Sulfur isotopes as a tracer for biogenic sulfate reduction in natural environments

A link between modern and ancient ecosystems

Marjolijn Stam

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Members of the dissertation committee

Dr. J. P. Amend
Earth and Planetary Sciences
Washington University in St. Louis
St. Louis, United States of America

Prof. dr. H. Strauss
Institut für Geologie und Paläontologie
Westfälische Wilhelms-Universität Münster
Münster, Germany

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Zürich, Switzerland

Prof. dr. H. J. Laanbroek
Netherlands Institute of Ecology (NIOO-KNAW)
Nieuwersluis, The Netherlands

Prof. dr. J. J. Middelburg
Faculty of Geosciences
Utrecht University
Utrecht, The Netherlands

Colofon

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Sulfur isotopes as a tracer for biogenic sulfate reduction in natural environments

A link between modern and ancient ecosystems

(with a summary in English)

Zwavelisotopen als indicator voor biogene sulfaatreductie onder natuurlijke omstandigheden
Hedendaagse bacteriën als gids voor de vroegste biologische sporen op Aarde

(met een samenvatting in het Nederlands)

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ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen
op donderdag 1 april 2010 des middags te 4.30 uur

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Marjolijn Christine Stam

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te Leiden

Promotoren:
Prof. dr. B.H.W.S. de Jong
Prof. dr. P. Van Cappellen

Co-promotor:
Dr. P.R.D. Mason

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Image of the Schelde Estuary, The Netherlands

Preface

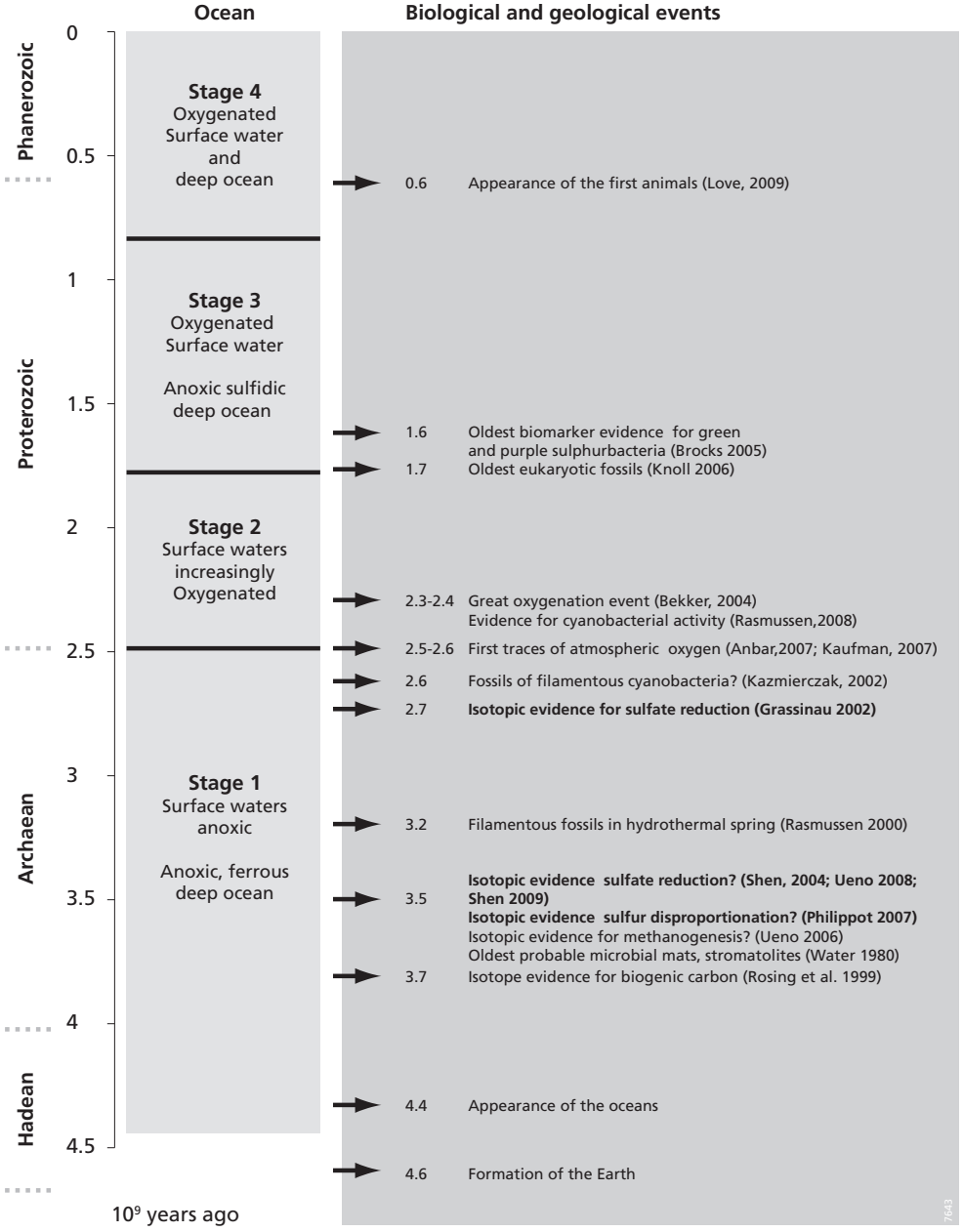
Tracing the appearance and the evolution of life on Earth remains one of the greatest challenges in the geological and biological sciences. Modern environments are an important analogue for the early Earth, and allow the direct study of microbial communities and the traces they can leave behind in sedimentary rocks. One of the most promising forms of life to link modern and ancient Earth ecosystems are the sulfate reducing prokaryotes for the following reasons: 1) microbiological studies indicate that they appeared very early during the Earth's biological evolution, 2) they are able to flourish under the oxygen-free and potentially high temperature conditions that are assumed to have been present on the early Earth, 3) they are ubiquitous in a large variety of modern environmental settings, and 4) the sulfur compounds that are used and produced by these microorganisms are preserved in rocks and minerals throughout the geological record and can be traced through distinctive fractionation in their stable isotopes.

Biogeochemical studies show that life probably appeared shortly after the formation of the Earth, possibly as early as 3.7 billion years ago (Figure P.1). Fixing this date is challenging since the amount of ancient well-preserved and unmetamorphosed sedimentary rocks is limited. It is difficult to constrain environmental conditions such as the Archean surface temperature, which may have fluctuated due to a changing atmosphere and volcanic activity. A variety of natural habitats could potentially have been occupied by microorganisms. Sulfur isotope fractionation, generated by and characteristic for specific microbial or abiotic processes, is well preserved from the Earth's earliest rocks, all the way through the geological record. It is of key importance to determine the range in isotope fractionation that can be produced by modern sulfate reducing prokaryotes so that we can interpret what traces have been left behind by this metabolism in the past. In addition, global sulfur cycling on both the modern and ancient Earth can be quantified using stable isotopes. This requires detailed study of microbial sulfate reduction that represents the dominant process in the biochemical part of the modern sulfur cycle.

This thesis investigates how biogenic sulfate reducing activity can be preserved in sulfur isotope fractionation for natural communities of sulfate reducing prokaryotes in sediments. Although many previous studies exist for individual strains of Bacteria and Archaea in pure culture, there are only a few limited data to show the bulk isotope signal for complex microbial communities that most closely represent what is recorded in sedimentary rocks. I have studied a number of different environments, varying from moderate to more extreme conditions that cover a range in temperature, salinity and alkalinity. ***Chapter 1*** gives a general overview of the nature and role of sulfate reducing prokaryotes, and reviews how their activity may be preserved, traced and detected using stable isotopes. Furthermore, the specific aims and scope of this thesis are described. The following five chapters discuss in detail the relationship between biogenic sulfate reduction and corresponding sulfur isotope fractionation effects, using intact natural sediment slices sampled from four distinct geochemical settings including a brackish estuarine site (Schelde Estuary, The Netherlands, ***Chapter 2***), a hypersaline soda lake (Mono Lake, California, USA, ***Chapter 3***), a shallow hydrothermal vent system (Vulcano Island, Italy, ***Chapter 4***) and a fresh water site (River Schelde, Belgium, ***Chapter 6***). For each setting, sediments were exposed to a variety laboratory

conditions to mimic those found in the natural environment. ***Chapter 5*** shows how sulfur isotope fractionation responds to stimulation from compounds that enhance or reduce sulfate reducing activity. ***Chapter 6*** compares the results from all of the sampling sites and shows how modern isotope fractionation effects can be used to argue for microbial processes in ancient rocks and sediments. This final chapter also outlines the general conclusions and implications of this thesis and provides some recommendations for further research directions.

My intention in writing this thesis is to set more solid constraints on the extent of sulfur isotope fractionation generated during microbial sulfate reduction by natural communities of sulfate reducing prokaryotes, and to show how this varies across different geochemical settings. A key goal is to provide a new database of isotope fractionation effect data that can be applied in studies that trace microbial activity on the early Earth, as well as studies that indentify the role of different processes in local and global sulfur cycling throughout the Earth's history, until the present day.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Biological and geological events
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Stage 4
Oxygenated
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Surface water
Anoxic sulfidic
deep ocean
Stage 2
Surface waters
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Oxygenated
Stage 1
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anoxic
Anoxic, ferrous
deep ocean
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3.2 Filamentous fossils in hydrothermal spring (Rasmussen 2000)
3.5 Isotopic evidence sulfate reduction? (Shen, 2004; Ueno 2008; Shen 2009)
Isotopic evidence sulfur disproportionation? (Philippot 2007)
Isotopic evidence for methanogenesis? (Ueno 2006)
Oldest probable microbial mats, stromatolites (Water 1980)
3.7 Isotope evidence for biogenic carbon (Rosing et al. 1999)
4.4 Appearance of the oceans
4.6 Formation of the Earth
10⁹ years ago
7643

Figure P.1: (see left page) Timeline of the main biological and geochemical events from the formation of the Earth, approximately 4.6 billion years ago (Ga), to the present day. This figure is redrawn from similar timelines compiled by Des Marais (2000), Brocks and Banfield (2009) and Lyons et al. (2009). Source data were obtained from Walter et al. (1980), Rosing, (1999), Rasmussen (2000), Grassineau et al. (2001), Kazmierczak and Altermann (2002) Bekker et al. (2004), Shen and Buick (2004), Brocks et al. (2005), Knoll et al. (2006), Ueno et al. (2006), Anbar et al. (2007), Kaufman et al. (2007), Philippot et al. (2007), Rasmussen et al. (2008), Ueno et al. (2008), Love et al. (2009) and Shen et al. (2009). The left panel shows the different Aeons, the middle panel the evolution of the redox state and oxygenation of the oceans, and the right panel shows isotope, fossil and biomarker evidence for the first appearance of different types of metabolic processes. The first stable isotope evidence for biogenic sulfate reduction is highlighted in bold. Question marks indicate the main places where the evidence is equivocal and highly debated in the literature. Since this thesis focuses on the first traces of biogenic sulfate reduction on Earth, the emphasis in the timeline is based on biological events appearing in the Archean and early Proterozoic and therefore most evidence for metabolisms after 1.6 Ga are excluded from this figure. A more complete overview for the Proterozoic and Phanerozoic is given by Brocks and Banfield (2009).

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Image of the summit of Vulcano Island, Italy

Chapter 1
Sulfur isotope fractionation by sulfate reducing prokaryotes

1

Introduction and aims of this study

1.1 Tracing microbiological activity in the geological record

Microorganisms from the domains of Bacteria and Archaea have an important role in controlling the global biogeochemical cycles of major and minor nutrients such as carbon, sulfur and iron. Prokaryotes were also amongst the first forms of life to appear on Earth possibly as early as 3.7 billion years ago (Ga) and almost certainly by 2.7 Ga. Identifying the existence and activity of microbes in past geological environments is essential to constrain the geological and biological evolution of the Earth.

The most straightforward way to identify microorganisms is to search for preserved remains (Westall, 2008). However, this approach is often difficult due to the lack of biomineralization in prokaryotes and poor preservation or absence of soft tissue in rocks. Most microbes have a simple morphology which is circular or rod-shaped and these features are difficult to discriminate from physical forms produced by abiotic processes in rocks and sediments (Schopf, 2002; Brasier et al., 2002). Another approach to find past traces of life is to search for distinctive molecular biomarkers (Summons and Walter, 1990; Brocks et al., 1999; Brocks and Pearson, 2005; Brocks and Banfield, 2009). Biomarkers are organic products produced by specific groups of microorganisms that are characteristic for specific metabolisms (Summons et al., 1999). Although this approach is a useful strategy in modern or very well-preserved sediments, molecular biomarkers are not resistant to temperature and pressure changes during metamorphism, and are thus not traceable in the earliest rock record (Shen and Buick, 2004). The most promising approach to detect the traces of past microbial activity is stable isotope fractionation. Kinetic isotope fractionation effects typically lead to enrichment of the lighter stable isotope and the magnitude of these changes potentially correlate with reaction rates (Fry, 2006). Stable isotope ratios can survive metamorphic and alteration events, depending upon the mineralogical or amorphous phase that hosts the element of interest. Mass balance calculations using stable isotope variations can also be used to constrain the amount of biomass and extent of the biosphere through Earth in space and time (Canfield et al., 2006b). The interpretation of stable isotope variations and the extent to which they can be attributed to microbial activity requires a thorough investigation of the main controls on isotope partitioning during the growth of natural communities of microorganisms. This introductory chapter explains the background behind the use of stable sulfur isotopes for investigating the activity of sulfate reducing prokaryotes (SRP) and concludes by outlining the main goals of this thesis.

1.2 Sulfur

Sulfur is a redox sensitive element that is abundant in all of the major reservoirs at the Earth's surface including the oceans, crust, atmosphere and biosphere (Figure 1.1). Rocks and sediments in the lithosphere include a large variety of sulfur bearing minerals or amorphous compounds. The oxidation state of sulfur varies from +VI in, for example, Gypsum ($CaSO_4$) to -II in Sulfides. Sulfur is soluble in water in both oxidized and reduced oxidation states,

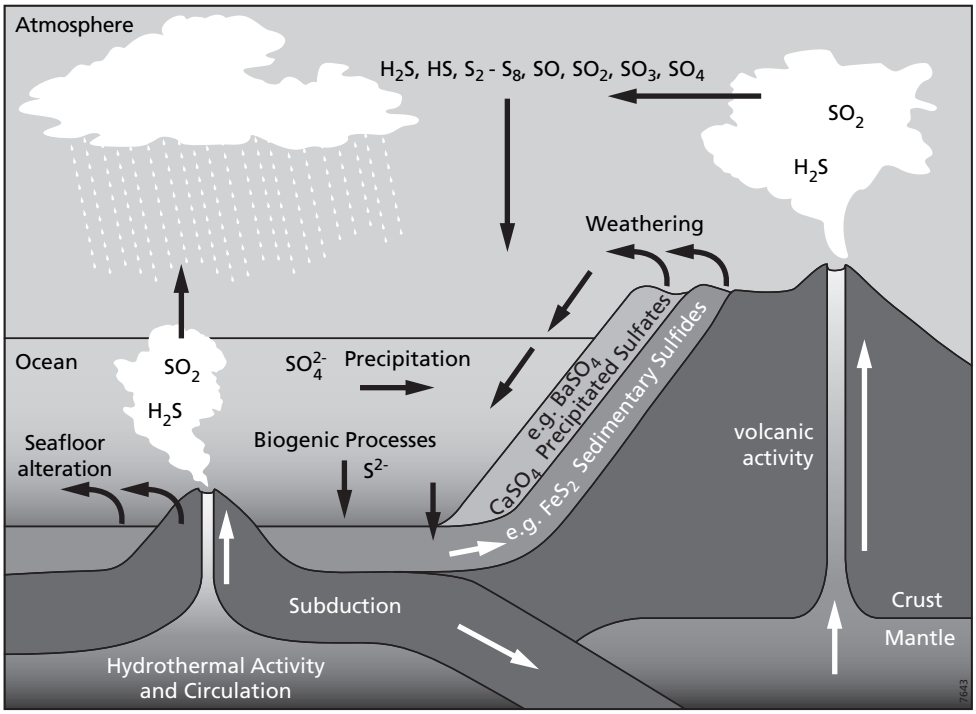

Figure 1.1: The global sulfur cycle showing biogenic and inorganic processes interacting between atmosphere, hydrosphere, lithosphere and biosphere. This figure is re-drawn after Canfield (2004) and Brimblecombe (2005).

occurring mainly as sulfate (SO_4^{2-}) and sulfide (S^{2-}). Dissolved forms of intermediate oxidation states e.g. sulfite (SO_3^{2-}), thiosulfate ($S_2O_3^{2-}$) and trithionate ($S_3O_6^{2-}$) and other polythionates are present in reduced amounts. Sulfur is emitted into surface reservoirs from the deeper mantle by volcanic or magmatic activity, primarily as SO_2 or H_2S. Mantle output adds only minor amounts of sulfur to the total budget on the modern Earth as most sulfur is recycled at the surface. Sulfur is an essential nutrient for all organisms and makes up on average 1 wt % of total dry biomass (Widdel, 1988; Canfield, 2001a). It is a key component in its most reduced (-II) form in the amino acids Cysteine and Methionine that are building blocks of proteins and is also assimilated into vitamins, coenzymes and electron carriers, which are essential in metabolic processes (Brock, 2006).

Sulfur compounds are used as a source of energy during microbial respiration in anoxic environments (Widdel, 1988). Sulfate reduction is the most dominant microbial pathway for sulfur on the modern Earth in which the sulfate to sulfide conversion is coupled to growth. Other redox transformations such as sulfide oxidation or elemental sulfur disproportionation are also energy yielding. The global sulfur cycle is driven by both biological and abiotic processes and is closely linked to the cycles of other elements including carbon, oxygen and nitrogen (Jørgensen, 1982; Canfield and Raiswell, 1999; Canfield et al., 2005; Bottrell and

Newton, 2006). Sulfur has four stable isotopes ^{32}S (95.02 %), ^{34}S (4.22 %), ^{33}S (0.76 %) and ^{36}S (0.0136 %) (Macnamara and Thode, 1950; Ohmoto and Goldhaber, 1997; Canfield, 2001a). The ratios of these isotopes can be modified by equilibrium or kinetic fractionation during microbial or abiotic redox reactions involving sulfur compounds as discussed below.

1.3 Sulfate reducing prokaryotes

Sulfate reducing prokaryotes (SRP) are defined here as microorganisms from both the Bacterial and Archaeal domains (Figure 1.2) (Woese et al., 1978; Winker and Woese, 1991; Doolittle, 1999; Woese, 2000) that reduce sulfate to sulfide for energy gain during respiration (Harrison & Thode, 1958; Kaplan & Rittenberg 1964; Rees, 1973; Canfield and Raiswell, 1999; Canfield, 2001a; Shen and Buick, 2004; Johnson et al., 2008; Muyzer & Stams, 2009). They occur in a large variety of anoxic marine and terrestrial settings, grow across a wide range of temperatures from below 0°C (Brüchert et al., 2001; Robador et al., 2009) up to 100°C (Jørgensen et al., 1992; Amend and Teske, 2005), and have a diverse phylogeny based on their ribosomal RNA (Wagner et al., 1998, Castro et al., 2000). SRP have been isolated from, or found to be active in anaerobic fresh water, brackish and marine coastal sites and lakes (Bak and Pfennig, 1991; Purdy et al., 2002; Roychoudhury et al., 2003; Pallud and Van Cappellen, 2006) as well as in environments that are considered extreme with respect to temperature (Knoblauch and Jørgensen, 1999, Brüchert et al., 2001; Rabus et al., 2002, Roychoudhury, 2004. Amend and Teske, 2005; Stetter, 2006a), pH (Koschorreck, 2008), and salinity (Oremland et al., 2000, Brandt et al., 2001; Detmers et al., 2001; Kulp et al., 2006; Pallud and Van Cappellen, 2006; Foti et al., 2007). The activity of the SRP can account for more than half of the total organic carbon mineralization in many environments and it has been estimated that up to 5×10^{12} kg year^{-1} of SO_4^{2-} is reduced by microorganisms on the modern Earth (Skyring, 1987). Only about 100 species of SRP have been isolated, cultured and characterized to date and their physiological and phylogenetic characterization is far from complete (Detmers et al., 2001).

Sulfate can be reduced by SRP through two distinct microbial processes termed assimilatory and dissimilatory reduction. In assimilatory sulfate reduction, sulfate is extracted from the environment and subsequently reduced, incorporated into the cell and used as an building block in biochemically important organic compounds (Canfield, 2001a). This process is energy consuming and is not specific for sulfate reducing prokaryotes but can also be performed by most pants, fungi and other types of prokaryotes such as cyanobacteria (Schiff, 1979; Frigaard and Dahl, 2008). The first step in assimilatory sulfate reduction is the transport of sulfate across the cell membrane which is performed by specific sulfate binding enzymes (Canfield, 2001a). This uptake is unidirectional, meaning that there is no exchange between internal and external sulfate (Cypionka, 1995). Once in the cell cytoplasm, sulfate can be reduced in two distinct pathways; the adenosine-5'-phosposulfate (APS) pathway and the phosphoadenosine-5'-phosphosulfate (PAPS) pathway. In both pathways, sulfate is attached to adenosine-5'-triphosphate (ATP) to form APS which is catalyzed by the enzyme

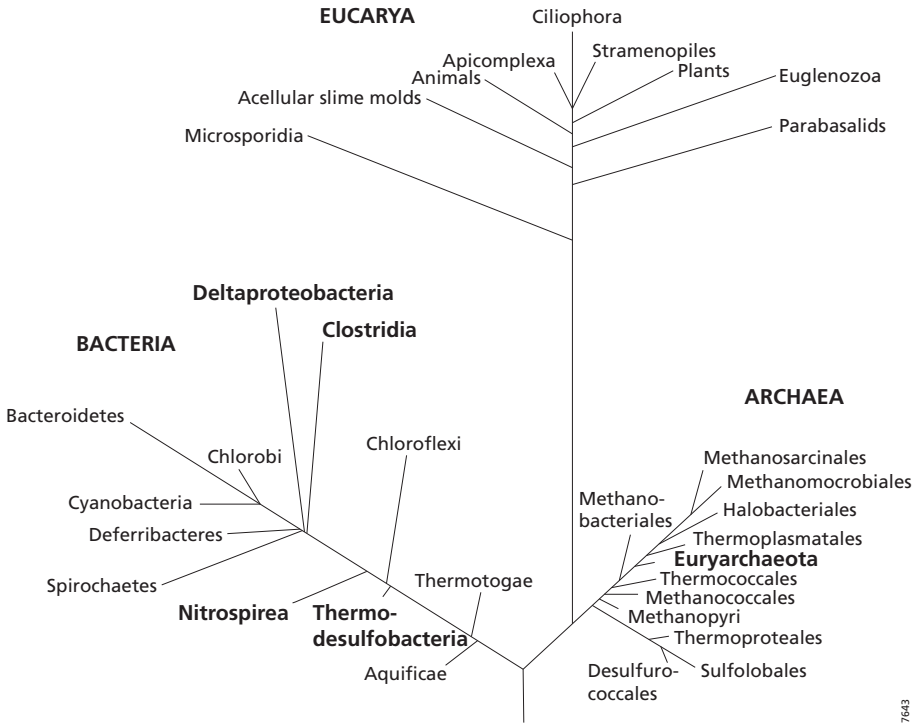

Figure 1.2: Phylogenetic tree indicating the position of the sulfate reducing prokaryotes (SRP) in bold. This figure is reconstructed from Shen and Buick (2004). Following the most recent literature review on sulfate reducing prokaryotes there are two additional groups: Thermodesulfobiaceae and Crenarchaeota (Muyzer and Stams, 2008). The positions of these groups are not indicated in this phylogenitic tree but belong to the Bacteria and Archaea respectively.

ATP-sulfurylase. In the APS pathway this compound can be further reduced to sulfide which is then cooperated into the Cysteine protein. APS can also be converted into PAPS to form sulfate containing esters. In the PAPS pathway, first an energy rich phosphoryl group (PP_i) is attached to the APS molecule to form PAPS which is then reduced to sulfite and finally to sulfide which is then used to form Cysteine. During assimilatory reduction no free sulfide is produced (Siegel, 1975).

Dissimilatory sulfate reduction is carried out exclusively by SRP and yields energy to support microbial growth and other cellular processes. Much more sulfide is produced than in assimilatory sulfate reduction as it is excreted from the cell (Widdel, 1988). The reduction of sulfate to sulfide is typically coupled with the oxidation of organic compounds such as lactate, acetate, ethanol (represented by equation 1, CH_2O is a general molecular formula used to indicate organic compounds) or inorganic compounds including H_2 and CO_2 (equation 2) (Widdel, 1988; Canfield, 2001a). Electrons are transferred from these compounds, which are termed *electron donors*, to the terminal *electron acceptor* which in this case is sulfate:

$$SO_4^{2-} + 2\ CH_2O \rightarrow H_2S + 2\ HCO_3^- \quad (1)$$
$$SO_4^{2-} + 4\ H_2 + 2\ H^+ \rightarrow H_2S + 4\ H_2O \quad (2)$$

The difference in reduction potential between electron donor and acceptor controls the amount of energy that can be liberated. For example energy yield is much higher with lactate than acetate as the electron donor. The standard Gibbs free energy (ΔG^0) for complete acetate oxidation is -47.7 kJ mol^{-1} whilst incomplete and complete lactate oxidation generate -160.1 kJ mol^{-1} and -255.3 kJ mol^{-1} respectively (Widdel, 1988; Oren, 1999; Amend and Shock, 2001; Detmers et al., 2001).

Most of the reduction steps in dissimilatory sulfate reduction (Figure 1.3) are similar to those in the assimilatory process. Sulfate is transported into the cell across the cell membrane. In contrast to assimilatory sulfate reduction, this step is reversible. However, transport out of the cell may be small since it reduces the membrane potential leading to less ATP synthesis (Cypionka, 1994; Brüchert, 2004). Once in the cell, sulfate needs to be activated before it can be reduced. Using ATP and the enzyme ATP-sulfurylase, sulfate is phosphorylated to form the high energy compound adenosine-5'-phosphosulfate (APS). As with assimilatory sulfate reduction, this step is an energy consuming or endergonic process. This reaction is made favorable by the hydrolysis of the product pyrophosphate (PP_i) to phosphate. The activated sulfate can then be reduced in two steps which are both exergonic, that replenish the energy spent on the activation of sulfate (Brüchert, 2004). Initially APS is reduced to sulfite, using the enzyme APS-reductase, which could then further be reduced to sulfide by the enzyme dissimilatory sulfate reductase (DSR) (Canfield, 2001a; Brüchert, 2004). Sulfite reduction to sulfide can proceed directly or through intermediate steps including the formation of trithionate ($S_3O_6^{2-}$) and thiosulfate ($S_2O_3^{2-}$) that is then finally reduced to sulfide (Shen and Buick, 2004; Brunner and Bernasconi, 2005) (Figure 1.3). Both reduction steps are reversible. The product sulfide is finally transported out of the cell which is considered in different models to be either a uni- (Rees, 1973) or bi-directional process (Brunner and Bernasconi, 2005).

1.4 Sulfur isotope fractionation

There are four naturally occurring stable isotopes of sulfur (Macnamara and Thode, 1950; Ohmoto and Goldhaber, 1997; Canfield, 2001a). Besides the most abundant isotopes ^{32}S (95.02 %) and ^{34}S (4.22 %) there are two minor isotopes, ^{33}S and ^{36}S with an abundance of 0.76 and 0.0136 % respectively. The ratio of abundance can change as an result of biogenic or abiotic reactions involving sulfur compounds (Ohmoto and Goldhaber, 1997). In general if reactants are present in excess, reaction products are mostly enriched in the lighter isotope since it is less energy costly to break sulfur bonds involving the lighter isotope compared to the heavier ones (Canfield, 2001a; Fry, 2006). The most abundant isotope, ^{32}S, is used as the reference point against which the magnitude of fractionation of the other isotopes is defined. Fractionation is calculated relative to the Vienna Canon Diablo troilite (V-CDT),

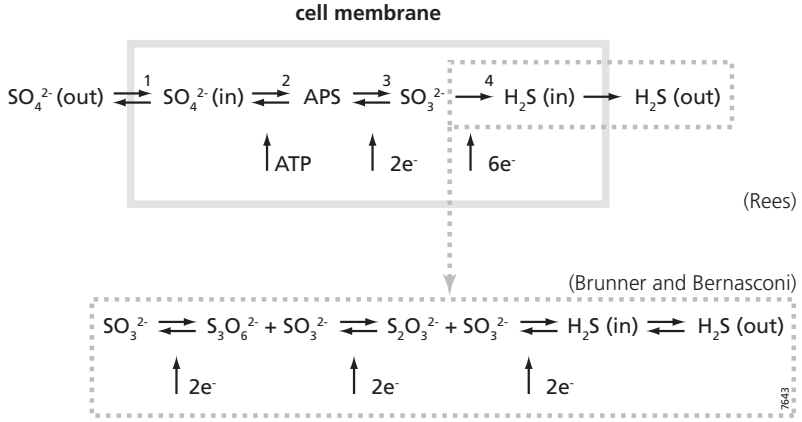

Figure 1.3: Schematic overview of the biogenic dissimilatory sulfate reduction pathways from Rees (1973) and Brunner and Bernasconi (2005).

representing meteoritic sulfur which is assumed to have a comparable signal to the bulk Earth. Differences in isotope fractionation is expressed per mil (‰) and presented in the δ-notation:

$$\delta^{34}S = [\{ (^{34}S/^{32}S)_{sample}/(^{34}S/^{32}S)_{V\text{-}CDT}\} - 1] \times 1000 \quad (3)$$

where ^{34}S could also be replaced by ^{33}S or ^{36}S to report fractionation of the minor isotopes.

Isotope fractionation can be produced by both equilibrium and kinetic processes (Ohmoto and Goldhaber, 1997; Canfield, 2001a; Fry, 2006). Equilibrium fractionation results from differences in bond strength between light and heavy isotopes, and occurs mainly as a result of physical effects such as temperature or variation in diffusion rates related to different reaction media (Ohmoto and Goldhaber, 1997; Fry, 2006). In contrast, kinetic fractionation is dependent on the reaction pathway and reflects the rate of chemical reactions. Most low temperature redox reactions involve kinetic isotope fractionation. $^{34}S/^{32}S$ isotope fractionation during sulfate reduction can be modeled as the interplay between two reactions:

$$^{32}SO_4^{2-} + 2\,H^+ \leftrightharpoons H_2{}^{32}S + 2\,O_2 \qquad K_{S32} = \frac{k_{32\ backward}}{k_{32\ forward}} \quad (4)$$

$$^{34}SO_4^{2-} + 2\,H^+ \leftrightharpoons H_2{}^{34}S + 2\,O_2 \qquad K_{S34} = \frac{k_{34\ backward}}{k_{34\ forward}} \quad (5)$$

Each reaction has its own equilibrium constants K_{32} and K_{34} which can be calculated from the rate constants, $k_{32\ forward}$, $k_{32\ backward}$, $k_{34\ forward}$, $k_{34\ backward}$. In a kinetic process, the backward rates of these reactions are so slow that they can be assumed unidirectional. Isotope fractionation arises since $k_{32\ forward}$ is larger than $k_{34\ forward}$ reflecting the fact that sulfur-oxygen bonds

involving the lighter isotopes are more easily broken and leading to the enrichment of H_2S in the lighter isotopes. In order to express an isotope effect the reaction should not go to completion.

Equilibrium fractionation is produced when exchange rates between reactant and product are in equilibrium and rate constants of backward and forward flows are similar for each isotope system. In the case of sulfate reduction and sulfide oxidation the above reactions could be combined:

$$^{32}SO_4^{2-} + H_2{}^{34}S \leftrightarrows H_2{}^{32}S + {}^{34}SO_4^{2-} \qquad (K_{eq}) \tag{6}$$

with the equilibrium constant (K_{eq}), defined as:

$$K_{eq} = \frac{K_{S32}}{K_{S34}} = \frac{(^{34}SO_4^{2-})\,(H_2{}^{32}S)}{(^{32}SO_4^{2-})\,(H_2{}^{34}S)} = \frac{\{(^{34}SO_4^{2-})/(^{32}SO_4^{2-})\}}{\{(H_2{}^{34}S)/(H_2{}^{32}S)\}} \tag{7}$$

An isotope fractionation factor (α) can be calculated from measured δ values for both kinetic and equilibrium fractionation, which defines the isotope relation between ratios of two coexisting sulfur species e.g. in this case SO_4 and H_2S:

$$\alpha_{SO4\text{-}H2S} = \frac{\text{Ratio } ^{34}S/^{32}S \text{ for } SO_4^{2-}}{\text{Ratio } ^{34}S/^{32}S \text{ for } H_2S} = \frac{(\delta^{34}SO_4 + 1000)}{(\delta^{34}H_2S + 1000)} \tag{8}$$

The fractionation factors of both processes are related by:

$$\alpha_{SO4 - H2S\ (equilibrium)} = \frac{\alpha_{H2S -> SO4\ (kinetic)}}{\alpha_{SO4 -> H2S\ (kinetic)}} \tag{9}$$

The difference in fractionation between the starting sulfate and the product sulfide is recorded as an isotope fractionation effect (ε), also termed the isotope enrichment factor, and is related to α by the following expression:

$$\varepsilon = 1000(\alpha_{SO4\text{-}H2S} - 1) \tag{10}$$

To calculate the amount of fractionation obtained during an experiment or under natural conditions it is important to determine the system as either open or closed. In an open system there is both mass and energy exchange with the outside and the reactant is continuously supplied in excess. In a closed system, however, there is only energy exchange and the reactants are not replenished and thus slowly consumed over time (t). A Rayleigh distillation model can be applied to calculate the amount of isotope fractionation (Canfield, 2001a). Since the starting material (t = 0) is consumed over time (t = x), the fractionation factor depends also on the fraction of the sulfate remaining relative to the starting concentration (*f*):

$$\frac{\text{Ratio } {}^{34}S/{}^{32}S \text{ for } SO_4^{2-}{}_{t=x}}{\text{Ratio } {}^{34}S/{}^{32}S \text{ for } SO_4^{2}{}_{t=0}} = f^{(1-\alpha)} \quad (11)$$

The amount of fractionation for the minor sulfur isotopes can be determined using the same procedure by substituting ^{34}S with ^{33}S or ^{36}S. Until recently, the minor isotopes were not routinely measured since it was widely assumed that the ^{33}S and ^{36}S abundances could be inferred from mass dependent correlations with $\delta^{34}S$. The mass dependent relationship is defined as $\delta^{33}S = 0.515 \times \delta^{34}S$ and $\delta^{36}S = 1.91 \times \delta^{34}S$. Recent studies have shown that all four sulfur isotopes can help to characterize the amount of fractionation within internal cell networks, and can help to distinguish microbial sulfate reduction from elemental sulfur disproportionation as well as help to distinguish microbial from abiotic sulfur cycling processes (Ono et al., 2003; Philippot et al., 2007; Johnston et al., 2007; Johnston et al., 2008; Shen et al., 2009).

Sulfur isotopes are fractionated by a variety of equilibrium or kinetic abiotic and biogenic reaction processes. During kinetic fractionation, the extent of fractionation is highly dependent on the type of reaction and the individual steps within a reaction chain. The rates and reversibility of these steps and the overall chemical reaction are affected by environmental conditions including temperature, pH, salinity, sulfate concentrations and substrate availability leading to a large range in sulfur isotope fractionation.

1.4.1 Sulfur isotope fractionation during microbial processes

Biogenic reactions are the main process to produce sulfur isotope fractionation at temperatures below 100°C (Clark and Fritz, 1997; Ohmoto and Goldhaber, 1997; Krouse and Mayer, 2000). Isotope fractionation is produced during assimilatory and dissimilatory sulfate reduction, sulfide reoxidation and elemental sulfur disproportion. The extent of fractionation associated with these processes under a variety of environmental conditions has been extensively studied in both pure culture experiments and for communities of SRP in natural sediments. The range in measured isotope fractionation, using $\delta^{34}S$ variations to calculate isotope fractionation effects (ε), varies from -3 to 47 ‰ (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Chambers et al., 1975; Habicht and Canfield, 1997; Canfield et al., 2000; Bolliger et al., 2001; Brüchert et al., 2001; Canfield, 2001b; Detmers et al., 2001; Habicht et al., 2002 Canfield et al., 2006a; Hoek et al., 2006; Farquhar et al., 2008; Mitchell et al., 2009). A theoretical fractionation model for biogenic sulfate reduction was developed explaining this range in fractionation and predicting the maximum ε value of 47 ‰ (Rees, 1973). The model assigns a fractionation up to -3 ‰ for transport of sulfate into the cell (Harrison and Thode, 1958; Rees, 1973), and values of 25 ‰ for both cellular sulfate and sulfite reduction. The amount of fractionation for the total reaction is determined in this model by the rates and reversibility for each individual step (Figure 1.3), which are influenced by experimental or environmental factors. The main principle in determining the overall amount of fractionation lies in fixing the rate limiting step. When the transport of sulfate into the cell is rate limiting then the overall fractionation is small since the downstream reactions are too fast to become reversible and to generate

additional fractionation (Brüchert, 2004). However, when the reduction of sulfite to sulfide becomes rate limiting, the maximum fractionation will be 47 ‰ (Rees, 1973).

Fractionation effects larger than 47 ‰ have been observed in natural environments and this has been explained by multiple cycling involving reoxidation and increasing enrichment in the lighter isotopes during subsequent reduction steps. Biogenic fractionation effects between 47 and 100 ‰ are often found when comparing $\delta^{34}S$ of the sulfates in pore waters and the coexisting sulfide present in the sediments of shallow marine environments with a high content of oxidizing species (Habicht and Canfield, 2001). However, differences in $\delta^{34}S$ between pore water sulfates and sedimentary sulfides larger than 47 ‰ were also observed in deep marine environments, which could not be readily addressed to cycles of reoxidation and reduction processes (Rudnicki et al., 2001; Wortmann et al., 2001). Therefore, a new model based on the Rees model was developed with additional fractionation addressed to the reduction of sulfite via trithionate ($S_3O_6^{2-}$) and thiosulfate ($S_2O_3^{2-}$) to sulfide (Figure 1.3), giving the possibility of biogenic fractionation of up to 70 ‰ during one step of sulfate reduction and thereby explaining part of these data (Brunner and Bernasconi, 2005). Although predicted by the new model, larger amounts of biogenic fractionation have not yet been found in pure culture or sediment incubation experiments. The principles of these reaction models and fractionation related to different steps within each metabolic process and the factors determining the amount of fractionation are discussed in more detail in sections 1.4.1a to 1.4.1d.

1.4.1a Assimilatory sulfate reduction

Isotope fractionation effects (ε) between cellular organic sulfide and the external sulfate pool during assimilatory sulfate reduction are relatively small from only -4.4 to 0.5 ‰ despite the fact that the reduction of sulfate to sulfide could yield fractionation effects up to 40 ‰ (Kaplan and Rittenberg, 1964; reviewed in Canfield, 2001a). This lack of fractionation is caused by the inability to exchange the intra- and extracellular sulfate pools and net fractionation is controlled by the uptake of sulfate into the cell, which is associated with a small fractionation effect of -3 ‰ (Rees, 1973). Since the sulfide is not excreted from the cell and the amount of sulfide produced is much smaller compared to that released by dissimilatory sulfate reduction, it is not likely that this process will significantly influence the sedimentary isotope record.

1.4.1b Dissimilatory sulfate reduction

The whole range of fractionation of -3 to 47 ‰ that is predicted by the Rees (1973) model has been measured during dissimilatory sulfate reduction in pure culture and sediment incubation experiments. The main difference compared with assimilatory sulfate reduction is that all reaction steps, except for the transport of sulfide out of the cell are reversible in the Rees model (Figure 1.3). The fractionation effect accompanying sulfate transport (-3 ‰), resulting from the transmembrane diffusion process, is much smaller than those effects observed for sulfate to sulfite (25 ‰) and sulfite to sulfide (25 ‰) reduction. These last two isotope effects are larger as these reactions involve the breaking of S-O bonds, which requires more energy

for the heavier isotope. During the transport of sulfide out of the cell no isotope fractionation is expected, since this step is unidirectional and not found to be rate determining under all environmental conditions explored. The more recently developed Brunner and Bernasconi (2005) model allows exchange of sulfide in both directions across the cell membrane and includes additional steps in the reduction of sulfite to sulfide.

The first pure culture experiments with sulfate reducing prokaryotes showed that the amount of fractionation depends on the sulfate reduction rate, and an inverse relationship is predicted by the Rees model between these two parameters (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Rees, 1973). When sulfate reduction is slow, there should be a large exchange between internal sulfate, sulfite and sulfide pools, leading to large fractionation effects. With increasing rate, the transport of sulfate across the cell membrane becomes rate limiting and the reduction of sulfate and sulfite becomes less reversible and fractionation should be reduced. Although this behavior was found in some natural sediment experiments (Habicht and Canfield, 1997; Canfield, 2001b), contradicting rate *versus* isotope fractionation relationships or their complete absence have been revealed as the amount of data has increased (Brüchert et al., 2001; Detmers et al., 2001; Canfield, 2001b; Canfield et al., 2006a; Hoek et al., 2006).

Deviations from the predicted rate *versus* isotope fractionation relationship can occur for several reasons. The first is a temperature effect. Fractionation can decrease when microorganisms are exposed to temperatures on the edges of their growth curves where low activity is measured (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968). This is especially the case at temperatures < 15°C, where increasing rigidity of the cell membrane makes transport in or out of the cell rate liming (Canfield, 2001b). More recent data have shown that a decrease in isotope fractionation is not always the case and fractionation can also increase on moving away from optimum growth conditions (Canfield et al., 2006a; Hoek et al., 2006). A second potential cause of suppressed fractionation is a low supply of sulfate. Isotope fractionation decreases to zero below sulfate concentrations of 200 μM, although there is not much evidence that fractionation decreases in a predictable way above this threshold value (Harrison and Thode, 1958; Habicht et al., 2002; Habicht et al., 2005). A final variable that could lead to a reduction in isotope fractionation is a change in electron donor. The metabolism of H_2 has been shown to lead to very small fractionation effects in comparison with those observed for the metabolism of organic electron donors by heterotrophic microorganisms (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Hoek et al., 2006).

