the hydrothermal vents should sample the microbial communities present within the rocks through which the fluids circulate. However, I was unable to successfully enrich organisms from the fluid samples. I believe this was due to the sample size. The rock-hosted habitats are likely to host only a very
small amount of biomass. The best approach for sampling fluids to increase the possibility of successful enrichment of microbial communities would have been to filter large amounts of fluid, concentrating the organisms on the filter, and inoculating culture experiments with the filter. Instead, I was given ~1 ml of unfiltered and not concentrated fluid. In this aliquot, the cell numbers were likely so low that there was little chance of anything surviving. In the future, I hope to have access to rocks from the subsurface, such as from drill cores into actively reacting systems, to attempt to enrich environmentally relevant microorganisms. Another attempt at culturing organisms from more appropriate samples would be well worthwhile. Similarly, it would also be worthwhile to attempt to enrich for the microbial communities in larger volumes of reacted fluids or filtered material.

6. Broader Implications

As detailed above, the work included in this dissertation constitutes important contributions to the fields of geomicrobiology and water-rock interactions. This work also has implications for the broader scientific community.

6.1 Life on early Earth

It is thought that the root of the ‘tree-of-life’ lies on the bacterial lineage and thermophilic Bacteria and Archaea occupy many of the deepest branches of the tree (Woese et al., 1990; Pace 1991, 1997; Reysenbach et al., 1999; Reysenbach and Shock 2002). These lineages are characteristically non-photosynthetic and instead use hydrogen as an electron donor in metabolic reduction-oxidation (redox) reactions. If deeply branching Bacteria and Archaea are thermophilic and use hydrogen as an electron donor it is likely that their last common ancestor also possessed these traits (Pace, 1997). Methanogenesis is considered to be one of the earliest metabolic
processes that functioned on Earth and is also predominately found in organisms that populate
the base of the tree of life (e.g. Woese et al., 1990; Pace, 1997; Reysenbach and Shock, 2002). It
has been postulated that these earliest methanogens inhabited, and perhaps originated in, rock-
hosted environments such as hydrothermal vent systems (e.g. Pace 1991, Martin et al., 2008).
Therefore, to understand early life on Earth it is essential to evaluate the potential for current H₂-
based environments to support extant life. Furthermore, assessing the potential for these habitats
to specifically support methanogenesis is especially relevant and applicable to considering life on
early Earth. Thus, the work embodied in this thesis, which critically evaluates the potential for
low temperature water-rock environments to support methanogenesis, is directly relevant to
understanding habitat availability and ecology of early Earth. The findings of this dissertation
support hypotheses that early life on Earth could have lived within (ultra)mafic rocks (e.g. Nisbet
and Fowler, 1996; Sleep and Bird, 2007).

6.2 Potential for biosignature formation

Beyond demonstrating that olivine-, pyroxene-, and peridotite-water reactions at low
temperatures produce enough H₂ to support methanogenesis, this work suggests that extant
organisms can affect the geochemical reaction pathways. Particularly, *M. thermoflexus*
influences the nature of the secondary minerals precipitated during the water-rock reactions. The
secondary mineral assemblage in presence of *M. thermoflexus* is unique to the biotic system and
as such it has the potential to be a biosignature indicative of the presence and activity of *M.
thermoflexus*. Mineral biosignatures, including Fe-bearing minerals (e.g. Fortin and Langley,
2005 and references therein), can be more robust than carbon-based materials that are fragile in
the face of common geologic process (e.g. weathering, diagenesis, hydrothermal alteration, and
metamorphism). Recently, Izawa et al. (2010) proposed that titanite in-fillings in putative
biogenic tubules in basalt preserve microbial trace fossils over geologic time scales. While the titanite itself is likely not formed by biological processes, it preserves trace fossils and because the titanite may persist over geologic time scales the fossils will also be retained over that time. Similarly, silicification of microorganisms can lead to their preservation as microfossils in a variety of natural environments (e.g. Schultze-Lam et al., 1995; Fortin et al., 1997 and references therein; Benning et al., 2004). Again, the silica may not precipitate as a direct result of microbial activity but it also persists over geologic time scales and thus preserves the cells over this time period. Because minerals are more easily preserved in the geologic record than other components of microbial communities (e.g. cells) they certainly have the potential to act as biosignatures of long gone ecosystems. The potential for the secondary mineral phases detected in this work to persist over geologic time scales was not assessed. Similarly, *M. thermoflexus* was not investigated for silicification. However, because these potential biosignatures are minerals they have a higher potential of being preserved over these time scales than many other potential biosignatures. And, in contrast to the titanite and silica mentioned above, the formation of these mineral phases is coeval with the activity of the microorganisms, not a later event. Therefore, they may record and preserve more information that would aid in unraveling their potentially biotic origin (e.g. Fe and Si isotope fractionation).

