Mineralogical and geochemical characterization of two Sulfolobales harboring hot spring systems : Rabbit Creek and Ragged Hills, Yellowstone National Park, WY, USA
by Braden Thomas Hanna

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Earth Sciences
Montana State University

Abstract:
The fine scale mineralogy of two Sulfolobus and Acidianus harboring acid sulfate geothermal areas in Yellowstone National Park was investigated to determine the interrelationships between these microbial populations and the ambient mineralogy. The two geothermal areas are a highly altered solfatara in Rabbit Creek, Midway Geyser. Basin and a less mature solfatara in Ragged Hills, Norris Geyser Basin. The fine scale mineralogy was determined using X-ray diffraction (XRD) in combination with scanning electron microscopy (SEM) equipped with energy dispersive spectroscopy (EDS). Cryogenic SEM was used to investigate in situ potential interactions between the mineralogy and microbial populations using both natural and cultured samples. Water chemistry of the natural springs was monitored over a one and a half year time span, and thermodynamic modeling was used to predict the equilibrium mineral assemblages.

The mineralogy of the two sites was found to be significantly different. Abundant sulfide and sulfate minerals were present at the Ragged Hills site, while no sulfate minerals and only trace amounts of sulfides were present at Rabbit Creek. Both sites contained kaolinite, quartz, a variety of paracrystalline silica phases, amorphous aluminum hydroxides, and a various residual volcanic phases. Rabbit Creek also contained hematite and halloysite. Elemental sulfur was only observed at one uniqe spring at the Ragged Hills site.

The mineralogy observed at these sites was found to influence the populations of sulfur metabolizing, acidophilic, hyperthermophiles in several ways. The microbes from high temperature springs at these sites were found to attach, both in situ and in culture, to a variety of mineral surfaces, including quartz, alunite, sulfur and kaolinite. Microbes from these springs were also found to consume elemental sulfur. It was determined that the kaolinite and amorphous aluminum hydroxide present at both sites are capable of buffering the pH in the range acceptable to sustain Sulfolobus and Acidianus. Observations indicate that elemental sulfur is potentially consumed by metabolic oxidation as rapidly as it forms.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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CHAPTER 1

PURPOSE OF STUDY

The primary purpose of this study was to determine the mineralogy of two geothermal systems in the Rabbit Creek (Midway Geyser Basin) and Ragged Hills (Norris Geyser Basin) areas of Yellowstone National Park, and to compare and contrast the older, more mature system (Rabbit Creek) with the younger, less mature system (Ragged Hills). Comparing and contrasting the older and younger systems provides the opportunity to determine the potential influences of the mineralogical evolution of the system on the microbiological populations in the system. This study also investigated the extent to which the mineralogy of each system influences and is influenced by the thermophilic microbial populations present in these springs) The potential relationships investigated include the attachment of these organisms to mineral surfaces, the use of certain minerals for energy or nutrients, and the implications of the buffering capacity of specific mineral precipitation reactions.

CHAPTER 2

BACKGROUND

Microorganisms Can Influence Their Environment

Microorganisms, such as bacteria and archaea, comprise the majority of the diversity of life, and perhaps the majority of Earth's biomass is composed of subterranean lithotrophs (Pace, 1997). They are found in nearly all environments, from underneath the surface of rocks and ice, to the depths of the oceans, to up to 3.5 kilometers beneath the surface of the Earths crust (Amend and Shock, 2001). In fact, they are capable of living in the most extreme environments of any known life forms. They are capable of altering their local environment, thereby influencing physical parameters such as pH, Eh, concentration of various aqueous elements and complexes, and precipitation and dissolution of minerals. Some of these microorganisms are chemotrophic, deriving their metabolic energy directly from the free energy associated with changes in the chemical state of their environment. These chemotrophic organisms allow ecosystems to develop where the energy source is from geologic processes rather than sunlight (Nealson and Stahl, 1997). In fact, all known hyperthermophiles and many thermophiles are chemosynthetic, using oxidation reduction reactions of inorganic or organic compounds (Amend and Shock, 2001).