Comparable sulfate reduction rates can thus produce very different fractionation effects. A modified version of the Rees fractionation model was developed in order to explain this behavior (Farquhar et al., 2003; Johnston et al., 2005; Canfield et al., 2006a; Hoek et al., 2006; Johnston et al., 2007; Farquhar et al., 2008). The modified model includes two branching points where the flow of sulfur through the cell can change direction. The first branching point, defined here as S(1) and equivalent to *f*3 in the Canfield et al (2006) model, compares the fraction of sulfide exiting against the sulfate entering the cell. The second branching point, defined here as S(2) and equivalent to *f*5 in the Canfield et al (2006) model, defines

the fraction of sulfate that is, via the formation of sulfite and other intermediates, reduced to sulfide compared to the total internal sulfate pool (Figure 1.4):

$$S(1) = \frac{SO_4^{2-}{}_{in}}{H_2S + SO_4^{2-}{}_{out}} \quad \text{and} \quad S(2) = \frac{SO_3^{2-}{}_{in}}{H_2S + SO_3^{2-}{}_{out}} \tag{12}$$

S(1) and S(2) have values ranging from 0 to 1. When values are close to zero steps are highly reversible and backward flows are larger than forward ones. When values approach 1 almost all of the reactants are consumed (e.g. almost all sulfate entering the cell is reduced to sulfide). The reversibility of the internal steps that process sulfur through the cell and hence the values of S(1) and S(2) are dependent on temperature, sulfate concentration, substrate and could differ between microorganisms. Different combinations of S(1) and S(2) could lead to identical fractionation effects (Figure 1.5).

The Rees model is consistent with this analysis. An inverse trend between isotope fractionation and sulfate reduction rates could be explained assuming a constant transport rate across the cell membrane (e.g. keeping S(1) fixed at a value of 0.5). Different relationships between fractionation and rate could be explained by changes in S(1) and S(2) that can be linked to experimental parameters for pure cultures of SRP such as temperature (Canfield et al., 2006a; Hoek et al., 2006; Mitchell et al., 2009).

1.4.1c Reoxidation reactions

The hydrogen sulfide (H_2S) produced by microbial activity can be readily buried into in the sedimentary rock record by incorporation into the mineral pyrite (FeS_2), discussed in more detail below. Despite this, sulfide can be readily reoxidized by microbial or abiotic processes (Jørgensen and Des Marais, 1986). In the modern oceans up to 90 % of the sulfide produced by the activity of SRP could be reoxidized (Jørgensen, 1982; Canfield and Teske, 1996). Products formed during reoxidation include S^0, SO_3^{2-}, $S_3O_6^{2-}$, $S_2O_3^{2-}$ or SO_4^{2-} which are often further transformed by microbial reduction, oxidation or disproportionation. The amount of

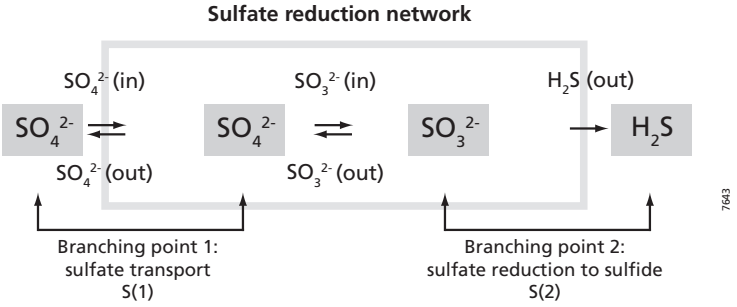

Figure 1.4: Branching points S(1) and S(2) used for predicting or explaining the extent of isotope fractionation (ε) obtained under specific environmental conditions, reconstructed from Canfield et al. (2006) and Hoek et al. (2006). In these papers S(1) is defined as f_3 and S(2) is defined as f_5.

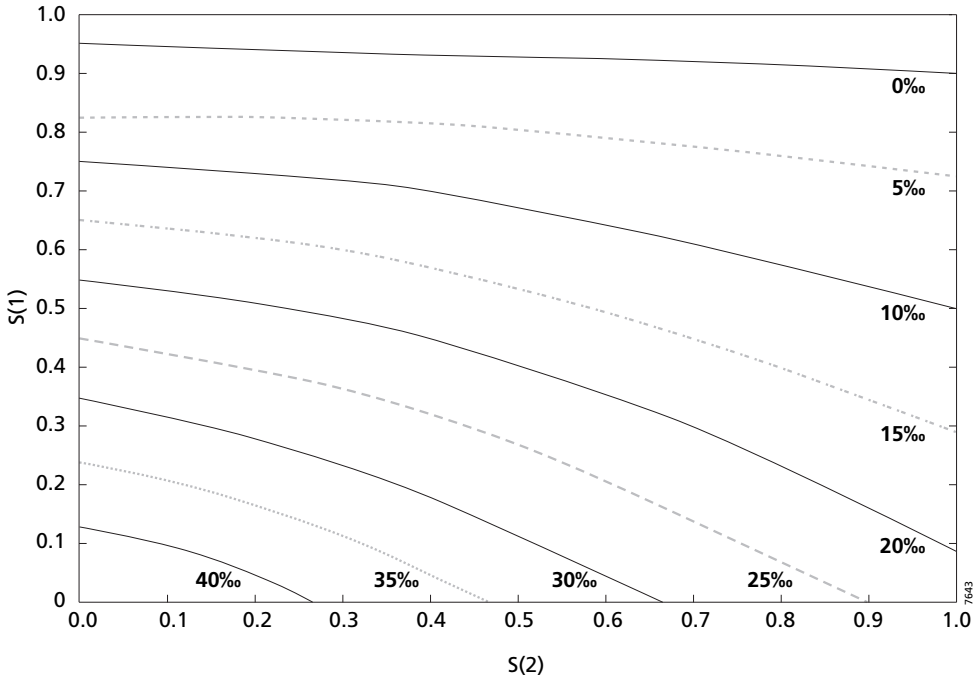

Figure 1.5: Different combinations of S(1) and S(2) could result in similar isotope fractionation (ε), reproduced from Canfield et al. (2006) and Hoek et al. (2006).

fractionation produced during reoxidation is relatively small, varying from -2 to 1 ‰ for microbial processes and 0 to 5 ‰ for abiotic oxidation (Fry et al., 1984; Fry et al., 1986; Fry et al., 1985; Fry et al., 1988 Canfield, 2001a; Zerkle et al., 2009). Large fractionation effects ranging from -19 to 10 ‰ were found in the biogenic reoxidation of sulfide to SO_4^{2-} and $S_2O_3^{2-}$ but only when these compounds were present in minor quantities in the reaction products (Kaplan and Rittenberg, 1964). Thus the oxidation of sulfide seems to have no significant effect on the modification of isotope signatures obtained from microbial sulfate reduction. However, this may not be the case during disproportionation.

1.4.1d Disproportionation

Compounds formed during reoxidation could be further oxidized or reduced through microbial disproportionation reactions to form sulfate and sulfide (Bak and Cypionka, 1987; Bak and Pfennig, 1987; Thamdrup et al., 1993; Finster et al., 1998; Finster, 2008). Disproportionation can be considered as a "fermentation"process in which a sulfur compound is converted or split into one oxidized and one reduced species. In this process electrons are not supplied from external substrates but part of the substrate molecule itself internally accepts electrons from the other side of the compound. No external electron donors or acceptors are needed to complete the metabolic process. The following disproportionation reactions can provide energy for microbial growth:

$$4SO_3^{2-} + 2\,H^+ \rightarrow H_2S + 3SO_4^{2-} \quad (13)$$
$$S_2O_3^{2-} + H_2O \rightarrow H_2S + SO_4^{2-} \quad (14)$$
$$4S^0 + 4\,H_2O \rightarrow 3H_2S + SO_4^{2-} + 2H^+ \quad (15)$$

These disproportionation reactions can be accompanied by large isotope fractionation effects (Canfield and Thamdrup, 1994; Canfield et al., 1998; Habicht et al., 1998; Böttcher and Thamdrup, 2001). Microbial elemental sulfur disproportionation in pure and mixed cultures produces a depletion of the sulfide in $\delta^{34}S$ of approximately 6 ‰ and an enrichment in the sulfate of between 18 ‰ and 35 ‰ (Böttcher et al., 2005). Disproportionation of sulfite produces larger depletions and enrichments in $\delta^{34}S$ of 37 and 12 ‰ for sulfide and sulfate respectively. The combination of microbial sulfate reduction with reoxidation and disproportionation reactions could lead to much larger fractionation effects in the sedimentary rock record then would be predicted from sulfate reduction alone (Habicht and Canfield, 2001). However, the range in fractionation possible with a single disproportionation reaction is comparable to that observed for a single step of sulfate reduction. To discriminate between the two biogenic processes in ancient rocks and sediments the minor ^{33}S and ^{36}S isotopes could be useful (Ono et al., 2006b). Recent studies suggest that microbial sulfur disproportionation may have been active in the geological record as far back as 3.5 Ga (Philippot et al., 2007; Philippot et al., 2008; Finster, 2008) although this has also been disputed (Bao et al., 2008; Shen et al., 2009).

1.4.2 Sulfur isotope fractionation during abiotic processes

Sulfur isotope fractionation is not exclusively related to microbiological processes and can also result from abiotic reactions. Abiotic sulfate reduction only readily occurs at temperatures higher than 200°C at neutral pH (Ohmoto and Goldhaber, 1997). The following redox reaction is possible at elevated temperature in geological environments in the presence of Fe^{2+}:

$$8\,Fe^{2+} + 10\,H^+ + SO_4^{2-} \rightarrow 8\,Fe^{3+} + H_2S + 4\,H_2O \quad (16)$$

Isotope fractionation most likely results from an equilibrium isotope fractionation effect which is temperature dependent ranging from 30 ‰ at 200°C to 20 ‰ at 300°C (Ohmoto and Goldhaber, 1997).

Sulfate can also be reduced thermochemically by hydrocarbons which could be present in the solid, liquid or gaseous phase (Shen and Buick, 2004):

$$4\,CH_2 + 3\,SO_4^{2-} + 6\,H^+ \rightarrow 3\,H_2S + 4\,HCO_3^- + 4\,H^+ \quad (17)$$

This process also occurs at elevated temperatures, between 80 and 200°C, mostly in deeply buried sedimentary rocks and shows a significant amount of isotope fractionation, which could be in the same range as biogenic sulfate reduction (Machel et al., 1995). Despite the large fractionation effect, little fractionation is typically seen in the product sulfide since this reaction usually proceeds to completion (Shen and Buick, 2004).

Isotopic fractionation can also occur as a result of magmatic hydrolysis of SO_2:

$$4\,SO_2 + 4\,H_2O \rightarrow H_2S + + 3\,H^+ + 3\,HSO_4^- \quad (18)$$

The dominant sulfur species in felsic magmatic fluids above 400°C is SO_2. As the fluid cools down to temperatures less than 400°C, the SO_2 could be hydrolyzed to form sulfide and sulfate. Isotope fractionation in the range of 15 to 20 ‰ has been found for this process (Ohmoto and Goldhaber, 1997).

Although these abiotic processes result in similar fractionation effects to the microbial ones discussed above, they do not occur at temperatures lower than 100°C which is close to the maximum temperature where microorganisms have found to be active (Jørgensen et al., 1992; Blöchl et al., 1997). It is important to take them into consideration when interpreting sulfur isotope fractionation in rocks that may have experienced elevated temperature during diagenesis, deformation and metamorphism.

1.5 Preservation of sulfur isotope fractionation in sediments and rocks

Sulfide produced during microbial activity can be trapped and preserved as pyrite in the sediment (Berner, 1984; Raiswell and Berner, 1985; Rickard, 1997; Raiswell and Canfield, 1998; Rickard and Luther III, 1997; Habicht and Canfield, 2001; Goldhaber, 2003; Popa et al., 2004; Bottrell and Newton, 2006). This occurs readily in environments with high concentrations of reactive iron:

$$Fe^{2+} + H_2S \rightarrow FeS + 2H^+ \quad (19)$$

Iron monosulfide is not very stable and typically reacts further to form pyrite through two possible pathways. In the first pathway iron monosulfide reacts with elemental sulfur (Wilkin and Barnes, 1996):

$$FeS + S^0 \rightarrow FeS_2 \quad (20)$$
$$FeS + S_x^{2-} \rightarrow FeS_2 \quad (21)$$

In the second pathway iron monosulfide reacts with sulfide (Rickard, 1997):

$$FeS + H_2S \rightarrow FeS_2 + H_2 \quad (22)$$

Both processes of pyrite formation do not lead to additional isotope fractionation indicating that the $\delta^{34}S$ of sedimentary and hydrothermal pyrite is a good approximation of the average sulfur isotopic composition of the dissolved sulfide sources from which the pyrite formed (Butler et al., 2004).

1.6 Sulfur isotopes throughout the geological record

Sulfate reducing prokaryotes are deep branching on the 16s-rRNA phylogenetic tree which suggests that they appeared early in biological evolution (Wagner et al., 1998; Klein et al., 2001; Stahl et al., 2002). Hyperthermophilic sulfate reducers could have been amongst the first forms of cellular life, which is likely to have begun in a high temperature hydrothermal setting (Martin et al., 2008; Nisbet and Sleep, 2001). Some studies have suggested that marine environments were warmer in the Archean than today as a result of more widespread volcanic activity and thermophilic SRP could have thrived in the early anoxic oceans (Nealson and Conrad, 1999; Nisbet and Sleep, 2001; Stetter, 2006b). A major limitation to the widespread activity of the SRP would have been the low concentration of SO_4^{2-} in the oceans prior to the onset of continental sulfide weathering during the great oxygenation event at approximately 2.4 Ga (Habicht et al., 2002; Pavlov and Kasting, 2002; Farquhar and Wing, 2003; Canfield, 2005; Holland, 2006; Kaufman et al., 2007).

The oldest least-metamorphosed and relatively un-deformed sedimentary rocks on Earth are exposed in the Pilbara block in Western Australia and the Barberton Greenstone Belt in South Africa. The oldest sediments from the Isua Greenstone Belt in Greenland are much more difficult to interpret and do not contain any sulfate minerals (Van Zuilen et al., 2002; Van Kranendonk, 2006; Westall, 2008). Both the Pilbara and Barberton areas contain both sulfate and sulfide minerals and differences in their sulfur isotope ratios have been used to argue for the presence of SRP as far back as 3.49 Ga (Shen et al., 2001; Shen & Buick, 2004; Ueno et al., 2008; Shen et al., 2009) as well as the appearance of sulfur disproportionating microorganisms (Philippot et al., 2007).

Temporal changes in $\delta^{34}S$ in both the marine sulfate reservoir and sedimentary pyrites are summarized in Figure 1.6, adapted from the compilation of Canfield (2005). Minor variations in isotope effects of 5 to 25 ‰ are found through the early-mid Archean, with the largest amounts of fractionation found for sulfides in close proximity to large barite deposits, suggesting a control by the locally high abundance of sulfate (Shen & Buick, 2004). A slightly larger range in $\delta^{34}S$ of 37 ‰ is found in late Archean shales of the Manjeri Formation at 2.7 Ga in the Belingwe Greenstone Belt, Zimbabwe (Grassineau et al., 2001) which is often taken to represent the first unequivocal appearance of SRP in the geological record. Larger $\delta^{34}S$ variations of up to 70 ‰ are observed relative to the seawater sulfate for sedimentary pyrite deposited in sediments younger than 2.3-2.4 Ga. This increase is interpreted by Habicht et al., (2002), and others, to represent a shift to microbial sulfate reduction under non-limiting sulfate conditions with larger amounts of fractionation above 47 ‰ attributed to oxidative recycling of sulfur coupled with microbial elemental sulfur disproportionation (Canfield and Thamdrup, 1994; Canfield and Teske, 1996).

Mass independent variations in the minor isotopes of sulfur support the appearance of atmospheric oxygen after 2.45 Ga (Farquhar et al., 2000; Kasting, 2001; Pavlov and Kasting, 2002). They also indicate the importance of atmospheric versus mantle sources of sulfur in Archean and early Proterozoic sediments (Farquhar and Wing, 2003; Mojzsis et al., 2003; Ono et al., 2006a; Kamber and Whitehouse, 2007) and are particularly useful to separate

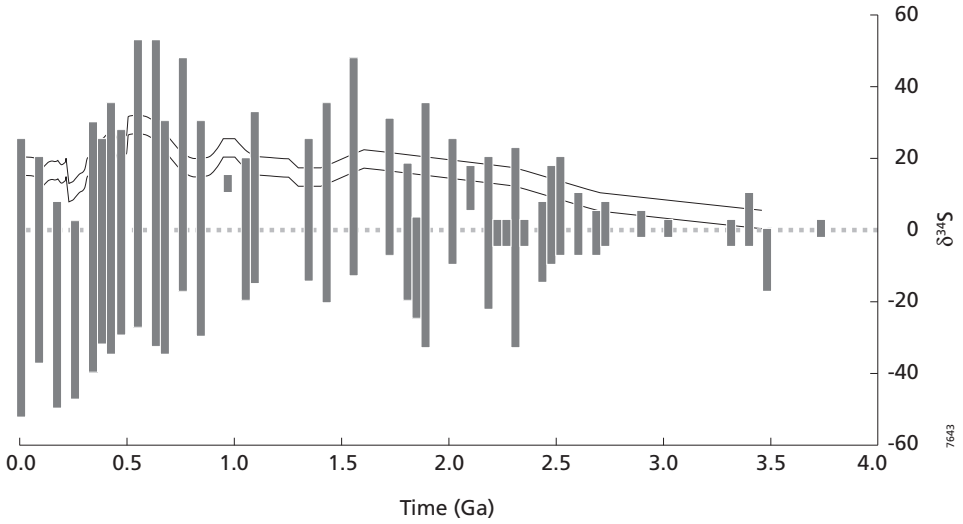

Figure 1.6: Variation in $\delta^{34}S$ of sedimentary sulfides and seawater sulfate through time from the early Archean to the present day. Bars indicate the range in fractionation for these sulfides for a specific time interval. The parallel lines show the reconstructed isotope composition of seawater sulfate with an error of 5 ‰. This figure is redrawn after Canfield (2005) and Canfield and Raiswell (1999).

mass dependent fractionation effects from mixing between different sulfide sources (Bao et al., 2008). The identification of different microbial pathways using minor sulfur isotope variations has not yet been attempted in the geological record and is expected to be difficult due to the small variations that accompany these processes which are potentially lost during diagenesis and burial.

1.7 New fractionation effect data for SRP using flow-through reactors

Although pure culture and mixed culture experiments are useful for investigating differences in metabolism between different SRP they do not provide information which can be directly applied to the interpretation of $\delta^{34}S$ variations in the geological record, since the pyrite buried into sediments records an integrated history of the activity of an entire community of SRP. Natural microbial communities can be studied through a variety of approaches: (1) Differences between sedimentary pyrite and associated pore water sulfate can be measured either *in situ* or in the laboratory to infer fractionation effects. This range typically exceeds maximum values predicted by fractionation models and those found in laboratory experiments (Habicht and Canfield, 2001), suggesting the presence of multiple reduction and oxidation cycles; (2) Batch sediment incubation experiments can be carried out under controlled laboratory conditions to measure a single step of sulfate reduction. The natural microbial community is initially identical to field conditions and the sediment forms the natural substrate for any microbial

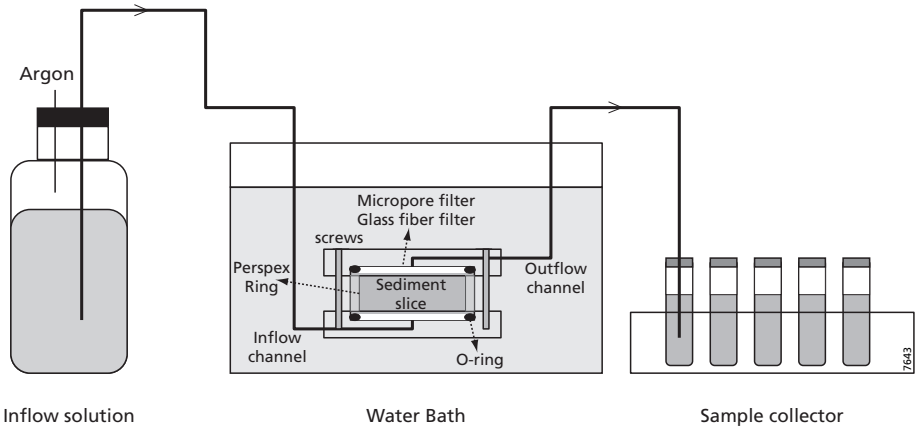

Figure 1.7: Schematic overview of the flow-through reactor system. The reactor has a length of 2 cm and an inside diameter of 4.2 cm.

reactions. The major disadvantage of this approach is that sulfate and other nutrients are supplied in a closed system and can become limiting during growth of the SRP. This can lead to anomalous isotope fractionation effects. Furthermore, sulfate reduction rates are often overestimated because of the loss of the sediment structure as it breaks up into a slurry, making nutrients more accessible and increasing growth rates compared to field conditions (Pallud and Van Cappellen, 2006). The final approach (3) is to use flow-through reactor experiments in which nutrients or substrates can be continuously supplied, and reaction products constantly removed from the sediment (Figure 1.7). This technique allows close to intact sediment slices to be incubated resulting in minimal disturbance of the sedimentary structure and microbial community during the experiment (Roychoudhury et al., 1998; Laverman et al., 2006; Pallud et al., 2007). Sulfate reduction rates and isotope fractionation effects can be readily measured for a single cycle of sulfate reduction under closely monitored and easily varied experimental conditions (e.g. temperature, substrate concentration, organic electron donor addition). Measured rates are potential rates, which are obtained with sulfate supplied in excess as the only terminal electron acceptor, and without competition from other metabolisms. Actual rates in the field site are expected to be lower than the laboratory potential rates.

1.8 Aims, scope and organization of this thesis

The activity of sulfate reducing prokaryotes has had a major impact on the global sulfur cycle through time, and is most easily studied using $\delta^{34}S$ variations in minerals, especially pyrite, and oxidized or reduced sulfur bearing sediments. Sulfur isotope data are also the best line of evidence for tracing the appearance of sulfate reducing prokaryotes in the earliest rock record. The interpretation of the magnitude of $\delta^{34}S$ variations relies heavily on the database

of sulfur fractionation effects for modern communities of SRP. This database is currently limited to a small number of batch incubation and flow-through reactor studies on a limited number of environments. Each experiment has been performed using a small number of sediment samples and none of the studies have been repeated with sufficient detail to investigate variability within the sampling site, such as location, depth or seasonality. Data to show how communities of SRP respond in extreme environments such as a high temperature and hypersalinity and high alkalinity are scarce. The relationships between microbial sulfur isotope variations and temperature, electron donor or sulfate reduction rate are not well constrained despite the widespread use of this isotope system to directly investigate or model these parameters.

The aim of this thesis is to explore biogenic sulfate reduction and corresponding $^{34}S/^{32}S$ isotope fractionation effects induced by natural communities of SRP isolated from three main geochemical settings: estuarine, hydrothermal and hypersaline. These environments are associated with sulfur deposition throughout the geological record and cover a range of physical and chemical conditions under which SRP can thrive. All experiments have been carried out using the same flow-through reactor methodology, which gives fractionation effect and rate data for a single step of sulfate reduction.

In ***Chapter 2*** sulfate reduction rates and fractionation effects obtained for sediments sampled in the Schelde Estuary in The Netherlands are discussed. Sediments were collected across horizontal and lateral profiles on the tidal flat and incubated across a range of temperatures. This site, with a high potential for sulfate reduction, was used to explore sulfur isotope fractionation in great detail under a large variety of experimental conditions. Results are compared to previous flow through reactor experiments performed with sediments sampled from a shallow marine tidal flat in Denmark. Similar sampling strategies were applied in ***Chapter 3*** and ***Chapter 4*** where sediments were collected from a location with extreme salinity and alkalinity at Mono Lake in California (***Chapter 3***) and across large temperature gradients in beach sediments surrounding the shallow marine hydrothermal system of Vulcano Island in Italy (***Chapter 4***). These chapters represent the first isotope fractionation data to be produced for a shallow marine hydrothermal system and an alkaline lake. Both environments are important for tracing sulfate reduction on the early Earth and these environments were present throughout the geological record up to the present. In ***Chapter 5*** a preliminary study is presented on the effects of inhibitors and enhancers of biogenic sulfate reduction on the extent of isotope fractionation. The aim was to investigate if these compounds could extend the range of measured SRR and induce greater ε variability. In ***Chapter 6*** all sulfate reduction rates and isotope data obtained in ***Chapters 2***, ***Chapter 3*** and ***Chapter 4*** are discussed and compared to published flow-through reactor data with a view to defining more general conclusions about the controls on microbial isotope fractionation in the natural environment. This chapter also includes data from a fourth locality, a fresh water site in the Schelde river in Belgium. Implications for tracing biogenic sulfate reduction in the geological record and recommendations for future research are discussed.

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Image of the sampling area in the Schelde Estuary, The Netherlands

Chapter 2

Controls on sulfate reduction and sulfur isotope fractionation by natural microbial communities in sediments from the Schelde Estuary, The Netherlands

2

This chapter is under review with Geochimica et Cosmochimica Acta:
Stam, M.C., Mason, P.R.D., Laverman, A.M. Pallud, C. & Van Cappellen, P. Controls on sulfate reduction and sulfur isotope fractionation by natural microbial communities in estuarine sediments

Abstract

Stable sulfur isotopes are potential tracers of microbial activity with numerous geological and environmental applications. Here, I report concurrent measurements of potential sulfate reduction rates (SRRs) and $^{34}S/^{32}S$ isotope fractionation effects (ε) obtained with flow-through reactors containing intact, 2 cm thick, sediment slices sampled from an unvegetated, intertidal site adjoining a salt marsh along the Schelde Estuary, The Netherlands. A total of 30 reactors were run with sediments sampled in February, May and October 2006. The effects of incubation temperature (10, 20, 30 and 50°C), sediment depth (0-2, 4-6 and 8-10 cm), distance from the vegetated marsh and sampling time were systematically investigated. Sulfate was supplied in non-limiting concentrations via the reactor inflow solutions. No external electron donor was supplied. Data analysis was restricted to SSR and isotope fractionation effects (ε) obtained under steady state conditions. Values of ε were derived from the measured differences in sulfate $\delta^{34}S$ between in- and outflow of the reactors. Potential SRRs varied over one order of magnitude (5 to 49 nmol cm^{-3} h^{-1}) and were highest in the 30°C incubations. SRRs systematically decreased with depth, and were highest in the sediments collected closest to the vegetated marsh. Steady state isotope fractionation effects (ε) ranged from 9 to 34 ‰ and exhibited an inverse relationship with SRR, as predicted by the standard fractionation model for enzymatic sulfate reduction of Rees (1973). The ε *versus* SRR relationship, however, varied between sampling times, with higher ε values measured in February, at comparable SRRs, than in May and October. The observed ε *versus* SRR relationships also deviated from the previously reported inverse trend for sediments collected in a marine lagoon in Denmark (Canfield, 2001b). Thus, isotope fractionation during sulfate reduction is not uniquely determined by SRR, but is site and season specific. Possible factors affecting the ε *versus* SRR relationship include the community structure and abundance of sulfate reducers, and the nature and accessibility of organic substrates. The data imply that small ranges in sulfur isotope fractionation (ε ≤ 15 ‰) observed in the environment may be indicative of biogenic processes, reflecting high sulfate reducing activity.

2.1 Introduction

Sulfur isotopes have been used as tracers of sources, mixing processes and transformations of sulfur compounds in a variety of modern environments, including soils, sediments, ground waters, rivers, estuaries, oceans and acid mine drainage areas (e.g. Brüchert and Pratt, 1999; Mandernack et al., 2003; Böttcher et al., 2004; Knöller et al., 2004; Vokal-Nemec et al., 2006; Gu et al., 2008). Stable sulfur isotopes in sedimentary rocks from the early Archean onwards have also yielded essential constraints on palaeo-environmental conditions in ancient

oceans (Habicht et al., 2002; Canfield, 2004; Johnston et al., 2006; Johnston et al., 2008b), the emergence and development of sulfur-based metabolisms (Shen et al., 2001; Farquhar and Wing, 2003b; Strauss, 2003; Shen and Buick, 2004; Philippot et al., 2007; Johnston et al., 2008a) and the evolution of atmospheric oxygen (Kasting, 2001; Pavlov and Kasting, 2002; Farquhar and Wing, 2003a; Kaufman et al., 2007).

Sulfur isotope fractionation can result from either biotic or abiotic processes, but microbial activity has been shown to be the dominant mechanism for fractionation in low temperature sedimentary and diagenetic environments (< 200°C) (Ohmoto and Goldhaber, 1997; Canfield and Raiswell, 1999; Newton and Bottrell, 2007). Sulfate reducing prokaryotes (SRP) gain energy for growth and maintenance under anaerobic conditions by reducing sulfate to sulfide during dissimilatory sulfate reduction (Widdel, 1988). Because of their ability to use both organic (e.g. acetate, lactate, ethanol) and inorganic (e.g. H_2 or CO) electron donors, and their adaptation to a wide range of environmental conditions, sulfate reducing microorganisms are found in many environmental settings, including littoral sediments (Isaksen et al., 1994; Pallud and Van Cappellen, 2006), saline lakes (Brandt et al., 2001; Scholten et al., 2005), subareal and submarine hydrothermal vents (Tor et al., 2003; Roychoudhury, 2004; Amend and Teske, 2005), freshwater sediments (Bak and Pfennig, 1991) and anthropogenically polluted environments (Kleikemper et al., 2002; Roychoudhury and Mccormick, 2006).

As sulfide produced by dissimilatory sulfate reduction is preferentially enriched in lighter ^{32}S, sulfur isotope fractionation in ancient sedimentary rocks should in principle provide information about past sulfate reducing activity (Shen et al., 2001; Canfield, 2001a). The interpretation of sulfur isotope signals, however, is complicated by environment-specific effects on fractionation due, for instance, to variations in temperature, pore water chemistry, substrate and nutrient availability, degree of anoxia, and the composition of the active sulfate reducing microbial community (Brüchert, 2004).

Numerous laboratory studies have been carried out to investigate sulfur isotope fractionation effects (ε) during microbial sulfate reduction, with most reported ε values, calculated from $\delta^{34}S$ differences between sulfate and sulfide, falling in the range -3 to 47 ‰ (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Chambers et al., 1975; Habicht and Canfield, 1997; Detmers et al., 2001; Canfield, 2001b; Brüchert et al., 2001; Brüchert, 2004). Early results with pure cultures of sulfate reducing prokaryotes showed an inverse correlation between the extent of isotope fractionation and the rate of sulfate reduction (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968), opening up the possibility that variations in ε could be used as a tracer of sulfate reducing activity. The observed range of experimental ε values, and the inverse relationship with the sulfate reduction rate, also led to the development of a conceptual model for isotope flow during dissimilatory sulfate reduction (Rees, 1973).

Several aspects of the original, or standard, Rees model for isotope fractionation during enzymatic sulfate reduction have been brought into question. First, more extreme variations in isotope fractionation, up to and exceeding 100 ‰, have been measured in sediment pore fluids (Rudnicki et al., 2001). Second, the inverse relationship between sulfur isotope fractionation and the rate of sulfate reduction in pure cultures was found to vary or be

completely absent when comparing one strain of sulfate reducer to another (Detmers et al., 2001; Canfield et al., 2006; Hoek et al., 2006; Mangalo et al., 2007). More recent studies considering the variations in all four stable sulfur isotopes by sulfate reducing and sulfur disproportionating microorganisms have provided new insights into the various enzymatic pathways and branching points involved in the flow of sulfur through the cells (Farquhar et al., 2003; Johnston et al., 2005; Farquhar et al., 2007; Johnston et al., 2007). These studies have resulted in modifications of the standard model, thereby allowing for values of ε in excess of 47 ‰ (Brunner and Bernasconi, 2005).

While many pure culture studies have been carried out, far fewer coupled measurements of sulfate reduction rates and sulfur isotope fractionation are available for natural sulfate reducing communities. In this chapter, I present such coupled measurements using flow-through reactors containing intact slices of intertidal estuarine sediments collected at a variety of depth intervals, distances from a vegetated salt marsh, and at various times throughout the year from the same brackish water location within the Schelde Estuary in The Netherlands. This flow-through reactor approach has been used previously to investigate microbial reaction kinetics in sediments (Roychoudhury et al., 1998; Brüchert and Arnosti, 2003; Roychoudhury et al., 2003; Weston and Joye, 2005; Laverman et al., 2006; Pallud and Van Cappellen, 2006; Abell et al., 2009), but also to establish the link between sulfate reducing activity and sulfur isotope fractionation effects (Canfield et al., 2000; Canfield, 2001b; Habicht et al., 2002; Farquhar et al., 2008). However, little is known about the spatio-temporal variations in sulfate reduction and coupled isotope fractionation for a single sampling site. The flow-through reactor approach avoids the build-up of dissolved reaction products in the reactor, as well as artifacts resulting from the disruption of the sediment structure in slurry incubations (Pallud and Van Cappellen, 2006). The resulting reaction parameters, including isotope fractionation, should therefore closely approach the corresponding *in situ* values (Pallud et al., 2007).

2.2 Sampling and experimental methodology

2.2.1 Sample selection and collection

Sediments were sampled in February, May and October 2006 from a brackish site located in the Schelde Estuary (51°24'04"N 04°07'04"E), close to the village of Waarde in The Netherlands (see Hyacinthe and Van Cappellen (2004) and Pallud and Van Cappellen (2006) for more specific details on the sampling site, pore water and sediment characteristics). Different depth intervals (0-2, 4-6 and 8-10 cm) were sampled at four locations along a 30 m transect from a mud-flat adjacent to the salt marsh, into the non-vegetated tidal flat of the estuary (Table 2.1). Most samples were collected in duplicate or triplicate (Table 2.1).

Intact sediment slices (2 cm thickness, 4.2 cm diameter) corresponding to different depth intervals were sampled directly into the Perspex reactor cells using a steel shuttle corer. Reactors were sealed with 0.2 μm pore size nitrocellulose filters and glass fiber filters at each end, to avoid sediment and bacterial outflow and to support radial flow in the reactor cell, respectively. Reactors were closed using O-rings and plastic caps which contained the

inflow and outflow channels. The sealed reactors were transported in anaerobic bags to the laboratory and were stored at 4°C for up to several days before starting the flow-through reactor experiments.

2.2.2 Flow-through reactor experiments

A detailed description of the flow-through reactor technique is given in Roychoudhury et al. (1998), Laverman et al. (2006), Pallud and Van Cappellen (2006) and Pallud et al. (2007). Inflow solutions consisted of deionized water containing 2 mM Na_2SO_4 and 180 mM NaCl, yielding sulfate and salt concentrations comparable to those at the sampling site (Pallud and Van Cappellen, 2006). The chosen sulfate concentration was in excess of the apparent sulfate half-saturation concentration, K_m, previously estimated to be in the range 0.37 to 0.87 mM (Pallud and Van Cappellen, 2006), in order to avoid anomalous isotopic effects associated with sulfate limitation (Kampara et al., 2008; Thullner et al., 2008). Bromide (2 mM NaBr) was used as a tracer to monitor fluid flow-through each reactor. Inflow solutions and tubing were purged with Argon before and during the experiments to maintain anaerobic conditions. The inflow solution was supplied using a peristaltic pump with a continuous flow rate of 1.0 ± 0.1 ml/h. Reactors and inflow solutions were kept in the dark during experimentation.

A range of incubation temperatures between 10 and 50°C was achieved using thermostatic water baths. In the February 2006 experiment, temperature was varied between reactors so that replicates were maintained at different but constant temperatures (10, 20, 30 and 50°C). In the May 2006 experiment, each reactor was successively exposed to different temperatures (10 → 20 → 30 → 50°C). Temperature was gradually increased, 5°C per day, only after outflow sulfate concentration had remained at steady state for at least 3 days.

Before starting the experiments, reactors were flushed for 24 h with 180 mM NaCl solution (corresponding to approximately 1.5 reactor pore space volumes) to remove the sampling site pore water remaining in the sediment. Outflow samples were collected every 2 h for the first 24 h of the experiment to obtain detailed breakthrough Br data. For the remainder of the experiment, outflow samples were collected every 12 h. One sample per 24 h sampling period was used for chemical and isotopic analysis and the other was stored at -18°C. All collection tubes were pre-filled with 2 ml zinc acetate (10 %) to trap sulfide as ZnS.

Effects of seasonal variations at the sampling site on sulfate reduction rates and isotope fractionation were assessed using a series of duplicate reactors containing sediment collected in February, May and October 2006 from the 0-2 cm depth interval at a distance of 30 m from the salt marsh (Table 2.1). These reactors were incubated at 20°C with remaining experimental conditions identical to the other experiments. Effects of sampling location were investigated for the 4-6 cm depth interval in February 2006 using reactors sampled 1, 10 and 20 m from the salt marsh (Table 2.1). These reactors were incubated at 20°C with remaining experimental conditions identical to the other reactors. Blank experiments were run using duplicate reactors sterilized with gamma radiation (25 kGy). Following irradiation, the reactors were stored at 4°C for 2 months to ensure the absence of enzymatic activity before starting the experiment. Irradiated reactors were run under similar experimental conditions to the other 20°C reactors.

2.2.3 Chemical analysis

SO_4^{2-} and Br^- concentrations were determined in the inflow and outflow solutions by ion chromatography (Dionex DX120 equipped with an AS14 column). The detection limit was < 5 μM with a mean precision of approximately 4 %. Sulfur isotope fractionation was measured in the same samples. Sulfate in the collection tubes was precipitated as $BaSO_4$ with $BaCl_2$ solution (10 %), rinsed with deionized water and dried for several days at 50˚C. $\delta^{34}S$ was measured using an elemental analyzer Na 1500NCS coupled to a Finnigan MAT (Delta +) gas source mass spectrometer. $BaSO_4$ was converted to SO_2 by flash combustion in a tungstic oxide, ultra pure copper quartz tube at 1050˚C (mean precision of approximately 0.5 ‰).

Sedimentary sulfide $\delta^{34}S$ was measured on sediments collected in May 2006 at 1 m and 30 m from the salt marsh for all depth intervals (0-2, 4-6 and 8-10 cm). Samples were taken next to the sediment cores which were used for the flow-through reactor experiments (Table 2.1). Approximately 2 g freeze dried sediment was distilled using a chromium reduction method to isolate the reduced sulfur compounds (Canfield et al., 1986; Fossing and Jørgensen, 1989). Sulfide produced during distillation was trapped as Ag_2S and analyzed using the techniques described above.

2.2.4 Sulfate reduction rates and isotope fractionation

Steady state potential sulfate reduction rates (SRRs) were calculated using equation 1:

$$SRR = \frac{\Delta C * Q}{V} \tag{1}$$

where Q represents the flow rate of the solution through the reactor in ml h^{-1}, ΔC is the difference between inflow (C_0) and outflow (C) sulfate concentration in mM and V is the volume of the sediment in cm^3, which for our reactor was 27.7 cm^3. A three-way analysis of variance (ANOVA) was performed for all steady state sulfate reduction rates to statistically investigate the effect of incubation temperature, sampling depth, and proximity to the salt marsh at the sampling site on SRR using the Sigmastat software package.

Isotope fractionation, expressed in isotope fractionation effects (ε), were derived using the Rayleigh distillation model assuming that laminar flow in the reactor approximated closed system behavior (Canfield, 2001b; Fry, 2006). Isotope fractionation effects (ε), approximately equal to the difference in fractionation between outflow sulfate and sulfide, were calculated from the fractionation factor (ε) and measured $\delta^{34}S$ values using equations 2 and 3:

$$\alpha = 1 + \frac{\left(\ln \delta_{SO4in} + 1000\right) - \left(\ln \delta_{SO4out} + 1000\right)}{\ln(f_{SO4})} \tag{2}$$

and

$$\varepsilon = 1000(\alpha - 1) \tag{3}$$

where $\delta_{SO4\text{-}in}$ represents the isotopic composition of the inflow solution, $\delta_{SO4\text{-}out}$ is the isotopic composition of the outflow solution, and f_{SO4} is the fraction of sulfate remaining in the outflow solution compared to the inflow solution,

$$f\,SO4 = \frac{[SO_{4out}]}{[SO_{4in}]} \tag{4}$$

The accurate determination of ε requires sufficient consumption of substrate in order to generate $\delta^{34}S$ values that are outside analytical error of $\delta^{34}S$ for the inflow sulfate. Excessive consumption of sulfate can also lead to inaccuracy since fractionation may be reduced at low substrate concentration. To take account of these problems ε was determined when *f* was between 10 and 90 %.

Equations (1), (2) and (3) could only be applied when outflow sulfate concentration was constant, implying that the system was thriving under non changing conditions. Therefore all sulfate reduction rates and isotope fractionation data presented in the results and discussion were obtained from these periods at (or near) steady state. In our experiments steady state was defined as the first time interval, after applying a new experimental parameter, where at least 3 measurements (across three days) showed a constant outflow sulfate concentration within a maximum error of approximately 10 %. Our measured sulfate reduction rates should be considered as potential rates since sulfate was the only electron acceptor supplied to the reactors, resulting in a probable, but small, overestimation compared with site representative values (Pallud and Van Cappellen, 2006).

2.3 Results

2.3.1 Reactor hydrodynamics and abiotic controls

Measured bromide breakthrough curves, i.e. outflow Br concentrations (C) normalized to the inflow Br concentration (C_0) plotted *versus* time (Figure 2.1), were in good agreement with theoretical curves predicted by a one-dimensional advective-dispersive model for a finite, radially homogeneous porous medium (Pallud and Van Cappellen, 2006; Pallud et al., 2007), with the exception of 5 out of the 30 reactor experiments. Results of these 5 flow-through reactors were not used in further data interpretation. Longitudinal dispersion coefficients (D) and pore water velocities (v) derived from the model fits fell in the ranges 0.017 to 0.14 $cm^2\ h^{-1}$ and 0.039 to 0.17 cm h^{-1}, respectively.

Sulfate concentrations in the outflow of the blank, gamma-irradiated, reactor experiments were indistinguishable from the inflow concentrations, implying the absence of sulfate reducing activity. The measured $\delta^{34}S$ of sulfate in inflow and outflow solutions were also identical, indicating the absence of abiotic sulfur isotope exchange between the inflow solution and pre-existing phases in the sediments.