6.3 Potential for rock-hosted H\textsubscript{2} driven habitats on other planets

The potential for rock-derived H\textsubscript{2} gas to support microbial life is not limited to Earth. The ingredients for hydrothermal systems including abundant volcanic activity and the presence of water are evident on Mars (e.g. McCollom and Hynek, 2005). Martian meteorites provide geochemical and isotopic data that suggests that hydrothermal systems exist or did exist on Mars (Shock, 1997; Fisk and Giovannoni, 1999). Mars has a crust composed primarily of mafic rocks
(basalts to olivine basalts (e.g. Hoefen et al., 2003). In fact, Fe(II)-bearing primary igneous olivines and pyroxenes, responsible for rock-derived H₂ production on Earth are present in the Martian crust and thought to be 3-6x more Fe-rich (30-60 mol% Fe vs. 10-20 mol% Fe) than the comparable minerals on Earth (e.g. Fisk and Giovannoni, 1999; Hoefen et al., 2003). Mars has had crustal water throughout its history and is likely to even have shallow aquifers at present (Malin et al., 2006; McEwen et al., 2011). Thus, the presence of olivine-bearing rocks beneath the surface of Mars may put them in contact with subsurface fluids (Fisk and Giovannoni, 1999; Oze and Sharma, 2005; Morris et al., 2006). It is possible that subsurface water-rock reactions such as serpentinization have occurred, and may even be occurring today, on Mars (Oze and Sharma, 2005; Schulte et al., 2006).

Somewhat controversial measurements of methane on Mars from multiple techniques and instruments (Mumma et al., 2009; Geminale et al., 2011) support the notion that methane may be produced at present on Mars. Boston et al. (1992) hypothesized that extant chemolithoautotrophic microbial ecosystems on Mars would be associated with subsurface volcanic and hydrothermal activity and suggested that methanogen and sulfur-based metabolisms would underpin these communities. Other researchers have concluded that the subsurface of Mars possesses all of the necessary ingredients for life (Shock, 1997; Fisk and Giovannoni, 1999) and specifically suggested serpentinization as the source of H₂ to support life on Mars (Schulte et al., 2006). Due to the high UV flux, thin atmosphere, and cold temperatures at the surface, it is likely that any extant life on Mars is likely relegated to the subsurface (e.g. Boston et al., 1992), understanding potential H₂ production from low temperature, subsurface water-rock reactions has important implications for understanding the potential for life to exist on Mars. Similar environments have been postulated to exist and support extraterrestrial microbial
methanogenesis on Europa and Venus, had it ever been clement (e.g. Jakosky and Shock, 1998; McCollom, 1999; Sleep and Bird, 2007). As we have not yet been able to find and identify specific sites of H₂ generation on other planets we must infer the geochemical conditions and potential to support life from what we know of similar sites on Earth.

7. Closing statement

Laboratory investigations of H₂ production from low temperature, anoxic water-rock reactions suggest that under suitable conditions, similar natural geologic systems can support H₂-metabolizing microbial life. A pure culture of a methanogen has been shown to be capable of influencing the geochemical reaction pathways in such systems. Future culturing work using more geologically relevant materials to produce H₂ will provide even greater insights into the interconnections between the geologic environment and the life it hosts. This has exciting implications for understanding how H₂-generating geochemical processes and H₂-metabolizing life may have coevolved over Earth’s history.
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Appendix A.

CHAPTER 2

Microscale imaging and identification of Fe speciation and distribution during progressive fluid-mineral reactions in highly reducing conditions

Supporting Information
Methods

Sample preparation.

The epoxy we used is a standard material used to impregnate geologic materials for both optical and chemical analyses. Buehler Epoxicure Resin and Hardener (Buehler, IL) was specifically chosen because it is a hard epoxy that seals the grains tightly and lessens the risk of air, water, and grinding and polishing compounds coming in to contact with the sample material anywhere except along the exposed surface. We have not conducted experiments on the effect of the epoxy specifically on our samples. It is possible that this process could have affected the Fe chemistry. However, the incorporation of basalt, olivine and Fe(III)-oxides, all known starting materials, in the fits of the end-member (EM) XANES from both samples suggests that there was little to no 1) change in speciation during the sample preparation process, 2) radiation induced changes to our samples during data collection, and 3) significant errors in fitting the μXANES spectra with model compounds collected on different beamlines.

μXANES analyses in the 48-hour sample.