Microbially induced chemical transformations can occur in aqueous, solid, or gaseous phases. Solid phase alteration has been well investigated with respect to the microbially induced oxidation of pyrite (FeS_2) and subsequent acidification in acid mine drainages (Bigham and Nordstrom, 2000). In this process the sulfur and iron in pyrite are microbially oxidized, leading to the dissolution of pyrite and the acidification of surface waters. Microbes can also indirectly mediate mineral dissolution or precipitation by depleting or increasing concentrations of various dissolved chemical species, driving dissolution and precipitation through equilibrium reactions (Fortin *et al.*, 1997). Microbial mediation occurs when these reactions are "thermodynamically favored but kinetically inhibited" (Amend and Shock, 2001), as in the oxidation of pyrite at surface conditions. Microorganisms are capable of directly mediating the precipitation of minerals such as iron and manganese oxides, various other metals, opaline silica, carbonates, on and or inside their cell perimeters (Tebo *et al.*, 1997, de Vrind-de Jong and de Vrind, 1997). Microbes have also been shown to selectively mediate mineral dissolution to gain access to limiting nutrients such as the phosphate contained in the mineral apatite ($Ca_5(PO_4)_3(F,Cl,OH)$) (Rogers *et al.*, 1999). Microbial populations have been shown to accelerate weathering rates of quartz and feldspar even when aqueous concentrations are at saturation (Heibert and Bennett, 1992). The coupling of high temperatures and microbial activity provides an opportunity for greatly accelerated weathering processes and microbially mediated mineral transformations.

The microbial alteration of physical conditions can have profound implications for the system. The oxidation state of metals such as iron and arsenic influence characteristics such as mobility (Xu *et al.*, 1991), toxicity (Stauffer *et al.*, 1980), and bioavailability (Banfield and Hamers, 1997). In order to understand the ramifications of microbially mediated physical environmental change, it is necessary to investigate the role that microorganisms play in influencing their environment, as well as the details of the physical environments they inhabit.

Mineralogy Can Influence Microbial Populations

Minerals can influence microbial populations by controlling physical and chemical processes in a variety of ways. In addition to providing a source of energy through potential oxidation-reduction pathways and a serving as a source of nutrients such as phosphorous, calcium, sodium, potassium, iron, etc, minerals can also influence microbial populations in other ways. The presence of certain minerals can buffer concentrations of chemical species. For example, the presence of calcite will buffer the pH of surface waters with which it is in contact at a pH of 8.3. The precipitation of minerals that contain certain metals will prevent the respective metals from exceeding a given aqueous concentration. Minerals such as clays are capable of retaining large amounts of water, as well as inhibiting the flow of groundwater. These properties directly influence the spatial distribution of organisms. Clays can also serve as a temporary reservoir for elements such as calcium, potassium, and other biological elements, thus keeping them available to organisms after their liberation from the host rock. Surface properties of minerals vary widely, and can provide conditions that enable microorganisms to attach to mineral surfaces.

Sulfur oxidizing organisms from hydrothermal environments have been shown to attach to a variety of surfaces (Guezennec *et al.*,1998). Dynamics of surficial attachment of microbes to minerals are diverse and complex. Attachment can range from electrostatic and transient to the permanent attachment of entire colonies via exopolymers produced by the organisms. Attachment and biofilm production can provide refuge in particularly dynamic environments by inhibiting transport across thermoclines and chemoclines (Little *et al.*, 1997). Attachment also enables colony forming organisms to generate localized physical conditions between mineral and biofilm surfaces. Rocks and soils with different mineralogies also have porosities and permeabilities that vary of several orders of magnitude. The mineralogy of an environment can drastically influence and even control the chemical composition of water, the processes of attachment and transport, and the availability of water, nutrients, and energy.