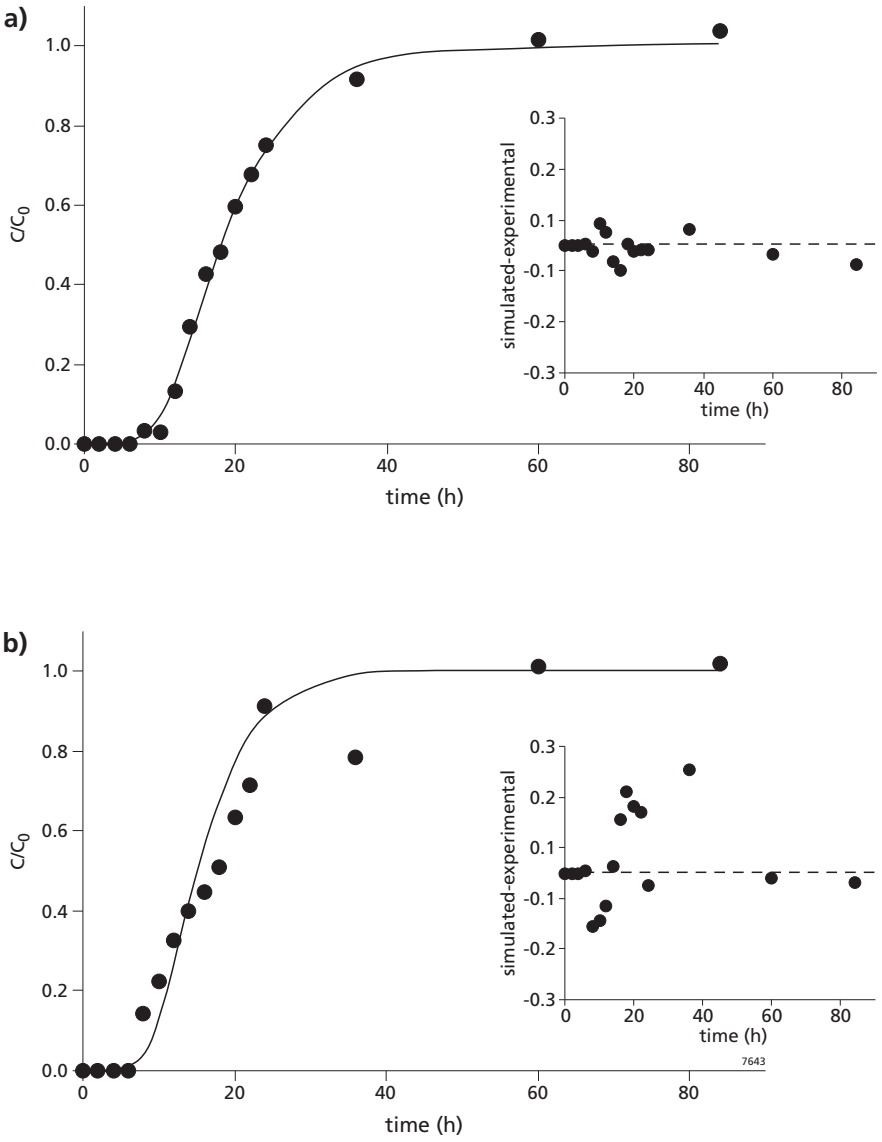

Figure 2.1: Comparison of simulated (line) and experimental (black circles) bromide breakthrough curves, with bromide concentrations (C) normalized to inflow concentrations (C_0). Insets indicate differences between simulated and experimental C/C_0. Panel 2.1a shows an example of data considered as acceptable, with a dispersion coefficient (D) of 0.019 $cm^2\ h^{-1}$ and a pore water velocity (ν) of 0.10 cm h^{-1}. Panel 2.1b shows an example of data considered not acceptable (D = 0.018 $cm^2\ h^{-1}$, ν = 0.12 cm h^{-1}).

2.3.2 Potential sulfate reduction rates

Steady state potential sulfate reduction rates (SRRs) ranged from 5 to 49 nmol cm^{-3} h^{-1} (Table 2.1). The rates listed in Table 2.1 are averages of rate determinations for duplicate or triplicate reactors, where available, that were run under identical experimental conditions. The corresponding relative standard deviations of the SRRs ranged from 1 to 30 %. It took typically 3 to 9 days for the outflow sulfate concentration to stabilize after a change in conditions, e.g., a change in temperature.

SRRs measured in the 0-2 cm interval reactors of February and May exhibited the same systematic response to temperature (Figure 2.2), despite the fact that a slightly different approach was used for comparing this parameter in the February and May experiments (see experimental section). The temperature dependence of SRR was also similar for the May reactors collected at the locations furthest (30 m) and closest (1 m) to the edge of the salt marsh (Figures 2.2a and 2.2b). For any given depth interval, the lowest SRR was typically measured at 10˚C and the highest at 30˚C. Increasing the temperature to 50˚C caused SRR to decrease to values similar to, or smaller than those measured at 20˚C on the same sediment slice.

Under optimum temperature conditions (20-30˚C), SSRs were highest in the top layer of sediment (0-2 cm) and generally decreased with increasing depth (Table 2.1 and Figure 2.2). Sulfate reducing activity was most sensitive to temperature in the 0-2 cm depth interval sediment slices. The difference between maximum and minimum SRRs decreased systematically with depth (Figure 2.2). Hence, it was not possibly to uniquely define an activation energy or Q_{10} value of sulfate reduction for the entire set of variable temperature experiments carried out.

A three-way ANOVA analysis was performed for all steady state SRRs to statistically investigate the effect of incubation temperature, sampling depth, time of the year when sampling took place and location with respect to the edge of the salt marsh. The analysis confirmed that incubation temperature ($P < 0.001$), sampling depth ($P < 0.001$) and sampling season ($P < 0.001$) had significant effects on SRRs, whereas location had no significant effect ($P = 1.00$).

2.3.3 Sulfur isotope fractionation effects

Sulfur isotope fractionation effects (ε), calculated using equations 2 and 3, ranged from 9 to 34‰ (Table 2.1). Note that only sulfate $\delta^{34}S$ was used to calculate ε. Relative errors on individual ε values were calculated using standard error propagation methods, and were typically on the order of approximately 12 % RSD.

Frequency distributions of ε values obtained at the four incubation temperatures are shown in Figure 2.3. Each panel combines data from February, May and October reactors and shows the May data separately. For 20 and 30˚C (Figures 2.3a and 2.3b), the distributions exhibit a well-defined maximum, less so at 10 and 50˚C (Figures 2.3c and 2.3d). This is also the case when considering only the May data, which represent the bulk of the data (13 out of 25 reactors). The mean ε values for the entire data set were 19, 18, 15 and 15 ‰ at 10, 20, 30 and 50˚C, respectively.

Table 2.1: Overview of samples collected and experiments performed in February, May and October 2006 showing average sulfate reduction rates (SRR) and corresponding isotope fractionation effects (ε). All data were produced under steady state conditions. Errors are reported for agreement between multiple reactors run under identical conditions, where appropriate. Where no replicates were made, errors represent agreement between measurements made within the steady state area of one reactor, typically for 3-5 data points.

Sampling Time	Temp. (°C)	Depth (cm)	Location distance from the salt marsh	# reactors	SRR (nmol $cm^{-3}h^{-1}$)	sd (nmol $cm^{-3}h^{-1}$)	ε (‰)	sd (‰)	Relevant Figures
February 2006	10	0-2	30 m	1	7	1	22	3	Figs 2.2, 2.3, 2.5
	20			1	24	1	17	1	
	30			3	43	2	17	2	
	50			2	26	2	13	2	
February 2006	20	4-6	1 m	1	43	4	18	3	Figs 2.3, 2.5
			10 m	1	10.2	0.3	30	5	
			20 m	1	11.0	0.6	34	5	
May 2006	10	0-2	30 m	2	11	1	20	3	Figs 2.2, 2.3, 2.4, 2.5, 2.6
	20			2	36	3	14.6	0.8	
	30			2	41	2	12.6	0.9	
	50			2	14	2	12.6	0.9	
May 2006	10	0-2	1 m	2	16	1	15	2	Figs 2.2, 2.3, 2.4, 2.5, 2.6
	20			2	43	3	12	2	
	30			2	49	2	10.7	0.6	
	50			2	12	4	19	6	
May 2006	10	4-6	30 m	1	9.2	0.8	15	1	Figs 2.2, 2.3, 2.4, 2.5
	20			2	15	6	20	4	
	30			2	18	1	17	1	
	50			2	18	3	13	2	
May 2006	10	4-6	1 m	2	8	1	21	6	Figs 2.2, 2.3, 2.4, 2.5
	20			2	22	3	16	4	
	30			2	34	1	12	1	
	50			2	24	2	12	2	
May 2006	10	8-10	30 m	1	4.6	0.3	22	3	Figs 2.2, 2.3, 2.4, 2.5
	20			1	12	1	21	2	
	30			1	18.5	0.4	15.7	0.8	
	50			1	11,4	0,9	18	5	
May 2006	10	8-10	1 m	0	no steady state data				Figs 2.2, 2.3, 2.4, 2.5
	20			2	13	6	19	3	
	30			2	22	5	18	4	
	50			1	13.6	0.7	9	4	
May 2006	20	0-2	30 m	2	45	1	16	2	Figs 2.3, 2.5
October 2006	20	0-2	30 m	2	35	3	15	1	Figs 2.3, 2.5

Isotope fractionation generally increased with depth in the sediment. This is illustrated in Figure 2.4a, where average ε values are plotted *versus* sampling depth for the May reactors collected 1 and 30 m away from the salt marsh edge. The large error bars are due to the fact that data from the four incubation temperatures (10, 20, 30 and 50˚C) were averaged together. As Figures 2.4a and 2.4b show, the increasing trend of ε with depth tracked a decrease in SSR. No systematic effect of sampling location on ε values was observed.

Isotope fractionation correlated negatively with SRR (Figure 2.5). Data collected for all the May reactors at 20 and 30˚C exhibited a single, near-linear ε *versus* SRR trend. The fractionation measured in the 20˚C October reactor experiment fell on the same trend. In contrast, the February reactors run at 20 and 30˚C, yielded ε values exceeding those measured in May at comparable SRRs. The 10 and 50˚C reactors defined a somewhat steeper inverse relationship between ε and SRR, at the lower end of SRRs.

Sedimentary sulfide, extracted from sediments collected in May 2006, gave $\delta^{34}S$ in the range of -15 to -20 ‰. There was no systematic variation in isotope fractionation observed in sediments collected at the different depth intervals. Average isotope fractionation of sediments sampled 1 m from the salt marsh showed $\delta^{34}S$ values which were approximately 2 ‰ lighter compared to the 30 m location.

2.4 Discussion

2.4.1 Sulfur isotope fractionation effects

Sulfur isotope fractionation effects (ε) during microbial sulfate reduction in brackish estuarine sediments were studied using an experimental flow-through reactor approach designed to preserve the original physical, geochemical and microbial structure of the sediment (Pallud et al., 2007). The work builds on an earlier detailed study of the kinetics of sulfate reduction in sediments from the same site (Pallud and Van Cappellen, 2006). As no external electron acceptor other than sulfate was supplied to the reactors, sulfate reduction to sulfide, coupled to the oxidation of naturally-occurring electron donors, was the predominant respiratory process taking place in the reactors. The experimental approach minimizes isotope effects due to sulfide reoxidation and sulfur disproportionation reactions. Abiotic controls with sterilized sediment further confirm that the observed isotope fractionation was due to the metabolic activity of microorganisms inhabiting the sediment.

By sampling various intertidal locations near the salt marsh, at different depths and different times of the year, and imposing a range of incubation temperatures, sulfur isotope fractionation was measured over a relatively large span of potential sulfate reduction rates (SRRs). Only SRRs and ε values corresponding to steady state conditions are included in the analysis. In the following sections, the results are compared to predictions of existing metabolic fractionation models, and to isotope fractionation obtained using the same experimental approach on sediments from a marine lagoon site in Denmark (Canfield, 2001b).

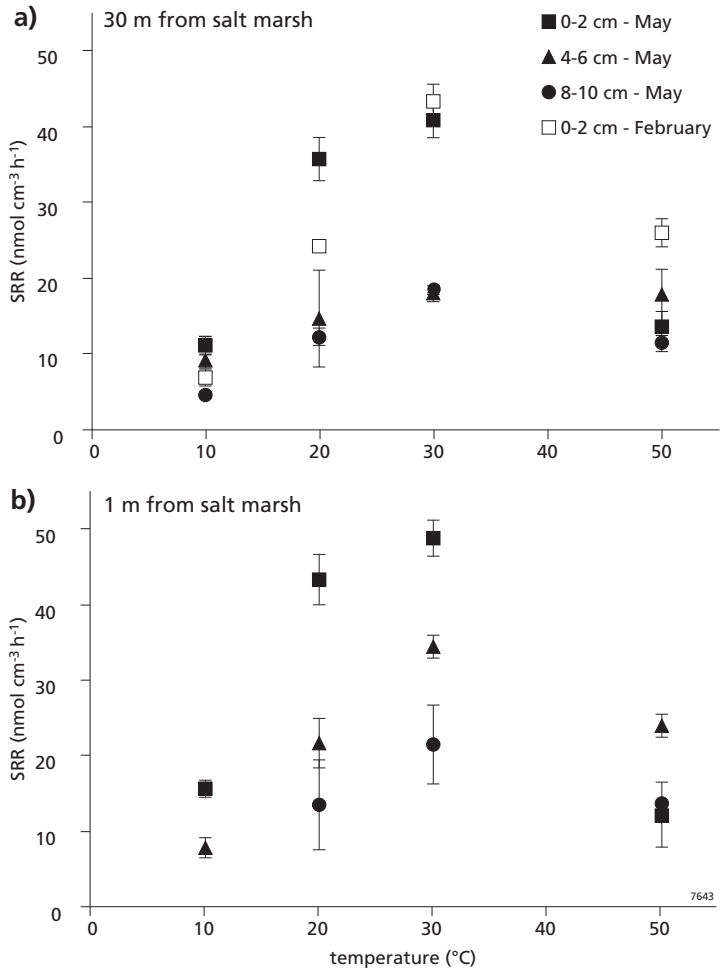

Figure 2.2: Effect of temperature on steady state potential sulfate reduction rates (SRR) measured with flow-through reactor experiments for different sediment depth intervals (squares: 0-2 cm, triangles: 4-6 cm and circles: 8-10 cm) sampled 30 m from the salt marsh in May (black symbols) and February (white symbols) 2006 (Panel 2.2a) and 1 m from the salt marsh in May 2006 (Panel 2.2b). Error bars represent the standard deviation calculated from replicate reactors, where available (Table 2.1), with a minimum of 3 measurements per reactor or error within a single reactor when no replicates were measured. When not visible, the size of the y-error bars fall within the size of the symbols.

The SRRs follow a temperature trend that would be expected for mesophilic micro-organisms, with the highest rates measured at 30°C and the lowest at 10 and 50°C. This trend is explained by a combination of 1) the physiological response of individual strains of sulfate reducing prokaryotes (SRP) to temperature, which affects metabolic rates such as

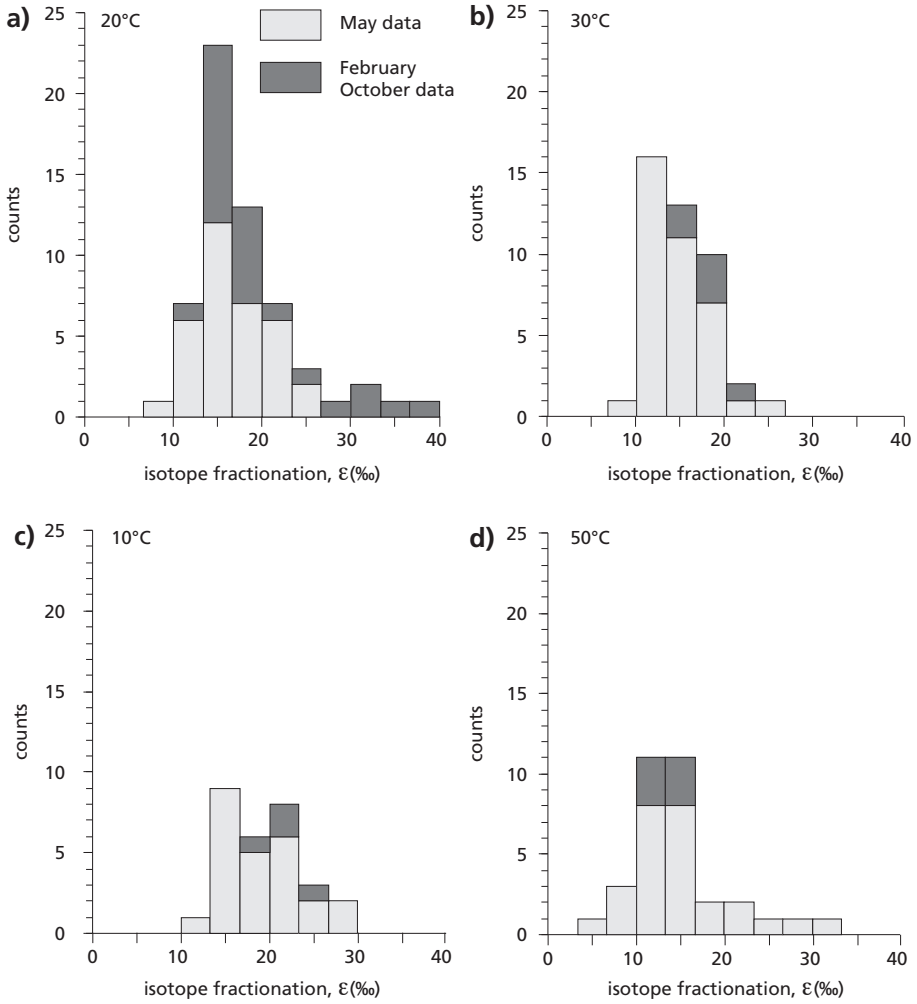

Figure 2.3: Distribution plots of isotope fractionation effects (ε) *versus* the number of samples analyzed for incubations at 20°C (Panel 2.3a), 30°C (Panel 2.3b), 10°C (Panel 2.3c) and 50°C (Panel 2.3d). Plots contain data from February, May and October 2006 (grey and black) and only May data (grey). These plots were made using the individual data points produced under steady state conditions.

reduction of cellular sulfite to sulfide, or the transport of substrates and nutrients through cell membranes (Brüchert et al., 2001; Canfield, 2001b; Rabus et al., 2002), 2) a change in (labile) electron donor supply, either released from the sediment or due to a temperature-dependent shift in the activity of fermenting microorganisms (Macdonald et al., 1995; Zogg et al., 1997; Andrews et al., 2000), and 3) the variable composition of the active part of the sulfate reducing microbial community as the growth *versus* temperature behavior is strain

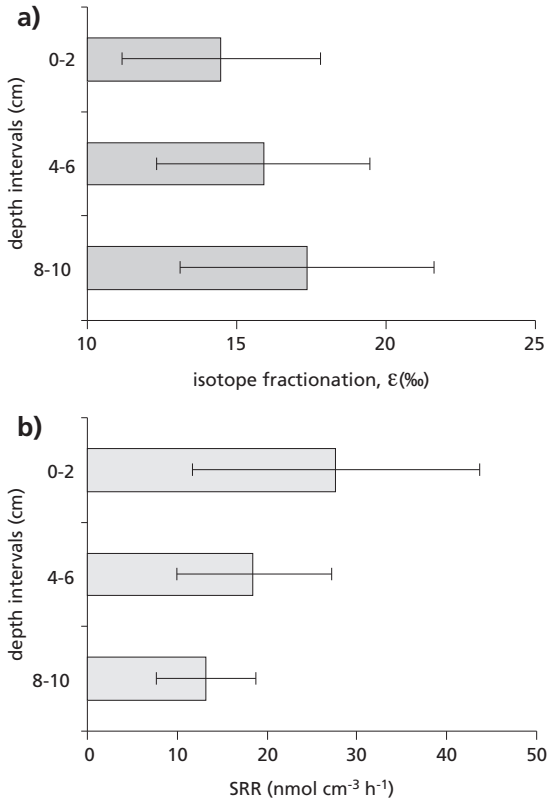

Figure 2.4: Isotope fractionation effects (ε) (Panel 2.4a) and steady state potential sulfate reduction rates (SRR) (Panel 2.4b) averaged for all temperatures selected by depth intervals (0-2, 4-6 and 8-10 cm) obtained for tidal flat sediments sampled in May 2006.

specific (Nedwell and Floodgate, 1971; Detmers et al., 2001). The transient times observed after switching to a new temperature are similar to those observed in previous studies with intertidal sediments (Nedwell and Floodgate, 1971; Canfield, 2001b). The results further indicate that an active community of sulfate reducers was present in all sediment samples and was able to respond immediately to changes in temperature.

Whereas the entire data set confirms the existence of a general inverse relationship between ε and SRR, a detailed comparison of the results from individual reactors also illustrates the natural variability of the relationship, even within a single environmental setting (Figure 2.5). Particularly at lower SRRs (≤ 15 nmol cm^{-3} h^{-1}), ε values tend to exhibit significant scatter (compare error bars on Figure 2.5). At 10°C, differences in ε of up to 30 % were observed between parallel reactors run under identical conditions. The spread in ε values among these reactors is real and not caused by analytical error. The correlation between ε and SRR is also weaker for the deeper sediment intervals sampled, where sulfate reduction rates were lower, most likely because of a drop in availability of organic substrates with depth in the sediment

(Pallud and Van Cappellen, 2006). A similar increasing spread of ε with decreasing SRR has been reported in other laboratory studies (see Habicht and Canfield (1997) and references therein).

The ε *versus* SRR relationship also appears to depend on the time of sampling during the year (Figure 2.5). Although the size of the February data set is limited, there is an offset to higher ε values, compared to the May and October data. Possibly, this offset was due to variations in the nature and supply of organic substrates (Brüchert et al., 2001; Canfield, 2001b), or in the composition and size of the active fraction of the sulfate reducing microbial community (Detmers et al., 2001). However, experimental artifacts may have also played a role, as it took longer to achieve steady state in the February than in the May experiments. In fact, for several February reactors steady state was not reached at all, and the corresponding data were rejected.

Although the isotope fractionation effects, derived from the $\delta^{34}S$ values of aqueous sulfate, show a relatively broad range in the experiments (9 to 34 ‰), most *in situ* sulfate reduction activity is likely restricted to the period from late spring to early fall, when temperatures in the field (12 to 23˚C) are closest to the optimum temperature (Figure 2.2). Thus, from among the entire data set, the ε values obtained for the May and October sediments at 20˚C are expected be the most representative of the *in situ* isotope fractionation due to sulfate reduction to sulfide at the site. The corresponding average ε value is 17 ± 3 ‰. A limited effective range of ε is consistent with the narrow range in $\delta^{34}S$ values determined on the extracted whole sediment pyrite fraction (-15 to -20 ‰). However, as is generally observed, the measured ε values in the flow-through reactor experiments and the $\delta^{34}S$ values of the sedimentary sulfides also imply that redox processes in addition to the microbial reduction of sulfate to sulfide are needed to explain the isotopic composition of early diagenetic pyrite (Goldhaber, 2003). The location water sulfate has a $\delta^{34}S$ value of approximately 20 ‰ suggesting ε values of approximately 40 ‰ which are a factor of two larger than the fractionation effects obtained in our experiments.

2.4.2 Isotope fractionation models

Microbial sulfate reduction can be divided into four steps: 1) the uptake of sulfate into the cell (Cypionka, 1995), 2) the reaction of sulfate with adenosine-5'-triphosphate (ATP) to form adenosine-3'-phosphate-5'-phosphosulfate (APS), 3) the reduction of APS to sulfite, and 4) the reduction of sulfite to sulfide with subsequent export from the cell (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Rees, 1973). Steps 1, 2 and 3 are reversible, while step 4 is believed to be irreversible, although reversibility has also been suggested (Brunner and Bernasconi, 2005). Large variations in ε can be produced within steps 3 (up to 25 ‰) and 4 (up to 25 ‰) whereas little or no fractionation is associated with steps 1 (up to -3 ‰) and 2 (0 ‰) (Rees, 1973). According to this standard model outlined by Rees (1973) based on experimental work by Harrison and Thode, 1958, Kaplan and Rittenberg, 1964 and Kemp and Thode, 1968, at low rates of sulfate reduction all backward and forward reactions are close to equilibrium, resulting in large isotope fractionation. As rate increases, cell sulfate demand increases, intermediate reactions become increasingly irreversible, exchange between internal sulfur pools is minimized, and transport of sulfate across the cell membrane ultimately

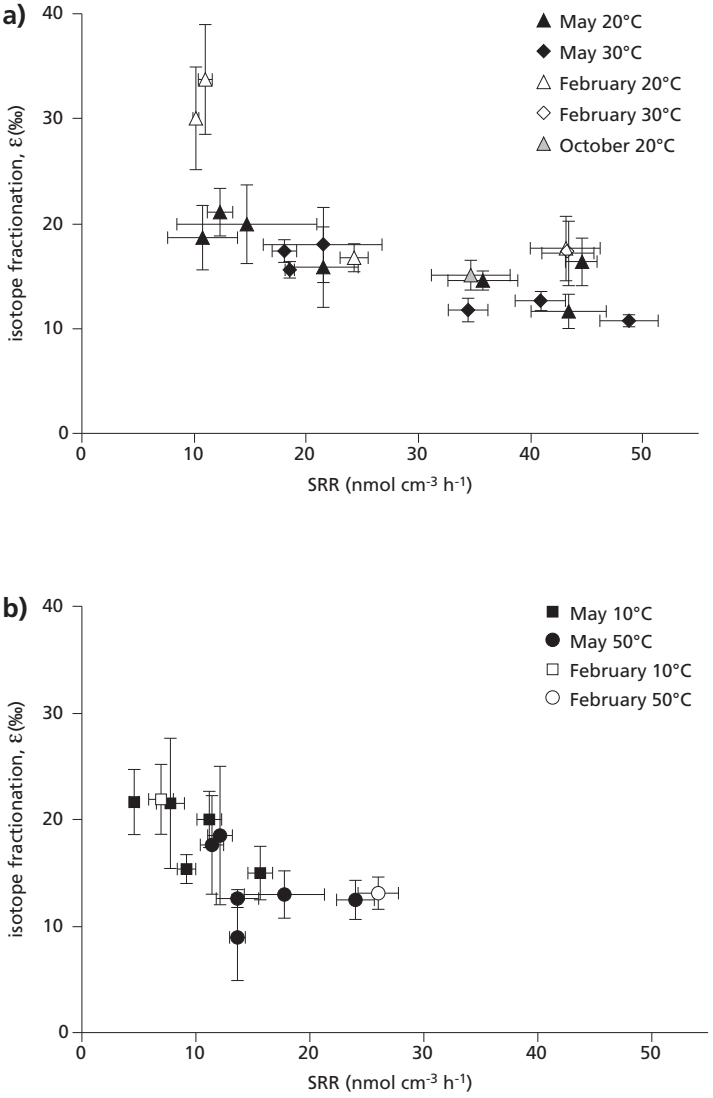

Figure 2.5: Relationship between isotope fractionation effects (ε) and steady state potential sulfate reduction rates (SRR). In Panel 2.5a, ε and SRR were measured at 20˚C (triangles) and 30˚C (diamonds) in February (white symbols), May (black symbols) and October (gray symbols) 2006 and in Panel 2.5b, ε and SRR were measured at 10˚C (squares) and 50˚C (circles) in February and May 2006. Error bars represent 1 standard deviation calculated from replicate reactors or from steady state areas within reactors where no replicates were made (Table 2.1).

becomes the rate limiting step, resulting in a decrease in fractionation. In general, the further along the reduction process the rate determining step is, the larger the expected fractionation (Rees, 1973; Brunner and Bernasconi, 2005). The standard model thus implies that the physiology of the cell, which is controlled by environmental parameters such as temperature and organic substrate availability, regulates the rate of sulfate reduction and the associated isotope fractionation in a predictable way. When the system is in complete equilibrium, the maximum fractionation should be about 47 ‰.

Most studies have yielded ε values less than or equal to 47 ‰, although more recent field observations show that sulfide formed as a result of microbial sulfate reduction has corresponding ε values exceeding 47 ‰ (Rudnicki et al., 2001; Wortmann et al., 2001). Brunner and Bernasconi (2005) therefore proposed a revised model in which the reduction of sulfite to sulfide proceeds not in a single step but in a series of reversible steps called the trithionate pathway. This more complex reaction network can potentially result in fractionation of up to 70 ‰.

Pure culture (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Chambers et al., 1975) and natural sediment studies (Habicht and Canfield, 1997; Canfield, 2001b; this study) have shown systematic inverse correlations between SRR and ε, in accordance with the standard model. However, other studies found that this relationship was either absent (Detmers et al., 2001; Brüchert et al., 2001; Mangalo et al., 2007) or more complex than expected from the standard fractionation model (Canfield, 2001b; Canfield et al., 2006; Hoek et al., 2006). For example, Canfield, 2001b measured small isotope fractionation at low temperatures and correspondingly low SRR. The low observed fractionation was explained by a reduction in the fluidity of the cell membrane, thereby rendering transport across the cell membrane rate limiting. Furthermore, Canfield et al. (2006) and Hoek et al. (2006) reported a positive relationship between rate and fractionation at low and high temperatures. These authors proposed a new model, built on the model introduced by Farquhar et al. (2003) and Johnston et al. (2005), in which they consider variations in mass flow and associated fractionation at two branching points: 1) transport of sulfate in and out the cell, and 2) sulfur exchange between the different internal sulfur pools. The magnitude and balance between these branching points at different temperatures could vary among different microorganisms leading to variable responses for different pure cultures or natural communities of sulfate reducing prokaryotes (Johnston et al., 2007).

Taken together, the data of the Schelde estuarine sediments are consistent with the standard model of Rees (1973). The measured values of ε of 9 to 34 ‰, fall within the permissible range of the standard model, and they correlate inversely with SRR (Rees, 1973). Though no evidence was found for the larger isotope fractionation suggested by the modified model of Brunner and Bernasconi (2005), this model cannot be excluded. To test which model is most suitable, additional information on $\Delta^{33}S$ and $\Delta^{36}S$ values produced during the experiments would be needed (Farquhar et al., 2008). A systematic decrease in fractionation at the lowest temperature (10˚C), which could indicate an effect of reduced fluidity of the cell membrane, was not clearly observed either.

2.4.3 Comparison with isotope fractionation in a Danish coastal sediment

Flow-through reactor experiments similar to the ones described here have been carried out with a small number of sediments sampled in a semi-enclosed marine lagoon at the northern tip of the Island of Fyn in Denmark (Canfield, 2001b; Farquhar et al., 2008). When no external electron donor was added, these experiments yielded comparable volume-based sulfate reduction rates as obtained in the present study of 2 to 38 nmol cm^{-3} h^{-1} (Canfield, 2001b) and 3 to 15 nmol cm^{-3} h^{-1} (Farquhar et al., 2008). Isotope fractionation effects, however, were substantially larger, ranging from 19 to 40 ‰ (Canfield, 2001b**)** and 37 to 45 ‰ (Farquhar et al., 2008).

The relationship between ε and SRR obtained by Canfield (2001b) with a single reactor, containing 0-2 cm depth interval sediment, is shown in Figure 2.6. The data points included are those in which the resident sulfate reducing prokaryotes utilize the naturally occurring electron donors present in the sediment (i.e., no external electron donor was supplied via the inflow solution). For comparison, the results from the multiple May reactors run at 20 and 30°C are also plotted. The figure implies that the ε *versus* SRR relationship is site-specific, and may reflect differences in the nature and availability of organic matter, or in the structure and abundance of the sulfate reducing community, between the two sites (Detmers et al., 2001). Similar considerations also apply to the differences in isotope fractionation observed among different sampling times at the Schelde Estuary site. In fact, the 20 and 30°C February data define a trend that is intermediate between the two relationships displayed on Figure 2.6.

As pointed out by Habicht and Canfield (1997), for comparative purposes it would make more sense to relate the isotope fractionation effects to cell-specific rates of sulfate reduction. This, however, requires accurate estimates of the *in situ* density of the active sulfate reducing community, which are not routinely accessible with currently available culturing and molecular techniques. Nonetheless, a quantitative characterization of natural sulfate reducing communities will be needed to fully interpret the observed variations in isotope fractionation in field settings, and to relate them to the large body of data available from laboratory experiments with pure cultures.

2.5 Conclusions

Potential sulfate reduction rates (SSRs) and corresponding $^{34}S/^{32}S$ isotope fractionation effects (ε) produced by natural sulfate reducing communities were measured under steady state conditions using flow-through reactors containing undisturbed slices of intertidal estuarine sediments collected next to a salt marsh in the Schelde Estuary (Waarde, The Netherlands). Isotope fractionation effects (ε) and SRRs correlate inversely. Their variations are mainly related to the incubation temperature, sediment depth and sampling time, while sampling location with respect to the adjacent salt marsh has little effect. The potential SRRs range from 5 to 49 nmol cm^{-3} h^{-1}, and exhibit an optimum temperature around 30°C. Isotope fractionation ranged from 9 to 34 ‰. The SRRs systematically decrease with depth in the sediments while ε values simultaneously increase.

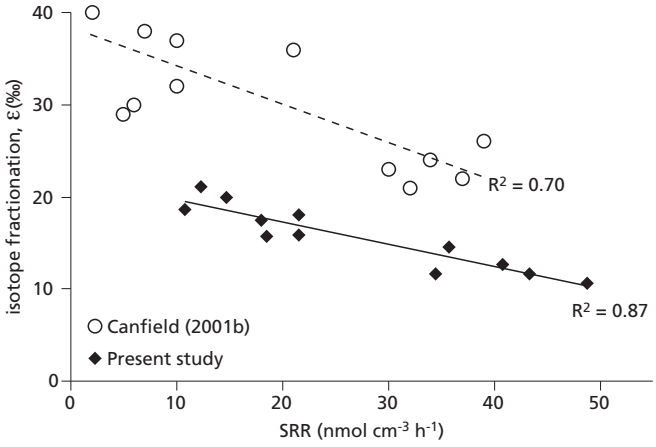

Figure 2.6: Sulfate reduction rate (SRR) *versus* isotope fractionation effects (ε) measured in the present study (black diamonds and solid line) compared to data obtained for a Danish coastal sediment by Canfield (2001b) (white circles and dotted line), for incubation temperatures ranging from 15 to 35°C.

The observed inverse relationship between ε and SRR is consistent with the standard Rees model of isotope fractionation during microbial sulfate reduction. The correlation is strongest for the data measured at 20 and 30°C, but weaker at suboptimal temperatures (10 and 50°C). In addition, the ε *versus* SRR relationship obtained for the sediments sampled in February shows a positive offset of several ‰, relative to the relationship obtained for the May and October sediments. The overall consequence is a range in ε values of about 20 ‰ when SRR drops below 15 nmol cm^{-3} h^{-1}. At higher SRRs, ε exhibits a narrower range (~5 ‰) around an average value of 17 ‰. The value of 17 ‰ is probably representative of the bulk *in situ* isotope fractionation in Schelde sediments produced by a single step of sulfate reduction.

At comparable SRRs, ε values in the present study are systematically lower than those measured previously by Canfield (2001b) in a near shore marine sediment in Denmark, using a similar flow-through reactor approach. Although in both cases ε and SRR are inversely related, the relationships are site-specific, possibly reflecting differences in the size and structure of the microbial communities, or in the nature and availability of electron donor substrates. Quantitative information on the composition and activity of the sulfate reducing community will be needed in order to fully elucidate the mechanisms controlling variations in biogenic sulfur isotope fractionation.

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Image of Mono Lake, California, USA

Chapter 3

Sulfate reducing activity and sulfur isotope fractionation by natural microbial communities in sediments of a hypersaline soda lake (Mono Lake, California)

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Abstract

Hypersaline soda lakes are extreme environments for microbial life as a result of their high salt and carbonate concentrations. Similar environments produced evaporate mineral deposits and sulfide-rich shales in the geological record and are often used to aid reconstruction of the sulfur isotopic composition of seawater through time. The relationship between microbial metabolisms and associated sulfur isotope effects is currently not well constrained in hypersaline environments. Sulfate reduction rates (SRRs) and sulfur isotope fractionation effects (ε) were measured using flow-through reactors containing intact sediment collected from three sites and two depth intervals (0-2 and 2-4 cm) along the littoral zone of Mono Lake, a hypersaline soda lake in California, USA. Incubation temperature (10, 20, 30, 40 and 50°C) and the inflow sulfate concentration (1, 2 and 3 mM) were varied. The resulting SRRs ranged from below detection limit to 62 nmol cm^{-3} h^{-1}, and the rates peaked at 40°C. The rates were also systematically lower in the deeper (2-4 cm) than in the shallower (0-2 cm) depth intervals. When inflow solutions were amended with lactate (10 mM), SRRs increased 2 to 5 fold, with a maximum value of 176 nmol cm^{-3} h^{-1}. Stable sulfur isotope fractionation was relatively small: ε values varied between 5 and 21 ‰ with an average value of 12 ‰. The general inverse relationship between ε and SRR predicted by the standard isotope fractionation model for sulfate reduction was not observed. In fact, at SRRs below approximately 25 nmol cm^{-3} h^{-1}, ε exhibited values correlated positively with SRR. This can be explained by a gradual increase in cellular energy available for the synthesis of adenosine-5'-phosphosulfate (APS) that leads to increased metabolic rates and more isotopic selectivity within the cell. At higher SRRs, isotope fractionation follows the standard fractionation model and the positive trend shifts towards a weak negative one. Decreased isotope fractionation at low rates of sulfate reduction may be a characteristic of halophilic sulfate reducers.

3.1 Introduction

Hypersaline soda lakes represent one of the most extreme environmental settings on the modern Earth (Baas Becking et al., 1960). Nonetheless, they are inhabited by active microbial communities that carry out similar metabolic processes as their counterparts in more moderate environments (Jones et al., 1998; Oren, 2002; Foti et al., 2008). Halophiles and alkaliphiles are found in the three kingdoms of Archaea, Bacteria and Eukarya (Oren, 2002; Oren, 2008). These microorganisms have developed adaptation strategies allowing them to survive under stressful living conditions (Oren, 1999, Oren, 2008). In particular, they must regulate the intracellular activity of water to resist the high osmotic pressure of the surrounding medium (Empadinhas and Da Costa, 2008). Because the processes regulating the osmotic pressure and ionic composition of the cytoplasm are energy-demanding, only microbes with relatively

high energy yielding metabolisms should thrive under extreme salt and alkalinity conditions (Oren, 2001; Oren, 2002).

Among alkaliphilic and hypersaline heterotrophs a variety of sulfate reducers have been identified (Welsh et al., 1996; Zhilina et al., 1997; Oremland et al., 2000; Brandt et al., 2001 Scholten et al., 2005; Foti et al., 2007; Sorokin et al., 2008). Most of them do not completely oxidize their organic substrates, a feature that has been rationalized in terms of the bioenergetic yields of the corresponding catabolic pathways (Oren, 1999). The presence of active sulfate reducing microorganisms in modern environments can be detected by measuring the distributions of sulfate and sulfide concentrations, by analyzing the microbial community structure or by tracking sulfur isotope signatures. Isotope fractionation is often the only way to trace biogenic sulfate reduction in ancient environments (Strauss, 2003; Shen and Buick, 2004).

When sulfate is non-limiting, microbial sulfate reduction leads to the production of sulfides enriched in the lighter ^{32}S isotope relative to the heavier isotope ^{34}S (Ohmoto and Goldhaber, 1997; Canfield, 2001a; Brüchert, 2004). A variety of studies have been performed to delineate the range of, and controls on, biogenic sulfur isotope fractionation (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Chambers et al., 1975; Habicht and Canfield, 1997; Detmers et al., 2001; Canfield, 2001b; Johnston et al., 2005; Hoek et al., 2006; Farquhar et al., 2008). Both experimental data and theoretical considerations imply that the extent of fractionation between ^{32}S and ^{34}S depends on the rate of sulfate reduction itself.

Limited data are available on sulfur isotope fractionation during microbial sulfate reduction under hypersaline and hyperalkaline conditions (Habicht and Canfield, 1996; Habicht and Canfield, 1997; Detmers et al., 2001). In this paper, rates of sulfate reduction (SRRs) and associated sulfur isotope fractionation effects (ε) are measured on intact sediment slices collected in Mono Lake, a hypersaline soda lake. The results provide new insight in the relationship between ε and SRR in this extreme environmental setting.

3.2 Sampling and experimental methodology

3.2.1 Site description and sample collection

Sediments were collected in Mono Lake, which is located on the arid eastern side of the Sierra Nevada Mountains in California, USA. The waters of this closed basin exhibit high alkalinity (pH 9.2-10) and salinity (75-100 g/l). The high sodium and (bi)carbonate concentrations are derived from weathering of the surrounding volcanic rocks and hydrothermal inflow (Oremland et al., 2000; Kulp et al., 2007). Mono Lake has an average depth of 17 m and occasionally experiences meromixis as a result of density controlled stratification of the water column (Kulp et al., 2006). Bottom waters contain elevated concentrations of reduced compounds, including sulfide (≥ 2 mM). High concentrations of inorganic arsenic oxyanions (200 μM) and sulfate (130 mM) have also been reported (Hoeft et al., 2004; Oremland et al., 2000). Mono Lake water further contains high levels of dissolved organic carbon (7 mM),

Table 3.1: Sampling locations, and sediment plus surface water characteristics (Mono Lake, July 2008). Sulfur isotope compositions and molar C/N ratios of the sediment in the reactors at the end of the flow-through experiments are also given. *Sediment C/N ratios could not be determined because the reactors were supplied with lactate-amended inflow solutions. **Precision about the mean isotope composition is given to indicate agreement between different reactors, and is in this case smaller than the external reproducibility of the analytical technique that is 0.5 per mil for $\delta^{34}S$ measurements.

Sampling Location			Sediment						Water				
Name	Sampling Time	GPS Coördinates	Characteristics	Sampling Depth	Molar C/N Ratios		Sedimentary Sulfides		Temp.	pH	Salinity	Sulfate	Chloride
				(cm)	start	end	start (‰)	end (‰)	(°C)		TDS (g/l)	(mM)	(mM)
Site 1 Boat Launche	20 July 2008	37°58′51″N 119°06′29″E	Soft sandy sediment	0-2	10.2	*	-15.4	-18 ± 2	22.6	9.8	80.9	98.1	458.8
			Abundant organic debris	2-4	7.9	*	-10.5	-23 ± 0.2**					
Site 2 Black Point	20 July 2008	38°01′04″N 119°07′09″E	Soft black (basaltic) sediment	0-2	5.7	6.5 ± 0.8	-4.7	-9 ± 2	23.8	10.8	85.8	37.6	186.8
			Covered with microbial matts (0.1 to 1 cm) Containing pink coloured sulfur bacteria Exposed areas covered with salt deposits	2-4	6.9	8.9 ± 0.9	-10.4	-13 ± 3					
Site 3 Navy Beach	21 July 2008	37°56′37″N 119°00′28″E	Soft sandy sediment	0-2	5.3	5.5 ± 0.01	-14.5	-17 ± 2	26.0	9.9	82.8	92.2	436.8
			Abundant organic debris	2-4	5.0	5.9 ± 0.5	-13.2	-19 ± 3					

although much of it is not bioavailable. Pulses of labile organic matter are released in spring and fall from the breakdown of single celled algae that inhabit the shallow lake water. Sulfate reduction accounts for 41 % of the mineralization of annual primary production in Mono Lake (Hoeft et al., 2004).