We designed the 48-hour experiment to have a limited number of Fe-phases to test the ability of our method to detect and locate known materials. We used the multiple energy (ME) principal component maps to select locations for μXANES analyses, distributing the μXANES locations across the components. We purposely chose three spots within each of the components for analysis and we expected overlap between spectra collected from a single component. In fact, upon visual inspection of the spectra, there appeared to be several unique spectral signatures (Figure A2). As expected, the spectra representative of component 1 (XANES 6-8) are all very similar to one another and spectra representative of component 2 (XANES 4, 5, and 9) are also all very similar to one another (Figure A2). In contrast, spectra collected from map areas strong in component 3 (XANES 1-3) are more diverse (Figure A2). Thus, qualitative comparison of the μXANES spectra illustrate that map components 1, 2, and 3 are truly distinct from one another in terms of Fe speciation, and any non-unique spectra are similar only to other spectra collected from within the same component. These qualitative observations are supported by the results of PCA of the XANES data. PCA guided selection of end-member XANES spectra identified representative spectra from each group of similar spectra as end-members. The fact that the
method presented resulted in the selection of a single spectrum from each group of similar spectra is testimony to the effectiveness and usefulness of the method.

**Cycle-fitting of sample XANES with Fe-model compounds.**

A library of appropriate model compounds (Table A1) was loaded into the program and all models were fit individually as 1-component fits. The $R^2$ values for each of the 1-component fits were reported and the model compound with the best quality of fit was included for the next series of 2-component fits. The cycle fit was repeated for 2-component fits using the selected model compound paired with each of the remaining model compounds. The model compound resulting in the most improvement in the quality of fit was selected and the cycle fit repeated for 3-component fits. This process was repeated until there was no longer an improvement in the best fit that decreased the $R^2$ value by 10% or greater. The sum of the components was not constrained to unity (mean sum = 1.035), negative fractions were not allowed, and energy was not allowed to shift during cycle fitting. Cycle fit results were verified by a final manual fit using the selected model compounds. For the final manual fit, energy was allowed to shift up to ~0.5 eV for end-member spectra to compensate for inherent discrepancies caused by collecting unknown data and models on different beam lines. End-member XANES spectra represent the entire variety of spectra present in the dataset. Therefore, all non end-member XANES spectra can be ‘internally’ fit using end-member XANES spectra (Figure A6), rather than Fe-model compounds, and thereby explain the entire diversity of the map area in an efficient and statistically valid manner. Energy was not allowed to shift for internal fits because both the unknowns and the end-members were collected on the same beam line at the same time under the same conditions.

**Testing of cycle fitting procedure and comparison to other methods.**

Commonly, target transformation (TT) of a model library run against XANES components is used to identify the subset of reference spectra that are sufficient to describe the full set of observed spectra. This results in sub-sampling the model library to be used in LSF. Sub-sampling a library is often necessary to make it feasible to manually fit spectra in a reasonably efficient manner by ‘floating’ all models (fitting with all models and removing those ≤10%). The advantage of cycle fitting over TT is the ability to test the entire model compound
library during fitting. We conducted a series of tests to determine if better fits were obtained from TT or cycle fitting. We found that TT directed subsampling of our library resulted in fits with much larger R² values than fits obtained using cycle fitting.

We also compared the cycle fit method to simultaneously floating the entire library of model compounds to verify its accuracy. Cycle fitting generally produced fits with the same results (within 10% error) as floating the full library. However, floating the entire library using a standard MacBook computer took much longer to execute. In addition, we tested matrix fitting (all 1 component fits, followed by all 2 component fits, followed by all 3 component fits, etc.) and were unable to produce results after allowing the program to run overnight.

We tested the cycle fitting procedure to ensure we obtained the best fit results possible. For example, we fit EM XANES 9 from the 48-hour sample a second time but chose the Fe-model compound with the 2nd best 1-component fit during the first cycle (instead of the Fe-model compound with the best 1-component fit). This approach produced a much worse fit (higher R² value). This exercise illustrates that using the cycle fit function as described above produces the best N-component fit.
Table A1. Library of iron model compounds used in cycle fitting of end-member XANES.