Site Selection

Two different geothermal areas within the Yellowstone caldera were chosen for this study. These two sites are referred to here as Rabbit Creek and Ragged Hills (see Figure 1). High resolution maps were created for each of these sites and are provided in the cd-rom insert.

The Rabbit Creek study site is located along the otherwise alkaline Rabbit Creek drainage in Midway Geyser Basin (UTM zone 12, 0515034E, 4929794N). A photograph of the Rabbit Creek thermal site is shown in Figure 2. This site is a relatively mature geothermal area and appears to be highly altered. No dead trees are remaining from the emergence of the thermal area, although a few stunted pines do continue to live in the thermal area. The presence of this acidic sulfate rich thermal site was recorded as early as 1935 (Allen and Day).

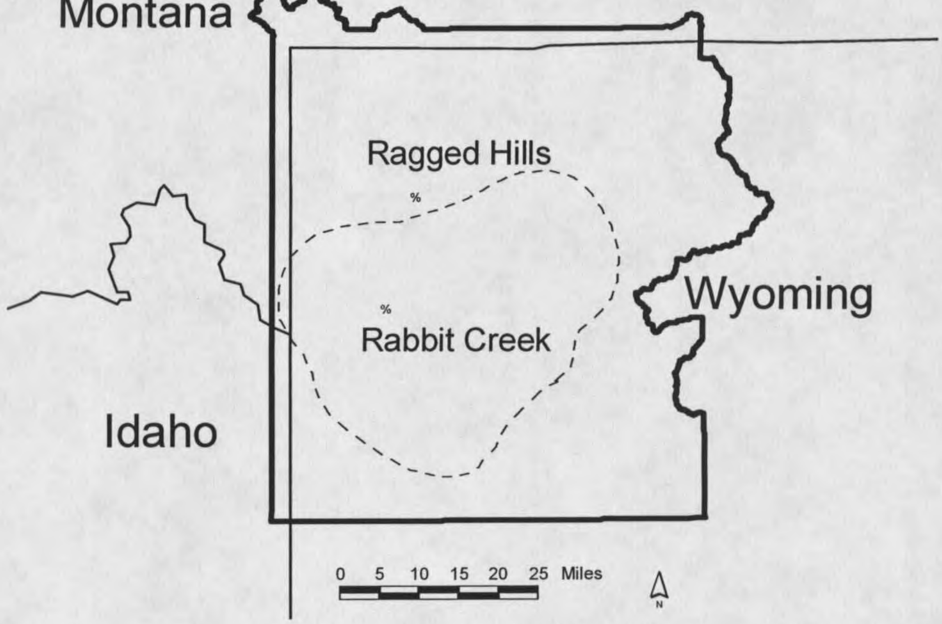

Figure 1: Map of study sites. Rabbit Creek and Ragged Hills study sites are shown as black circles. Boundary of Yellowstone National Park is marked with the thick black line, state borders are shown in thin black (from Spatial Analysis Center, Yellowstone National Park, 1995), and the most recent caldera limits (from Hildreth *et al.*, 1991) are marked with the dashed line.

The second study site, Ragged Hills, is located in the Ragged Hills area of Norris Geyser Basin (UTM zone 12, 521000 E, 4952543 N). A photograph of the Ragged Hills site is shown in Figure 3. Ragged Hills is composed of silica cemented glacial gravels and as of 1935 there was no evidence of any past thermal activity (Allen and Day, 1935). This site is a much less mature geothermal area than Rabbit Creek. There are abundant dead trees, killed off from the recent emergence of the thermal area, and the emergence of new springs at Ragged Hills over the 3 year interval from 1999-2001 has been recently noted (Nordstrom *et al.*, 2001). Both sites have a variety of geothermal features including mud pots, thermal springs, outflow channels, and fumaroles. Both sites contain springs which maintain pH in the range of 2-4, and temperatures in the range of 65 °C to 90 °C. In addition, they both have alteration mineral assemblages derived from similar underlying rhyolite flows.