Sediment cores were collected in July 2008 at three locations along the littoral zone of Mono Lake (sites 1, 2 and 3, see Table 3.1), 1 to 2 m away from the shore at water depths of about 0.5 m. Sediments from the 0-2 cm and 2-4 cm depth intervals were sampled directly into Perspex reactor cells (2 cm thickness, 4.2 cm inside diameter) using a shuttle corer. Each sediment slice was covered by a glass fiber filter and 0.2 μm pore size nitrocellulose filter at either end. Reactors were sealed with O-rings to prevent leakage and they were stored anaerobically at 4˚C until starting the experiments. The first experiments were started within 2 weeks after sample collection. Detailed information on the sediment sampling and flow-through reactor technique can be found in Roychoudhury et al., (1998), Pallud and Van Cappellen, (2006), Laverman et al., (2006) and Pallud et al., (2007).

3.2.2 Flow-through reactor experiments

Flow-through reactors containing intact sediment slices were placed in a thermostatic water bath. In a first set of experiments performed in August 2008, replicate reactors from sites 2 and 3 were successively run at 10, 20, 30, 40 and 50 ˚C, whereby the temperature was increased every 8 to 10 days (Table 3.2). The inflow solutions contained 2 mM sulfate and no electron donor. In a second series of experiments carried out in October 2008, reactors from site 1 were run at a constant temperature (30˚C). Sulfate concentrations in the inflow solutions of 1, 2 and 3 mM were used. In a number of experiments 10 mM of lactate and 10 mM sulfate were supplied via the inflow solutions (Table 3.2). In both sets of experiments, temperature or inflow solution composition were changed only after the outflow sulfate concentration remained constant within 10 % for at least three consecutive days.

The artificial inflow solutions consisted of 0.047 M Na_2CO_3, 0.28 M $NaHCO_3$ and 0.51 M NaCl, in order to match the lake bottom water composition (Oremland et al., 2000; Pikuta et al., 2003). Sulfate was supplied as Na_2SO_4 and lactate as $NaC_3H_6O_3$. Bromide was used as an inert flow tracer at a concentration of 2 mM (NaBr); pH was adjusted to 9.8 using 1M NaOH.

Reactors were flushed for 24 hours at the start of each experiment (approximately 1.5 pore space volumes) with a salt solution containing 0.047 M Na_2CO_3, 0.28 M $NaHCO_3$ and 0.51 M NaCl to remove any remaining pore water sulfate. Experiments were run under anaerobic conditions. Tygon tubing, having low oxygen permeability, was used throughout, whilst inflow and outflow solutions were maintained under argon atmosphere. Inflow solutions were introduced with a peristaltic pump at a constant flow rate of 1 ± 0.1 ml h^{-1}. Outflow collection tubes were prefilled with 10 ml zinc acetate (1 M) solution to precipitate sulfide as ZnS. Since carbonate also precipitates with zinc ($ZnCO_3$), it was necessary to use a high concentration of zinc acetate to ensure complete precipitation of CO_3^{-2} and S^{2-}. Collection tubes were changed every 24 h and then stored at -18˚C prior to chemical and isotopic analysis.

Table 3.2: Overview of average sulfate reduction rates (SRRs) and corresponding sulfur isotope fractionation effects (ε) obtained in experiments performed in August and October 2008 with Mono Lake sediments. All values listed correspond to steady state conditions. Each SRR and ε value is an average of 3 to 5 measurements within a given steady state period of an individual reactor, with variability expressed as standard deviations. *In the lactate-amended experiments, the inflow solution contained 10 mM of sulfate. **For these ε values the standard deviation reported between steady state measurements within the individual reactor is smaller than the external reproducibility of the analytical technique that is typically 0.5 per mil for $\delta^{34}S$ measurements (see experimental methodology). (n.d.l. near detection limit)

Location	Reactor name	Temp. (°C)	Depth (cm)	inflow SO_4 (mM)	Natural Substrate				Lactate addition			
					SRR	sd	ε	sd	SRR*	sd	ε	sd
Site 1	1A 1	30	0-2	2	37,6	0,4	13	2	84	6	12	2
									144	6	10	1
	1A 2	30	0-2	2	42	1	13.8	0.2**	130	5	14	2
									176	5	12	1
	1A 3	30	0-2	2	20	1	17	9	60	4	21	10
	1B 1	30	2-4	2	17	2	18	1	51	4	13	2
	1B 2	30	2-4	2	25.2	0.03	18	3	53	1	no isotope data	
Site 1	1A 1	30	0-2	1	16	1	16	1				
	1A 2	30	0-2	2	23	1	17	1				
	1A 3	30	0-2	3	24	1	15	1				
	1B 1	30	2-4	1	14	1	18	3				
	1B 2	30	2-4	2	22	2	20	4				
Site 2	2A 1	10	0-2	2	n.d.l.							
	2A 2	10	0-2	2	n.d.l.							
	2A 1	20	0-2	2	15	1	11	1				
	2A 2	20	0-2	2	11	1	5	0.4**				
	2A 1	30	0-2	2	32	1	14	1				
	2A 2	30	0-2	2	21,3	0.1	11.0	0.1**				
	2A 1	40	0-2	2	40	2	18	1				
	2A 2	40	0-2	2	no steady state data							
	2A 1	50	0-2	2	n.d.l.							
	2A 2	50	0-2	2	n.d.l.							
Site 2	2B 1	10	2-4	2	n.d.l.							
	2B 2	10	2-4	2	n.d.l.							
	2B 1	20	2-4	2	6.7	0.2						
	2B 2	20	2-4	2	n.d.l.							
	2B 1	30	2-4	2	14	1	9	2				
	2B 2	30	2-4	2	8	1	7	3				
	2B 1	40	2-4	2	16,9	0,4	10	1				
	2B 2	40	2-4	2	no steady state data							
	2B 1	50	2-4	2	n.d.l.							
	2B 2	50	2-4	2	n.d.l.							

Location	Reactor name	Temp. (°C)	Depth (cm)	inflow SO_4 (mM)	Natural Substrate				Lactate addition			
					SRR	sd	ε	sd	SRR*	sd	ε	sd
Site 3	3A 1	10	0-2	2	n.d.l.							
	3A 2	10	0-2	2	n.d.l.							
	3A 1	20	0-2	2	16.7	0.2	14	3				
	3A 2	20	0-2	2	10	1	17	4				
	3A 1	30	0-2	2	30	1	20	1				
	3A 2	30	0-2	2	22	2	15	2				
	3A 1	40	0-2	2	62	1	8	1				
	3A 2	40	0-2	2	41	2	9	1				
	3A 1	50	0-2	2	n.d.l.							
	3A 2	50	0-2	2	n.d.l.							
Site 3	3B 1	10	2-4	2	n.d.l.							
	3B 2	10	2-4	2	n.d.l.							
	3B 1	20	2-4	2	11	1	8	2				
	3B 2	20	2-4	2	7.9	0.1	no isotope data					
	3B 1	30	2-4	2	28	1	12	2				
	3B 2	30	2-4	2	16.4	0.3	6	5				
	3B 1	40	2-4	2	33	1	9	1				
	3B 2	40	2-4	2	33	3	6.1	0.3**				
	3B 1	50	2-4	2	n.d.l.							
	3B 2	50	2-4	2	n.d.l.							

3.2.3 Chemical and isotopic analyses

Sediment molar C/N ratios were determined using a Carlo Erba CN analyzer on freeze dried and decalcified sediment samples collected from cores taken in the immediate vicinity of the flow-through reactor sediments. Inflow and outflow concentrations of SO_4^{2-} and Br^- were determined by ion chromatography using a Dionex DX120 equipped with an AS14 column, with a detection limit of < 5 μM and a mean precision of approximately 4 %. Sulfur isotope ratios were measured in the sulfate fraction of the inflow and outflow solutions, through precipitation as $BaSO_4$ using 10 % $BaCl_2$ solution followed by drying at 50°C. Isotope ratios ($^{34}S/^{32}S$) were determined by elemental analyzer gas source mass spectrometry, using a Na 1500NCS coupled to a Finnigan MAT (Delta +) in which $BaSO_4$ was converted to SO_2 by flash combustion in a tungstic oxide, ultra pure copper quartz tube at 1050°C. Measurements had a mean precision of approximately 0.5 ‰. The sulfur isotope composition of sulfides present in homogenized freeze dried sediment collected at the site or from the reactors at end of the experiments were determined following sulfide extraction using the standard chromium reduction and 6 M HCl acid distillation technique (Canfield et al., 1986; Fossing and Jørgensen, 1989).

3.2.4 Sulfate reduction rates and sulfur isotope fractionation effects

Potential sulfate reduction rates (SRRs) and isotope fractionation effects (ε) were determined for the time intervals when the outflow sulfate concentration remained constant, that is when

the sulfate reducing activity was at (or near) steady state. Steady state sulfate reduction rates were then calculated using equation 1:

$$SRR = \frac{\Delta C * Q}{V} \quad (1)$$

where Q represents the flow rate in ml h^{-1}, ΔC is the difference between inflow and outflow sulfate concentration in mM and V is the bulk volume of sediment in the reactor in cm^3 which has a fixed value of 27.7 cm^3. All rates should be considered as potential sulfate reduction rates, because sulfate is the only electron acceptor supplied to the flow-through reactors. Isotope fractionation effects, representing the difference in $\delta^{34}S$ between inflow and outflow sulfate, were calculated using a Rayleigh distillation model following the approach of Canfield, 2001b:

$$\alpha = 1 + \frac{(\ln \delta_{SO4in} + 1000) - (\ln \delta_{SO4out} + 1000)}{\ln(f_{SO4})} \quad (2)$$

and

$$\varepsilon = 1000(\alpha - 1) \quad (3)$$

where $\delta_{SO4\text{-}in}$ represents the isotopic composition of the sulfate in the inflow and $\delta_{SO4\text{-}out}$ is the isotopic composition of the sulfate in the outflow solution. f_{SO4} is the fraction of sulfate remaining in the outflow compared to the inflow solution. Note that the use of equation (2) requires the reacting system to be at steady state.

3.3 Results

3.3.1 Site and sediment characteristics

Table 3.1 provides relevant characteristics of the sediments and lake water at the three sampling sites. Concentrations of SO_4^{2-} and Cl^- and salinity were comparable for sites 1 and 3, but lower for site 2, whilst pH was constant across the three sites. Molar C/N ratios of starting sediment (0-2 and 2-4 cm depth intervals) were highest at site 1, followed by sites 2 and 3. The C/N ratios of the sediments increased during the flow-through reactor experiments (Table 3.1). Sedimentary sulfide $\delta^{34}S$ values measured in the starting sediment were in the range -5 to -15 ‰, and were generally more negative at the end of the flow-through experiments.

3.3.2 Potential sulfate reduction rates

Outflow sulfate concentrations typically reached steady state within 3 to 5 days after changing temperature or inflow solution composition (Figure 3.1). The inflow sulfate concentration had no significant effect on the potential sulfate reduction rates (SRRs), with identical rates measured when sulfate was provided at concentrations of 2 and 3 mM, implying apparent

sulfate half-saturation concentrations of less than 2 mM (Pallud and Van Cappellen, 2006; Pallud et al., 2007) (Table 3.2). SRRs measured in the absence of lactate amendment (natural substrate experiments) ranged from below the detection limit to 62 nmol cm^{3-} h^{-1}, and increased to values between 51 and 176 nmol cm^{3-} h^{-1} when lactate was supplied as external electron donor (Table 3.2).

Figure 3.2 shows the effects of incubation temperature, in the range 10 to 50°C, on SRRs in the 0-2 cm and 2-4 cm sediment slices collected from sites 2 and 3. Agreement in absolute SRR values between replicate reactors ranged from 3 to 36 %, and was generally poorer at 30 and 40°C. Despite this, the SRR *versus* temperature trends were similar between duplicate reactors (Figure 3.2, Table 3.2). Sediments from the 0-2 cm depth interval consistently gave higher SRRs compared to the 2-4 cm sediments. At 30°C, SRRs at 0-2 cm were comparable for sites 2 and 3, whereas rates for site 1 were significantly higher for the same depth interval (Figure 3.3). Similar SRRs were observed between site 1 and 3 for the 2-4 cm depth interval, whereas rates were significantly lower for this depth interval at site 2 (Figure 3.3).

The addition of 10 mM lactate as an electron donor resulted in a 2 to 5 fold increase in SRR (Table 3.2). In two reactors, an additional intermediate period of steady state was observed for 48 to 72 hours following lactate amendment, with rates in between the values for the natural substrate and the higher rates observed subsequently.

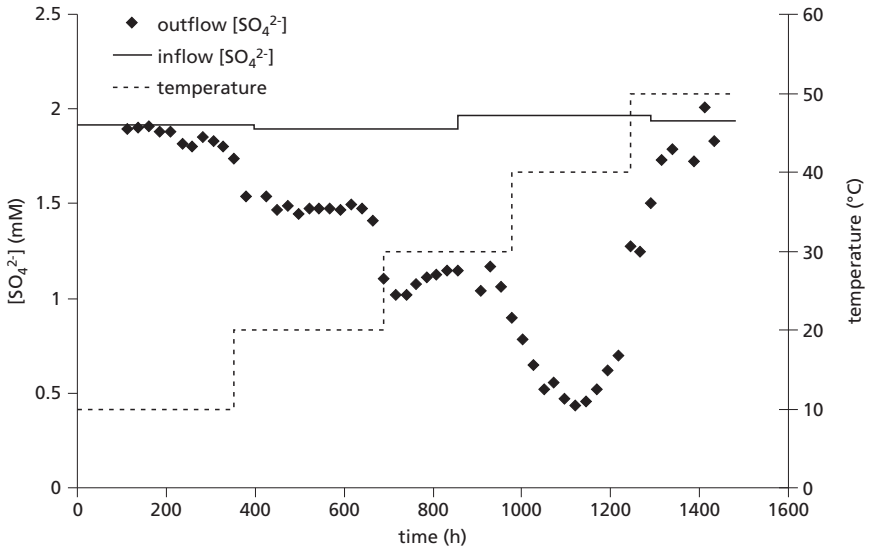

Figure 3.1: Example of sulfate concentrations measured in the outflow of a flow-through reactor experiment (0-2 cm depth, site 3). Symbols represent the measured outflow sulfate concentrations, the solid line represents the imposed inflow sulfate concentration and the dotted line the temperature of the water bath.

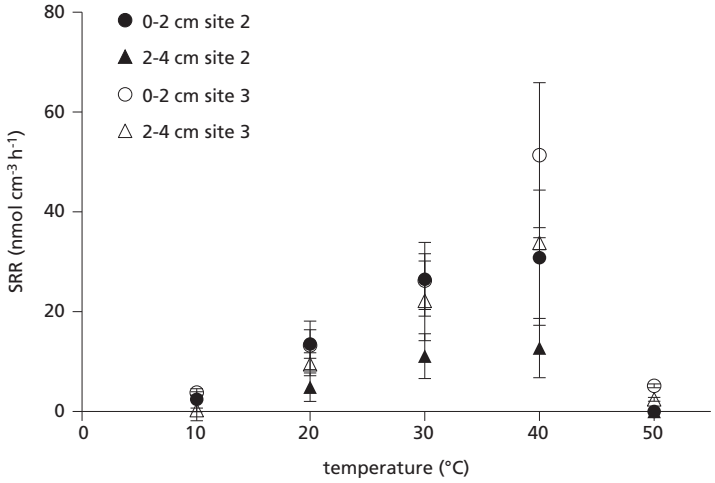

Figure 3.2: Steady state potential sulfate reduction rates (SRRs) measured using flow-through reactor experiments at variable temperatures, for sediments from the 0-2 cm (circles) and 2-4 cm (triangles) depth intervals collected at site 2 (black) and site 3 (open). Error bars represent standard deviations calculated from data of duplicate reactors (Table 3.2). When error bars are not visible, the error falls within the size of the symbols.

3.3.3 Sulfur isotope fractionation effects

Sulfur isotope fractionation effects (ε) measured during periods of steady state sulfate reducing activity ranged from 5 to 21 ‰ with an average value of 12 ‰ when combining the data for the three sites (Table 3.2). No change in ε was observed when increasing the inflow sulfate concentration from 2 to 3 mM. Statistical distribution plots of all ε values are shown in Figure 3.4. The mean ε value of 14 ‰ at 30°C was slightly higher than the values of 11 and 10 ‰ at 20°C and 40°C, respectively (Figure 3.4a). Although lactate addition resulted in a large increase in SRR, it did not significantly change the frequency distribution of ε. The mean ε value for the lactate-amended experiments was 14 ‰, compared to 12 ‰ for the experiments with only natural sediment electron donor substrates (Figure 3.4b).

No clear correlation between ε and SRR was observed when considering the entire data set obtained with the natural sediment substrates (Figure 3.5). A more detailed analysis, however, shows some weak correlations between ε and SRR for individual incubation temperatures or sampling sites (Figure 3.6). Positive ε *versus* SRR trends were found at 20 and 30°C and for sites 2 and 3, at SRRs below 20-25 nmol cm^{-3} h^{-1} (Figure 3.6a and 3.6b). At higher SRRs no consistent trends emerged for the non-amended reactor experiments. SRR and ε values obtained with lactate amended reactors showed a weak inverse correlation, although the data set was rather limited. In individual reactors, with two exceptions, ε decreased by 2 to 5 ‰ when lactate was added (Table 3.2). However, the mean isotope fractionation effects obtained at 30°C with and without lactate amendment were both 14 ‰ and close to the overall average of 12 ‰.

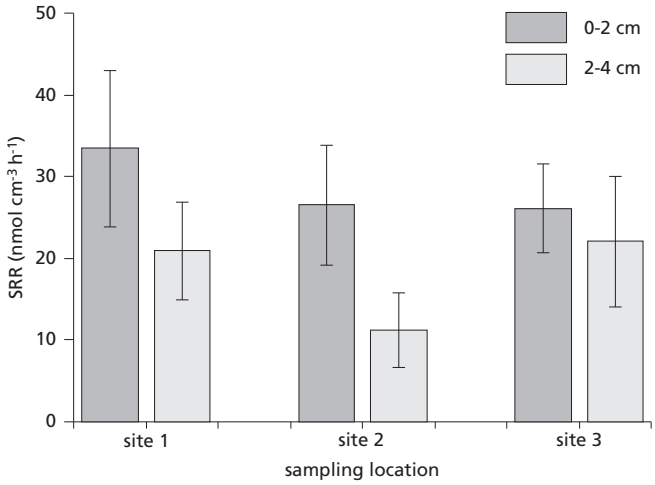

Figure 3.3: Effect of sampling depth and location on steady state potential sulfate reduction rates (SRRs) measured at 30°C. Error bars represent standard deviations calculated for SRRs within a given steady state period.

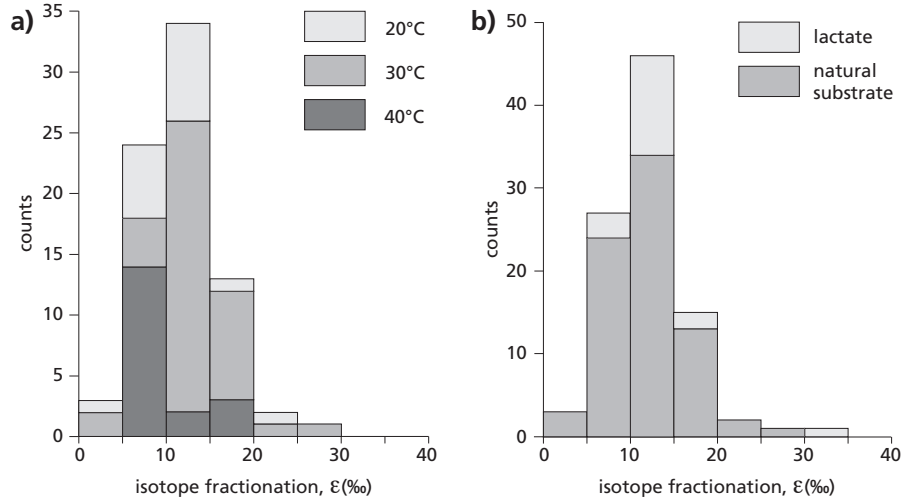

Figure 3.4: Distribution plots of sulfur isotope fractionation effects (ε) for various temperatures (Panel 3.4a) and with or without lactate amendment (Panel 3.4b). Each count refers to a SRR determination within a steady state period.

3.4 Discussion

Previous studies of Mono Lake sediments have demonstrated the presence of a diverse and active mesophilic sulfate reducing community adapted to hypersaline and high alkaline conditions (Oremland et al., 2000; Hollibaugh et al., 2001; Humayoun et al., 2003; Scholten et al., 2005; Kulp et al., 2006). The observed consumption of sulfate in the flow-through reactors is consistent with this earlier work (Figure 3.1). Furthermore, the highest SRRs measured in the present study in the absence of lactate addition (62 nmol cm^{-3} h^{-1}) are of the same magnitude as the highest rates measured in Mono Lake sediments by Kulp et al. (2006). Note that, because sulfate is the only electron acceptor supplied to the flow-through reactors, the measured rates should be considered potential sulfate reduction rates.

The experimental results show that sulfate reducing activity in Mono Lake sediments is similar in many respects to that observed in other mesophilic environments, including marine, estuarine and freshwater sediments (Westrich and Berner, 1984; Canfield, 2001b; Pallud and Van Cappellen, 2006, and references therein). The SRRs fall within the range of values reported for depositional environments of lower salinity (see Table 3 in Pallud and Van Cappellen (2006), for a review), the optimum temperature is on the order of 30 to 40°C (Figure 3.2), the sulfate half-saturation concentration is well below 2 mM, and the potential rates are controlled by the electron donor availability. The latter is supported by the response of the sulfate reducing activity to the addition of lactate, and by the drop in potential SRRs with depth in the sediment (Figure 3.3), as labile organic matter input to the sediments is predominantly through deposition of algal matter from the water column. Coupling of dissimilatory sulfate reduction to the decomposition of algal organic matter also explains the increase in molar C/N ratio of the sediment during the flow-through experiments (Table 3.1).

Sulfur isotope fractionation effects (ε) obtained for the Mono Lake sediments (5 to 21 ‰) are within the range of previously measured values of -3 to 47 ‰ (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Chambers et al., 1975; Habicht and Canfield, 1997; Detmers et al., 2001; Canfield, 2001b; Johnston et al., 2005; Hoek et al., 2006; Farquhar et al., 2008), and within the theoretical range predicted by the standard fractionation model for sulfate reduction of Rees (1973). They also agree with sulfur isotope fractionation observed in pure cultures of sulfate reducers isolated from hypersaline mats and alkaline lake environments (2 to 19 ‰, Detmers et al., 2001), although values obtained for a hypersaline mat from Solar Lake, Sinai, were higher (16 to 39 ‰) at equivalent to higher rates of sulfate reduction (12 to 1125 nmol cm^{-3} h^{-1}) (Habicht and Canfield, 1996; Habicht and Canfield, 1997).One key aspect in which sulfate reduction in Mono Lake sediments deviates significantly from that in less extreme environments is the relationship between ε and SRR (Figures 3.5 and 3.6). Incubations with sediments from marine and continental settings typically exhibit an inverse trend between ε and SRR (Habicht and Canfield, 1997; Canfield, 2001b; *Chapter 2*). That is, in contrast to the data for Mono Lake sediments, the highest ε values are found when sulfate reducing activity is lowest, for example, when the labile organic substrates are depleted (Canfield, 2001b; Brüchert, 2004; Habicht et al., 2005;

Hoek et al., 2006). An inverse relationship is also predicted by the standard fractionation model for sulfate reduction and its various derivatives (Rees, 1973; Brunner and Bernasconi, 2005; Farquhar and Wing, 2003; Canfield et al., 2006; Johnston et al., 2007; Farquhar et al., 2008).

Within the framework of the standard model, variations in fractionation are explained by the differences in rate and reversibility of the individual steps in the microbial sulfate reduction process. The slowest or rate determining step controls the net amount of fractionation. Sulfate reduction is initiated by the transport of sulfate though the cell membrane. Subsequently, the sulfate is activated by adenosine-5'-triphosphate (ATP) to form adenosine-5'-phosphosulfate (APS), which is then reduced to sulfite. Further reduction of sulfite to sulfide results in a large energy yield, which drives the overall dissimilatory process. Following the standard fractionation model, at low specific rates of sulfate reduction the intracellular reduction of sulfite to sulfide is rate limiting, leading to a relatively large pool of APS and large isotope fractionation effects. As the rate increases, transport of sulfate through the cell membrane becomes rate determining leading to lower fractionation. Deviation from the standard inverse ε *versus* SRR trend can be induced by low temperatures (especially below 15°C), which decrease the fluidity of the cell membrane, thereby hindering sulfate transport and causing a simultaneous drop in ε values and SRR (Canfield, 2001b). Such a temperature effect, however, does not explain the positive ε *versus* SRR relationship observed for the 20 and 30°C data of Mono Lake sediments, at SRR below 25 nmol cm^{-3} h^{-1} (Figure 3.6a).

Detmers et al. (2001) reported the lack of an inverse ε *versus* SRR relationship for a large number of pure cultures of halophilic and haloalkaliphilic sulfate reducing microorganisms

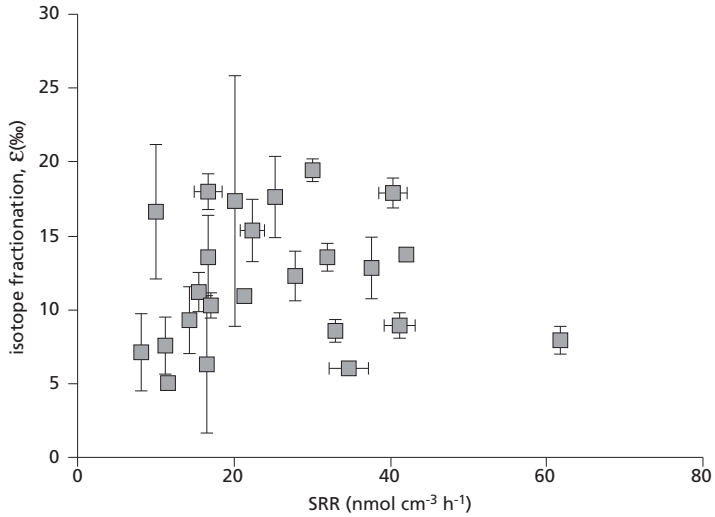

Figure 3.5: Relationship between sulfur isotope fractionation effects (ε) and potential sulfate reduction rates (SRRs) for Mono Lake sediments, under steady state conditions. Error bars represent standard deviations calculated from SRRs and ε within a given steady state period of an individual reactor (Table 3.2). All individual ε and SRR measurements for the three sites are combined in the plot.

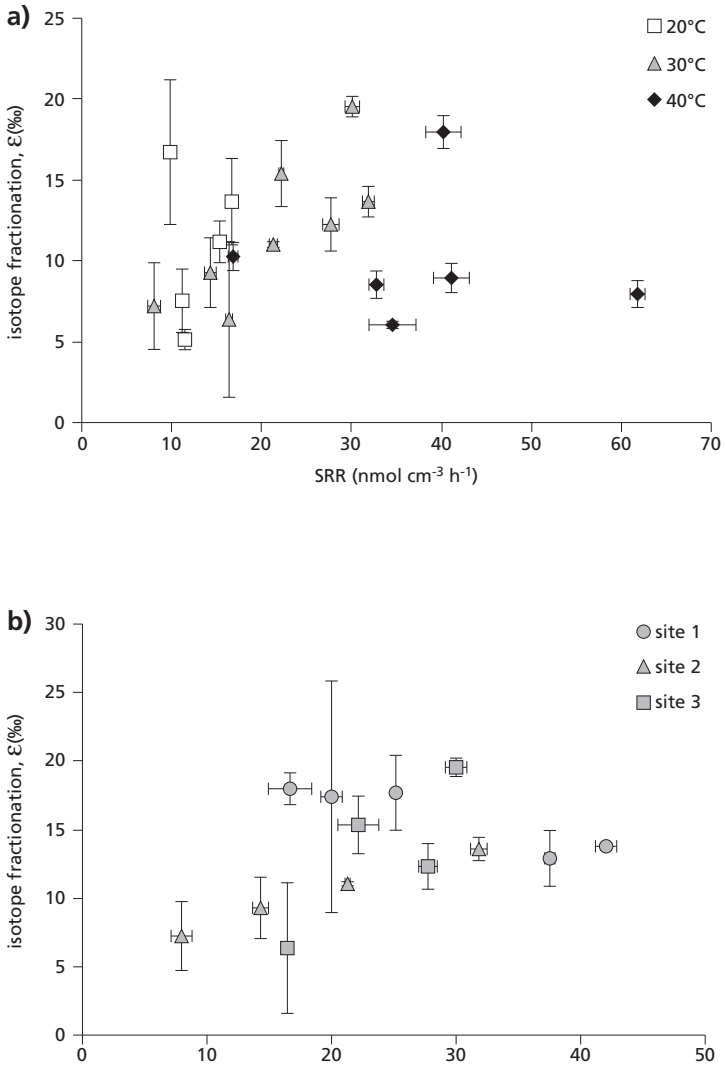

Figure 3.6: Effects of temperature (Panel 3.6a) and sampling location (Panel 3.6b) on the trends between sulfur isotope fractionation effects (ε) and potential sulfate reduction rates (SRRs). Error bars represent standard deviations calculated from variations in SRR and ε within a given steady state period of an individual reactor (Table 3.2).

grown under optimum conditions. A similar observation was also made for the natural community of sulfate reducers inhabiting a cyanobacterial mat from the hypersaline Solar Lake (Habicht and Canfield, 1997). Based on the available data, it would thus appear that sulfur isotope fractionation during dissimilatory sulfate reduction in hypersaline environments does not follow the standard inverse ε *versus* SRR relationship. One possible explanation is

Table 3.3: Concentrations and activities of solute species in pore waters of Mono Lake sediments (upper 5 cm). Values between brackets represent observed concentration ranges. Average values are used in the calculation of Gibbs energy yields of reactions (4) and (5) in the text. The concentrations listed are obtained from a variety of published and unpublished sources. No information on lactate and acetate concentrations could be found, however. The concentrations listed for these two organic acids are typical for marine and estuarine sediments. Activity coefficients are assumed to be the same for all monovalent and divalent ionic species. Although this is not strictly true, it does not affect the conclusions of this study. The concentration of bicarbonate ions is derived from the alkalinity and pH using a second acid dissociation constant pK_2 of 10.329.

pH	= 9.8	(9.2–10)
salinity	= 85 g L-1	(75–100 g L-1)
alkalinity	= 0.8 Eq L-1	(0.7–0.9 L-1)
[HCO3-]	= 0.18 M	(0.1–0.3 M)
[SO42-]	= 100 mM	(20–120 mM)
[HS-]	= 45 mM M	(2–100 mM)
[lactate]	= 20 µM	
[acetate]	= 10 µM	
γ(monovalent)	= 0.54	
γ(divalent)	= 0.09	

related to the high energetic cost of maintaining cellular activity at high salt concentration (and high pH).

While a variety of adaptive mechanisms allow microorganisms to survive under extreme salt and alkalinity conditions, they all require a continuous supply of energy (Welsh et al., 1996; Oren, 1999; Oren, 2001; Oren, 2002; Boltyanskaya et al., 2005; Sorokin et al., 2008). Thus, at a given cell specific rate of sulfate reduction, a sulfate reducing microorganism in Mono Lake must divert more of its respiratory ATP production to maintaining intracellular osmotic pressure and pH than a counterpart living in a less extreme environment. As a consequence, less ATP is available to produce APS. The smaller APS reservoir also means there is less discrimination between ^{32}S and ^{34}S during reduction to sulfite. As the specific rate of sulfate reduction increases, a larger part of the generated energy is available for APS production and higher ε values are generated. However, when the rate increases above a certain threshold, the size of the APS pool becomes again limited by sulfate transport into the cell. From then on, fractionation follows the standard model of Rees (1973), resulting in a reversal of the ε *versus* SRR relationship. The threshold SRR likely varies among various sulfate reducers, and may further be affected by temperature and the type of substrate (Macdonald et al., 1995; Zogg et al., 1997), thereby explaining the scatter in the ε *versus* SRR trends in Figures 3.5 and Figure 3.6.

Nearly all sulfate reducing organisms isolated so far from hypersaline environments do not completely oxidize their organic substrates (Oren, 1999; Detmers et al., 2001). Furthermore, incomplete oxidizers typically yield sulfur isotope fractionation below 19 ‰ (Detmers et al., 2001; Brüchert, 2004), consistent with the ε values obtained for Mono Lake sediments (Figure 3.5). A predominance of incomplete oxidizers may seem counterintuitive when considering

standard Gibbs energies of reaction. Using lactate as the energy substrate, complete and incomplete oxidation in the presence of sulfate can be illustrated by the following reactions:

$$\text{lactate} + \frac{3}{2}SO_4^{2-} \rightarrow 3HCO_3^- + \frac{3}{2}HS^- + \frac{1}{2}H^+ \quad (4)$$

and

$$\text{lactate} + \frac{1}{2}SO_4^{2-} \rightarrow \text{acetate} + HCO_3^- + \frac{1}{2}HS^- + \frac{1}{2}H^+ \quad (5)$$

Under standard conditions, the Gibbs energies of reaction (ΔG^0) are -112.6 and -80.1 kJ mol^{-1} for reactions (4) and (5), respectively (Oyekola et al., 2009), thus suggesting a large energetic advantage of complete oxidation of the organic substrate into inorganic carbon. However, the actual energetic advantage is significantly smaller at the high alkalinities and high aqueous sulfide concentrations characterizing the pore waters of Mono Lake sediments (Table 3.3). For example, using the typical concentrations and activity coefficients given in Table 3.3, the Gibbs energies (ΔG) for reactions (4) and (5) at 25°C are -110.1 and -95.0 kJ mol^{-1}. Thus, the energy yields of both reactions are of comparable magnitude. Furthermore, per mole of substrate utilized, incomplete oxidizers need to export far less inorganic carbon and sulfide against steep concentration gradients, thereby providing them with an additional advantage under the conditions encountered in Mono Lake sediments.

3.5 Conclusions

Despite the extreme environmental conditions, Mono Lake sediments exhibit high potential rates of sulfate reduction (SRRs up to 62 nmol cm^{-3} h^{-1}). The corresponding sulfur isotope fractionation effects (ε) are relatively small (≤ 20 ‰), and do not follow the standard inverse relationship with SRR. Instead, ε values peak at SRRs around 20-30 nmol cm^{-3} h^{-1}. The ε *versus* SRR trend is attributed to the large energy cost of life under high salt and high pH conditions. At low specific rates of sulfate reduction, the demand for ATP to maintain intracellular osmotic pressure and pH decreases the production and life-time of APS, hence limiting the extent of sulfur isotope fractionation. The overall low values of ε are also consistent with a sulfate reducing community dominated by incomplete oxidizers. It would thus appear that the dependence of sulfur isotope fractionation on the rate of dissimilatory sulfate reduction is fundamentally different in soda lakes than in normal marine and continental environments.

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Image of Vulcano Island, Italy

Chapter 4

Sulfur isotope fractionation by natural communities of sulfate reducing prokaryotes in the shallow marine hydrothermal vent system of Vulcano Island, Italy

This chapter is submitted to Geobiology:
Stam, M.C., Mason, P.R.D. & Amend, J.P. Controls on sulfate reduction and sulfur isotope fractionation by natural microbial communities in estuarine sediments

Abstract

Shallow marine hydrothermal systems provide energy and nutrients to support numerous types of microbial metabolic processes and are analogue environments for sites on the early Earth where life may have begun. Microbial sulfate reduction is a deep branching metabolism that can thrive at elevated temperatures in close proximity to hydrothermal vents, and can be recorded geochemically through the fractionation of stable sulfur isotope ratios. Despite this, little is known about the degree of sulfur isotope fractionation imparted by natural communities of sulfate reducing (hyper)thermophilic microorganisms that inhabit hydrothermally-influenced sediments. In this study, potential sulfate reduction rates (SRRs) and corresponding sulfur isotope fractionation effects were determined during laboratory incubations of beach sediments collected adjacent to the shallow marine hydrothermal vent system of Baia di Levante, Vulcano Island, Italy. Intact sediments were incubated using flow-through reactors at 30, 60 and 85°C, with sulfate continuously supplied in excess and the electron donor either provided from the natural substrate or by amending inflow solutions with lactate. No measurable SRRs were detected when incubating with the natural sediment substrate, due to low concentrations of labile organic matter and consequent electron donor limitation. However, high SRRs of 78 to 167 nmol cm^{-3} h^{-1} were achieved at all three incubation temperatures when amending with lactate. Corresponding isotope fractionation effects (ε), calculated from differences in $\delta^{34}S$ between sulfate and sulfide were relatively small, ranging from 6 to 16 ‰, suggesting a dominant role for microorganisms that do not completely oxidize their carbon source. No clear relationship was observed between SRR and ε, although the investigated range in both parameters was relatively small. The observation that small amounts of fractionation reflect high sulfate reducing activity implies that low ocean sulfate concentrations are not a unique explanation for the limited degrees of $\delta^{34}S$ variation in the terrestrial geological record prior to 2.4 Ga. A consequence of these observations is that microbial sulfate reduction may be difficult to identify using $\delta^{34}S$ variations in some hydrothermally-modified areas since the expected range of fractionation could be easily overprinted by mixing between distinctive sulfur sources as well as abiotic fractionation processes.

4.1 Introduction

Hydrothermal vents and their surrounding sediments are highly dynamic systems, with rapid fluctuations and large gradients in physical and chemical conditions. Despite the high temperatures and extreme variability these sites host diverse microbial communities (Segerer et al., 1993; Zierenberg, 2000; Kelley et al., 2002) and may play a key role in linking biogenic activity on the modern world to microorganisms that thrived in paleo-environments on

the anoxic and potentially warmer early Earth (Farmer, 2000; Kasting, 2001; Nisbet and Sleep, 2001; Pavlov and Kasting, 2002; Farquhar and Wing, 2003; Stetter, 2006; Kaufman et al., 2007; Van Kranendonk, 2006; Martin et al., 2008; Burns et al., 2009). Sulfate reducing prokaryotes (SRP), including both Bacteria and Archaea, are ubiquitously present in high temperature environments (e.g. Zeikus, 1983; Jørgensen et al., 1992; Dhillon et al., 2003; Ferris et al., 2003; Fishbain et al., 2003; Nakagawa et al., 2004; Roychoudhury, 2004; Amend and Teske, 2005; Meyer-Dombard et al., 2005; Dillon et al., 2007; Wagner and Wiegel, 2008; Amend, 2009) whilst phylogenetic studies, based on 16s-rRNA subunits, show that several strains of (hyper)thermophilic SRP are deeply branching in the phylogenetic tree suggesting their early appearance in microbial evolution (Wagner et al., 1998; Klein et al., 2001; Stahl et al., 2002; Blank, 2009). Stable sulfur isotope ratios, under non-limited sulfate concentrations, have the potential to record the metabolic activity of SRP, due to kinetic effects that favor the enrichment of lighter ^{32}S isotope into the sulfide formed during dissimilatory sulfate reduction. Incorporation of sulfide into pyrite in sedimentary rocks provides a link to past sulfate reducing activity in the geological record.

Sulfur isotope fractionation during microbial metabolism has been extensively studied using laboratory experiments with pure cultures, and to a more limited extent with natural communities of SRP in sediment samples (e.g. Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Chambers et al., 1975; Habicht and Canfield, 1997 Bolliger et al., 2001; Canfield, 2001; Detmers et al., 2001; Kleikemper et al., 2004; Hoek et al., 2006; Johnston et al., 2007; Farquhar et al., 2008). Biogenic isotope fractionation effects (ε) between sulfate and sulfide measured in these experiments range between -3 and 47 ‰. The main controls on the magnitude of this fractionation effect are the incubation temperature, the type and availability of electron donor, the concentration of the electron acceptor and the type of microorganism or community structure. Sulfate availability exerts a strong control on fractionation and ε decreases to zero below concentrations of 200 μM (Habicht et al., 2002; Habicht et al., 2005). Most literature data were collected using cultured strains or sediments isolated from marine and terrestrial environments at temperatures ranging from 20 to 40°C, with incubation under conditions favorable for mesophilic microorganisms. A more limited number of studies were carried out with SRP obtained from hydrothermal environments and cultured under (hyper)thermophilic conditions (Böttcher et al., 1999; Canfield et al., 2000; Detmers et al., 2001; Habicht et al., 2002; Habicht et al., 2005; Hoek et al., 2006; Davidson et al., 2009; Mitchell et al., 2009). These indicate fractionation ranging from 1 to 37 ‰ which is comparable to that obtained for mesophilic communities, although in most cases fractionation was less than 25 ‰ (Table 4.1). Isotope fractionation effects generally increase under electron donor substrate limiting conditions (Böttcher et al., 1999; Detmers et al., 2001; Habicht et al., 2005; Hoek et al., 2006; Davidson et al., 2009), but this was less pronounced in natural sediments isolated from the hydrothermal system of Guyamas Basin (Table 4.1) (Canfield et al., 2000). Although much is already known about the mechanisms and processes involved in microbial sulfate reduction, there are limited data available to show the response of natural communities of SRP in sediments from natural

Table 4.1: Overview of pure culture and natural sediment incubation studies of biogenic sulfur isotope fractionation by (hyper)thermophilic sulfate reducing prokaryotes. Pure culture data are obtained from Böttcher et al. (1999), Detmers et al. (2001), Habicht et al. (2002), Habicht et al. (2005), Hoek et al. (2006), Davidson (2009) and Mitchell et al. (2009). Natural sediment data are obtained from Canfield et al. (2000).

	isolated from	Temp. (°C)
Pure Cultures		
Archaeoglobus fulgidus strain Z	Submarine hot spring	80
		80
		80
		80
		80
Archaeoglobus fulgidus strain Z and VC-16		80
Archaeoglobus fulgidus strain Z and VC-16		80
Thermodesulfovibrio Yellowstonii	Thermal vent water	60
Desulfotomaculum thermocisternum	Oil reservoir	60
Desulfotomaculum geothermicum	Aquifer	50
Thermodesulfobacterium commune	Thermal spring	60
Gram negative SRB (strain MT-96)	Shallow water hydrothermal vent	60
Desulfotomaculum thermocisternum putei	Thermal vent water?	65
		65
Thermodesulfotator indicus	Hydrothermal vent	70
		70
		70
Natural communities		
sediments	Guyamas Basin	50-75
	Deep sea hydrothermal vent	50-75
		≥ 80
		≥ 80
		50-75
		≥ 80

hydrothermally-modified environments (Canfield et al., 2000), which may have been one of the first environments to be colonized by microbial life on the early Earth.