<table>
<thead>
<tr>
<th>Model compound</th>
<th>Theoretical Formula</th>
<th>Mineral group</th>
<th>Mineral source</th>
<th>Author</th>
<th>Synch/BL</th>
<th>Data type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankerite</td>
<td>CaFe$^{2+}<em>{0.6}$Mg$^{2+}</em>{0.3}$Mn$^{2+}_{0.1}$(CO$_3$)$_2$</td>
<td>carbonate</td>
<td>UCLA Mineral Collection</td>
<td>Templeton Group</td>
<td>SSRL 4-1</td>
<td>fl</td>
</tr>
<tr>
<td>Antigorite</td>
<td>Mg$<em>{2.25}$Fe$^{2+}</em>{0.75}$(Si$_2$O$_5$)(OH)$_4$</td>
<td>serpentine</td>
<td>Smithsonian NMNH # 157139</td>
<td>Templeton Group</td>
<td>SSRL 4-1</td>
<td>tr</td>
</tr>
<tr>
<td>Augite</td>
<td>Ca$<em>{0.9}$Na$</em>{0.1}$Mg$<em>{0.9}$Fe$</em>{0.2}$Al$<em>{0.4}$Ti$</em>{0.1}$Si$_{1.9}$O$_6$</td>
<td>clinopyroxene</td>
<td>Ward’s</td>
<td>Templeton Group</td>
<td>SSRL 4-1</td>
<td>fl</td>
</tr>
<tr>
<td>Awaruite</td>
<td>Ni$_{2.3}$Fe</td>
<td>alloy</td>
<td>Mineralogical Research Company</td>
<td>Marcus et al., 2008</td>
<td>ALS 10.3.2</td>
<td>fl</td>
</tr>
<tr>
<td>Basalt</td>
<td></td>
<td>Igneous rock</td>
<td>Quenched Hawaiian lava</td>
<td>Templeton Group</td>
<td>SSRL 11-2</td>
<td>tr</td>
</tr>
<tr>
<td>Brucite</td>
<td>Mg(OH)$_2$</td>
<td>hydroxide</td>
<td>Smithsonian NMNH # Cl1795-1</td>
<td>Templeton Group</td>
<td>SSRL 4-1</td>
<td>tr</td>
</tr>
<tr>
<td>Chamosite</td>
<td>Fe$<em>{3.3}$Mg$</em>{1.7}$Si$<em>3$Al$</em>{12}$(OH)$_6$</td>
<td>chlorite</td>
<td>Smithsonian NMNH # B17943</td>
<td>Templeton Group</td>
<td>SSRL 4-1</td>
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<td>Templeton Group</td>
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<td>chlorite</td>
<td>Smithsonian NMNH # 85790</td>
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<td>Cronstedtite</td>
<td>Fe$^{2+}$Si$_3$O$_5$(OH)$_4$</td>
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<td>Excalibur Minerals</td>
<td>Templeton Group</td>
<td>SSRL 4-1</td>
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<tr>
<td>Fe$^{0+}$</td>
<td>Fe$^0$</td>
<td>Metal</td>
<td>Acros organics</td>
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<tr>
<td>Ferrihydrite</td>
<td>Fe$^{3+}$_2O$_6$$•$0.5(H$_2$O)</td>
<td>hydroxide</td>
<td>synthesized</td>
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<td>Marcus et al., 2008</td>
<td>ALS 10.3.2</td>
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<td>Hansel et al., 2003</td>
<td>SSRL 4-1/4-3</td>
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<tr>
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<td>synthesized</td>
<td>Hansel et al., 2003</td>
<td>SSRL 4-1/4-3</td>
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<td>SSRL 4-1/4-3</td>
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<td>Chemical Formula</td>
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<td>Source</td>
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<td>Code</td>
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<td>Lizardite</td>
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<td>sulfide</td>
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<td>fl</td>
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<td>oxide</td>
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<td>SSRL 4-1/4-3</td>
<td>fl</td>
</tr>
<tr>
<td>Minnesotaite</td>
<td>Fe₇₋₅Mg₄.₅Si₄O₇(OH)₂</td>
<td>talc</td>
<td>Excalibur Minerals</td>
<td>Templeton Group</td>
<td>SSRL 4-1</td>
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</tr>
<tr>
<td>Olivine (San Carlos)</td>
<td>Mg₁.₈Fe₀.₂SiO₄</td>
<td>Olivine</td>
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<td>Templeton Group</td>
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<td>fl</td>
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<tr>
<td>Olivine (Sapat Valley)</td>
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<td>APS 13-IDC</td>
<td>fl</td>
<td></td>
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<tr>
<td>Pentlandite</td>
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<td>sulfide</td>
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<td>Templeton Group</td>
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<tr>
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<td>FeS₂</td>
<td>sulfide</td>
<td>synthesized</td>
<td>Hansel et al., 2003</td>
<td>SSRL 4-1/4-3</td>
<td>fl</td>
</tr>
<tr>
<td>Saponite</td>
<td>Ca₀.₁Na₀.₁Mg₂.₅Fe₀.₇₅Si₃AIO₁₀(OH)₂</td>
<td>Ferroan smectite montmorillonite</td>
<td>Excalibur</td>
<td>Templeton Group</td>
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<td>tr</td>
</tr>
<tr>
<td>Smectite (SAz-1)</td>
<td>Ca₀.₃₉Na₀.₃₆K₀.₀₁₂Al₂.₇₈Fe₀.₁₂Mg₁.₁₁Mn₀.₀₁</td>
<td>clay</td>
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<td>SSRL 4-1/4-3</td>
<td>fl</td>
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<tr>
<td>Siderite</td>
<td>FeCO₃</td>
<td>carbonate</td>
<td>Stanford University Mineral Collection</td>
<td>Hansel et al., 2003</td>
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<td>fl</td>
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<tr>
<td>Vivianite</td>
<td>Fe₅(PO₄)₂·₈(H₂O)</td>
<td>phosphate</td>
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<td>Hansel et al., 2003</td>
<td>SSRL 4-1/4-3</td>
<td>fl</td>
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</table>
Table A2. Normalized fluorescence values of XANES spectra used in XANES fitting of multiple energy maps for the 48-hour sample.