The geomicrobiological aspect of this study focuses on the highly acidic (pH 2-4), high temperature (65 °C to 90 °C) springs. The organisms that inhabit this environment are referred to as acidophilic hyperthermophiles (Barns and Nierzwicki-Bauer, 1997). Although numerous types of organisms do inhabit this niche, it has much less diversity than other less extreme habitats. While this study focuses on the sulfur oxidizing organisms *Sulfolobus and Acidianus*, it is important to note that other acidophilic thermophiles are undoubtedly present at these sites. However, the microbial diversity and potential role of other microbial organisms at these sites was beyond the scope of this study.

Extremophiles

Extremophiles, organisms that grow in environments that are inhospitable to most living things, have recently become an important topic for research. Categories of extremophiles include thermophiles and hyperthermophiles (which grow optimally at >80C), psychrophiles (which grow optimally at <15C) halophiles (which grow in high salinity), endoliths (which grow inside rocks), barophiles (which grow at high hydrostatic pressure), acidophiles (which grow at low pH), as well as many others. Extremophiles can also demonstrate the ability to grow multiple extreme environments such as thermophilic acidiophiles.

The construction of a universal phylogenetic tree using evolutionary distances between different organisms based on their 18S and16S rDNA sequences (Figure 4) has indicated that there are three major domains of life, or urkingdoms (Woese, 1987). The hypothetical last common ancestor is represented by the root of the tree. Theoretically, the closer an organism is to the root of the tree, the closer it is to the last common ancestor (Woese, 1987). The fact that extremophiles plot close to the base of the tree is evidence that they may serve as analogues of extraterrestrial life and of early life on Earth as well.

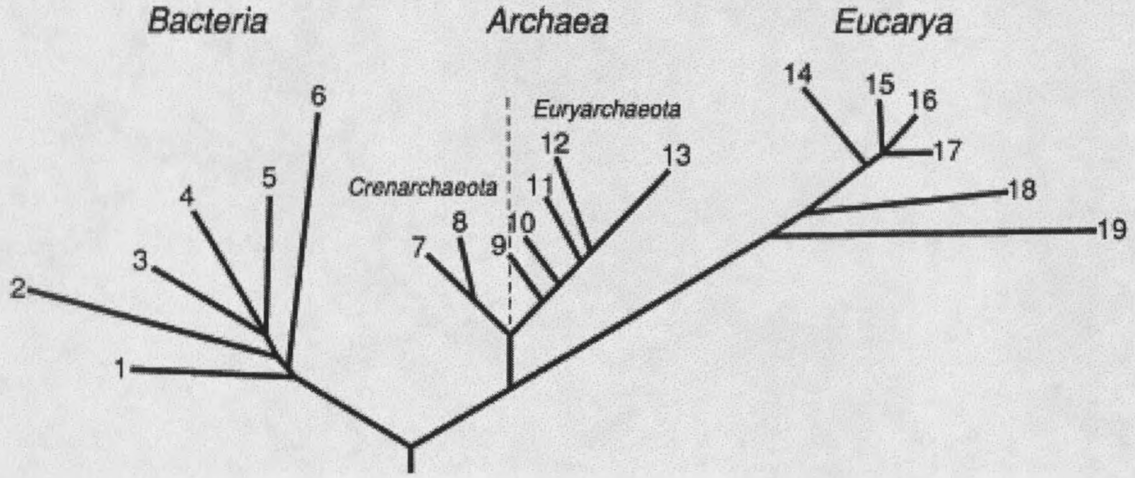

Figure 4: Phylogenetic tree of life. *Sulfolobus* lies in the group labeled 8 on this tree. Branching order and length are based on rRNA sequences. Length of branches and distance from the base of the tree are proportional to evolutionary distance from the hypothetical common ancestor. Numbers on the tree represent the following groups: 1, the Thermotogales; 2, the flavobacteria; 3, the cyanobacteria; 4, the purple bacteria; 5, the Gram-positive bacteria; 6, the green nonsulur bacteria; 7, *Pyrodictum*; 8, *Thermoproteus*; 9, the Thermococcales; 10, the Methanococcales; 11, the Methanobacteriales; 12, the Methanomicrobiales; 13, the extreme halophiles; 14, the animals; 15, the ciliates; 16, the green plants; 17, the fungi; 18, the flagellates; and 19, the microsporidia. (From Woese *et al*., 1990).