One of the best studied marine shallow hydrothermal vent systems is situated in Baia di Levante on Vulcano Island, which is part of the volcanically active Aeolian Archipelago situated to the north of Sicily. This shallow hydrothermal system harbors the majority of known and cultured hyperthermophiles to date (Amend, 2009). Previous studies have focused on the chemical composition of vent fluids and sediments (Gugliandolo et al., 1999; Rogers and Amend, 2006; Skoog et al., 2007; Rogers et al., 2007), the microbial community composition (Amend and Teske, 2005; Rogers and Amend, 2005; Rusch et al., 2005; Rusch

substrate	growth conditions	ε (‰)	reference
lactate (incomplete oxidation)	optimum	17	Detmers et al. (2001)
lactate (incomplete oxidation)	optimum	20 to 26	Habicht et al. (2002)
lactate (incomplete oxidation)	sulfate limiting	8 to 20	
lactate (incomplete oxidation)	optimum	20 to 26	Habicht et al. (2005)
lactate (incomplete oxidation)	sulfate limiting	14 to 20	
lactate (incomplete, complete oxidation)	optimum	10 to 14	Mitchell et al. (2009)
lactate (incomplete, complete oxidation)	outsite optimum temperature	12 to 25	
lactate (incomplete oxidation)	optimum	17	Detmers et al. (2001)
lactate (incomplete oxidation)	optimum	15	Detmers et al. (2001)
lactate (incomplete oxidation)	optimum	12.5	Detmers et al. (2001)
lactate (incomplete oxidation)	optimum	5	Detmers et al. (2001)
lactate (complete oxidation)	optimum	19	Böttcher et al. (1999)
Lactate	optimum	10	Davidson et al. (2009)
Lactate	substrate limitation	21	
H_2	optimum	1 to 6	Hoek et al. (2006)
H_2	substrate limitation	24 to 37	
H_2	outsite optimum temperature	1 to 10	
lactate		13 to 28	Canfield et al. (2000)
lactate	lactate limitating substrate	13 to 24	
lactate		8 to 27	
lactate	lactate limitating substrate	7 to 24	
ethanol		23 to 25	
acetate		25	

and Amend, 2008) and the energetics of putative metabolic pathways (Amend and Shock, 2001; Amend et al., 2003; Rogers and Amend, 2005; Rogers et al., 2007). Sulfate reduction was shown to be one of the dominant active microbial processes in this environment (Tor et al., 2003; Amend, 2009) as supported by the experimental isolation of a large variety of hyperthermophilic sulfate reducers (Stetter, 1987; Stetter, 1988; Zellner et al., 1989). In addition, extensive theoretical calculations combined with experimental observations have established that these microorganisms are energetically able to thrive on a wide range of inorganic compounds such as H_2 and CO (Amend and Shock, 2001) and organic compounds including fermentation products including acetate or lactate (Tor et al., 2003; Rogers et al.,

2007), amino acids (Svensson et al., 2004) and volatile fatty acids (Amend et al., 1998). These are all present in variable concentrations in the vent fluids and sediment pore waters sampled at Vulcano.

This study investigates biogenic sulfur isotope fractionation imparted by natural communities of SRP in a shallow marine hydrothermal setting for the first time, using sediments collected in close proximity to hydrothermal vents on the beach in Baia di Levante. The focus is on the effect of temperature (30, 60 to 85°C) on potential sulfate reduction rates (SRRs) and corresponding isotope fractionation effects (ε) between sulfate and sulfide. Flow-through reactor experiments were used to incubate intact sediments, under controlled laboratory conditions (Roychoudhury et al., 1998; Laverman et al., 2006; Pallud and Van Cappellen, 2006). The results contribute to the detection and interpretation of biogenic activity in modern and ancient hydrothermally-influenced ecosystems.

4.2 Sampling and experimental methodology

4.2.1 Sample collection

Sediment samples were collected in June 2007 from Stinky Surf Rock (38°25'05.50"N, 14°57'35.20"E), a location in Baia di Levante, on the eastern side of Vulcano Island, Italy (Rogers and Amend, 2006; Rogers et al., 2007). Sediments were collected at three different positions within this site: 1) in the surf zone of the beach, 2) several meters further along the beach in the surf zone close to an active gas emitting vent and 3) approximately 20 m offshore at a depth of 1.5 m below the water line (Table 4.2). Hydrothermal vents were located using a temperature probe or by observing gas bubbles on the seafloor or in the beach sand. Sediment slices (2 cm thickness with a diameter of 4.2 cm) were sampled using a metal shuttle corer stacked with Perspex rings at a depth of 8 to 10 cm from the sediment surface. Sediments were enclosed in plastic caps containing an O-ring, and sealed with glass fiber filters and 0.2 μm micro pore filters on both sites to support radial flow-through the reactor and to prevent bacterial outflow, respectively. Further aspects of the flow-through reactor technique are given in Roychoudhury et al., 1998, Pallud and Van Cappellen, 2006 and Laverman et al., 2006. Reactors were stored in anaerobic bags and kept at 4°C during transport and storage before experimentation. Flow-through reactor experiments were started within 2 weeks after sample collection.

4.2.2 Flow-through reactor experiments

The majority of reactors, total of 4, were incubated sequentially at 30, 60 and 85°C using a thermostatic water bath (Table 4.2). Reactor materials and tubing were stable to a maximum temperature of 85°C, thereby limiting the maximum incubation temperature. The temperature was kept constant until a steady state outflow SO_4^{-2} concentration was measured, which was defined as a constant outflow of SO_4^{-2} and corresponding potential sulfate reduction rate (SRR) for at least three subsequent days with a maximum standard deviation of 10 %. After obtaining steady state SRR, the temperature was increased gradually (10°C per day) until

Table 4.2: Overview of samples, experimental conditions, average potential sulfate reduction rate (SRR) and isotopic fractionation effects (ε) for sediments collected in June 2007 in Baia di Levant, Vulcano Island, Italy. SRR and ε values are calculated from an average of 3 to 5 outflow solutions with identical sulfate concentrations within an individual reactor, which indicated steady state behavior. Variability is given as the standard deviation around the mean. *For these ε values the standard deviation reported between steady state measurements within the individual reactor is smaller than the external reproducibility of the analytical technique that is typically 0.5 per mil for $\delta^{34}S$ measurements (see experimental methodology).

location	reactor name	Temp. (°C)	Depth (cm)	SO_4^{2-} (mM)	SRR (nmol cm^{-3} h^{-1})	sd	ε (‰)	sd
surf zone by vent	1a	30	8-10	7	126	5	12	1
surf zone by vent	1b	30	8-10	7	158	6	13	1
surf zone	2a	30	8-10	7	127	2	15	1
surf zone	2b	30	8-10	7	143	3	13	1
surf zone by vent	1a	60	8-10	7	97	5	14	1
surf zone by vent	1b	60	8-10	7	142	14	6	0.2
surf zone	2a	60	8-10	7	78	5	16	2
surf zone	2b	60	8-10	3.5	82	2	9	0.3
surf zone by vent	1a	85	8-10	7	164	3	9	1
surf zone by vent	1b	85	8-10	7	164	3	8	0.5
surf zone	2a	85	8-10	7	run out of sulfate			
surf zone	2b	85	8-10	7	167	2	7	1
20 m offshore, 1.5 m below water line	3	85	8-10	3.5	85	2	10	1

the new incubation condition was reached. One reactor was incubated directly at 85°C, without temperature changes throughout the experiment. For all reactors artificial inflow solutions were prepared containing 10 mM of lactic acid and 3.5 or 7 mM $MgSO_4$ (Table 4.2). Salinity was adjusted to that of Baia di Levante seawater concentrations, with 32 mM $MgCl_2 \cdot 6H_2O$ and 430 mM NaCl. Bromide (2 mM NaBr) was added as a flow tracer to monitor homogeneous flow within the reactor during the course of the experiment. Before connecting the inflow solutions, reactors were flushed for 24 h (approximately 1.5 pore space volumes) with a solution containing 32 mM $MgCl_2 \cdot 6H_2O$ and 430 mM NaCl to replace the site pore water. Inflow solutions were introduced at 0.9 ± 0.1 mL/h using a peristaltic pump with Tygon tubing of negligible oxygen permeability. Outflow solutions were collected in 50 ml tubes, prefilled with 4 ml 20 % zinc acetate solution to trap sulfide as ZnS. Tubes were changed manually every 24 h. After collection, tubes were stored immediately at -18°C until chemical and isotopic analysis could be performed. The whole system, including sampling tubes, was pressurized under an argon atmosphere. Reactors remained in the dark during the incubation experiments, which ran for up to 1500 h. Following incubation, the sediments were freeze dried and stored for bulk isotopic analysis.

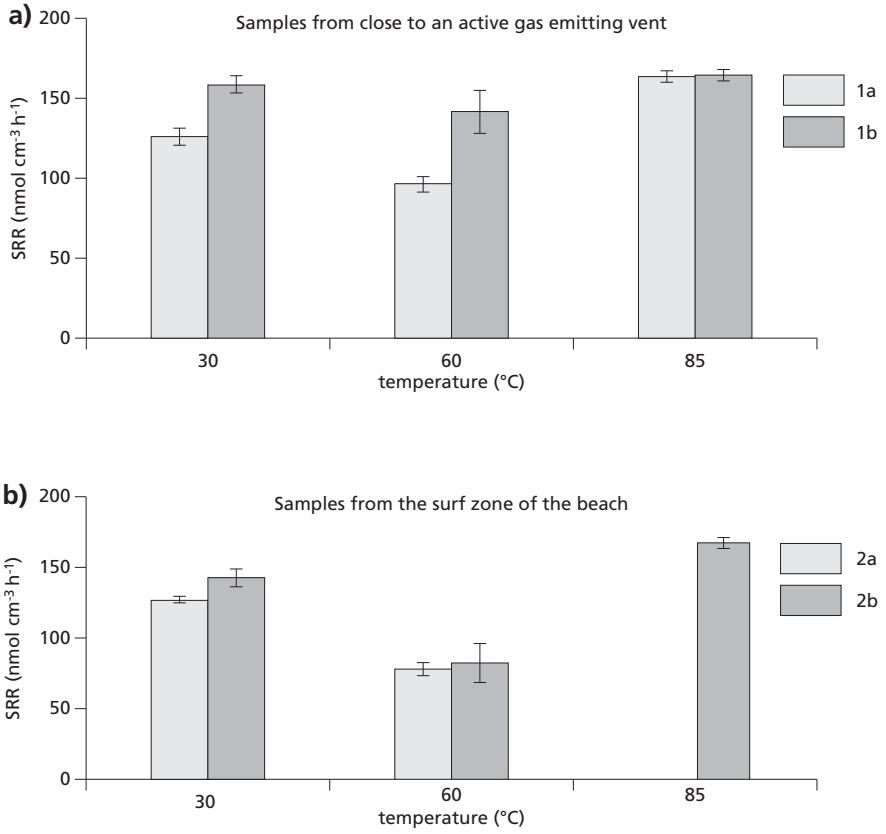

Figure 4.1: Potential sulfate reduction rates (SRRs) obtained at 30, 60 and 85°C for sediments collected on the beach at Baia di Levante (Panel 1a) and on the beach close to a gas emitting vent (Panel 1b). Error bars represent the variability in steady state SSR expressed as the standard deviation (Table 4.2).

4.2.3 Analytical techniques and calculation of reduction rates and isotope fractionation

SO_4^{2-} and Br^- concentrations were determined by ion chromatography (Dionex DX120 equipped with an AS14 column). The detection limit was < 5 µM with a mean precision of approximately 4 %. Steady state sulfate reduction rates were calculated using:

$$SRR = \frac{Q\ C}{V} \qquad (1)$$

where Q represent the flow rate in ml/h, ΔC is the difference between input and output sulfate concentration in mM and V is the volume of the sediment in the reactor in cm^3 which is 27.7 cm^3. All measured sulfate reduction rates should be considered as potential rates, because sulfate is the only electron acceptor supplied to the flow-through reactors.

Isotope measurements were made on samples selected from (near) steady state areas. Sulfate in the inflow and outflow solutions was precipitated as $BaSO_4$ with $BaCl_2$ solution (10 %). After washing with deionized water, the precipitate was dried for several days at 50°C. $\delta^{34}S$ was measured using an elemental analyzer Na 1500NCS coupled to a Finnigan MAT (Delta +) gas source mass spectrometer. $BaSO_4$ was converted to SO_2 by flash combustion in a tungstic oxide, ultra pure copper quartz tube at 1050°C. (mean precision of approximately 0.5 ‰). Isotope fractionation was calculated following a Rayleigh distillation model assuming homogeneous flow-through the reactors (Canfield, 2001). Isotope fractionation effects (ε) were calculated from the delta notation (δ) using:

$$\alpha = \left(\frac{1 + (\ln\delta_{SO4\text{-}in} + 1000) - (\ln\delta_{SO4\text{-}out} + 1000)}{\ln(f_{SO4})} \right) \quad (2)$$

and

$$\varepsilon = 1000(\alpha - 1) \quad (3)$$

where $\delta_{SO4\text{-}in}$ and $\delta_{SO4\text{-}out}$ represents the isotopic composition of the inflow and outflow solution, respectively, and f_{SO4} is the fraction of sulfate remaining in the outflow solution compared to the inflow concentration. Sedimentary sulfide $\delta^{34}S$ was measured on freeze dried starting sedimentary material, collected next to the sediment cores used for the flow-through

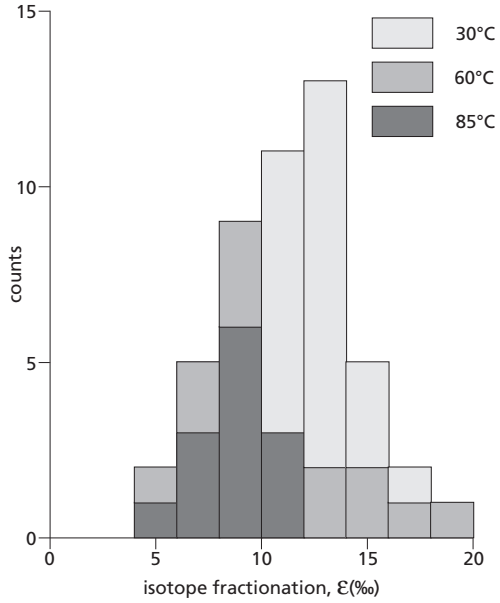

Figure 4.2: Distribution plot of isotope fractionation (ε) *versus* the number of samples selected by temperature (30, 60, 85°C; 23, 12 and 13 data points, respectively). This plot was made using the individual data points produced under steady state conditions.

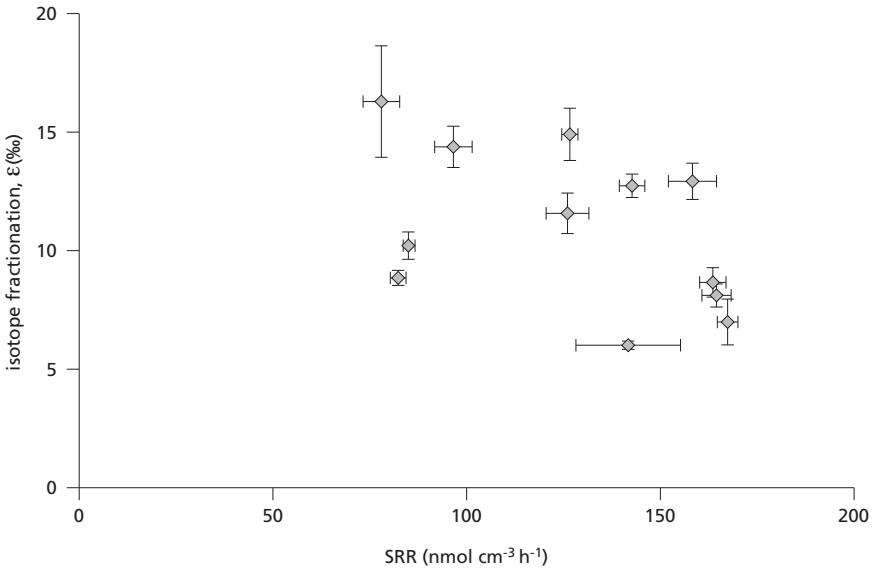

Figure 4.3: Isotope fractionation effects (ε) *versus* steady state sulfate reduction rates (SRR) for Vulcano sediments. Error bars represent standard deviation calculated from variation in SRR and ε within the steady state regions of individual reactors (Table 4.2).

reactors, and in freeze dried samples of all sediments used in the flow-through reactors after finishing the experiments. Approximately 2 g of these sediments were distilled using a chromium reduction method to separate out reduced sulfur compounds (Canfield et al., 1986; Fossing and Jørgensen, 1989). Sulfide produced during distillation was trapped as Ag_2S and measured using the same analytical approach as for the sulfates.

Molar C/N ratios of freeze dried and decalcified starting sediments were determined by measuring the organic carbon (C) plus total nitrogen (N) content using a Carlo Erba CN analyzer. Sediment samples for this additional analysis were collected next to the sediment cores that were used for flow-through reactor experiments.

4.3 Results

4.3.1 Potential sulfate reduction rates

No measurable potential sulfate reduction rates (SRRs) were obtained when using the natural substrate, when electron donors for sulfate reduction could only be supplied from within the sediment. High SRRs varying from 78 to 168 nmol cm^{-3} h^{-1} were readily achieved during lactate amendment, across the complete temperature range from 30, 60 to 85°C and for all sampling locations (Table 4.2, Figure 4.1). All sediments collected on the beach, in proximity to or away from a gas emitting source, showed a similar SRR response to incubation temperature with the highest rates obtained at 30 and 85°C and lower rates at 60°C (Figure

4.1) The sediment collected 20 m offshore showed a lower maximum rate at 85°C of 85 nmol cm^{-3} h^{-1} compared to the sediments sampled on the beach with an average value of 165 nmol cm^{-3} h^{-1}. In all cases, steady state SRR ware reached within 3 to 5 days after a temperature change.

4.3.2 Sulfur isotope fractionation effects

Isotope fractionation effects (ε) ranged from 6 to 16 ‰ (Table 4.2). At 60°C there was a larger range in fractionation compared to 30 and 85°C (Figure 4.2). Average fractionation decreased with increasing temperature from 13 to 11 and 9 ‰ respectively. A very weak negative ε *versus* SRR trend with a R^2 of 0.21 was found when considering all data together (Figure 4.3). However, within individual reactors this relationship was highly variable with the measurement of strong inverse (R^2 = 0.99) to weak positive (R^2 = 0.28) trends. In non-amended reactors, run with the natural substrate, where rates were close to zero, $\delta^{34}S$ values of the inflow solution were identical to those of the outflow solution. For samples yielding high SRRs, the $\delta^{34}S$ signal of sulfides in the final sediment following incubation was enriched in the lighter ^{32}S isotope by 1 to 3 ‰ compared to the starting sediment.

4.4 Discussion

This study presents the first sulfur isotope data related to microbial activity in a shallow marine hydrothermal environment, using sediments sampled from the previously well-studied area close to active vents of Vulcano Island, Italy. It is assumed that all fractionation effects in these experimental data are of biogenic origin since potential sulfate reduction rates (SRRs) were not detectable and $\delta^{34}S$ values remained unchanged when incubating sediments with a non-amended natural substrate. The apparent absence of abiotic processes capable of significantly reducing sulfate or modifying sulfur isotope ratios above background levels was observed across the whole experimental temperature range.

4.4.1 Potential sulfate reduction rates

The necessity for lactate addition to initiate sulfate reduction during sediment incubation suggests substrate limitation in the sediment. Low levels of labile organic matter in Vulcano sediments were confirmed by C/N ratio measurements in which the nitrogen content was below detection limit and the percentage of organic carbon was approximately 0.05 %. This was at least 20 to 100 times lower when compared to more typical present day sites of microbial sulfate reduction including mesophilic fresh water, brackish to marine localities (Pallud and Van Cappellen, 2006). Lactate addition resulted in high SRRs, up to 167 nmol cm^{-3} h^{-1}, over the complete temperature range of 30 to 85°C applied in this study. A previous study on biogenic sulfate reduction in sediments from Vulcano, which were incubated at 90°C, used acetate as the organic substrate (Tor et al., 2003). Maximum rates were around 3 nmol cm^{-3} h^{-1} which was much lower compared to this study. This indicates that lactate is a more efficient electron donor at this site. The preference of SRP for lactate may explain the

relative high *in situ* concentrations of 7.0 μM acetate measured by Rusch et al. (2005), which could reflect the accumulation of metabolic products from the incomplete oxidation of lactate. Although lactate concentrations (2.8 μM) in the field are lower compared to acetate, they are relatively high compared to other small organic compounds including formate and propionate with *in situ* concentrations of less than 0.8 μM and 0.6 μM respectively (Rusch et al., 2005). However, under *in situ* conditions H_2 may also have been an important electron donor, since measured H_2 pulses in Vulcano fluids are very variable ranging from below detection limit (Gugliandolo et al., 1999; Capasso et al., 2001) to values between 570 to 1840 vppm (Rogers et al., 2007). These fluctuations in concentration may partly be explained by the fact that many microorganisms, especially (hyper)thermophiles, are able to metabolize with H_2 as an electron donor (Hoek et al., 2006; Childs et al., 2008; D'imperio et al., 2008). Furthermore, both hydrogen and lactate are among the most efficient electron donors during sulfate reduction, since oxidation of these substrates releases the most energy at moderate (Oren, 1999; Detmers et al., 2001) and (hyper)thermophilic conditions (Amend and Shock, 2001;). Thus high sulfate reducing activity may well occur within the natural substrate in the Baia di Levante despite the fact that it was not observed in the flow-through reactor experiments of this study. A preference for high energy yielding substrates by the (hyper)thermophile community is likely due to the energetically costly adaptation strategies that are required to make cell membranes, internal proteins and enzymes heat resistant (Stetter, 1999; Albers et al., 2000; Berry, 2002; Minic, 2009). A similar observation with respect to energetics was made for mesophiles thriving in environments of hypersalinity or high alkalinity (***Chapter 3***).

The high SRRs obtained within a single lactate amended sediment slice over the complete temperature range from 30°C, 60°C to 85°C, indicate the presence of mesophilic, thermophilic and hyperthermophilic species. The presence of mesophiles in sediments that periodically experience high temperatures and (hyper)thermophiles that persist into low temperature environments are not uncommon (Isaksen et al., 1994; Elsgaard et al., 1995). Although all these microorganisms have their own optimum growth temperature, they could survive in a dormant state and become active when favorable conditions are experienced. This may especially be the case for the Vulcano hydrothermal vent system where communities should be able to quickly adapt to larger ranges and faster fluctuations in temperatures compared to low temperature marine environments. For example in an estuarine site the annual fluctuation in temperature varies from 2 to 30°C (Cho et al., 2005) whereas fluctuation in hydrothermal vent systems may vary from 3 to 120°C within several days (Elsgaard et al., 1994). Furthermore, Rusch et al. (2005) measured large fluctuations in temperature from 30 to 60°C within a small spatial lateral interval (top 0-2 cm) of Vulcano sediments.

The range in SRRs of 78 to 167 nmol cm^{-3} h^{-1} obtained for Vulcano sediments is much higher compared to some other hydrothermal vent systems (Jørgensen et al., 1992; Elsgaard et al., 1994; Roychoudhury, 2004) but several studies have found similar rates (Weber and Jørgensen, 2002; Kallmeyer and Boetius, 2004; Dillon et al., 2007). In a flow-through reactor study using lactate amended sediment collected from the Guaymas basin, rates exceeded 80 nmol cm^{-3} h^{-1} for only a restricted period of time, up to a maximum of 174 nmol cm^{-3} h^{-1} and only at a temperature of 75°C. At all other temperatures explored, ranging from 50 to 88°C,

rates were much lower fluctuating between 5 to 60 nmol cm^{-3} h^{-1} (Canfield et al., 2000). In the sediments from Vulcano, continuously high rates were achieved at temperatures ranging from 30 to 85°C. These differences between sites are most likely a result of site specific community structures in both the SRP and coexisting microorganisms e.g. fermenters. The availability of substrates may also have played a role if the sediment was heterogeneous with respect to organic matter content. These effects may also explain the difference in SRR observed in this study between different temperatures, and the difference in SRR between sediments collected on the beach or offshore (Figure 4.1). However, the difference in rate may also be partly an effect of experimental approach as the offshore sediment was immediately incubated at 85°C, whilst the beach sediment was gradually heated to 85°C within several weeks, giving the hyperthermophilic community the ability to slowly grow and adapt to high temperatures. The consistent drop to slightly lower SRR at 60°C (Figure 4.1) may also be a result of a change in community composition. Whereas at 30°C and 85°C only mesophiles or hyperthermophiles are active some hyperthermophiles may show low growth rates at the intermediate temperature (60°C) that suppresses the average rate to lower values. For example *Archeoglobus Fulgidus* showed growth activity at temperatures as low as 45°C while its optimum growth temperature is around 80°C (Mitchell et al., 2009).

4.4.2 Sulfur isotope fractionation effects

The average isotope fractionation effect within individual reactors obtained with Vulcano sediments was relatively small ranging from 6 to 16 ‰, compared to the complete range of fractionation of -3 to 47 ‰ previously observed in pure culture and natural sediment experiments (e.g. Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Habicht and Canfield, 1997; Detmers et al., 2001; Canfield, 2001; Johnston et al., 2007; Farquhar et al., 2008). Substrate limitation is often assumed to be the reason for larger amounts of fractionation (Canfield et al., 2000), and the small fractionation effects could be a result of amendment with lactate. Alternatively, the type of metabolic process may account for the overall fractionation (Detmers et al., 2001; Brüchert, 2004). Complete oxidizers have been shown under optimum growth conditions in pure culture to fractionate above 15 ‰ whereas incomplete oxidation resulted in ε values smaller than 19 ‰ (Detmers et al., 2001; Hoek et al., 2006; Davidson et al., 2009). Incomplete oxidizers might be dominant at Vulcano and the decrease in average fractionation (13, 11 to 9 ‰) from mesophilic towards hyperthermophilic conditions would be consistent with a shift to more incomplete oxidation at higher temperatures. Detailed molecular biological studies are required to investigate this possibility.

Relatively small fractionation effects, consistent with ε values obtained for Vulcano sediments, have been found in many pure cultures of thermophile to hyperthermophile microorganisms (Detmers et al., 2001). However, the most well studied hyperthermophilic sulfate reducer *Archaeoglobus*, originally isolated from Vulcano sediments (Stetter, 1987), can produce, under optimum conditions with lactate as an electron donor, fractionation up to 26 ‰ (Habicht et al., 2002; Habicht et al., 2005) (Table 4.1). Microbial community analysis of sediments from Baia di Levante showed that *Archaeoglobus* was not found to be dominant

(Rusch and Amend, 2008). This suggests that the relatively small fractionation effects in this study were produced by other types of hyperthermophilic SRP. Similar flow-through reactor experiments with Guaymas Basin sediments under non-limiting substrate conditions yielded much higher fractionation compared to this study (Canfield et al., 2000). This was especially the case at high SRR, where average fractionation was approximately 25 ‰. The difference in fractionation effects could be a result of changes in microbial community structure, especially the ratio of complete to incomplete oxidizers.

4.4.3 Implications for tracing high temperature SRP in the geological record

Relatively minor amounts of sulfur isotope fractionation are observed in the earliest terrestrial rock record, especially in the Archean, with only a few localities showing significant deviations below inferred seawater values of 3 to 4 ‰ (Shen et al., 2001; Shen and Buick, 2004; Philippot et al., 2007; Johnston et al., 2008b; Shen et al., 2009). The generally small variation in $\delta^{34}S$ in sedimentary rocks at that time has been interpreted to reflect a lack of isotope fractionation due to sulfate limiting conditions in the Archean oceans (Habicht et al., 2002). Thus if microbial sulfate reduction had evolved, as suggested by variations in pyrite $\delta^{34}S$ included in or in close proximity to Archean barite deposits (Shen and Buick, 2004), it would not have been recorded by isotope variations in an open marine setting. However, data obtained in this study open the possibility that biogenic sulfate reduction could have been more prevalent on the early Earth than previously thought, especially in areas of abundant sulfate close to hydrothermal vent systems where fractionation effects may have been small. Low sulfate concentrations, although certainly important, may not have been uniform across early Earth environments and are not an exclusive solution to the lack of sulfur isotope variation at this time. Unfortunately, in hydrothermal systems where temperatures above 200°C can be reached, the biogenic $\delta^{34}S$ signal maybe overprinted by that obtained from inorganic processes e.g. thermal sulfate reduction, which can show a similar range in $\delta^{34}S$ values (Ohmoto and Goldhaber, 1997). Recent developments in using $\Delta^{33}S$ and $\Delta^{36}S$ as a tracer of biogenic activity may help to resolve these problems (Ono et al., 2007; Johnston et al., 2008a; Ono, 2008).

4.5 Conclusions

Microbial communities thriving in the sediments of the shallow submarine hydrothermal vent system of Vulcano Island (Italy) contain a dynamic microbial sulfate reducing community showing consistently high sulfate reduction rates during lactate amendment of up to 167 nmol cm^{-3} h^{-1}, over a broad temperature range (30 to 85°C). Sulfate reduction was not detectible when electron donors were only available from the natural substrate, most likely the result of low organic matter content in the sediment. Sulfur isotope fractionation effects (ε) were small ranging from 6 to 16 ‰ suggesting that the majority of sulfate reducers are incompletely oxidizing their substrate. This study shows that small isotope fractionation effects, often found in Archean rocks and sediments, are not only explained by sulfate limiting

conditions, but may alternatively reflect high biogenic sulfate reducing activity of specific microbial communities thriving on a non-limiting sulfate pool.

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Image of flow-through reactors

Chapter 5

Sulfur isotope fraction in an estuarine sediment during enhancement or partial inhibition of microbial sulfate reduction

A preliminary study

Abstract

Large microbial sulfur isotope effects of up to 70 ‰ between coexisting sulfate and sulfide reservoirs have been observed in the nature but cannot be reproduced in sediment incubation and pure culture experiments which fractionate up to only 47 ‰. The origin of the excess fractionation in nature is unclear but may be linked to very low rates of sulfate reduction, geochemical variability in for instance type of electron donor, or repeated cycles of oxidation and reduction. In this study the range of measured sulfate reduction rates at a brackish tidal estuary in the Netherlands is expanded by adding compounds that are known to enhance or inhibit microbial sulfate reduction, to investigate the potential for more isotopic variability than found under the site optimum conditions that were used in previous experiments (*Chapter 2*). Electron donors for sulfate reducing prokaryotes, lactate and acetate (10 mM), were used to increase potential sulfate reduction rates (SRRs), whilst rate reductions were achieved by adding variable concentrations of the group VI oxidized anions chromate, selenate, molybdate and tungstate (0 to 10 mM). Sediments were incubated in flow-through reactors at temperatures from 10 to 30°C. Lactate addition resulted in a 14 fold increase in SRR, whilst isotope fractionation remained comparable to values obtained for the natural substrate. Acetate addition had a negligible effect on SRR but gave more variability, up to 8 ‰, in isotope fractionation when compared against the natural substrate data. Inhibition of SRR with SeO_4^{2-}, MoO_4^{2-} and WO_4^{2-} was complete at concentrations above 5 mM where no isotope effects could be measured. Isotope fractionation was suppressed with a maximum of 12 and 18 ‰ with increasing concentrations from 0 to 1 mM of MoO_4^{2-} and SeO_4^{2-} respectively, whilst WO_4^{2-} and CrO_4^{2-} showed smaller changes in SRR and isotope fractionation due to strong adsorption of these compounds into the sediment. The total variability in isotope data induced by enhancers and inhibitors of sulfate reduction, 5 to 32 ‰, does not extend the range that is possible across the SRR that would be normally experienced in this sedimentary environment.

5.1 Introduction

Sulfur isotope fractionation during microbial sulfate reduction has been extensively studied in experiments with both pure cultures and microbial communities hosted in sediments, and reveals a range in $\delta^{34}S$ between reactant sulfate and product sulfide of up to 47 ‰ (Kaplan and Rittenberg, 1964; Rees, 1973; Chambers et al., 1975; Habicht and Canfield, 1997; Canfield, 2001; Detmers et al., 2001; Canfield et al., 2006; Farquhar et al., 2008; *Chapters 2, 3* and 4). In contrast, natural sedimentary sulfides are often depleted in $\delta^{34}S$ by up to 70 ‰ with respect to seawater from which their sulfur was derived (Strauss, 1997). This mismatch between experimental and natural variability has been attributed to oxidative recycling of sulfur coupled to microbial elemental sulfur disproportionation, leading to additional fractionation effects (Canfield and Thamdrup, 1994; Canfield and Teske, 1996). Identification of this process in the geological record is important since it places constrains on the redox level of depositional environments through time as well as the evolution of different microbial

metabolisms. However, more recent studies have suggested that reoxidation is not necessary, as large isotope fractionation effects may be possible within the cellular sulfate reduction process (Wortmann et al., 2001; Rudnicki et al., 2001; Brunner and Bernasconi, 2005; Davidson et al., 2009). Fractionation exceeding 47 ‰ could occur at very low rates of sulfate reduction or at high concentrations of pore water sulfide as suggested for deep sea sediments (Rudnicki et al., 2001; Wortmann et al., 2001). Multiple sulfur isotope data (^{32}S, ^{33}S, ^{34}S, ^{36}S) have been used to distinguish oxidative pathways from intracellular fractionation effects (Johnston et al., 2005; Ono, 2008) and data for natural samples suggest that another, yet undetermined factor, may have influenced and thereby altered the sulfur isotope composition of sedimentary sulfides (Ono et al., 2006; Ono et al., 2007; Rouxel et al., 2008).

This study is designed to investigate whether sulfur isotope fractionation shows strong variability at high and low sulfate reduction rates, when amending sediments with chemical compounds which are known to strongly enhance or inhibit the metabolism of sulfate by microorganisms. Although it is well known that rates of microbial sulfate reduction and corresponding isotope fractionation effects (ε) are influenced by the type and concentration of the organic substrate, the exact magnitude of these effects is not well constrained (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Canfield, 2001; Brüchert, 2004; Hoek et al., 2006). Furthermore, sulfur isotope fractionation associated with partial inhibition by the presence of chemical compounds that block the processing of sulfate through the cell has not been extensively investigated and has been shown in only one study to date (Stogbauer et al., 2004).

Sulfate reducing prokaryotes (SRP) can metabolize with diverse electron donors including both small and large organic molecules (e.g. ethanol, acetate, lactate, formate, propionate, fatty acids, sugars, hydrocarbons) or inorganic species (e.g. H_2 or CO) (Liamleam and Annachhatre, 2007). Both lactate and acetate are commonly used to promote sulfate reduction in pure culture and sediment incubation experiments (Widdel, 1988; Muyzer and Stams, 2008). In sediments, the natural substrate concentration is difficult to estimate and could fluctuate considerably depending on metabolic rates through the community of SRP as well as on the episodic inflow of degraded plant and animal material or dissolved inorganic compounds (Westrich and Berner, 1984; Middelburg et al., 1996; Zogg et al., 1997; Kostka et al., 2002; Weston and Joye, 2005). Substrate limitation in sediment incubation experiments has been shown to result in smaller SRR and increased ε (Canfield, 2001; Hoek et al., 2006) which is in agreement with the standard isotope fractionation model (Rees, 1973). However, there are indications that not only rates but also the physiology and the metabolic pathway used by the SRP can determine ε, where for instance complete organic substrate oxidation leads to fractionations greater than 15 ‰ compared to less than 19 ‰ for incomplete oxidizers (Detmers et al., 2001; Brüchert, 2004). In summary, addition of an excess of electron donor is thus expected to increase SRR and reduce the observed amount of fractionation relative to the natural substrate whereas the absolute magnitude of ε in the product sulfide is likely to be dependent on the type of substrate and the metabolic pathway of the sulfate reduction process.

Sulfate reduction is inhibited by the presence of group VI oxidized anions (e.g. chromate, selenate, molybdate and tungstate) that block key cellular enzymatic steps (Frausto Da Silva

and Williams, 1991). These compounds are effective in suppressing the sulfate reduction process since their stereo chemical structure is similar to that of sulfate (Oremland and Capone, 1988). The first step in sulfate reduction process is the formation of adenosine-5'-phosphosulfate (APS) from adenosine-5'-triphosphate (ATP) and SO_4^{2-}. All four inhibitors can substitute for the sulfate thereby blocking the formation of APS and sequential steps cannot occur. Only selenate can form a stable APSe complex whereas APCr, APMo and APW are unstable and are quickly broken down to adenosine mono phosphate (AMP) and the inhibitor itself. This process leads to the depletion in ATP which is only recovered by the biogenic reduction of sulfite to sulfide (Taylor and Oremland, 1979). All compounds are competitive inhibitors because they bind to the same position on the enzymes as the sulfate ion. The degree of inhibition is therefore also strongly dependent on the sulfate concentration (Banat and Nedwell, 1984). Efficiency in inhibition decreases in the following order: chromate > molybdate = tungstate > selenate (Oremland and Capone, 1988). In sediment incubation experiment this order may be different due to adsorption onto the sediment or respiration or assimilation of some of these compounds by the diverse microbial community. Increasing amounts of inhibitor addition should lead to a progressive reduction in SRR until complete inhibition is observed. In the only study to date investigating the effects of a group VI oxyanion inhibitor, molybdate, on sulfur isotope fractionation, a decrease in ε of up to 6 ‰ during partial inhibition of sulfate reduction was observed (Stogbauer et al., 2004).

Flow-through reactor experiments provide comprehensive isotope fractionation effect data for the microbial reduction of sulfate to sulfide (Canfield, 2001; Farquhar et al., 2008; *Chapters 2, Chapter 3* and *Chapter 4*) that fall within the range predicted by the standard fractionation model (Rees, 1973). Experiments using sediments from the Schelde Estuary (*Chapter 2*), Mono Lake, California (*Chapter **3***) and Vulcano Island, Italy (*Chapter 4*), reveal that the bulk of isotope fractionation in natural communities gives a $\delta^{34}S$ offset into sulfide of less than 20 ‰. However, the bulk of these experiments have been performed under close to optimum, site matched conditions with electron donors for sulfate reduction obtained from within the natural substrate. In this study, experiments that were done in *Chapter 2*, were expanded to more extreme SRR values, in order to investigate whether a significant change in isotope fractionation effect can be induced by fluctuations in the chemical environment. Sediments were incubated with 1) the natural substrate, 2) acetate or lactate as enhancers of sulfate reduction and 3) different concentrations of CrO_4^{2-}, SeO_4^{2-}, MoO_4^{2-} or WO_4^{2-} to inhibit sulfate reduction. The effects on SRR and ε are explored and compared to those observed using the natural substrate.

5.2 Sampling and experimental methodology

5.2.1 Sample collection

Sediment samples were collected from the tidal flat of the Schelde Estuary (51°24'04"N 04°07'04"E) close to the village of Waarde in The Netherlands. Samples were taken approximately 30 m from the border of vegetated salt marsh during three field seasons in

February 2006 (experiments with acetate), October 2006 (experiments with SeO_4^{2-} and MoO_4^{2-}) and April 2007 (experiments with lactate and CrO_4^{2-} and WO_4^{2-}), most of which were collected at the same time as those described in *Chapter 2*. Sediments slices (2 cm thickness and a diameter of 4.2 cm) were sampled from the 0-2 cm depth interval using a shuttle corer packed with 2 cm Perspex rings and were immediately closed between two plastic caps, containing centered inflow and outflow channels, to complete the flow-through reactor. The caps were prefilled with an O-ring, a glass fiber filter and a 0.2 µm nitrocellulose filter to prevent leakage and outflow of microorganisms and sedimentary material. Reactors were sealed in anaerobic bags and transported to the lab, where they were stored at 4°C prior to experimentation that began within 5 days of sampling. More detailed information of the sampling site and flow-through experiments can be found in Roychoudhury et al. (1998), Pallud and Van Cappellen (2006) and Laverman et al. (2006).

5.2.2 Flow-through reactor experiments

Artificial inflow solutions were prepared with 2 mM Na_2SO_4, a site-adjusted salinity of 180 mM NaCl and 2 mM NaBr as a flow tracer. For the enhancement experiments 10 mM acetate or 10 mM lactate were added (Table 5.1). In the case of lactate amendment, the sulfate concentration was increased to 10 mM to prevent sulfate limitation. For the inhibition experiments variable concentrations of Na_2CrO_4, Na_2SeO_4, Na_2MoO_4 and Na_2WO_4, ranging from 0 to 10 mM, were added to inflow solutions (Table 5.1). Each concentration was supplied to a different reactor. Control reactors, to which no external electron donor was added, were run in parallel during the October 2006 and April 2007 experiments. For the acetate experiment (February 2006) reactors at 10, 20 and 30°C were first run with the natural substrate and after a steady state constant sulfate outflow concentration was reached, reactors were amended with 10 mM of acetate.

Inflow solutions and collection tubes were connected to the flow-through reactor using Tygon tubing and the experimental set up was pressurized under an argon atmosphere to maintain anoxic conditions. Reactors were kept in the dark during experimentation.

Table 5.1: Overview of concentrations of inhibitors (CrO_4^{2-}, SeO_4^{2-}, MoO_4^{2-}, WO_4^{2-}) and enhancers (lactate and acetate) of microbial sulfate reduction. Each compound and concentration was supplied to a separate reactor.

concentration (mM)	Inhibitors				Enhancers	
	CrO_4	SeO_4	MoO_4	WO_4	Acetate	Lactate
0.005	x	x	x	x		
0.01	x	x	x	x		
0.1	x	x	x	x		
0.5	x	x	x	x		
1	x	x	x	x		
5		x	x	x		
10		x	x		x	x

Incubation, using a thermostatic water bath, was carried out at 20°C, except for reactors amended with acetate that were run at 10, 20 and 30°C. Solutions were introduced using a peristaltic pump with a flow rate of 0.9 ± 0.1 ml/h and outflow samples were collected using an autosampler. Reactors were initially flushed for 24 hours with a 180 mM NaCl solution to remove the pre-existing pore water. Inflow solutions with NaCl, Na_2SO_4, NaBr and the inhibitor or enhancer were then connected. Samples were initially collected every 2 hours, for the first 26 hours, followed by every 6 hours for the next 24 hours. Sediments were further incubated between 300 and 1000 hours whilst outflow solutions were collected every 12 hours in 15 ml tubes prefilled with 2 ml 1% zinc acetate solution to trap the product sulfide as ZnS. After collection samples were stored at -18°C until chemical or isotopic analysis could be performed.