<table>
<thead>
<tr>
<th>Energy (eV)</th>
<th>Normalized fluorescence</th>
<th>XANES 2</th>
<th>XANES 3</th>
<th>XANES 7</th>
<th>XANES 9</th>
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<tr>
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<td>Ferrihydrite</td>
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<tr>
<td>7123</td>
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<td>0.99</td>
<td>0.99</td>
<td>0.96</td>
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<tr>
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<td>0.65</td>
<td>1.48</td>
<td>1.48</td>
<td>1.25</td>
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<tr>
<td>7128</td>
<td>1.29</td>
<td>0.93</td>
<td>1.54</td>
<td>1.50</td>
<td>1.27</td>
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<tr>
<td>7130</td>
<td>1.26</td>
<td>1.17</td>
<td>1.57</td>
<td>1.40</td>
<td>1.24</td>
</tr>
<tr>
<td>7133</td>
<td>1.17</td>
<td>1.31</td>
<td>1.18</td>
<td>1.12</td>
<td>1.19</td>
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Table A3. Normalized fluorescence values of XANES spectra used in XANES fitting of multiple energy maps for the 10-month sample.

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<tr>
<th>Energy (eV)</th>
<th>XANES 5</th>
<th>XANES 7</th>
<th>XANES 14</th>
<th>XANES 15</th>
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<td>1.10</td>
<td>1.29</td>
<td>1.58</td>
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<tr>
<td>7130</td>
<td>1.65</td>
<td>1.32</td>
<td>1.30</td>
<td>1.44</td>
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<tr>
<td>7133</td>
<td>1.28</td>
<td>1.39</td>
<td>1.18</td>
<td>1.12</td>
</tr>
<tr>
<td>Sample/End-member</td>
<td>Model</td>
<td>Fraction</td>
<td>$R^2$ value</td>
<td>E shift</td>
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<td>-------</td>
<td>----------</td>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>48/2</td>
<td>Brucite</td>
<td>0.38</td>
<td>0.008576</td>
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<td></td>
<td>SV Olivine</td>
<td>0.36</td>
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<tr>
<td></td>
<td>SC Olivine</td>
<td>0.26</td>
<td></td>
<td></td>
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<td>48/9</td>
<td>Fe(III) clay</td>
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<td>0.0007109</td>
<td>-0.1</td>
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<tr>
<td></td>
<td>Hematite*</td>
<td>0.33</td>
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<td></td>
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<td></td>
<td>Ferrihydrite*</td>
<td>0.21</td>
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<td>Fe(III)PO$_4$**</td>
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<td>Basalt</td>
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<td>0.000817</td>
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<td>Green-rust sulfate*</td>
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<tr>
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<td>Ferrihydrite*</td>
<td>0.42</td>
<td>0.000617</td>
<td>0.46</td>
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<td>Fe(III) clay</td>
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<td></td>
<td>Hematite*</td>
<td>0.19</td>
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<td></td>
<td>Fe(III)PO$_4$**</td>
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<td>0.0004599</td>
<td>0.52</td>
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<tr>
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<td>Brucite</td>
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<td></td>
<td>Clinochlore</td>
<td>0.38</td>
<td></td>
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*Hansel et al., 2003 **O’Day et al., 2004; SV olivine is from the Sapat Valley, Iran; SC olivine is from San Carlos, AZ, USA
Table A5. Concentrations of aqueous\(^a\) and gaseous\(^b\) species of interest.