Research focus on these organisms has grown dramatically in recent years. They are important for a number of reasons. Their environments serve as analogues for potential extraterrestrial ecosystems. In fact, hot springs are a primary potential site for the origin of life on Earth and are currently a primary target for the search for life and fossils of past life outside of Earth (Farmer, 2000). They are also very important for industrial and biomedical applications because of the novel proteins they use to catalyze reactions under such unique conditions (Madigan and Marrs, 1997). The extremophiles of direct interest in this study are acidophilic hyperthermophiles.

Hyperthermophilic Sulfur Metabolizing Organisms

The domain archaea consists of two phyla, the Crenarchaeota and the Euryarchaeota. The phylum Crenarchaeota is composed of acidophilic, obligately thermophilic (Garrity, G.M., 2001), sulfur-dependent organisms (Howland, 2000). Most cultured Crenarchaeotes are hyperthermophilic (Snyder and Young, in press). Within the phylum Crenarchaeota, the sole class, *Thermoprotei,* has three orders: *Thermoproteales, Desulfurococcales*, and *Sulfolobales* (Garrity, G.M., 2001). Among these, the sulfur reducers, *Thermoproteales*, and the sulfur oxidizers, *Sulfolobales* are the most widely studied (Snyder and Young, in press). Two genus from the family *Sulfolobaceae* order *Sulfolobales, Sulfolobus* and *Acidianus*, have been isolated from the high temperature areas of the Rabbit Creek and Ragged Hills sites (although a true *Acidianus* type-member has not yet been isolated from Rabbit Creek) (Rice *et al.*, 2001).

The first acidophilic hyperthermophile to be characterized was the Crenarchaeote *Sulfolobus* (Brock *et al*., 1972), found in Yellowstone National Park. Since then, a variety of acidophilic hyperthermophiles have been identified in solfatara environments around the world. There are currently six recognized species of the genus *Sulfolobus*: *S. acidocaldarius, S. solfataricus, S. hakonensis, S. metallicus, S. shibatae, and S. yangmingensis* (as well as two other proposed species, S. islandicus and S. thuringiensis) (Garrity, G.M., 2001). The *Sulfolobus* species isolated from Rabbit Creek and Ragged Hills most closely resemble *S. acidocaldarius* and *S. solfataricus*, based on 16S rRNA sequencing (Rice *et al*., 2001).

Sulfolobus cells are coccoid in shape and can be highly lobed or spherical (Garrity, G.M., 2001). They have a diameter ranging from 0.7 to 2 microns, can be either motile or immotile, and flagella, pillus-like, and pseudopodium-like structures have all been observed. They live in a pH range from 1- 5.5 and a temperature range from 65 to 85°C. *Sulfolobus* is a facultative chemolithoautotroph, meaning it can use inorganic or organic carbon sources and attains energy from oxidation reduction reactions of organic molecules or inorganic sulfur and iron compounds. All species are obligate aerobes, requiring oxygen (although some species can use Fe^{3+}, or MoO_4^{2-}) as electron acceptors. Lithotrophic growth can occur using sulfide ores, sulfide, elemental sulfur, tetrathionate, or Fe^{2+} ions as electron donors. Organotrophic growth occurs using complex organic materials, sugars, or amino acids. (Garrity, G.M., 2001).

Although *Sulfolobus* is one of the most widely studied archaebacteria, its identification and characterization is often confounding. In fact, widely available