5.2.3 Chemical and isotopic analysis

Sulfate and Br^- concentrations were measured in the outflow and inflow solutions by standard ion chromatography techniques using a Dionex DX120 equipped with an AS14 column. The detection limit was < 5 µM with a mean precision of approximately 4 %. Concentrations of Cr, Se, Mo and W in the inflow and outflow solution were measured by ICP-OES. Sulfate was precipitated from the outflow and inflow solutions as $BaSO_4$ using a 10 % w/v $BaCl_2$ solution. Precipitates were rinsed with deionized water and dried for several days at 50°C. $\delta^{34}S$ was measured using a Na 1500NCS elemental analyzer coupled to a Finnigan MAT Delta+ gas source mass spectrometer, in which $BaSO_4$ was converted to SO_2 by flash combustion in a tungstic oxide, ultra pure copper quartz tube at 1050°C. Mean precision of the $\delta^{34}S$ measurements was approximately 0.5 ‰. Sulfate reduction rates (SRR) and isotope fractionation effects (ε) were calculated from areas where outflow sulfate concentration was constant for at least 3 subsequent days within a maximum error of approximately 10 %. SRR and ε were calculated as shown in *Chapters 2*, *Chapter 3* and *Chapter 4*.

5.3 Results

An overview of sulfate reduction rates (SRRs) and corresponding sulfur isotope fractionation effects (ε) is given in Table 5.2, broken down by organic substrate (Table 5.2a) and inhibitor (Table 5.2b and Table 5.2c) concentrations. Using the natural substrate, SRR ranged from 7 to 43 nmol cm^{-3} h^{-1} with the lowest and highest rates achieved at 10 and 30°C. Rates obtained at 20°C varied from 11 to 25 nmol cm^{-3} h^{-1} depending on the period in which the samples were collected at the field site. Steady state SRR were obtained at 20 and 30°C for a relatively short period of time of only 3 to 5 days. ε varied between 17 and 23 ‰ (Table 5.2).

Amending with lactate resulted in a more than 14 fold increase in SRR whereas with acetate rates were similar to those obtained with the natural substrate (Table 5.2a and Figure 5.1). The effect of adding an organic substrate was observed immediately but 2 to 3 days were required to reach a steady state outflow sulfate concentration, which then remained until the end of the experiment. Beside a large increase in SRR, lactate addition resulted in a decrease

in ε of 3 ‰. With acetate, a drop in ε (6 to 8 ‰) was observed at 10 and 30˚C whereas fractionation at 20˚C was comparable for the amended and non-amended substrates (Table 5.2a). The addition of lactate or acetate resulted in fractionation effects (ε) ranging from 9 to 18 ‰.

Significant inhibition of sulfate reduction (> 15 %) started at concentrations of 0.005 mM SeO_4^{2-}, 0.01 mM MoO_4^{2-}, 0.1 mM WO_4^{2-} or 0.5 mM CrO_4^{2-} (Table 5.2b and 5.2c and Figure 5.2). Complete inhibition with SeO_4^{2-}, MoO_4^{2-} or WO_4^{2-} was found at concentrations above 5 mM. Isotope fractionation during partial inhibition ranged from 5 to 32 ‰. Fractionation obtained with CrO_4^{2-} and WO_4^{2-} was more comparable to values obtained with the natural substrate with an excursion (maximum 12 ‰) towards higher values whereas with SeO_4^{2-} and MoO_4^{2-} fractionation was significantly suppressed up to 12 and 18 ‰ respectively (Table 5.2b and 5.2c and Figure 5.2). For all inhibitors the concentration in the outflow solutions were lower compared to the inflow concentrations (Figure 5.3). For Cr, W, Se and Mo only 0-6 %, 13-20 %, 12-35 % and 39-55 % of the original concentration was recovered, respectively.

5.4 Discussion

The addition of chemical compounds, known to enhance or inhibit microbial growth, to flow-through reactor inflow solutions resulted in a significant increase in the range of sulfate reduction rates (SRRs) measured in sediments from the Schelde Estuary (compare with *Chapter 2*). In the following discussion the variability in isotope fractionation at high and low extremes of SRR and the specific effects of inhibitor compounds on cellular processes during

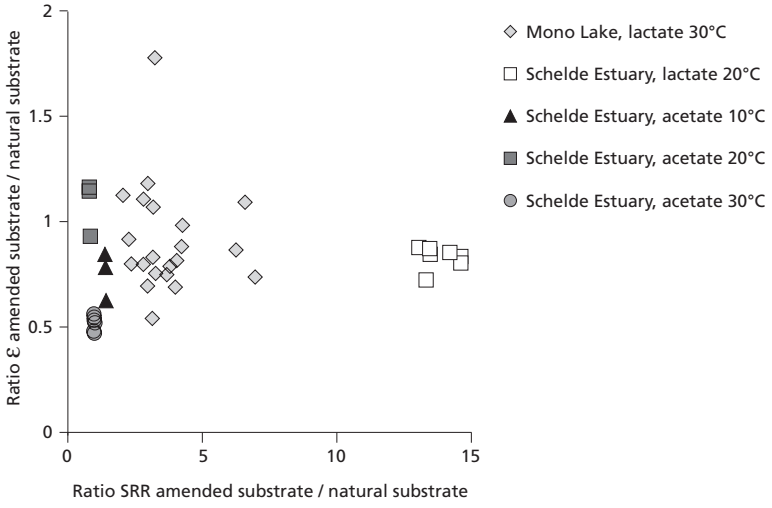

Figure 5.1: Decrease or increase in potential sulfate reduction rate (SRR) or sulfur isotope fractionation (ε) relative to the natural substrate for acetate and lactate. Data obtained from Mono Lake sediments (*Chapter 3*) are also shown.

Table 5.2: Potential sulfate reduction rates (SRRs), sulfur isotope fractionation effects (ε) and percentage (%) of inhibition and enhancement relative to the natural substrate obtained with the addition of acetate or lactate (Table 5.2a) and chromate, selenate, molybdate or tungstate (Table 5.2b and 5.2c). ε values indicated with "*"were obtained at rates < 5 nmol cm^{3-} h^{-1} and should be considered as an indicative value only, due to relatively large analytical error. The value of 20 ‰ indicated "**"with a standard deviation of 5 ‰ for the control reactor was estimated from Figure 2.5 in *Chapter 2* since isotope measurements from the control reactor were unsuccessful.

Table 2a: Enhancers of sulfate reduction

		Lactate					Acetate				
concentration (mM)	Temp. (°C)	SRR (nmol $cm^{-3}h^{-1}$)	sd	ε (‰)	sd	Enhancement to control (%)**	SRR (nmol $cm^{-3}h^{-1}$)	sd	ε (‰)	sd	Enhancement to control (%)**
0	10						7	1	22	3	
10	10						9.7	0.1	16	2	140
0	20	11.1	1.1	20*	5		24	1	17	1	
10	20	156	9	17	1	1410	19.7	0.5	18	2	81
0	30						43.4	0.7	17.4	0.6	
10	30						43	2	9	2	98

Table 2b: inhibitors of sulfate reduction CrO_4^{2-} and WO_4^{2-}

	CrO_4					WO_4				
concentration (mM)	SRR (nmol $cm^{-3}h^{-1}$)	sd	ε (‰)	sd	Inhibition to control (%)**	SRR (nmol $cm^{-3}h^{-1}$)	sd	ε (‰)	sd	Inhibition to control (%)
0	11	1	20**	5		11.1	1.1	20*	5	
0.005	9.8	0.9	21	3	11	11.3	0.5	28	2	-2
0.01	10.2	0.9	22	3	8	12	1	25	1	-11
0.1	11.0	0.6	25	5	1	7.2	0.7	32	5	35
0.5	9	1	26	6	20	2	1	no isotope data		86
1	4.8	0.7	21	3	57	3	2	no isotope data		75
5						0.0	0.0	no isotope data		100

Table 2c: inhibitors of sulfate reduction SeO_4^{2-} and MoO_4^{2-}

	SeO_4					MoO_4				
concentration (mM)	SRR (nmol $cm^{-3}h^{-1}$)	sd	ε (‰)	sd	Inhibition to control (%)	SRR (nmol $cm^{-3}h^{-1}$)	sd	ε (‰)	sd	Inhibition to control (%)***
0	25	9	23	6		25	9	23	6	
0.005	18	2	19	1	30	27	1	15.0	0.9	-6
0.01	15.4	0.3	18.3	0.7	39	21	1	11	2	16
0.1	2.4	0.4	15	9	91	11.3	0.5	5	1	56
0.5	2.1	0.3	11	2	92	8.5	0.9	no isotope data		67
1	2.4	0.3	14	4	91	5	1	no isotope data		82
5	-0.8	0.2	no isotope data		103	0.4	0.9	no isotope data		98
10	-0.6	0.9	no isotope data		102	-0.4	2.5	no isotope data		102

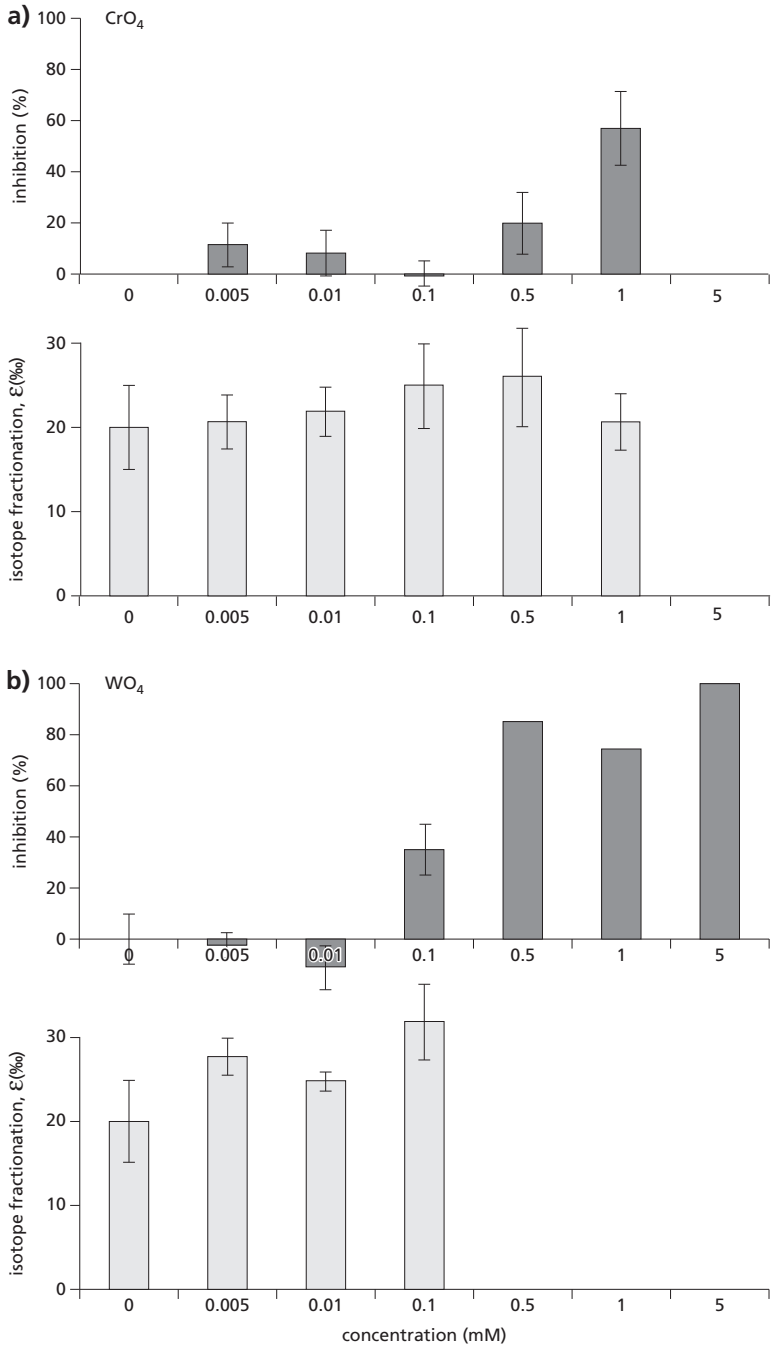

Figure 5.2: Percentage (%) of inhibition of potential sulfate reduction rate (SRR) relative to the control reactor and corresponding sulfur isotope fractionation effects (ε) for CrO_4^{2-} (Panel 5.2a), SeO_4^{2-} (Panel 5.2b), WO_4^{2-} (Panel 5.2c) and MoO_4^{2-} (Panel 2d). Vertical error bars represent standard deviations calculated from 3 to 5 measurement points per reactor.

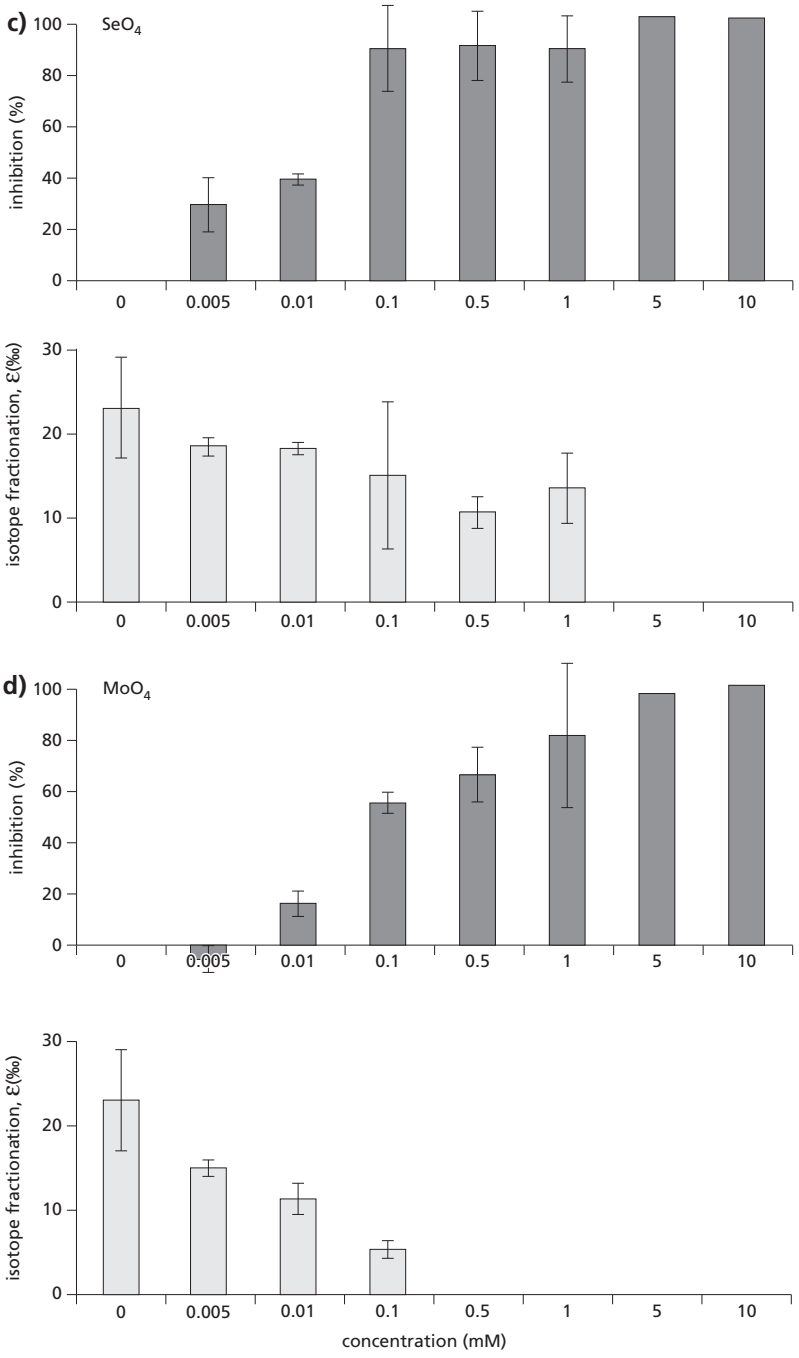

Figure 5.1: Continued

microbial sulfate reduction are explored. Although fractionation was affected by the addition of lactate, acetate or low concentrations of inhibitor compounds, ε values (5 to 32 ‰) fell within the previously measured range of fractionation for this site and are in agreement with theoretically predicted values from the standard fractionation model (Rees, 1973).

The large increase in SRR with lactate (Figure 5.1) confirms published observations that the electron donor supply is limiting under site-matched conditions for this part of the Schelde Estuary (Pallud and Van Cappellen, 2006). The rate increase was much larger than that experienced for Mono Lake sediments where the enhancement was only a factor of 3 to 5 (*Chapter 3*). The greater response to lactate than acetate also confirms the widespread observation that lactate is a more efficient electron donor during microbial sulfate reduction (Widdel, 1988; Canfield, 2001; Pallud and Van Cappellen, 2006), although acetate is also found to be a key substrate for sulfate reducing prokaryotes in nature (Sorensen et al., 1981; Tor et al., 2003). Increased rates with lactate were accompanied by a small decrease in isotope fractionation, which is consistent with the predictions of the standard Rees model and confirms the trend between SRR and ε found in *Chapter 2*.

Rate increases with acetate were small and in some experiments, especially those at 20°C, there was little effect or even a slight decrease on average, when compared to the natural substrate (Table 5.2a). In most acetate experiments there was an inverse relationship between SRR and ε again confirming the standard Rees model. The 30°C acetate experiments were an exception, since amended rates were similar to those measured with the natural substrate, whilst a significant drop in ε from 17 to 9 ‰ was observed (Table 5.2a). This indicates that a change in electron donor can also control the extent of fractionation, as has been previously observed in pure culture data. For example, the oxidation of H_2 by SRP leads to reduced fractionation, smaller than 14 ‰ (Detmers et al., 2001; Hoek et al., 2006), whereas complete oxidation during heterotrophic metabolisms will lead to greater fractionation than observed for incomplete oxidation with identical electron donor compounds (Detmers et al., 2001; Brüchert, 2004). The drop in ε during acetate addition is difficult to explain, since acetate is a substrate which undergoes only complete oxidation, which should result in principle in ε values of greater than 15 ‰. However, experimental results are derived from a mixed signal for the whole microbial community, of which only a small part may respond to acetate amendment. Furthermore, acetate could be preferentially used in other metabolic processes (e.g. methanogenesis) and converted to compounds such as CO_2 or CH_4 or H_2 which are then sequentially used by the SRP (Muyzer and Stams, 2008). The fact that the shift in isotope fractionation, independent of SRR, was only seen at 30°C and not at the other temperatures may be a result of a differential release of labile organic matter or activation of different microorganisms within the community (Macdonald et al., 1995; Zogg et al., 1997). Further investigation of the underlying process requires development of suitable microbiological techniques to determine the active part of the community and its response to complex external factors. In summary, both acetate and lactate amendment led to changes in isotope fractionation that, in most cases, followed the predictions of the standard fractionation model where an increase in SRR was related to a decrease in ε (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Rees, 1973).

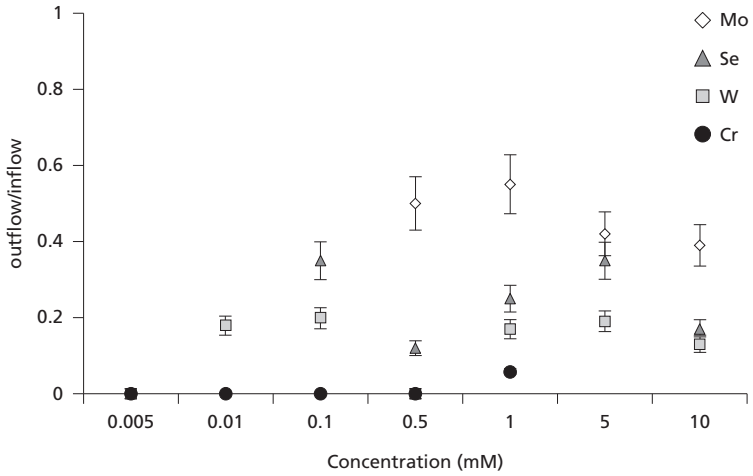

Figure 5.3: Ratios of concentrations of Cr (filled circles), W (gray square), Se (gray triangle), Mo (open diamonds) detected in the outflow solution relative to the inflow solution. External measurement precision was 14 % calculated at the 1 standard deviation level.

The addition of chromate, selenate, molybdate, and tungstate resulted in a progressive reduction in SRR with increasing concentration, with complete inhibition of microbial activity observed above 5 mM for all oxyanions, except for chromate where a maximum concentration of 1 mM was used. All inhibitor oxyanions were measured at much lower concentration in the outflow relative to the inflow solutions (Figure 5.3) in the following order: Cr < W < Se < Mo. Microbial inhibition should not result in a large concentration decrease in the outflow solution due to the nature of the intracellular blocking process, as discussed below (Taylor and Oremland, 1979). Both CrO_4^{2-} and WO_4^{2-} can readily adsorb onto the surface of sediment particles, especially organic matter and iron oxy-hydroxide minerals (Mayer and Schick, 1981; Losi et al., 1994; Kimbrough et al., 1999; Ding et al., 2000; Xu et al., 2006). Due to significant loss by possible adsorption or precipitation, sulfur isotope effects obtained in the experiments with WO_4^{2-} and CrO_4^{2-} will not be further discussed.

Besides absorbing onto the sediment, some of the inhibitor oxyanions could also have been metabolized. Selenate can be used as a terminal electron acceptor in dissimilatory respiration where it is converted to selenite or elemental selenium (Oremland et al., 1994; Ike et al., 2000; Blum et al., 2001). A red selenium precipitate was measured in the sediment, suggesting that the low selenate yield could have been largely caused by microbial reduction. Selenate is a relatively conservative species in solution, whereas selenite can readily adsorb onto particles or react abiotically with free sulfide to form elemental selenium. Chromate is another species that could have produced low yields in the outflow solutions because of microbial reduction (Cervantes, 1991; Smith and Gadd, 2000; Cheung and Gu, 2003; Battaglia-Brunet et al., 2007). Molybdate is not readily metabolized, except through assimilation as a micronutrient, and does not adsorb into sediments, being one of the most conservative and abundant transition metals in seawater (Lyons et al., 2009). It can however react with free H_2S where

it is converted to particle-reactive oxythiomolybdate ions ($MoO_xS_{4-x}^{2-}$) (Helz et al., 1996; Erickson and Helz, 2000). These ions then react with, and are sequestered by, sulfide minerals or organic matter (Helz et al., 1996; Erickson and Helz, 2000; Tribovillard et al., 2004). The low yields for molybdate in the outflow solutions are likely to have been caused by this process. To summarize, the results show that communities of microorganisms are potentially buffered from changes in inhibitor concentrations due to abiotic processes or symbiotic activity that may detoxify the surroundings.

The inhibitory effect on sulfate reduction by the group VI oxidized anions is caused by a similar stereo chemical structure to sulfate. These compounds compete with sulfate to attach themselves to adenosine-5'-triphosphate (ATP) and block the formation of adenosine-5'-phosphosulfate (APS) (Taylor and Oremland, 1979; Banat and Nedwell, 1984; Oremland and Capone, 1988). This leads to the formation APSe, APMo, APW and APCr complexes. Except for APSe, these complexes are not stable and are quickly broken down in adenosine monophosphate (APM) and the original inhibitor species, which is then flushed out of the reactor (Oremland and Capone, 1988). The amount of inhibition also depends on the ratio of inhibitor to sulfate concentration (Banat and Nedwell, 1984).

Selenate or molybdate addition led to decreased isotope fractionation relative to the control experiments (Figure 5.2c and 5.2d). This is comparable to previous experiments with a pure culture and an enrichment culture where addition of 0.01 mM Molybdate resulted in a 6 ‰ reduction in fractionation (Stogbauer et al., 2004). Although the decrease in fractionation during inhibition is of a similar magnitude to the one observed for acetate and lactate addition, the reduction results from a completely different cellular process. As the inhibitor concentration increases, smaller amounts of APS are able to form. This results in less discrimination between the light and heavy isotopes in the sulfate pool represented by the decrease in ε (Stogbauer et al., 2004).

Although an inhibitor or enhancer of sulfate reduction could locally affect isotope fractionation, values are within the range obtained using a single step of sulfate reduction (Rees, 1973) and their effects are not likely to be expressed in the bulk isotope signature for the site. Furthermore, no indications were found that these variations in the geochemical environment could result in exceptionally high ε values as found in the deep marine subsurface (Rudnicki et al., 2001; Wortmann et al., 2001). Since highly elevated concentrations of inhibitor oxyanions are unlikely to occur in nature, with the exception of a few anthropogenically polluted niche environments, the results are expected to have only minor significance for the wider interpretation of sulfur isotope ratios through the geological record.

5.5 Conclusions

The addition of a chemical enhancer or inhibitor of microbial sulfate reduction resulted in a decrease in isotope fractionation of between 6 and 18 ‰ compared to results obtained with the natural substrate. Reduced fractionation resulted from two different processes: a change in the rate in which internal sulfate is reduced to sulfide in the case of lactate and acetate amendment; or blocking of the formation of APS in the case of inhibitor addition. The range in fractionation (5 to 32 ‰) was within values predicted by the standard Rees fractionation model and lies within the range found for flow-through reactors from this sampling site that were previously incubated under optimum site-matched conditions.

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Image of the tufa formations in Mono Lake, California, USA. These sedimentary rocks were formed by precipitation of carbonate rich minerals and appeared above the water surface due to evaporation of the lake water.

Chapter 6

General trends in sulfur isotope fractionation produced by natural communities of sulfate reducing prokaryotes studied in flow-through reactors

Implications for the interpretation of the geological record

Abstract

Interpretation of sulfur isotope variations throughout the geological record relies heavily on biogenic isotope fractionation effect data obtained for modern environments. A consistent set of flow-through reactor experiments have been completed throughout this study to determine sulfate reduction rates (SRRs) and sulfur isotope fractionation effects (ε) produced by natural communities of sulfate reducing prokaryotes (SRP) hosted in sediments from new and diverse geochemical settings. These include a brackish tidal estuary, a hypersaline soda lake and a shallow marine hydrothermal system. Data from each of these sites are reviewed and compared in this chapter. Additional new data from a fourth site in the freshwater area of the River Schelde in Belgium are also presented. When considering all sites together SRR ranged from 5 to 179 nmol cm^{-3} h^{-1}, with corresponding isotope fractionation effects (ε), measured using the difference in $\delta^{34}S$ between sulfate and the corresponding sulfide, of 5 to 43 ‰ that fall within the range predicted by the standard fractionation model of Rees (1973). Isotope fractionation is distinct at each sampling site and differences are most likely linked to electron donor availability and microbial community size and structure, although salinity and cellular energetics may also play a role. Although no clear relationship was found overall between SRR and ε, greater isotopic variability was found at relatively low SRR below 20 nmol cm^{-3} h^{-1}. At high SRR isotope fractionation reaches a minimum of between 5 and 15 ‰ and does not fall to the smaller values expected from the Rees model. The compiled data indicate that relatively small amounts of isotope fractionation (< 20 ‰) are common for microbial communities in sediments in the absence of competition from other metabolic processes, especially under conditions of high sulfate reducing activity. A relatively small isotope effect for microbial sulfate reduction under these close to optimum conditions conflicts with large variations in $\delta^{34}S$ measured in sedimentary rocks through time. The larger natural variability thus requires additional explanation such as cycles of oxidation and reduction or may imply that the optimum growth conditions of laboratory experiments are not representative of most sedimentary environments. Microbial sulfate reduction may have been more widespread than previously thought on the early Archean Earth where a lack of competition from other heterotrophic metabolisms enabled optimum growth of the SRP at high SRR, with correspondingly small amounts of isotope fractionation that are now difficult to detect.

6.1 Introduction

Biogenic sulfate reduction has been suggested as one of the oldest metabolic processes on Earth, appearing somewhere between 3.5 and 2.7 Ga, as inferred from sulfur isotope variations in the Archean rock record (Shen et al., 2001; Grassineau et al., 2001; Shen and Buick, 2004; Johnston et al., 2008a; Ono, 2008; Ueno et al., 2008; Shen et al., 2009). Sulfate reducing prokaryotes (SRP) have exerted a major control on the global sulfur cycle up until the present day and are able to thrive in a wide variety of natural environments (Canfield and Raiswell, 1999; Canfield et al., 2000; Detmers et al., 2001; Johnston et al., 2005; Johnston et al., 2007). Models for the evolution of the sulfur cycle through time and arguments for

the emergence and evolution of this microbial metabolism require information about the magnitude of sulfur isotope fractionation effects (ε) between coexisting sulfate and sulfide from modern experimental data. Since the isotope signature preserved in the geological record most likely originates from a community of sulfate reducing microorganisms within the original sediments, the flow-through reactor data presented in this thesis make an important link between previous pure culture studies and the interpretation of $\delta^{34}S$ variations in sedimentary rocks. This chapter provides an overview of the flow-through reactor data obtained for the different sites presented in *Chapter 2, Chapter 3* and *Chapter 4,* with the addition of new data from a freshwater river site. General trends in the data are discussed and compared against literature data that were also collected using a similar flow-through reactor technique. Implications for the interpretation of the sulfur isotope record through time are discussed and suggestions are made for future research directions.

6.2 Overview of new and published flow-through reactor data

This thesis presents new isotope fractionation and SRR data for four different sites which are summarized in Tables 6.1 and Table 6.2 and Figure 6.1 and Figure 6.2. Average data for the brackish estuary (Schelde Estuary, The Netherlands), hypersaline soda lake (Mono Lake, USA) and shallow marine hydrothermal system (Vulcano Island, Italy) are given, along with new data for sediments sampled from the River Schelde in Belgium, close to the village of Appels (51°02´55,92”N 4°04´12,73”E, previously studied in Pallud and Van Cappellen (2006)). Flow-through reactors were collected and incubated using identical techniques to those described in detail in *Chapter 2, Chapter 3* and *Chapter 4*. Sediments from the River Schelde were incubated at 20°C and with the electron donor derived from the natural sediment substrate. An overview of flow-through reactor data from previously published studies is given in Table 6.3.

Figure 6.1 shows clear differences in the relationship between SRR and ε between the sampling sites. Some positive and negative trends were observed within sub-sets of this data as described in *Chapters 2, 3* and *4*, but no overall consistent relationship is observed. The range in fractionation (5 to 43 ‰) is larger below a SRR of 20 nmol $cm^{-3}h^{-1}$ than above (6 to 20 ‰) (Figure 6.1 and Figure 6.2). SRR data higher than 70 nmol $cm^{-3}h^{-1}$ were only obtained with lactate as an electron donor. The freshwater site is distinct with relatively large amounts of fractionation of around 30 ‰ (Figure 6.1 and Figure 6.2).

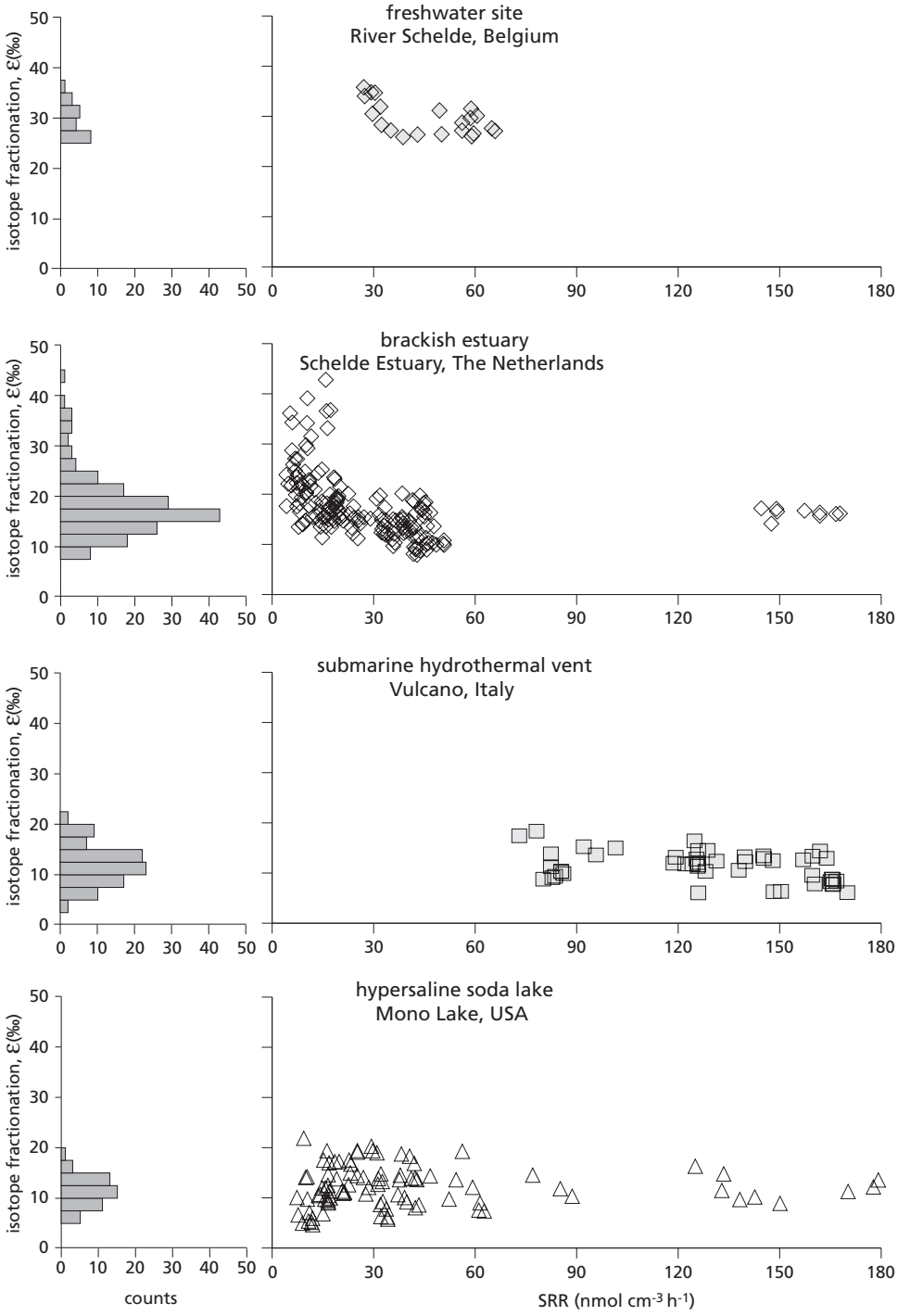
6.3 Controls on isotope fractionation effects

The range in isotope fractionation and its relationship to sulfate reduction rate is slightly different in each of the environments that were studied in this thesis, and also differs from published flow-through reactor data (Table 6.2 and Table 6.3). Despite this, some general trends were consistent and independent of sampling site. The total variability of 5 to 44 ‰ in

Table 6.1: Description of sampling sites with sampling characteristics and experimental conditions. Data were collected under steady state conditions for a minimum of 3 data points. This explains the larger number of data points than reactors.

Evironmental setting	Sampling site	GPS coördinates	Sampling period	Sampling depth (cm)	# Sampling locations	Temperatures (°C)	Substrate	# Reactors	# Data points
freshwater site	River Schelde Estuary, Belgium	51°02′55,92″N 4°04′12,73″E	August 2007	0-2	1	20	natural	4	21
brackish esturary	Schelde Estuary, The Netherlands	51°24′04″N 04°07′04″E	February, May, October 2006, April 2007	0-2, 4-6, 8-10	4	10, 20. 30	natural, acetate, lactate	42	171
hypersaline soda lake	Mono Lake, USA	37°56′37″N 119°00′28″E	July 2008	0-2, 2-4	3	20, 30, 40	natural, lactate	14	89
submarine hydrothemal vent	Vulcano Island, Italy	38°25′05.50″N 14°57′35.20″E	June 2007	8-10	2	30, 60, 90	lactate	5	48

Figure 6.1: (see right page) The left panel shows distribution plots of sulfur isotope fractionation effects (ε) for all sites. In the right panel sulfur isotope fractionation effects (ε) *versus* potential sulfate reduction rates (SRRs) of the individual data points for the different sampling sites are shown. These data include the freshwater site of the River Schelde in Belgium (21 data points obtained at 20°C), the brackish estuary of the Schelde Estuary in the Netherlands (171 data points obtained at 10, 20, 30°C), the shallow marine hydrothermal vent system of Vulcano Island in Italy (48 data points obtained at 30, 60, 85°C) and the hypersaline soda lake Mono Lake in California, USA (89 data points obtained at 20, 30, 40°C).


freshwater site
River Schelde, Belgium
brackish estuary
Schelde Estuary, The Netherlands
submarine hydrothermal vent
Vulcano, Italy
hypersaline soda lake
Mono Lake, USA
isotope fractionation, ε(‰)
counts
SRR (nmol cm-3 h-1)

both this study and the published sites falls within the range predicted by both the standard fractionation model of Rees (1973) and the larger range predicted by Brunner and Bernasconi (2005). On the basis of the flow-through reactor results it is not possible to distinguish between these two models. The absence of large fractionation effects in the incubated sediment data and also in published pure culture data (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Brüchert et al., 2001; Canfield, 2001b; Detmers et al., 2001; Brüchert, 2004; Canfield et al., 2006a; Hoek et al., 2006) do not require the additional intracellular steps of isotope fractionation suggested in the Brunner and Bernasconi, 2005) model. However, ^{33}S and ^{36}S isotope data obtained during laboratory incubations with sediments from a marine lagoon in Denmark (Farquhar et al., 2008)) support the modified Brunner and Bernasconi (2005) model and an expansion of this study to include these additional isotopes is required to resolve these two models here (see section 6.5.3 below).

Differences in isotope fractionation between sites, both those presented in this thesis and the previously published data, could be caused by a variety of factors. Electron donor limitation is common in natural environments (Pallud and Van Cappellen, 2006) and can result in increased isotope fractionation (Canfield, 2001b; Brüchert, 2004; Hoek et al., 2006). The type of electron donor and the metabolic pathway may also change the magnitude of a fractionation effect at a specific SRR (Detmers et al., 2001; Brüchert, 2004). For example, growth with hydrogen in excess produces fractionation effects smaller than 10 ‰, whilst the use of organic substrates under similar growth conditions gives much larger ε values ranging from 25 to 35 ‰ (Kaplan and Rittenberg, 1964; Hoek et al., 2006). The composition of the microbial community in the sediment may also differ. A difference in fractionation between hydrothermally-influenced sediments from Vulcano and the Guaymas basin (Table 6.2 and Table 6.3) most likely corresponds to the previous observation that these have significantly different community structures (Dhillon et al., 2003; Rusch and Amend, 2008). However, comparisons between this study and published data should also take into account the limited number of reactors that were incubated in previous studies. For example, Canfield (2001) based his interpretations on a single reactor, and some intra-site variability similar to that described in *Chapter 2* might reveal reduced fractionation elsewhere within his sampling site.

Isotope fractionation effects determined in this study were on average 10 ‰ smaller than for similar sites in the previously published data (Table 6.3), except for the freshwater site where relatively high values were observed (Table 6.3 and Figure 6.2). This increased level of fractionation in the River Schelde site occurred at similar SRRs, comparable organic matter concentrations (Pallud and Van Cappellen, 2006) and at an identical incubation temperature to the Schelde Estuary data. Microorganisms that grow under freshwater conditions have been shown to fractionate in similar ranges to their marine counterparts (Detmers et al., 2001; Brüchert, 2004). Despite this, salinity may have controlled the difference in fractionation as the uptake of sulfate into the cell can change as the environmental salinity falls (Detmers et al., 2001; Brüchert, 2004). In a freshwater environment, intracellular sulfate concentrations can be up to 5000 times higher than in the surrounding environment (Cypionka, 1989; Kreke and Cypionka, 1992; Detmers et al., 2001). Sulfate uptake is then not likely to be rate limiting, across a range of SRR, possibly leading to increased fractionation.

A major problem in comparing fractionation effects between sites is the use of volume based SRR rather than cell-specific SRR that are typically employed in pure culture studies (Habicht and Canfield, 1997). Similar volume based SRR may result from a relatively small community of SRP producing high cell-specific SRR or a large community metabolizing at low cell-specific SRR. Since isotope fractionation is controlled at the cellular level this could readily explain the discrepancy between sites, as already suggested in *Chapter 2*. Current techniques for determining community size such as most probable number (MPN) counting or direct counting using molecular probes or by using fluorescent in situ hybridization (FISH) are not sufficiently accurate and precise to determine *in situ* the extent of the active portion of the sulfate reducing microbial community.

At low SRR, isotope fractionation is much more variable than at high SRR (Figure 6.2). This behavior has been previously reported in both sediment incubation (Habicht and Canfield, 1997) and pure culture studies (Kaplan and Rittenberg, 1964) and is apparent in the compilation previously published flow-through reactor data shown in Table 6.3 where a range of 8 to 44 ‰ at low SRR contrasts with more constant values of 15 to 34 ‰ at higher rates. Fractionation is predicted to decrease with increasing rate by the standard fractionation model of Rees (1973), up to a threshold above which no further SRR increase can be achieved, resulting in a minimum fractionation of -3 ‰ (Figure 6.3). The deviation from this model at low SRR towards small amounts of fractionation could be controlled by temperature. Many of the low SRR data for obtained for the Schelde Estuary (*Chapter 2*), below 20 nmol cm^{-3} h^{-1} (Figure 6.1), were produced during incubation at 10°C. The cell membrane has been shown to be less flexible below 15°C, making the sulfate transport step rate determining which results in a decrease in fractionation (Canfield, 2001b). Similar reduced fractionation effects were also achieved when strains of SRP were exposed to temperatures at the lower and higher ends of their growth optimum (Kaplan and Rittenberg, 1964; Johnston et al., 2007; Mitchell et al., 2009), although this effect is not reproducible in other studies that found an increase in fractionation (Canfield et al., 2006a; Hoek et al., 2006) or found ε to be independent of temperature (Brüchert et al., 2001).

Laboratory investigations at optimized growth conditions suggest that energy supply might be important in controlling isotope fractionation (Detmers et al., 2001). Flow-through reactor data for the highly saline and hyperalkaline Mono Lake (*Chapter 3*) show a distinct relationship between SRR and isotope fractionation effects, possibly due to the large amounts of energy that are needed to sustain adaptation processes for the microorganisms that thrive in these environments. Adenosine-5'-triphosphate (ATP) is invested to maintain cellular osmotic pressure and to avoid sodium ions entering the cell. As a result less ATP is available for the generation of Adenosine-5'-phosphosulfate (APS), resulting in a smaller APS-pool and thereby less possibility for the microorganism to discriminate between the heavy and light isotope when reducing sulfate. The small fractionation obtained with Vulcano sediments could also partly be explained in this way since energy is also invested for cellular adaptation processes to sustain high temperature (*Chapter 4*), although small fractionation at high rates is also consistent with the Rees model and data for the mesophilic microbial community in sediments collected from the Schelde Estuary.