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<th>Species</th>
<th>Concentration (umol/L)</th>
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<th>72/96 hrs</th>
<th>10 months</th>
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<td>([\text{Si}]_{aq})</td>
<td>74</td>
<td>459</td>
<td>536</td>
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<tr>
<td>([\text{Fe}]_{aq})</td>
<td>16</td>
<td>101</td>
<td>95</td>
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<tr>
<td>([\text{Mn}]_{aq})</td>
<td>16</td>
<td>24</td>
<td>14</td>
<td></td>
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<tr>
<td>([\text{Mg}]_{aq})</td>
<td>35580</td>
<td>35995</td>
<td>33201</td>
<td></td>
</tr>
<tr>
<td>([\text{Ca}]_{aq})</td>
<td>11685</td>
<td>11985</td>
<td>12835</td>
<td></td>
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<tr>
<td>([\text{SO}<em>4^{2-}]</em>{aq})</td>
<td>683</td>
<td>665</td>
<td>705</td>
<td></td>
</tr>
<tr>
<td>([\text{P}]_{aq})</td>
<td>~300</td>
<td>-</td>
<td>-</td>
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<td>pH</td>
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<td>6.5</td>
<td>7.5</td>
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<tr>
<td>([\text{H}_2(g)]^\dagger)</td>
<td>-</td>
<td>157</td>
<td>3357</td>
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<tr>
<td>([\text{CO}_2(g)]^\dagger)</td>
<td>-</td>
<td>4633</td>
<td>1857</td>
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</table>

\(^a\) aqueous data was collected at 0 and 72 hours – ICP-AES measurements were conducted on filtered, acidified aliquots of experimental media at the Laboratory for Environmental Geoscience at the University of Colorado – Boulder.

\(^b\) gas data was collected at 96 hours and 10-months after starting with a N\(_2\):CO\(_2\) headspace with no initial H\(_2\). All measurements were conducted with an SRI Gas Chromatograph.

References for Appendix A – Supporting Information Chapter 2


Figure A1. The intensity of iron within the 48 hour sample mapped at 5 different energies a) 7123, b) 7126, c) 7128, d) 7130, and e) 7133 eV respectively. Scale bar is equal to 40 μm.
Figure A2. Overplot of all XANES spectra collected from the 48 hour sample. Spectra outlined in thicker lines indicate spectra defined as end-members. The overlap between spectra collected from particles present within a single map component is apparent. Lines indicate energies at which uXRF data was collected.
Figure A3. Normalized XANES spectra of basalt and ferrihydrite, used as the ‘standards’ for Fe(II) and Fe(III), respectively. Lines correspond to energies at which uXRF data were collected.
Figure A4. a-d) Distribution of distinct components (1-3 respectively) determined from PCA of the 5 energy Fe K-edge maps of the 10 month sample. PCA results in pixels with both positive and negative values, represented by red and blue respectively. e) Map of total iron in the sample. Numbers correspond to location of microXANES analyses. f) Optical image taken in plane polarized light at 10x magnification. Scale bars = 50 um.
Figure A5. Stackplot of all Fe-model compounds invoked in least squares fits of end-member XANES spectra. The similarity in Fe(III) oxide, clay, and phosphate spectra is apparent and illustrates the difficulty in discerning between the species using XANES. Each model is offset by 1 from the model below for clarity. Lines indicate energies at which uXRF data were collected.
Figure A6. Plots of internal fits of non end-member spectra. Data is in blue, fit is in red, residual is in yellow. Other colors correspond to the contribution of each end-member XANES spectra to the overall fit. Where a fit consists of only a single end-member XANES it is labeled as such. R² values are shown in the upper right-hand corner of each plot. All graphs plot normalized intensity (y-axis) versus energy (eV) (x-axis).
Appendix B.

CHAPTER 3

The effect of methanogenesis on the geochemistry of low temperature water-basalt-Fe$^0$ reactions

Supporting Information
Figure B1. Stack plot of Fe K-edge XANES model spectra used in least squares fits of unknown end-member XANES spectra.
Figure B2. Field-emission scanning electron microscope images of *M. thermoflexus* in association with mineral particles in the culture experiment.
Appendix C

CHAPTER 4

H₂ production from abiotic water-rock reactions at low temperatures

Supporting Information
Table C1. Library of iron model compounds used in cycle fitting of end-member XANES spectra. Not all models were included for all spectra. Spectra of starting substrates were only included in libraries for the appropriate reacted materials (e.g. petedunnite was only used to fit end-members from the reacted petedunnite sample).