Table 6.2: Overview of averages and ranges in potential sulfate reduction rates (SRR) and sulfur isotope fractionation effects (ε) for the different sampling sites. Data are divided by low (0 to 20 nmol cm^{-3} h^{-1}), intermediate (20 to 70 nmol cm^{-3} h^{-1}) and high (70 to 180 nmol cm^{-3} h^{-1}) SRRs. Within each rate interval, data are separated by temperature ((psychrophilic (10°C), mesophilic (20, 30, 40°C), thermophilic (60°C) and hyperthermophilic (85°C)) and electron donor (natural substrate, acetate, lactate).

	Environmental conditions	Sampling site	# Data points	SRR (noml $cm^{-3}h^{-1}$)		
				range	average	sd
Low rates						
rates < 20 nmol $cm^{-3}h^{-1}$	20, 30 and 40°C	hypersaline soda lake	32	7 to 19	14	3
	10°C	brackish estuary	29	5 to 18	10	4
	20 and 30°C	brackish estuary	43	7 to 20	15	4
	20°C actetate	brackish estuary	10	6 to 20	14	6
	10°C acetate	brackish estuary	3	9.5 to 9.8	9.7	0.1
total			**117**	**5 to 20**	**13**	**2**
Intermediate rates						
20-70 nmol $cm^{-3}h^{-1}$	30 and 40°C	hypersaline soda lake	40	20 to 63	33	10
	20 and 30 °C actetate	brackish estuary	12	20 to 44	35	8
	20 and 30°C	brackish estuary	65	21 to 49	38	8
	30°C lactate	hypersaline soda lake	5	47 to 59	54	5
	20°C	freahwater site	21	27 to 65	46	14
total			**143**	**20 to 65**	**41**	**8**
High rates						
> 70 nmol $cm^{-3}h^{-1}$	85°C lactate	submarine hydrothermal vent	13	83 to 170	140	39
	60°C lactate	submarine hydrothermal vent	12	73 to 151	100	27
	30°C lactate	hypersaline soda lake	12	77 to 179	133	35
	30°C lactate	submarine hydrothermal vent	23	120 to 164	136	14
	20°C lactate	brackish estuary	9	144 to 162	156	9
total			**69**	**73 to 179**	**133**	**21**

ε (‰)			Type ε-SRR relationship	R^2
range	average	sd		
5 to 19	11	4	weak positive	0.1
12 to 29	19	5	weak inverse	0.35
15 to 43	23	7	weak inverse	0.097
13 to 37	24	7	inverse	0.65
14 to 18	16	3		
5 to 43	**19**	**5**		
6 to 20	13	4	weak inverse	0.13
8 to 20	13	5	inverse	0.57
9 to 20	14	3	very weak inverse	0.045
10 to 19	14	4	no	0.008
26 to 35	30	3	weak inverse	0.33
6 to 35	**17**	**7**		
6 to 11	9	1	inverse	0.68
6 to 18	11	4	inverse	0.46
9 to 16	12	2	no	0.019
10 to 16	13	1	no	0.029
15 to 18	17	1	no	0.007
6 to 18	**12**	**3**		

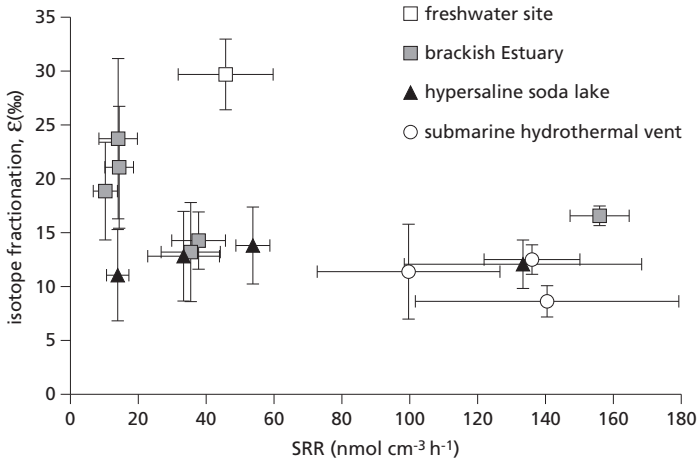


Figure 6.2: Potential sulfate reduction rates (SRRs) *versus* sulfur isotope fractionation effects (ε) for the different sampling sites including average data and standard deviations as presented in Table 6.2. Open square (freshwater site), gray squares (brackish estuary), black triangles (hypersaline soda lake) and open circles (shallow marine hydrothermal vent system). At low rates, ≤ 20 nmol cm^{-3} h^{-1}, there is a larger range in isotope fractionation of 5 to 43 ‰ compared to rates > 20 nmol cm^{-3} h^{-1} where the range is relatively limited (6 to 20 ‰).

At high rates (> 70 nmol cm^{-3} h^{-1}) fractionation effects within the data obtained in this study are similar, regardless of the sampling site, with an average of approximately 12 ‰ (Figure 6.2 and Figure 6.3). The minimum value of -3 ‰ predicted by the Rees model, based on a single measurement from a pure culture study (Harrison and Thode, 1958), has not yet been observed for communities of SRP in natural sediments (Habicht and Canfield, 1997; Canfield et al., 2000; Canfield, 2001b; Habicht et al., 2002; *Chapter 2, Chapter 3* and *Chapter 4*). All high SRR data were produced with lactate as electron donor. Sulfate was present in excess and the transport of sulfate across the cell membrane was not rate-limiting. This implies that the rate of electron supply, which is substrate dependent, may constrain the extent of fractionation at high rates, resulting in the elevated minimum ε value. Smaller fractionation effects at similar SRR have previously been found for H_2 compared to organic substrates as the electron donor (Hoek et al., 2006). The difference in fractionation was explained by a greater supply of electrons for the reduction of APS to sulfite through an efficient operation of the hydrogenase enzymes compared to the electron fluxes supplied by the degradation of organic substrates (Rees, 1973; Canfield, 2001a; Brüchert, 2004; Hoek et al., 2006). A greater supply of electrons should result in an increase in the APS to sulfite reduction rate, a smaller APS pool and reduced fractionation effects. Similar behavior was found by Habicht et al. (1997) where fractionation reached a higher minimum value of 25 ‰ at rates greater than 800 nmol cm^{-3} h^{-1}, when consuming the natural substrate. As lactate and hydrogen produce smaller minimum fractionation effects at high rates this amount of fractionation may be characteristic for a different type of organic substrate.

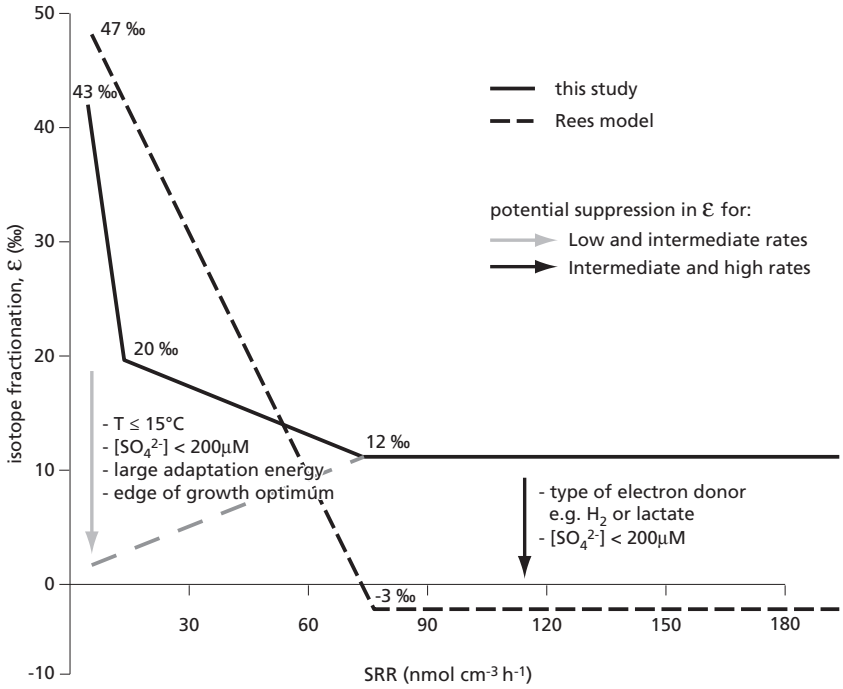


Figure 6.3: The range in sulfur isotope fractionation effects (ε) and type of ε-SRR relationship predicted from the Rees (1973) Model and as observed in this study. Errors indicate possible environmental conditions that could result in deviations from the standard model, at low and intermediate rates (gray error) and at intermediate and high rates (black error). The rates corresponding to the different branching points are extracted from the complete data set but these could vary between sites. The branching point of the Rees model is set arbitrarily at 70 nmol cm^{-3} h^{-1} which is similar to the branching point in this study.

The compiled data-set confirms the previous conclusion made by Detmers et al. (2001) that SRR cannot be inferred from absolute amounts of isotope fractionation. This is only possible within sub-sets of the data, such as the May 2006 data for the Schelde Estuary presented in *Chapter 2* or for the positive trends observed under specific conditions at Mono Lake (*Chapter 3*). Despite the clear relationships in these sub-sets, external parameters such as temperature and organic matter availability make extrapolation across each site difficult, if not impossible. The fractionation model developed by Canfield (2006) and Hoek (2006) shows that a unique SRR *versus* ε relationship is not possible if the relative flow of sulfur changes at the two branching points given in the standard Rees model. Using this model, the relatively small fractionation effects obtained in this study (less than 20 ‰ in most cases), imply that the sulfate transport S(1) is not very reversible and that the reversibility of the reduction of sulfate to sulfide S(2) is the main control on net fractionation. However, the model is difficult to apply to mixed communities where it is not possible to apply specific constraints on S(1)

Table 6.3: Ranges in potential sulfate reduction rates (SRRs) and sulfur isotope fractionation effects (ε) obtained by previous studies using flow-through reactors containing sediment from a fresh water lake (Habicht et al., 2002), a marine lagoon (Feallestrand, Denmark, Canfield, 2001b; Habicht et al., 2002; Farquhar et al., 2008) and a hydrothermal vent system (Guaymas Basin, Gulf of California, USA, Canfield et al., 2000). (n.a. not available)

	Study	Site	Temp. (°C)
Low rates	Farquhar et al. 2008	marine lagoon, Feallestrand, Denmark	25
≤ 20 nmol $cm^{-3}h^{-1}$	Canfield. 2001	marine lagoon, Feallestrand, Denmark	25
			15
	Canfield et al. 2000	Guaymas Basin, Gulf of California, USA	50
			75
			88
			60
			80
total			
Intermediate rates	Habicht et al. 2002	freshwater lake	17
20-70 nmol $cm^{-3}h^{-1}$		coastal marine sediment	17
	Canfield. 2001	marine Lagoon, Feallestrand, Denmark	25
			25
			25
			35
	Canfield et al. 2000	Guaymas Basin, Gulf of California, USA	60
			55 to 60
			70 to 75
			80 to 85
total			
High rates	Canfield. 2001	marine lagoon, Feallestrand Denmark	25
70-180 nmol $cm^{-3}h^{-1}$	Canfield et al. 2000	Guaymas Basin, Gulf of California, USA	75
total			

Substrate	SRR (nmol $cm^{-3}h^{-1}$)	ε (‰)	# Data points
natural substrate	3 to 15	37 to 44	46
natural substrate	10	30 to 35	3
natural substrate	5	40	3
6.3 mM lactate, 28 mM Sulfate	4 to 15	14 to 17	6
6.3 mM lactate, 28 mM Sulfate	5 to 9	24 to 27	4
6.3 mM lactate, 28 mM Sulfate	12 to 17	8 to 10	2
10 mM Ethanol, 28 mM Sulfate	3	23 to 25	3
10 mM Acetate, 28 mM Sulfate	5	25	2
	3 to 17	**8 to 44**	**69**
1 mM lactate, ≥ 1 mM Sulfate	n.a.	28 ± 6	4
1 mM lactate, ≥ 1 mM sulfate	n.a.	28 ± 6	1
10 mM acetate	25-30	18 to 25	4
10 mM ethanol	65-75	15 to 18	4
10 mM lactate	50	20	2
natural substrate	25-35	22 to 28	5
10 mM Ethanol, 28 mM Sulfate	25 to 26	15	2
6.3 mM lactate, 28 mM Sulfate	21 to 33	15 to 20	4
6.3 mM lactate, 28 mM Sulfate	43 to 62	26 to 28	3
6.3 mM lactate, 28 mM Sulfate	27 to 46	20 to 27	3
	21 to 75	**15 to 34**	**27**
10 mM lactate	100 to 150	12 to 20	3
6.3 mM lactate, 28 mM Sulfate	86 to 174	23 to 26	3
	86 to 174	**12 to 26**	**6**

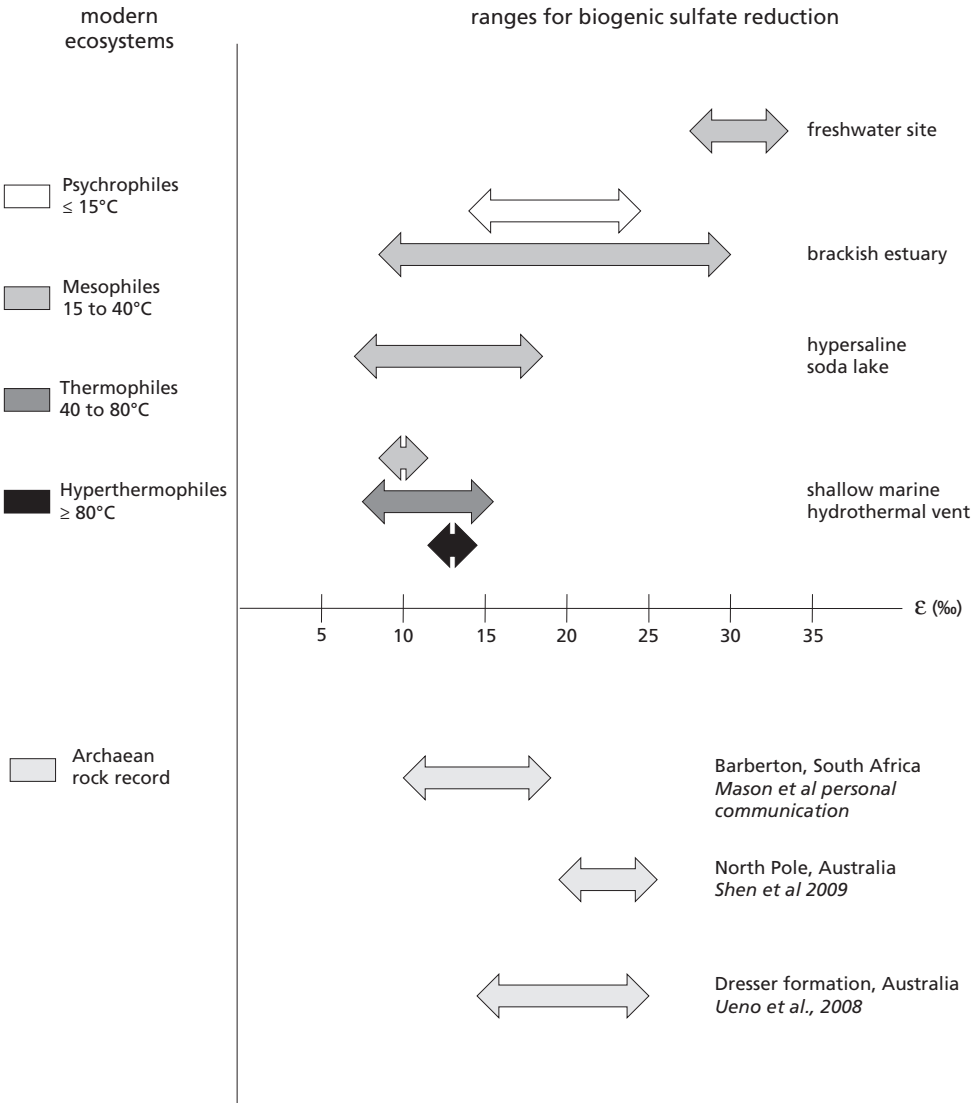


Figure 6.4: Ranges in isotope fractionation effects (ε) observed for the different sampling sites presented in this study compared to fractionation effects related to biogenic sulfate reduction preserved in Archean Rocks. Data are shown for the Barberton Greenstone Belt in South Africa (Mason et al. personal communication) and the North Pole barite deposit in the Pilbara Block, Australia (Ueno et al., 2008; Shen et al., 2009). The modern sites include all data presented in Table 6.2. Data is arranged per site and separated by ranges in temperature related to growth conditions for specific groups of microorganisms (Psychrophiles ≤ 15°C, Mesophiles 15 to 40°C, Thermophiles 40 to 80°C and hyperthermophiles ≥ 80 °C). For the Archean data, only ranges in measured $\delta^{34}S$ from the Archean rock record are presented where $\Delta^{33}S$ *versus* $\delta^{34}S$ relationships argue for a mass dependent process that could be biogenic sulfate reduction.

and S(2) due to the activity of multiple strains of SRP. Given the fact that different strains within a community are likely to have different responses, it is surprising that a strong ε *versus* SRR relationship exists within parts of the data in some of the sites studied here (Table 6.2).

6.4 Implications for interpreting the geological record

The range of $\delta^{34}S$ in sedimentary pyrites and sulfate deposits is highly variable through the geological record, with differences in fractionation increasing from small values in the Archean to as large as 80 ‰ in Proterozoic and Phanerozoic times (Figure 1.6 of *Chapter 1*, Canfield and Raiswell, 1999; Canfield, 2005). The experimental data presented in this thesis suggest that relatively small isotope fractionation effects should normally be expected between co-exisiting sulfate and sulfide geochemical reservoirs, if the two are related by a single step of microbial sulfate reduction. The larger fractionation effects observed after 2.4 Ga must therefore be a result of multiple cycles of reduction and oxidation (Canfield and Teske, 1996; Habicht and Canfield, 2001), or alternatively could reflect conditions that were not explored in this study such as low energy supply for microbial sulfate reduction coupled with hypersulfidic conditions as suggested by Brunner and Bernasconi (2005). An important consideration when applying flow-through reactor data to natural environments is that they were produced under close to optimum conditions in most cases, especially with respect to the electron acceptor, sulfate. Competition between different metabolic processes for the substrate and complications such as slow rates of nutrient diffusion or fluctuating physical and chemical environmental conditions could make *in situ* rates smaller. Reduced SRR could lead to elevated isotope fractionation, although this does not always lead to an increase in fractionation as seen for the hypersaline soda lake (*Chapter 3*).

The minor $\delta^{34}S$ variations in Archean rocks have not been widely linked to microbial sulfate reduction, except in relation to barite deposits (Shen et al., 2001; Ueno et al., 2008; Shen et al., 2009) where there is evidence for higher sulfate concentrations than assumed for the Archean oceans (Habicht et al., 2002). Correlations between $\delta^{34}S$ and $\Delta^{33}S$ or $\Delta^{36}S$, enable mass dependent isotope effects to be resolved from overprinting by mixing in the sulfide reservoir or post-deposition modification of pyrite minerals (e.g. Farquhar et al., 2000; Farquhar and Wing, 2003; Mojzsis et al., 2003; Ono et al., 2003; Johnston et al., 2005; Farquhar and Wing, 2005; Johnston et al., 2006; Papineau and Mojzsis, 2006; Ono et al., 2006; Johnston et al., 2007; Bao et al., 2007; Kamber and Whitehouse, 2007; Johnston et al., 2008b; Ono, 2008; Ueno et al., 2008; Shen et al., 2009; Zerkle et al., 2009). The amount of isotope fractionation that can be assigned to possible biogenic sulfate reduction close to the barite deposits is approximately 10 to 25 ‰ (Ueno et al., 2008; Shen et al., 2009; Mason et al. in preparation), which is consistent with the laboratory flow-through reactor data of this study for a single step of sulfate reduction (Figure 6.4). $\delta^{34}S$ variations in shales and pyrites from lithologies not in close contact with the barite show a much more limited range of only 5 to 10 ‰ lighter than the value assumed for seawater at this time. This variability is similar to that expected for $\delta^{34}S$ variations in magmatic fluids emitted from mid-oceanic

ridges or other volcanic sources. Small amounts of fractionation in Archean times have been attributed to low ocean sulfate concentrations (Habicht et al., 2002), so that microbial sulfate reduction may have been active without leaving a significant isotopic trace. Other studies have argued that the Archean oceans had closer to present day sulfate concentrations and that the minor variations in $\delta^{34}S$ are due to uniformly high SRRs (Ohmoto et al., 1993). It is likely that there were few heterotrophic metabolisms that could have competed with microbial sulfate reduction in the Archean ocean (Canfield et al., 2006b) which may have enabled the suggested high SRRs. However, primary production was likely to have been much smaller than in the modern ocean (Kharecha et al., 2005; Canfield et al., 2006b) and the corresponding organic substrate limitation could have conversely resulted in increased isotope fractionation.

My data do not support of reject the role of low sulfate concentrations or high SRR in influencing $\delta^{34}S$ variations in Archean rocks, but open up the additional possibility that small fractionation effects may be associated with more variable rates of biogenic activity in specific environmental settings, such as found in the hypersaline Mono lake and in the shallow marine hydrothermal system at Vulcano. Microbial sulfate reduction could thus have been more widespread than currently thought but this will be difficult to test as the small expected variations will be difficult to resolve from background variation in $\delta^{34}S$ related to magmatic and hydrothermal processes during the early Archean.

6.5 Suggestions for future research directions

6.5.1 New experimental conditions during flow-through reactor experiments

Experiments should be performed further away from the optimum conditions that have been used in the flow-through reactor experiments of this study in order to further test whether the predictions of the Brunner and Bernasconi (2005) model can be observed in the laboratory. This may help to explain the increased ε values estimated for modern anoxic deep marine settings (Rudnicki et al., 2001; Wortmann et al., 2001) as well as the large variability in $\delta^{34}S$ through the Proterozoic and Phanerozoic. A key parameter to test would be the effect of high concentrations of sulfide, which are known to inhibit the sulfate reduction process and which are expected to lead to increased fractionation. The preliminary study in *Chapter 5* shows that inhibitors which block the formation of ATP are unlikely to induce such large amounts of fractionation.

The isotopic response to competition between sulfate reduction and other metabolisms for the available organic and inorganic substrates should also be investigated. This is particularly relevant in modern ecosystems where heterotropic metabolisms are in competition (Canfield et al., 2006b). Flow-through reactor experiments similar to those performed in *Chapter 2* could be carried out with the addition of various concentrations of different electron acceptors such as nitrate. Many facultative microorganisms are for instance capable of growing with either nitrate or sulfate (Dalsgaard and Bak, 1994; Muyzer and Stams, 2008).

The effect of a wider range of electron donors, mainly organic compounds, but also notably H_2 should be further tested in new flow-through reactor experiments. Although difficult to measure, the concentration of organic compounds in the outflow solutions of the reactors may reveal the types of organic substrates that are used during sulfate reduction and could help to distinguish between complete or incomplete oxidation of the electron donor(s). This is valuable information as the type of substrate and the metabolic pathway can have a significant effect on isotope fractionation (Detmers et al., 2001; Brüchert, 2004; *Chapter 2, Chapter 3* and *Chapter 4*). Further research is required test the hypothesis that fractionation at high rates is limited by the supply of electrons, which is dependent on the type of electron donor, and not the transport rate of sulfate across the cell membrane. Unfortunately experiments with electron donors in natural communities could be complicated by the presence of other microorganisms such as the fermenters that are likely to compete for the substrate.

Experiments should be repeated with the natural non-amended substrate for the hydrothermally influenced sediments from Vulcano Island (***Chapter 4***). The addition of H_2 as an electron donor is recommended due to the fact that this is high in abundance at the site, although current pure culture data for H_2 (Kaplan and Rittenberg, 1964; Hoek et al., 2006) suggest that it is not likely to increase the amount of fractionation so far observed at this site.

6.5.2 New molecular biological techniques

New biological techniques are required to determine the composition, size and activity of the SRP within the total microbial community with greater ease, and with more specificity than is currently possible. Molecular biological techniques are laborious and time consuming and DNA extractions give information about the total community of SRP, rather than the portion which is active. Linking the active portion of the community to specific SRR and isotope fractionation effects will provide more direct process information and will enable extrapolation between different sites once the microbial community structure has been identified. This information is also necessary to convert volume based SRR into cell-specific SRR (Habicht and Canfield, 1997) and vice versa and explore the presence or absence of a relationship between SRR and ε that underpins the standard fractionation model of Rees (1973).

6.5.3 Additional stable isotope measurements

Deviations from mass dependant isotope fractionation, recorded by $\Delta^{33}S$ and $\Delta^{36}S$ variations have a high potential for discriminating between microbial sulfate reduction, elemental sulfur disproportionation and abiotic processes (Farquhar and Wing, 2003; Ono et al., 2006; Philippot et al., 2007; Ueno et al., 2008 Johnston et al., 2008a; Thomazo et al., 2009). Combined measurements of sulfur and oxygen isotopes have been shown to place constraints on the proportion of sulfate recycled from the cell and the surrounding solution (78 – 96%) (Farquhar et al., 2008). In addition, it is possible to calculate the proportion of intermediate sulfite that is recycled through APS to sulfate and released back to the external sulfate pool, as well as the fraction of the sulfur intermediates between sulfite and sulfide that are recycled to sulfate. It is a straightforward and logical extension to measure the minor sulfur and oxygen

isotopes in the outflow solutions produced during this study, as already shown for flow-through reactor experiments with Danish marine lagoon sediments (Farquhar et al., 2008).

The flow-through reactor technique could also be applied to the study of stable isotope systems other than sulfur, when fractionation is expected during a dissimilatory metabolism. Examples include the measurement of nitrogen isotope fractionation during nitrate reduction or selenium isotope fractionation during selenate reduction. This could be important for a number of stable isotope systems including N, Se and possibly Fe that are investigated in the geological record and which reflect an integrated isotopic signal produced by a diverse community of microorganisms in the precursor sedimentary environment.

6.5.5 Study of new environments

The flow-through reactor technique could also be extended to new environments on Earth where sulfur isotope fractionation is distinctive and as yet untested. Further work should be carried out with deep marine sediments. Another environment that requires attention is the hyperacidic one, such as the Rio Tinto river in Spain (Fernández-Remolar et al., 2005), which may be an important analogue for Mars where a high abundance of Jarosite was recently discovered by remote sensing.

6.6 Conclusions

Flow-through reactor data give a range in isotope fractionation effect data of 5 to 43 ‰ which is consistent with the standard fractionation model of Rees (1973). Most environments result in relatively small bulk isotope fractionation effects of less than 20 ‰ under close to optimum incubation conditions. The range in isotope fractionation is larger at low SRR below 20 nmol cm^{-3} h^{-1}. The greater variability in ε at low SRR could result from low temperature, low sulfate concentration, a large energy investment in cellular adaptation strategies under extreme environmental conditions, or microorganisms thriving on the edges of their optimum growth conditions. Consistently small fractionation effects with an average of 12 ‰ were achieved at SRR above 70 nmol $cm^{-3}h^{-1}$. Our data shows that small fractionation effects could be produced with sulfate in excess over a large range in sulfate reducing activity. These new data should be implemented in models that study sulfur cycling in modern and ancient geochemical settings.

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Image of the sunset at Mono Lake, California, USA

Glossary

List of abbreviations

Glossary

Abiotic:	non biogenic processes.
Alkaliphile:	a microorganism that grows best at high pH (> 8).
Alkalinity:	the capacity of a substance in solution to neutralize acids by combining with hydrogen ions, mostly expressed as equivalents of carbonate or bicarbonate ions.
Amorphous material:	a solid in which there is no long-range order of the positions of the atoms.
Anaerobe:	a microorganism that does not require oxygen for growth.
Anaerobic/ Anoxic:	an environment in which oxygen is absent or a process that could only proceed in the absence of oxygen.
Archaea:	one of the domains that divide cellular life into three main branches of evolutionary descent: Archaea, Bacteria, and Eukaryotes. Archaea and Bacteria belong to the group of the prokaryotes. Although Bacteria and Archaea are quite similar in size and shape, Archaea contain genes and perform several metabolic pathways that are more closely related to those of eukaryotes. The domain of the Archaea are believed to contain most of the extremophilic microorganisms.
Archean:	one of the tree time periods in the evolution of the Earth in which the Precambrian is divided. The Precambrian spans from the formation of the Earth, around 4.6 Ga, to 0.542 Ga and is subdivided into the Hadean (3.8 to 4.6 Ga), Archean (2.5 to 3.8 Ga) and the Proterozoic (2.5 to 0.542 Ga). The period from the present day to 0.542 Ga is the Phanerozoic.
Assimilatory sulfate:	the reduction of sulfate to sulfide. The produced sulfide is then incorporated as a building block in biochemical organic compounds. This process is carried out reduction by several organisms.

Atmosphere: the layer of gases surrounding the Earth that is retained by Earth's gravity and protects life on Earth by absorbing ultraviolet solar radiation, warming the surface, and reducing temperature extremes between day and night. The composition of the atmosphere has evolved over time. Most noticable is the evolution from an anoxic atmosphere, present until approximately 2.5 Ga, to an oxygenated atmosphere with close to the present day oxygen concentrations. The atmosphere becomes thinner with increasing altitude, with no precise upper limit (however, for practical reason this boundary set around 200 km).

Autotroph: a microorganism that is able to use CO_2 or bicarbonate as a carbon source and that could in this way produce complex organic molecules. The organism could obtain energy for metabolism from light or inorganic chemical reactions. These type of organisms provide organic matter that could be converted by the heterotrophs to generate energy for metabolism.

Bacteria: one of the domains that divide cellular life into three main branches of evolutionary descent: Archaea, Bacteria, and Eukaryotes. Archaea and Bacteria belong to the group of the prokaryotes. Bacteria are mostly single cellular organisms and are ubiquitous on Earth.

Barite: a mineral with the composition $BaSO_4$.

Batch experiment: experimental closed system with a fixed volume which is used for the growth of pure cultures or natural communities of microorganisms. Metabolic products accumulate in the system and reactants and nutrients are mostly only added before starting the experiment and are consumed over time.

Biogenic: produced by microorganisms.

Biomarker: a biological entity e.g. a cellular excretion product or a structure of a cellular component, that is indicative for the presence of a specific microbial process. Earth science research mostly focuses on biomarkers that could be preserved, for a short or long time period, in the sedimentary rock record.

Biosphere:	the part of the Earth's environment or the global ecological system, in which living organisms are found.
Biotic:	involving living organisms.
Cell membrane:	a layer of lipids which serves as a selective barrier around the cell that remains impermeable to specific particles, molecules, or substances.
Chemolithotroph:	a microorganism that obtains energy by oxidizing inorganic compounds.
Chemo-organotroph:	a microorganism that obtains energy by oxidizing organic compounds.
Chemotroph:	a microorganism that obtains energy through the oxidation of electron donating molecules. These compounds could be organic (organotrophs) or inorganic (lithotrophs).
Complete oxidation:	the oxidation of organic compounds by microorganisms to CO_2.
Cyano bacteria:	a phylogenic group of microorganisms that obtain their energy through photosynthesis.
Cytoplasm:	the part of a cell that is enclosed within the cell membrane.
Diagenesis:	a chemical, physical, or biological change undergone by a sediment after its initial deposition.
Disproportionation:	a chemical reaction in which a compound is simultaneously reduced and oxidized and forms two different products from one substrate.
Dissimilatory:	the microbial process that generates energy during the reduction of sulfate to sulfate sulfide. The energy is used for metabolic reactions. This process is only reduction performed by sulfate reducing microorganisms.
Ecosystem:	an area within the natural environment in which physical (abiotic) factors of the environment, such as rocks and soil, function together with (biotic) organisms, such as plants and animals, within the same habitat.

Electron acceptor: a compound that is reduced in a chemical or biochemical reaction by accepting or gaining electrons from another molecule. This compound could be inorganic or organic.

Electron a compound that is oxidized in a chemical or biochemical reaction by donating donor: or losing electrons to another species. This compound can be inorganic or organic.

Endergonic: a reaction process that subtracts energy from the environment.

Enrichment culture: the use of selective conditions to favor the growth of a specific strain or group of microorganisms that enables the microorganism to out compete the growth of the other microorganisms present in the sediment or solution.

Enzyme: a protein that catalyzes and increases the rate of a chemical reaction.

Estuary: a coastal area where mixing of fresh water and marine water occurs.

Eukaryotes: one of the domains that divide cellular life into three main branches of evolutionary descent: Archaea, Bacteria, and Eukaryotes. Eukaryotes contain a nucleus and most cells also contain other membrane-bound organelles (cellular subunits with a specific function). This domain contains almost all species of large, multicellular organisms, including animals, plants and fungi, although many eukaryotic species are single celled microorganisms.

Evaporitic lake: a lake with a high salt concentration resulting from high evaporation rate relative to water supply.

Exergonic: a reaction that releases energy to the environment.

Extracellular: outside the cell.

Extremophile: an organism that thrives in or requires extreme physical or chemical conditions under which the majority of life on Earth is not able to flourish. Extreme conditions could include high or low temperature, salinity, alkalinity or pH.

Felsic: a geological term referring to silicate minerals, magma, and rocks which are enriched in the lighter elements such as silicon, oxygen, aluminium, sodium, and potassium.

Fermentation: a process performed by microorganisms in which energy is derived from the oxidation of organic compounds with the use of an endogenous (a substance that originate from within the organism) electron acceptor,

Fermenter: a microorganism that performs fermentation reactions in which organic compounds are used as the primary electron donor and the ultimate electron acceptor.

Flow-through reactor: a experimental technique to study microbial processes in intact sediment slices with the continuous supply of substrate. Experimental conditions e.g. temperature or inflow solution are easily changed.

Fossil: preserved remains or traces of an animal, plant or another organism from the remote past. The preserved fingerprint usually consists of that portion that was partially mineralized during life, such as the bones and teeth. Preservation of soft tissues is rare in the fossil record.

Genus: a part of the biological classification system used to group and categorize organisms. Genus comes above species and below family.

Gypsum: a mineral with the composition $CaSO_4$.

Halophile: a salt tolerant or salt requiring microorganism.

Heterotroph: a microorganism that uses organic carbon as a nutrient for growth and is not able to directly use CO_2, which contrasts with autotrophic microorganisms.

Hydrolysis: a chemical process in which a molecule is cleaved into two parts by the addition of a molecule of water.

Hydrosphere: the region of Earth covered with water in the form of a liquid, ice or water vapor. This area includes groundwater, surface water and atmospheric moisture.

Hydrothermal vent system: a fissure in the crust from which geothermally heated water rises and mixes with the surrounding water. Hydrothermal vents are commonly found near active volcanism, mid-ocean ridges, ocean basins and hotspots. Hydrothermal vents could be shallow or deep marine. These types of high temperature environments are assumed to have been widely present on the early Earth.

Hypersaline: containing high concentrations of sodium chloride or other mineral salts.

Hyperthermophile: a microorganism that has an optimum growth temperature above 80°C.

Incomplete oxidation: the oxidation of organic compounds to smaller organic molecules. This process contrasts with complete oxidation where all organic carbon is converted to CO_2.

Inhibition: the reduction in activity of a metabolic process.

In situ: occurring in the original environment or setting.

Intertidal zone: region of the shoreline that is exposed to alternating periods of flooding and drying out at high and low tides respectively. This area can include many different types of habitats, e.g. steep rocky cliffs, sandy beaches or wetlands (e.g. mudflats). The area can be a narrow strip or can include many meters of shoreline.

Intracellular: inside the cell.

Irreversible reaction: a reaction that runs in only one direction.

Isotopes: different types of atoms of the same chemical element, each having a different number of neutrons. Isotopes thus differ in mass. Sulfur for example has four naturally occurring isotopes, in order of abundance ^{32}S, ^{34}S, ^{33}S and ^{36}S.

Isotope fractionation: the separation of stable isotopes from each other by their mass during biogenic or abiotic chemical or physical processes.

Isotope fractionation effect: the difference in isotope fractionation between two different chemical species of the same element which can be produced during microbial or abiotic processes.

Jarosite:	a potassium iron sulfate hydroxide mineral with the general chemical formula of $KFe_3(OH)_6(SO4)_2$
Labile:	organic matter that is readily available for consumption by microorganisms. organic matter
Lithology:	the description of rock composition (what it's made of) and texture.
Lithosphere:	The upper (oceanic and continental) layer of the solid Earth, comprising all crustal rocks and the brittle part of the uppermost mantle. It is generally considered to deform by brittle fracture and if subjected to stresses of the order of 100 MPa.
Littoral zone:	region of the shoreline from the highest level reached by the body of water to areas that are permanently submerged. This zone is generally shallow in depth and light often penetrates to the bottom of the water. This zone also includes the intertidal zone.
Marine:	refers to things which are related to the sea or ocean.
Mesophile:	a microorganism that has an optimum growth temperature between 15 and 40°C.
Metabolism:	the collection of biochemical reactions within a cell that generate or require energy, classified as either anabolic reactions (use energy to synthesize molecules) or catabolic reactions (carry out the breakdown of molecules with often results in the release of energy).
Metamorphism:	the solid-state recrystallization of pre-existing rocks and sediments due to changes in physical and chemical conditions, mainly heat, pressure, and the introduction of chemically active fluids.
Methanogenesis:	the formation of methane by microbial processes.
Microbe:	a microscopic organism that exists mainly as a single cell or in an aggregate of cells.

Microbial:	a group of microorganisms that are living together and interacting with each Community other within a specific habitat or environment.
Microbial:	an increase in the number of microorganisms relative to the staring microbial growth population.
Microbial respiration:	the process in which nutrients are converted into energy which is subsequently used in other cellular processes.
Microorganism:	a microscopic organism that exists mainly as a single cell or in an aggregate of cells.
Mineral:	naturally occurring solid formed through geological processes that has a characteristic chemical composition, a highly ordered atomic structure, and specific physical properties.
Morphology:	shape.
Most probable number:	a standardized procedure for estimating the concentration of microorganisms.
Natural conditions:	conditions which are similar to the environmental conditions in the field.
Natural substrate:	substrate present in the sediment that can be used by microorganisms for metabolism. In this theses it mostly refers to the electron donors available from within the sediment.
Nutrient:	a chemical that is used by a microorganism and is essential for its metabolism.
Optimum growth: temperature	the temperature at which growth of a particular microorganism is most rapid. At this temperature enzymatic reactions occur at the maximum rate.
Organic compound:	a chemical that contains carbon. A few types of compounds such as carbonates or simple oxides e.g. CO_2 are considered as inorganic.

Osmosis:	diffusion of water across the semi-permeable cell membrane. Water flows from a region of low solute concentration (high water concentration) to a region of high solute concentration (low water concentration).
Oxic:	containing oxygen.
Oxidant:	a substance that gains electrons in a redox reaction.
Oxidation:	a chemical or biochemical reaction in which the reactant looses electrons.
Oxyanion:	a chemical compound with the generic formula $A_xO_y^{z-}$
Paleo-environment:	ancient environment.
Phanerozoic:	one of the time periods in the evolution of the Earth, indicating the period from the present day to 0.542 Ga.
Phototroph:	a microorganism that is capable of using light as an energy source.
Phylogenetic:	use of genetic traits to classify the relatedness of organisms.
Phylogenetic tree:	a schematic "tree"showing the evolutionary relationships among various biological species that are known to have a common ancestor. The tree is constructed based on analysis of the 16s-rRNA subunit of the organisms.
Phylogeny:	the study of evolutionary relatedness among various groups of organisms.
Physiology:	the study of how living organisms function.
Plankton:	very small, often microscopic plants and animals that mostly inhabit the surface level of a water body.
Primary production:	the production of organic compounds from atmospheric or aquatic carbon dioxide by microorganisms. This process is performed by autotrophic microorganisms and energy is obtained from light or inorganic chemical reactions.

Prokaryotes: the group of microorganisms that contain the Archaea and the Bacteria. These organisms are mostly unicellular and they lack a cell membrane or other membrane-bound organelles (cellular subunits with a specific function).

Protein: an organic compound that is made of amino acids arranged in a linear chain and folded into a globular form. Many proteins are enzymes that catalyze biochemical reactions. Proteins also have structural or mechanical functions.

Proterozoic: one of the three time periods in the evolution of the Earth in which the Precambrian is divided. The Precambrian spans from the formation of the Earth, around 4.6 Ga, to 0.542 Ga and is subdivided into the Hadean (3.8 to 4.6 Ga), Archean (2.5 to 3.8 Ga) and the Proterozoic (2.5 to 0.542 Ga). The period from the present day to 0.542 Ga is the Phanerozoic.

Psychrophile: a microorganism that has an optimum growth temperature less than 15°C.

Pure culture: experiments used for multiplying a single strain of microorganism by letting experiments it reproduce in a predetermined culture media under controlled laboratory conditions. In the experiments, the effect of different growth conditions could be tested on a specific type of microorganism.

Pyrite: a mineral with the composition FeS_2.

Q_{10} value: the change in enzymatic activity caused by a 10°C temperature rise.

Rate constant: quantifies the speed of a chemical reaction and is temperature dependent.

Redox state: refers to the reduction-oxidation state of the chemical compounds present in a specific environment.

Reductant:	a substance that looses electrons in a redox reaction.
Reduction:	a chemical or biochemical reaction in which the reactant gains electrons.
Reversible reaction:	a reaction that could run in both forward and backward directions.
Shale:	a fine-grained, sedimentary rock composed of flakes of clay minerals and tiny fragments of other minerals, especially quartz and calcite. The ratio of clay to other minerals is variable.
Slurry:	a suspension of sediment in solution.
Species:	part of the biological classification system used to group and categorize organisms. Species comes below genus. Species contain a collection of strains that show the same major properties.
Stable isotopes:	isotopes that are not radioactive and whose abundance does not change due to radioactive decay.
Sedimentary rock:	a rock that is formed by sedimentation of material at the Earth's surface.
Sedimentation:	the tendency for particles in suspension to settle or precipitate out of a solution.
Strain:	part of the biological classification system used to group and categorize organisms. A strain comes below species. In a microbial population of a specific strain all cells are descendants from the same microorganism.
Steady state:	a condition in which a microbial process is supposed to proceed at a (near) constant rate.
Substrate:	a compound that serves as a nutrient for growth.

Sulfate reducing: prokaryotes	A morphologically diverse groups of prokaryotes that use sulfate as a terminal electron acceptor. These microorganisms grow mostly under anoxic conditions and are ubiquitous on the modern Earth. They are able to flourish in a large variety of moderate to extreme geochemical settings.
Sulfate reduction rate:	the rate at which sulfate is reduced to sulfide.
Sulfur cycle:	biogeochemical process involving the transformation of sulfur through a series of oxidation states.
Thermophile:	a microorganism with an optimum growth temperature of between 40˚C and 80˚C.
Terminal acceptor:	compound at the end of a series of oxidation reduction reactions that becomes electron reduced as a result of gaining electrons.
Terrestrial:	refers to things which are related the land or the Earth.

Definitions are derived from:

Stenzenbach, L. D. and Yates, M. V., (2003) The dictionary of environmental microbiology. Elsevier Ltd

Allaby, A. and Allaby, M., (2003) A Dictionary of Earth Sciences. *Oxford University Press*, 640.

List of abbreviations

α	isotope fractionation factor
APS	adenosine-5'-phosposulfate
ATP	adenosine-5'-triphosphate
$BaSO_4$	barite
$CaSO_4$	gypsum
C/N ratio	Carbon Nitrate ratio
CrO_4^{2-}	Chromate
DSR	dissimilatory sulfate reductase
$\delta^{34}S$	isotope fractionation of the $^{34}S/^{32}S$ isotope system in a particular compound
ΔG^0	standard Gibbs free energy
ΔG	Gibs free energy
$\Delta^{33}S$	deviation of $\delta^{33}S$ from the mass dependent fractionation line
$\Delta^{36}S$	deviation of $\delta^{36}S$ from the mass dependent fractionation line
ε	isotope fractionation effect
FeS_2	pyrite
FISH	Fluorescent in situ hybridization
Ga	109 years
H_2S	hydrogen sulfide
k	rate constant
K	equilibrium constant
Km	half-saturation concentration
kJ	kilo Joule
mM	millimolar
MPN	Most probable number
MoO_4^{2-}	Molybdate
PAPS	phosphoadenosine-5'-phosphosulfate
PPi	pyrophosphate
ppm	parts per million
rRNA	ribosomal Ribo Nucleic Acid
S0	elemental sulfur
S_2^-	sulfide
SO_2	sulfur dioxide
SO_4^{2-}	sulfate
SO_3^{2-}	sulfite
$S_2O_3^{2-}$	thiosulfate
$S_3O_6^{2-}$	trithionate
SeO_4^{2-}	selenate

SRP	sulfate reducing prokaryotes
SRR	potential sulfate reduction rate
μM	micro molar
vppm	volume parts per million
WO_4^{2-}	tungstate

Image of Mono Lake, California, USA

English summary

Sulfur isotopes have been extensively used to trace the activity of sulfate reducing prokaryotes (SRP) in a large variety of modern and ancient geochemical settings on Earth and to estimate the role of this microbial metabolism in global sulfur cycling. Extensive pure culture and sediment incubation data have been produced to date, providing a detailed insight into the mechanisms involved in microbial sulfate reduction at the cellular level. However, studies on natural communities of sulfate reducers in close to intact sediments are required for interpreting variations in $\delta^{34}S$ in sedimentary rocks, and these are much more limited. This thesis uses a flow-through reactor technique to fill this gap, and describes ranges in potential sulfate reduction rates (SRRs) and sulfur isotope fractionation effects (ε) for sediments sampled from diverse modern geochemical settings. Sulfur isotope fractionation effects were calculated as the difference in $\delta^{34}S$ between co-existing sulfate and sulfide, under a variety of experimental conditions with incubation temperature and electron donor concentration as the main variables.

Four geochemical settings have been investigated, including a brackish estuary (Schelde Estuary, The Netherlands), a hypersaline soda lake (Mono Lake, California, USA), a shallow marine hydrothermal vent system (Vulcano, Italy) and a freshwater river site (River Schelde, Belgium). Sediments were sampled as 2 cm thick slices using the flow-through reactor approach that is designed to preserve the original physical, geochemical and microbial structure. Laboratory incubations were carried out for between 3 and 12 weeks, under anoxic conditions with SRRs and ε values determined from the difference in sulfate concentrations and isotope ratios measured in the reactor inflow and outflow solutions. Sulfate was supplied in non-limiting concentrations via the reactor inflow solutions. No external electron donor was amended during the majority of experiments, but was supplied by the natural sediment substrate. Some reactors were amended with lactate as external electron donor. Data analysis was restricted to SSR and ε values obtained under steady state conditions. The total range in SRR varied over more than an order of magnitude from 5 to 179 nmol cm^{-3} h^{-1}, with corresponding ε values of 5 to 43 ‰. This range in isotope fractionation effects is similar to the total variability found in other sediment incubation and pure culture experiments.

The Schelde Estuary site was used to carry out a detailed study that explored the effect of sampling during different seasons, as well as taking samples from lateral and vertical spatial profiles with respect to the boundary between the tidal flat and a more organic matter-rich salt marsh (***Chapter 2***). Isotope fractionation effects ranged from 9 to 34 ‰. A clear inverse SRR *versus* ε relationship was observed within data collected in May 2006, which follows the standard fractionation model of Rees (1973). The relationship was not consistent between different sampling periods and contrasted with previously published data for a marine lagoon in Denmark that was offset towards larger fractionation effects (Canfield, 2001). Seasonal

and site related variations in labile organic matter availability or differences in the microbial community most likely controlled these differences.

Similar experiments were performed with sediments collected from the littoral zone of the hypersaline soda lake at Mono Lake in California, USA (***Chapter 3***). In contrast to the estuarine site, the general inverse relationship between ε and SRR predicted by the standard isotope fractionation model for sulfate reduction was not observed. In fact, at low SRRs of below approximately 25 nmol cm^{-3} h^{-1}, ε values correlated positively with SRR. This can be explained by a gradual increase in cellular energy available for the synthesis of adenosine-5'-phosphosulfate (APS) that leads to increased metabolic rates and more isotopic selectivity at the cellular level. At higher SRRs, isotope fractionation followed the standard fractionation model and the positive trend shifted towards a weak negative one. Isotope fractionation effects for this site were relatively small, below 21 ‰. Decreased isotope fractionation at low rates of sulfate reduction may be a general characteristic of halophilic sulfate reducers.

The same experimental approach was also used to incubate sediments that were collected in the shallow marine hydrothermal vent system of Vulcano, Italy (***Chapter 4***). This site contained large temperature fluctuations, ranging from 20 to 90°C. No measurable SRRs were detected when incubating with the natural sediment substrate, due to low concentrations of labile organic matter and consequent electron donor limitation. However, high SRRs of 78 to 167 nmol cm^{-3} h^{-1} were achieved when amending with lactate, across the full range of incubation temperatures at 30°C, 60°C and 85°C. Corresponding isotope fractionation effects were relatively small, ranging from 6 to 16 ‰, suggesting a dominant role for microorganisms that do not completely oxidize their carbon source. No clear relationship was observed between SRR and ε, although both parameters were investigated at relatively high rates.

Experiments were performed with the addition of the electron donors acetate and lactate and variable concentrations of compounds that inhibit microbial sulfate reduction in order to expand the range of SRR measured for the Schelde Estuary site and investigate if fractionation effects could vary outside the range predicted by the Rees model (***Chapter 5***). Group VI oxyanions were used for inhibition. This preliminary study showed either no deviation from the Rees model or resulted in a slight suppression in ε for a particular value of SRR. The total variability in isotope data induced by enhancers and inhibitors of sulfate reduction did not extend the range that was normally experienced in this sedimentary environment.

In general, the flow-through reactor experiments resulted in a range in isotope fractionation effect data of 5 to 43 ‰ which is consistent with the standard fractionation model of Rees (1973) (***Chapter 6***). Most experiments resulted in relatively small average isotope fractionation effects of less than 20 ‰ under close to optimum incubation conditions across the full range of SRR. Isotope fractionation was distinct at each sampling site and differences were most likely controlled by electron donor availability and microbial community size and structure, although salinity and cellular energetics may have also played a role. No overall clear relationship was found for all sites between SRR and ε, except within subsets of data at the Schelde Estuary and Mono Lake locations. The range in isotope fractionation was generally larger at low SRRs of below 20 nmol cm^{-3} h^{-1}, which could have resulted from the reduced reversibility of sulfate transport into the cell at low temperature, the large energy

investment in cellular adaptation strategies required for the more extreme environmental sites, or anomalous isotope effects induced by microorganisms thriving on the edges of their optimum growth conditions. Consistently small fractionation effects with an average of 12 ‰ were achieved at SRR above 70 nmol $cm^{-3}h^{-1}$ which is higher than the minimum predicted by the Rees model. The data show that small fractionation effects could be produced over a large range in sulfate reducing activity when sulfate is present in excess.

Large fractionation effects in the rock record younger than 2.4 Ga are thus most likely an effect of additional reoxidation or reduction cycles as previously suggested (Canfield and Thamdrup, 1994; Canfield and Teske, 1996), since isotope fractionation never exceeds 47 ‰ during a single step of sulfate reduction in natural communities of SRP. Limited $\delta^{34}S$ variations in the earliest rock record from Archean times could be linked to microbial sulfate reduction if this occurred at high SRR or under specific environmental conditions such as those experienced at the Mono Lake and Vulcano sites. Low ocean sulfate concentrations, as suggested by Habicht et al., 2002, are thus not a unique explanation for the limited degrees of $\delta^{34}S$ variation in the terrestrial geological record prior to 2.4 Ga. A major potential problem in tracing small $\delta^{34}S$ variations caused by microbial sulfate reduction is the fact that they could be easily overprinted by mixing between distinctive sulfur sources as well as abiotic fractionation processes. The new data presented in this thesis should be further implemented in models that study sulfur cycling in modern and ancient geochemical settings.

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Samenvatting in het Nederlands

Hoe zag de Aarde er miljarden jaren geleden uit en hoe is zij in de loop der tijd veranderd? Wat voor levensvormen waren er toen op de Aarde aanwezig, zijn die er nu nog steeds, en hoe en wanneer zijn deze levensvormen ontstaan? Dit zijn vragen waar wetenschappers van verschillende disciplines zich al eeuwenlang mee bezig houden. Dat de Aarde er miljarden jaren geleden heel anders uitzag, staat vast. Er was veel meer vulkanische activiteit, maar ook de samenstelling van de atmosfeer was heel anders. Zo was er tot zo'n 2,4 miljard jaar geleden geen zuurstof in de lucht. Dit zorgde ervoor dat er maar een beperkt aantal soorten organismen deze "extreme"omstandigheden op Aarde konden overleven. Een van de soorten zijn de sulfaatreducerende prokaryoten. Dit zijn eencellige micro-organismen die sulfaat gebruiken als energiebron voor het functioneren van hun celprocessen (zoals wij zuurstof gebruiken). Bacteriën behoren ook tot de groep van de prokaryoten. Sulfaatreducerende micro-organismen komen nu nog steeds in grote aantallen en op veel verschillende plaatsen voor op de Aarde. Hun aanwezigheid kan worden aangetoond met behulp van stabiele zwavelisotopen die ook goed bewaard kunnen worden in gesteente, zelfs van miljarden jaren oud. Isotopen zijn atomen van hetzelfde element, in dit geval zwavel, die alleen verschillen in hun massa. Zwavel heeft vier stabiele isotopen te weten ^{32}S, ^{33}S, ^{34}S en ^{36}S, waarvan ^{32}S en ^{34}S met respectievelijk 95 en 4 % de meest voorkomende zijn. Tijdens het genereren van energie wordt het opgenomen sulfaat in de cel omgezet in sulfide. Hierdoor verandert de isotoopverhouding omdat het lichte sulfaatmolecuul makkelijker te consumeren is dan het zware. Met andere woorden het kost minder energie om de zwavel-zuurstof bindingen in het lichte sulfaatmolecuul te breken en daardoor bevat een groter deel van het gevormde sulfide de lichte zwavelatomen. Door de verandering en mogelijke variatie in isotoopverhouding, ook wel isotoopfractionering of isotoopeffecten genoemd, te meten en die te vergelijken met waarden in het gesteente zou je dus kunnen bepalen waar en wanneer deze sulfaatreducerende prokaryoten op Aarde aanwezig waren. Voor grotere organismen worden hiervoor vaak fossielen gebruikt maar het probleem is dat fossielen van micro-organismen moeilijk te bewaren zijn. Ten eerste omdat ze zo klein zijn maar ook omdat ze geen botstructuur hebben. Het gebruik van isotopen kan daarom een uitkomst bieden. Er zijn al verschillende studies gedaan met isolaties van één enkele soort van dit type prokaryoten om de variatie in isotoopeffecten vast te stellen, maar voor de interpretatie van sporen in het gesteente is het juist belangrijk om te weten wat voor signaal een populatie bestaande uit verschillende soorten achterlaat. Hier is tot op dit moment weinig over bekend.

Dit proefschrift beschrijft de variatie in $^{34}S/^{32}S$ zwavelisotoopverhoudingen geproduceerd door actieve populaties van sulfaatreducerende prokaryoten in hun natuurlijke leefomgeving. Sedimenten die grote aantallen en verschillende soorten van dit type micro-organismen bevatten, zijn bestudeerd en de resultaten zijn met elkaar vergeleken maar ook met

isotoopsporen beschreven in andere literatuurstudies van sedimenten en gesteenten van 0 tot 3,5 miljard jaar oud. De onderzochte sedimenten zijn afkomstig van verschillende locaties op de "moderne"Aarde, variërend van de Nederlandse kust tot locaties die zich kenmerken door meer "extreme"leefomstandigheden zoals het vulkanisch eiland "Vulcano"in Italië of "Mono Lake", een meer in de Verenigde Staten met extreem hoge zoutconcentratie en hoge pH. De uitkomsten van dit promotieonderzoek zijn niet alleen belangrijk voor de zoektocht naar sporen van de vroegste levensvormen op Aarde in oud gesteente, maar geven daarnaast ook inzicht in de variatie in isotoopeffecten gerelateerd aan biologische activiteit onder verschillende natuurlijke omstandigheden, welke gebruikt kunnen worden in geologische modellen die de bronnen en de cyclus van het element zwavel op de Aarde, zowel op kleine als grote schaal, bestuderen.

In ***Chapter 1*** wordt een overzicht en introductie gegeven over sulfaatreducerende prokaryoten en zwavelisotoopfractionering. In detail wordt het sulfaatreductieproces in de cel besproken met de nadruk op de stappen in dit proces die leiden tot isotoopfractionering en de isotoopeffecten die in eerdere literatuurstudies zijn gevonden voor zowel isolaties van één soort als populaties van meerdere soorten sulfaatreducerende prokaryoten. Al is er over de isotoopeffecten geproduceerd door populaties, naast de resultaten die nu beschreven staan in dit proefschrift, nog relatief weinig bekend. De zwavelisotoopeffecten gerelateerd aan biologische processen dit tot nu tot zijn gemeten liggen tussen de -3 en 47 per mil. Er zijn suggesties dat de snelheid van de sulfaatreductie bepalend is voor het isotoopeffect. Gaat een reactie snel, dan is er weinig "tijd"voor de prokaryoten om te selecteren voor de lichte isotoop en dan zal er weinig isotoopfractionering optreden, maar gaat de reactie langzaam dan is er meer "tijd"voor selectie en wordt de isotoopfractionering groter. Er zou dus een omgekeerd evenredige relatie zijn tussen de sulfaatreductiesnelheid en de corresponderende zwavelisotoopfractionering. Dit wordt weergegeven in het model van Rees (1973). Dit type relatie is echter niet in alle studies gevonden. Soms komen er geen of positieve relaties naar voren. Dit heeft geleid tot de ontwikkeling van nieuwe modellen (Farquhar et al., 2003; Johnston et al., 2005; Canfield et al., 2006). Verder kan een tekort aan sulfaat ook leiden tot kleinere of geen isotoopeffecten omdat er te weinig sulfaat aanwezig is voor de sulfaatreducerende prokaryoten om tussen het lichte of zware sulfaatmolecuul te "kiezen". Daarom zijn alle experimenten beschreven in dit proefschrift uitgevoerd met een overmaat aan sulfaat. Niet alleen biologische processen kunnen zwavelisotopen fractioneren maar ook anorganische processen, vooral bij temperaturen hoger dan 200 graden Celsius, kunnen isotoopeffecten veroorzaken. Vooral bij de interpretatie van isotoopsporen in oud gesteente moet rekening gehouden worden met dit soort niet-biologische processen. Deze processen staan ook beschreven in ***Chapter 1***. Tot slot wordt in ***Chapter 1*** de *flow-through reactor* techniek beschreven. Deze techniek is in alle experimenten toegepast en biedt de mogelijkheid om de sulfaatreducerende populaties te bestuderen onder condities die zo dicht mogelijk liggen bij hun natuurlijke leefomstandigheden. Schijfjes sediment van 2 cm breed en met een diameter van 4,2 cm worden direct vanuit het veld in de reactor gestopt. Hierdoor wordt de sedimentstructuur niet verstoord en de verdeling van de natuurlijke microbiële populatie en

het organisch materiaal in het sediment behouden. Organisch materiaal is belangrijk voor het functioneren van sulfaatreducerende prokaryoten omdat deze moleculen de elektronen leveren die nodig zijn om het sulfaatreductieproces te laten verlopen. In omgevingen waar relatief weinig organisch materiaal aanwezig is zoals in de meeste vulkanische systemen kunnen anorganische moleculen als waterstofgas (H_2) ook de elektronen leveren. Tijdens het experiment stroomt er een oplossing langzaam door het sediment. Deze oplossing bevat het sulfaat en is verder aangepast aan de samenstelling van het water dat op de locatie aanwezig is. Het organisch materiaal halen de prokaryoten in de meeste gevallen uit het sediment zelf. Soms is er lactaat of acetaat toegevoegd. De temperatuur is een van de hoofdvariabelen in deze experimenten en kan makkelijk worden aangepast met behulp van een thermostatisch waterbad. Ook bevat ***Chapter 1*** de onderzoeksdoelen van dit proefschrift.

De resultaten van uitgebreide studies van populaties van sulfaatreducerende prokaryoten in vier geochemisch verschillende locaties worden uitgebreid beschreven in ***Chapter 2***, ***Chapter 3***, ***Chapter 4***, ***Chapter 5*** en ***Chapter 6***. Ook de effecten van variaties in fysische en chemische omgevingsfactoren zoals temperatuur en de hoeveelheid en type aanwezig organisch materiaal worden beschreven.

In ***Chapter 2*** wordt een gedetailleerde studie beschreven van populaties van sulfaatreducerende prokaryoten aanwezig in sedimenten van het Schelde-gebied aan de Nederlandse kust. Deze locatie is een goed uitgangspunt voor het onderzoek omdat eerdere studies de aanwezigheid van grote populaties sulfaatreducerende prokaryoten hebben aangetoond. Verder zijn er ook enkele gegevens bekend over de grootte van de sulfaatreductiesnelheden. Sedimenten zijn verzameld van verschillende plekken en dieptes binnen deze locatie maar ook gedurende verschillende periodes van het jaar, te weten februari, mei en oktober 2006. Gedurende de experimenten is de temperatuur veranderd van 10 naar 20, 30 en 50 graden Celsius. Sulfaatreductiesnelheden gemeten in deze experimenten variëren van 5 tot 49 nmol cm^{3-} h^{-1}. De bijbehorende isotoopeffecten liggen tussen de 9 en 34 per mil. Er wordt een duidelijke omgekeerd evenredige relatie gevonden tussen de sulfaatreductiesnelheid en de hoeveelheid isotoopfractionering. Deze relatie volgt daarbij het Rees (1973) model. Ook al was er een duidelijke relatie te vinden, deze was verschillend voor sedimenten verzameld in februari en mei. Ook was er een verschil met een eerdere literatuurstudie van Canfield (2001), die vergelijkbare experimenten heeft uitgevoerd met sediment van de Deense kust. De sulfaatreductiesnelheden en de inverse relatie met de zwavelisotoopfractionering gevonden voor beide locaties waren vergelijkbaar, maar de hoeveelheid fractionering in de experimenten met Schelde-sediment was een stuk lager. Het verschil wordt meest waarschijnlijk veroorzaakt door variaties in de samenstelling van de sulfaatreducerende populatie maar ook door variaties in het type en concentraties van het aanwezige organisch materiaal. Deze factoren kunnen zowel variëren voor verschillende periodes van het jaar in sedimenten van dezelfde locatie maar ook tussen verschillende geologische locaties van vergelijkbaar geochemisch karakter.

In ***Chapter 3*** wordt een vergelijkbare studie als in ***Chapter 2*** beschreven maar dan met sedimenten van "Mono Lake", een meer in Californië in de Verenigde Staten, dat zich kenmerkt door hoge zoutconcentraties en pH. Hierdoor wordt deze locatie ook wel gezien als een "extreme"omgeving waar alleen bepaalde soorten organismen voorkomen. Micro-organismen die onder deze omstandigheden kunnen leven worden meestal alkalofiele of halofiele organismen genoemd. Eerdere literatuurstudies hebben de aanwezigheid van bepaalde soorten alkalofiele en halofiele sulfaatreducerende prokaryoten in Mono Lake beschreven. De sulfaatreductiesnelheden variëren van onder detectielimiet, bijna 0, tot 62 nmol cm^{-3} h^{-1}. Aan sommige sedimenten is ook lactaat toegevoegd. Dit resulteerde in een twee tot vijfvoudige toename van de sulfaatreductiesnelheden met een maximum van 176 nmol cm^{-3} h^{-1}. In tegenstelling tot het Schelde-gebied zijn de isotoopeffecten voor deze locatie relatief klein, variërend van 5 tot 21 per mil. Ook wordt er voor deze locatie geen duidelijke relatie gevonden tussen zwavelisotoopfractionering en sulfaatreductiesnelheden die overeenkomt met de inverse relatie voorspeld door het standaard model van Rees (1973). Voor reductiesnelheden onder de 25 nmol cm^{-3} h^{-1} wordt er een positieve relatie gevonden die bij snelheden daarboven verandert in een zwakke inverse relatie. De verschuiving van de isotoopfractionering naar lagere waarden bij lage sulfaatreductiesnelheden zou karakteristiek kunnen zijn voor halofiele en alkalofiele sulfaatreducerende prokaryoten. De oorsprong ligt waarschijnlijk in het feit dat deze micro-organismen een groot deel van de geproduceerde energie moeten investeren in aanpassingsstrategieën aan de "extreme"omstandigheden van dit meer. Daardoor blijft er minder over voor het sulfaatreductieproces en zijn bij lagere sulfaatreductiesnelheden de isotoopeffecten kleiner. De overgang naar een inverse relatie bij hogere snelheden duidt erop dat dit proces daar een kleinere rol speelt.

In ***Chapter 4*** worden *flow-through reactor* experimenten beschreven die zijn uitgevoerd met sedimenten van het vulkanisch eiland "Vulcano"in Italië. Deze locatie kan ook "extreem"worden genoemd vanwege de grote fluctuatie in temperatuur in het sediment, variërend van 20 tot 90 graden Celsius. Micro-organismen die onder hoge temperaturen kunnen leven worden ook wel thermofiel (temperaturen groter dan 40 graden Celsius) of hyperthermofiel (temperaturen groter dan 80 graden Celsius) genoemd. Ook in dit type omgeving zijn verschillende soorten thermofiele en hyperthermofiele sulfaatreducerende prokaryoten aangetroffen. Experimenten zijn uitgevoerd bij verschillende temperaturen (30, 60 en 85 graden Celsius). In deze experimenten werd bij geen van de temperaturen sulfaatreductie gemeten door gebruik te maken van het in het sediment aanwezige organisch materiaal. Pas wanneer lactaat werd toegevoegd werden er hoge reductiesnelheden gemeten. De meest waarschijnlijke oorzaken hiervan zijn dat of de concentraties van het organisch materiaal in deze sedimenten erg laag zijn of dat de prokaryoten in dit type sediment hoofdzakelijk sulfaat reduceren in combinatie met H_2 als de meest belangrijke elektronenbron voor de reactie. H_2 wordt in hoofdzaak gegenereerd door het actieve vulkanische systeem ter plaatse en is niet opgeslagen in het sediment. Met toevoeging van lactaat werden hoge reductiesnelheden gevonden variërend van 78 tot 167 nmol $cm^{-3}h^{-1}$ voor alle drie de onderzochte temperaturen. Ook voor deze "extreme"sedimenten waren de isotoopeffecten

relatief klein, variërend van 6 to 16 per mil. Er werd verder geen relatie gevonden tussen de reductiesnelheden en de hoeveelheid isotoopfractionering.

In ***Chapter 5*** worden de effecten van verschillende types organische componenten, die elektronen genereren voor het sulfaatreductie proces, op reductiesnelheden en isotoopeffecten beschreven. In deze studie worden acetaat en lactaat gebruikt. Deze moleculen staan erom bekend de snelheid van de sulfaatreductie aanzienlijk te vergroten. Ook worden de effecten van verschillende groep VI oxyanionen (Chromaat, Selenaat, Molybdaat en Wolframaat) beschreven. Deze moleculen hebben stereometrisch dezelfde structuur als sulfaat. Daardoor kunnen ze concurreren in het sulfaatreductieproces en als gevolg daarvan de sulfaatreductie snelheid verlagen of zelfs bij hoge concentraties compleet stoppen. De sedimenten die in deze oriënterende studie worden gebruikt komen van dezelfde locatie in het Schelde-gebied als de sedimenten beschreven in ***Chapter 2***. Het doel van deze studie is niet alleen het uitbreiden van de sulfaatreductiesnelheden naar lagere en hogere waarden dan die worden gevonden in ***Chapter 2*** maar ook om te kijken of de toevoeging van speciale componenten zoals oxyanionen kan leiden tot isotoopeffecten groter dan 47 per mil. Bij bestudering van sedimenten en gesteenten, vooral jonger dan 2,4 miljard jaar oud, worden grotere isotoopeffecten gevonden dan die voorspeld door experimenten met sulfaatreducerende prokaryoten. De waarden kunnen zelfs oplopen tot groter dan 100 per mil. Deze grote isotoopeffecten worden meestal verklaard door meerdere cycli van sulfaatreductie. Het gevormde sulfide kan in sommige gevallen weer terug reageren, ook wel oxideren, naar sulfaat door bijvoorbeeld de aanwezigheid van andere types prokaryoten maar ook door de reactie met zuurstof aanwezig in de lucht of in het water of sediment waar het sulfide gevormd wordt. Op deze manier kan lichter sulfaat gevormd worden dan het oorspronkelijke sulfaat. Als het gevormde lichte sulfaat dan weer wordt gereduceerd, dan kan het sulfide nog "lichter"worden. Maar er zijn ook een aantal gevallen bekend waar het niet waarschijnlijk is dat dit proces verantwoordelijk is voor de grote isotoopeffecten en de werkelijke oorzaak nog onbekend is. Deze studie heeft laten zien dat zowel de toevoeging van acetaat, lactaat of van groep VI oxyanionen aan populaties van sulfaatreducerende prokaryoten niet resulteren in extreme waarden in isotoopfractionering en daarbij binnen de waarden vallen die worden voorspeld door het Rees (1973) model.

In ***Chapter 6*** worden de resultaten van de voorafgaande studies met elkaar vergeleken. Ook worden de resultaten besproken die zijn verkregen met sedimenten van een vierde onderzoekslocatie. Dit betreft een zoetwatergebied in het Schelde-gebied in België. Ook worden de belangrijkste conclusies gegeven, en aanbevelingen voor vervolgonderzoek. Alle reactorexperimenten leiden tot isotoopeffecten variërend van 5 tot 43 per mil. Deze waarden vallen binnen de maximumwaarden van -3 tot 47 per mil gepubliceerd in voorafgaande literatuurstudies en de waarden die voorspeld worden door het standaard zwavelisotoopfractioneringsmodel van Rees (1973). De meeste experimenten resulteerden in relatief kleine isotoopeffecten, meestal kleiner dan 20 per mil. De hoeveelheid fractionering was verschillend voor iedere onderzochte locatie. Deze verschillen worden meest waarschijnlijk veroorzaakt door locatiespecifieke variaties in organisch materiaal dat elektronen genereert

voor het sulfaatreductieproces maar ook verschil in samenstelling van de sulfaatreducerende populatie speelt een belangrijke rol. Verder kunnen het zoutgehalte en energie-investering in adaptatieprocessen in de cel aan extreme omgevingsomstandigheden ook resulteren in beperkte isotoopeffecten. Wanneer de resultaten van alle locaties samen worden bekeken, wordt geen duidelijke relatie gevonden tussen sulfaatreductiesnelheid en isotoopfractionering. Een relatie tussen deze twee factoren komt alleen tot uiting onder bepaalde omstandigheden in data verkregen met Mono Lake en Schelde-gebied sedimenten. Over het algemeen kan wel gezegd worden dat de variatie in isotoopeffecten groter is bij reductiesnelheden die kleiner zijn dan 20 nmol cm^{-3} h^{-1} in vergelijking tot snelheden daarboven. Dit kan verschillende oorzaken hebben. Ten eerste kunnen temperaturen kleiner dan 15 graden Celsius het sulfaattransport in de cel moeilijker maken en daarbij de isotoopeffecten verlagen. Ook grote energie-investeringen in adaptatieprocessen in sulfaatreducerende prokaryoten levend onder meer "extreme"omgevingsomstandigheden kunnen leiden tot dit effect. Afwijkende isotoopeffecten naar hogere of lagere waarden kunnen voorkomen als sulfaatreducerende prokaryoten niet functioneren onder optimale omstandigheden. Bij hoge sulfaatreductiesnelheden, groter dan 70 nmol cm^{-3} h^{-1} worden voor alle locaties isotoopeffecten gevonden die laag zijn met een gemiddelde waarde van 12 per mil. Deze minimum waarde is hoger dan de minimum waarde van -3 per mil voorspeld in het Rees (1973) model.

In ***Chapter 6*** wordt ook een vergelijking gemaakt tussen de resultaten beschreven in dit proefschrift voor locaties op de "moderne"Aarde en waarden die zijn gemeten in sedimenten en gesteenten variërend van 0 tot 3,5 miljard jaar oud. De isotoopeffecten die in dit promotieonderzoek worden gevonden komen overeen met de relatief lage waarden (5 tot 20 per mil) gevonden in gesteente van ongeveer 3,5 miljard jaar oud. Dit zou er dus op duiden dat dit soort micro-organismen rond die tijd al op Aarde aanwezig waren. Het grote probleem is echter dat op basis van alleen het $^{34}S/^{32}S$ zwavelisotoopsysteem de sporen van het biologische sulfaatreductieproces moeilijk zijn te onderscheiden van isotoopeffecten die worden veroorzaakt door niet-biologische processen. Deze processen genereren meestal ook waarden die rond de 20 per mil zijn en dus overlappen met isotoopeffecten van biogene sulfaatreductie. Gelukkig hebben recente ontwikkelingen laten zien dat het gebruik van de $^{33}S/^{32}S$ en $^{36}S/^{32}S$ zwavelisotoopsystemen in combinatie met het meer conventionele $^{34}S/^{32}S$ systeem een oplossing kan beiden voor dit probleem. Eén van de aanbevelingen die in dit proefschrift wordt gegeven is dan ook om in vervolgexperimenten alle stabiele zwavelisotopen te meten. Verder kan uit de resultaten van dit promotieonderzoek worden opgemaakt dat de grotere isotoopeffecten, vaak veel groter dan 47 per mil, die worden gevonden is sedimenten jonger dan 2,4 miljard jaar oud, meest waarschijnlijk worden veroorzaakt door, zoals eerder in de literatuur is gesuggereerd door Canfield and Thamdrup, (1994) en Canfield and Teske, (1996), opeenvolgende cycli van sulfaatreductie en sulfideoxidatiereacties. Alhoewel het ook zeker is aan te bevelen om verder onderzoek te doen naar andere extreme omgevingsomstandigheden, zoals hoge sulfideconcentraties, die zouden kunnen leiden tot grotere isotoopeffecten.

Dit proefschrift laat dus zien dat de variatie in zwavelisotoopeffecten geproduceerd door hedendaagse micro-organismen in hun natuurlijke leefomgeving overeenkomt met sporen die worden gevonden in gesteente van ongeveer 3,5 miljard jaar oud, en dat kleine isotoopeffecten dus niet alleen worden geproduceerd onder lage sulfaatconcentraties zoals gesuggereerd door Habicht et al. (2002). Er is dus een goede mogelijkheid dat deze sulfaatreducerende prokaryoten toen al aanwezig waren op Aarde. Omdat niet-biologische processen de isotoopfractionering in oud gesteente ook kunnen beïnvloeden is het aan te bevelen de resultaten van dit onderzoek niet alleen te combineren met andere stabiele zwavelisotopen maar ook met andere methodes die ook sporen van leven kunnen opsporen zoals stabiele isotoopsystemen van andere elementen. Verder is de variatie in isotoopeffecten in gesteente en sedimenten jonger dan 2,4 miljard jaar oud veel groter dan die in dit onderzoek worden gemeten voor populaties van sulfaatreducerende prokaryoten. Om deze grotere waarden te kunnen verklaren zijn er dus andere processen nodig die in combinatie met deze resultaten de isotoopeffecten kunnen verhogen.

De waarden gevonden in dit promotieonderzoek zijn niet alleen belangrijk voor de zoektocht naar biologische sporen in gesteente maar ook zeker zo belangrijk om te gebruiken in geologische modellen die de bronnen en de cyclus van het element zwavel op de Aarde, zowel nu als vroeger, op grote en kleine schaal, bestuderen. De resultaten van dit onderzoek geven minimale en maximale waarden die worden geproduceerd door de aanwezige sulfaatreducerende populatie in een grote verscheidenheid aan locaties op Aarde die variëren in hun geochemische samenstelling.

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Dankwoord

Voilà! Hier is het dan mijn proefschrift, het resultaat van jaren hard werk. Ik ben erg blij het boekje nu echt in handen te hebben. Als je begint met promotieonderzoek heb je het idee dat je zeeën van tijd hebt maar al snel begint alles toch in een stroomversnelling te raken. De eerste resultaten komen binnen met goed of minder goed resultaat en al snel is de tijd te kort. Soms zit het onderzoek mee en soms valt het tegen. Er zijn van die dagen waarop je jezelf afvraagt of het werk ooit nog wel af komt, maar het volgende moment kun je toch weer zo enthousiast zijn over een goed resultaat, een leuke samenwerking of een nieuwe onderzoeksinvalshoek dat je hier weer zoveel energie van krijgt om met goede moed verder te gaan. Doorzettingsvermogen is gewoon erg belangrijk. Het is waar, wetenschap is nooit af en ook de resultaten van dit proefschrift roepen weer vele vragen op om verder te onderzoeken maar dat maakt wetenschappelijk onderzoek ook zo leuk. Ook al is dit mijn eigen project, onderzoek doe je niet alleen. Zowel samenwerking tijdens het werk als activiteiten en contacten buiten het werk zijn erg belangrijk. Ik wil daarom van deze gelegenheid gebruik maken om een aantal mensen te bedanken.

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Marjolijn

Curriculum Vitae in English

Marjolijn Christine Stam was born on April the 1st, 1980 in Leiden, The Netherlands. She grew up in Baarn where she passed her high school exam at *Het Baarnsch Lyceum* in 1998. In the same year she started her degree in Chemistry at Utrecht University.

During her study she was awarded a grant from the *Vereniging Nederlandse Chemische Industrie (VNCI)*, the organisation for the Dutch chemical industry, for excellent study results obtained during the first year. During 2000-2001 she was vice-chairman of the *Utrechtse Scheikundige Studievereniging Proton*, the chemistry-students' association for which she also actively participated in various committees throughout her student years. From 2001-2002 she participated in the organization of the *Bèta Bedrijvendagen*, Utrecht University's employment fair. During her study she also attended a three-month course on Chemistry Didactics.

Her Master research project, titled "1-Butyl-3-Methyl Imidazolium Substituted Tetraphenylborate Ionic Liquids, Synthesis and Properties", was done with the group Chemical Biology and Organic Chemistry under supervision of dr. J. van den Broeke and prof. dr. G. van Koten. This research project resulted in a publication in the "European Journal of Inorganic Chemistry".

In 2002-2003, she completed a five-month internship at science center NEMO in Amsterdam where she also started her professional career in March 2003. For more than two years she worked in the Education Department at NEMO coaching trainees and coordinating the public chemical laboratory where visitors can independently carry out experiments. She also developed teaching materials and educational projects for primary and high school students.

She obtained her Masters degree in June 2003 with honours (*judicium met genoegen)*.

In August 2005 she started her doctoral research at Utrecht University's Faculty of Geosciences under supervision of dr. P. Mason, prof. dr. P. van Cappellen and prof. dr. B. de Jong which resulted in this thesis. Besides her research she was also involved in teaching, supervising Bachelor and Master students and performing fieldwork in The Netherlands, Italy and the USA. Furthermore she attended an Astrobiology summer school in Austria which was organized, among others, by the ESA. The results of Marjolijn's PhD research were published and presented at various national and international meetings and conferences, including the Goldschmidt conferences in Melbourne, Australia (2006), Cologne, Germany (2007), Vancouver, Canada (2008) and Davos, Switzerland (2009) and meetings of the ESF network "Archean Environmental Studies"in Spain and Austria.

Curriculum Vitae in het Nederlands

Marjolijn Christine Stam werd geboren op 1 april 1980 te Leiden. Ze groeide op in Baarn waar zij in 1998 haar VWO-diploma behaalde aan Het Baarnsch Lyceum. In datzelfde jaar begon zij de studie Scheikunde aan de Universiteit Utrecht.

Tijdens haar studie ontving zij een studiebeurs van de Vereniging Nederlandse Chemische Industrie (VNCI) voor uitmuntende studieresultaten, behaald gedurende het propedeusejaar. Van 2000-2001 was zij vice-voorzitter van het bestuur van de Utrechtse Scheikundige Studievereniging Proton voor welke zij ook gedurende haar hele studieperiode actief was in verschillende commissies. In 2001-2002 nam zij deel aan de organisatie van de Bèta Bedrijvendagen van de Universiteit Utrecht. Tijdens haar studie volgde zij ook een drie maanden durende cursus in Chemie Didactiek.

Ze deed haar afstudeeronderzoek, getiteld "1-Butyl-3-Methyl Imidazolium Substituted Tetraphenylborate Ionic Liquids, Synthesis and Properties", bij de groep Chemische Biologie en Organische Chemie onder leiding van dr. J. van den Broeke en prof. dr. G. van Koten. Dit onderzoek resulteerde in een publicatie in het internationale vaktijdschrift "European Journal of Inorganic Chemistry".

In 2002-2003 liep zij stage bij science center NEMO te Amsterdam waar in maart 2003 haar professionele carrière startte op de afdeling Educatie. Haar functie bestond uit het begeleiden van stagiaires, het coördineren van NEMO's scheikundelaboratorium waar bezoekers zelfstandig experimenten kunnen uitvoeren en het ontwikkelen van lesmateriaal en educatieve projecten voor leerlingen van basis- en middelbare scholen.

In juni 2003 behaalde ze haar doctoraalexamen, judicium met genoegen.

Na ruim twee jaar werkzaam te zijn geweest bij NEMO begon zij in augustus 2005 aan haar promotieonderzoek aan de faculteit Geowetenschappen van de Universiteit Utrecht onder leiding van dr. P. Mason, prof. dr. P. van Cappellen en prof. dr. B. de Jong dat resulteerde in dit proefschrift. Naast het verrichten van wetenschappelijk onderzoek heeft zij tijdens haar promotietraject ook ervaring opgedaan met het geven van onderwijs, het begeleiden van Bachelor- en Masterstudenten en het uitvoeren van veldwerk in Nederland, Italië en de Verenigde Staten. Verder heeft zij in Oostenrijk een zomerschool in Astrobiologie gevolgd, georganiseerd door onder andere de ESA. Haar promotieonderzoek heeft geleid tot wetenschappelijke publicaties en is gepresenteerd op verschillende nationale en internationale bijeenkomsten en conferenties, waaronder de Goldschmidt conferenties in Melbourne, Australië (2006), Keulen, Duitsland (2007), Vancouver, Canada (2008) en Davos, Zwitserland (2009) en bijeenkomsten van het ESF netwerk "Archean Environmental Studies"in Spanje en Oostenrijk.

List of publications

Published abstracts

Controls on microbial sulfur isotope fractionation in littoral sediments M.C. Stam[1], P.R.D. Mason[1], A.M. Laverman[2], C. Pallud[3], P. Van Cappellen[1,4] Geochimica et Cosmochimica Acta, Volume 73, Issue 13, A1263 (2009); Oral presentation at the Goldschmidt Conference in Davos Switzerland, 21st to the 26th of June 2009.

Microbial $\delta^{34}S$ fractionation in a shallow submarine hydrothermal vent system, Vulcano, Italy M.C. Stam[1], P.R.D. Mason[1],.P. Amend[5], M. Viñas[6], J. Gerritse[6] Geochimica et Cosmochimica Acta, Volume 72, Issue 12, A891 (2008); Oral presentation at the Goldschmidt Conference in Vancouver Canada, 13th to the 18th of July 2008.

What controls sulfur isotope fractionation in modern estuarine sediments? M.C. Stam[1], P.R.D. Mason[1], A.M. Laverman[2], C. Pallud[3], P. Van Cappellen[1,4] Geochimica et Cosmochimica Acta, Volume 71, Issue 15, A966 (2007); Oral presentation at the Goldschmidt Conference in Cologne Germany, 19th to the 24th of August 2007.

Does sulfur isotope fractionation in natural sediments record sulfate reducing activity? M.C. Stam[1], P.R.D. Mason[1], A.M. Laverman[2], C. Pallud[3], P. Van Cappellen[1,4] Geochimica et Cosmochimica Acta, Volume 70, Issue 18, A610 (2006); Oral presentation at the Goldschmidt Conference in Melbourne Australia, 27th of Augustus to the 1st of September 2006.

Submitted manuscripts

Controls on sulfate reduction and sulfur isotope fractionation by natural microbial communities in estuarine sediments Marjolijn C. Stam[1], Paul R.D. Mason[1], Anniet M. Laverman[2], Céline Pallud[3], Philippe Van Cappellen[1,4] Submitted to Geochimica et Cosmochimica Acta (*Chapter 2* of this thesis)

Sulfate reducing activity and sulfur isotope fractionation by natural microbial communities in sediments from a hypersaline soda lake (Mono Lake, California) Marjolijn C. Stam[1], Paul R.D. Mason[1], Céline Pallud[3], Philippe Van Cappellen[1,4] to be submitted to Chemical Geology (*Chapter 3* of this thesis)

Sulfur isotope fractionation by natural communities of sulfate reducing prokaryotes in the shallow marine hydrothermal vent system of Vulcano Island, Italy Marjolijn C. Stam[1], Paul R.D. Mason[1], Jan P. Amend[5] to be submitted to Geobiology (*Chapter 4* of this thesis)

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1 Department of Earth Sciences, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The Netherlands

2 UMR Sisyphe7619, Université Pierre et Marie Curie, 75005 Paris, France

3 Department of Environmental Science, Policy and Management, UC Berkeley, CA 94720, USA

4 School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA

[5] Department of Earth and Planetary Sciences and Division of Biology and Biomedical Sciences, Washington University, St. Louis, MO 63130, USA
[6] Deltares-TNO, Unit Subsurface and Groundwater Systems, Princetonlaan 6, 3508 TA Utrecht, The Netherlands