<table>
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<th>Model compound</th>
<th>Theoretical Formula</th>
<th>Mineral group</th>
<th>Mineral source</th>
<th>Author</th>
<th>Synch/BL</th>
<th>Data type</th>
</tr>
</thead>
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<td>Ankerite</td>
<td>CaFe$^{2+}$&lt;sub&gt;0.6&lt;/sub&gt;Mg&lt;sub&gt;0.3&lt;/sub&gt;Mn$^{2+}$&lt;sub&gt;0.1&lt;/sub&gt;(CO&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>carbonate</td>
<td>UCLA Mineral Collection</td>
<td>Templeton Group</td>
<td>SSRL 4-1</td>
<td>fl</td>
</tr>
<tr>
<td>Antigorite</td>
<td>Mg&lt;sub&gt;2.25&lt;/sub&gt;Fe$^{2+}$&lt;sub&gt;0.75&lt;/sub&gt;(Si&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;)(OH)&lt;sub&gt;4&lt;/sub&gt;</td>
<td>serpentine</td>
<td>Smithsonian NMNH # 157139</td>
<td>Templeton Group</td>
<td>SSRL 4-1</td>
<td>tr</td>
</tr>
<tr>
<td>Augite</td>
<td>Ca&lt;sub&gt;0.9&lt;/sub&gt;Na&lt;sub&gt;0.1&lt;/sub&gt;Mg&lt;sub&gt;0.9&lt;/sub&gt;Fe&lt;sub&gt;0.2&lt;/sub&gt;Al&lt;sub&gt;0.4&lt;/sub&gt;Ti&lt;sub&gt;0.1&lt;/sub&gt;Si&lt;sub&gt;1.9&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;</td>
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<td>Ward’s</td>
<td>Templeton Group</td>
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<td>fl</td>
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<tr>
<td>Awaruite</td>
<td>Ni&lt;sub&gt;2.2&lt;/sub&gt;Fe</td>
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<td>Mineralogical Research Company</td>
<td>Marcus et al., 2008</td>
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<td>fl</td>
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<td>Mg(OH)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>hydroxide</td>
<td>Quenched Hawaiian lava</td>
<td>Templeton Group</td>
<td>SSRL 11-2</td>
<td>tr</td>
</tr>
<tr>
<td>Brucite</td>
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<td>hydroxide</td>
<td>Smithsonian NMNH # C1795-1</td>
<td>Templeton Group</td>
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<tr>
<td>Chamosite</td>
<td>Fe&lt;sub&gt;3&lt;/sub&gt;Mg&lt;sub&gt;1.5&lt;/sub&gt;Si&lt;sub&gt;3&lt;/sub&gt;AlO&lt;sub&gt;12&lt;/sub&gt;(OH)&lt;sub&gt;6&lt;/sub&gt;</td>
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<td>Smithsonian NMNH # B17943</td>
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<td>spinel</td>
<td>Smithsonian NMNH # 85790</td>
<td>Templeton Group</td>
<td>SSRL 4-1</td>
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<tr>
<td>Clinochlore</td>
<td>Fe$^{2+}$&lt;sub&gt;1.25&lt;/sub&gt;Mg&lt;sub&gt;0.75&lt;/sub&gt;Si&lt;sub&gt;3&lt;/sub&gt;AlO&lt;sub&gt;10&lt;/sub&gt;(OH)&lt;sub&gt;8&lt;/sub&gt;</td>
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<td>Templeton Group</td>
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239

Fe3O4

Fe2+2.5Mg0.5Si4O10(OH)2

Magnetite

Minnesotaite

Mg1.8Fe0.2SiO4
Fe4.5Ni4.5S8

NA

Ca0.9Na0.1Zn0.4Fe2+0.2Mn2+0.2Mg0.1Fe3+0.1Si2O6

FeS2

Ca0.1Na0.1Mg2.2Fe0.75Si3AlO10(OH)2

Ca0.39Na0.36K0.02Al2.71Fe0.12Mg1.11Mn0.01Ti0.03Si
8O20(OH)4
FeCO3

Olivine (San Carlos)
Pentlandite

Peridotite (San
Carlos)
Petedunnite

Pyrite

Saponite

Smectite (SAz-1)

Ferroan smectite
montmorillonite
clay

sulfide

clinopyroxene

Igneous rock

Olivine
sulfide

clay

oxide

Excalibur Minerals

synthesized

Excalibur Minerals

Ward’s
Smithsonian
NMNH # 128239
Ward’s

Stanford University
Mineral Collection
Forysthe fayalite
Mine - Ward’s
Excalibur Minerals

synthesized

Excalibur Minerals

Clay Minerals
Society
Siderite
carbonate
Stanford University
Mineral Collection
Vivianite
Fe3(PO4)2·8(H2O)
phosphate
Stanford University
Mineral Collection
* clay was reduced prior to analysis and sample was in an anaerobic sample holder during analysis

Na0.3Fe

Nontronite*

4(H2O)

talc

Fe3O4

Magnetite

2Si3AlO10(OH)2•

oxide

(Fe,Ni)1 + xS

Mackinawite

3+

spinel

Fe2+Al2O4

Hercynite
sulfide

oxide

Fe2O3

Hematite

clinopyroxene

CaFe2+Si2O6

Hedenbergite

fl
fl

SSRL 4-1/ 4-3
SSRL 4-1/ 4-3

Hansel et al.,
2003
Hansel et al.,
2003

fl

tr

fl

fl

tr

fl
tr

tr

tr

tr

fl

fl

fl

fl

fl

SSRL 4-1/ 4-3

SSRL 4-1

SSRL 4-1/ 4-3

SSRL 4-1

SSRL 4-1

SSRL 11-2
SSRL 4-1

SSRL 4-1

SSRL 4-1

SSRL 4-1

SSRL 4-1/ 4-3

SSRL 4-1/ 4-3

ALS 10.3.2

SSRL 4-1/ 4-3

SSRL 4-1

O’Day et al., 2004

Hansel et al.,
2003
Templeton Group

Templeton Group

Templeton Group

Templeton Group
Templeton Group

Templeton Group

Templeton Group

Hansel et al.,
2003
Marcus et al.,
2008
Hansel et al.,
2003
Hansel et al.,
2003
Templeton Group

Templeton Group


Table C2. Results of least squared fits of end-member XANES spectra.

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<tr>
<th>Sample/End-member</th>
<th>Model</th>
<th>Normalized proportion</th>
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<th>eV shift</th>
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Unreacted (UR) hedenbergite

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reacted hedenbergite

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Unreacted (UR) San Carlos peridotites*

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*Fits to the end-member spectra were not accurate due to self-adsorption issues. Contents were inferred from comparison to spectra from the corresponding reacted substrate and results from other analytical techniques.
Figure C1. Plot of end-member XANES spectra identified in the reacted San Carlos peridotite. Spectra are offset by 1 for clarity. Vertical lines are at 727.5 eV, the peak position of EM 24, and 7130.25, the peak position of EM 13, to show the offset in peak positions between the spectra.
Figure C2. XANES spectra of Fe-bearing brucite and talc model compounds overplotted with ferrihydrite (an Fe(III)-oxide). The offset in the adsorption edge and peak positions illustrates the Fe(II)-rich character of the Fe-bearing brucite and talc.
The data presented below, unless otherwise noted, was obtained from experiments using 53-212 μm substrates reacted with artificial seawater in an 80% N₂ 20% CO₂ headspace.

Table C3. Minerals and rocks tested for H₂ generation potential but not included in Chapter 4.

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<th>Substrate</th>
<th>Theoretical Composition</th>
<th>Experimental Composition*</th>
<th>Source</th>
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<td>(Ca,Na)(Mg,Fe,Al,Ti)(Si,Al)₂O₆</td>
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<td>Ward’s Scientific</td>
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<td>basalt</td>
<td>CaMgSi₂O₆</td>
<td>See Chapter 3</td>
<td>Hawaii lava</td>
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<td>diopside</td>
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<td>enstatite</td>
<td>Mg₂Si₂O₆</td>
<td>Not determined</td>
<td>Ward’s Scientific</td>
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</table>

* mineral formulas calculated from electron microprobe measurements of weight percent oxides
Figure C3. H$_2$ production from different mineral and rock substrates at 55, 80, and 100°C. a) augite, b) basalt, c) diopside, d) enstatite. (a, c, d) are the average of two replicates per temperature and (b) is the average of 3 replicates per temperature. Experiments were run at 55°C (solid lines, circles) and 80°C (dashed lines, triangles). Error bars are equal to one standard deviation.
Figure C4. H₂ production from San Carlos olivine (triangles), peridotite (circles), and glassy Hawaiian basalt (squares) as temperature was increased from 50 to 80°C at 2600 hours and from 80 to 100°C at 7900 hours. Error bars are one standard deviation.
Figure C5. H$_2$ production from San Carlos olivine at 55°C at a water-rock ratio of 17.5 (circles) and 7 (squares).
Figure C6. H₂ production from San Carlos olivine at 55 (solid lines) and 100°C (dashed lines) under a 100% N₂ headspace (circles) and an 80% N₂, 20% CO₂ headspace (squares). Error bars are one standard deviation.