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).

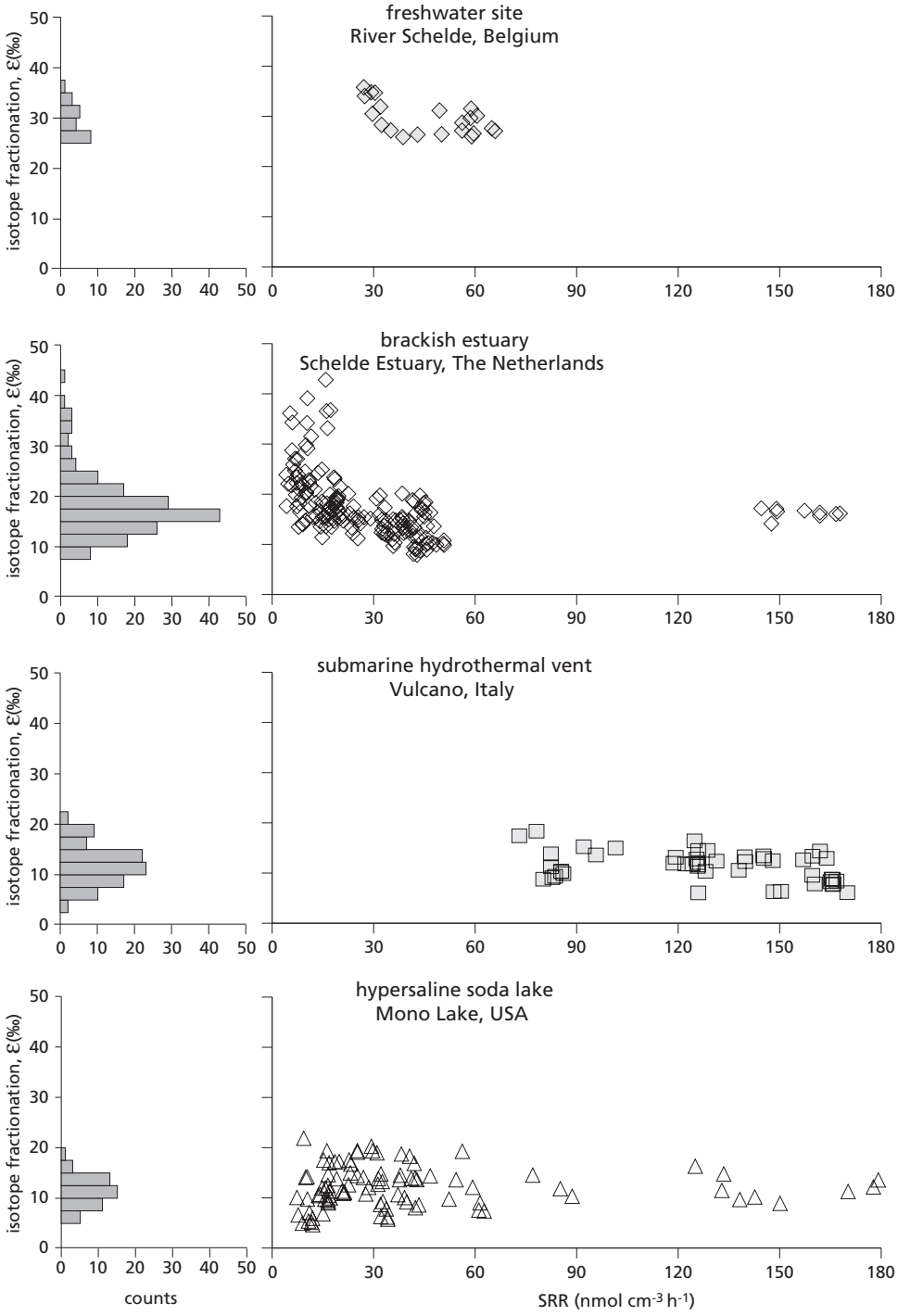
6.3 Controls on isotope fractionation effects

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**		

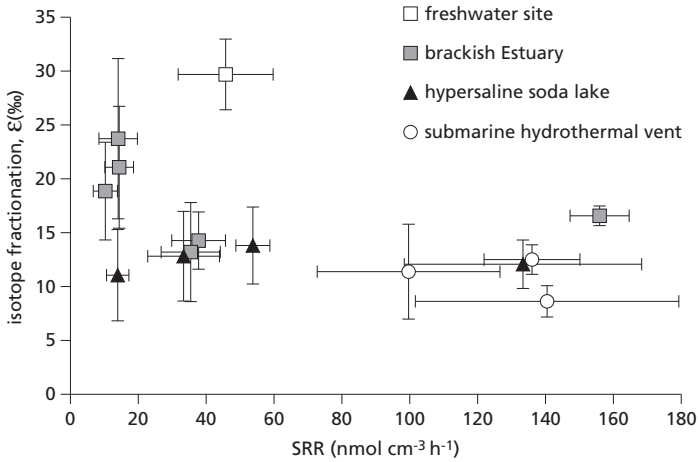


Figure 6.2: Potential sulfate reduction rates (SRRs) *versus* sulfur isotope fractionation effects (ε) for the different sampling sites including average data and standard deviations as presented in Table 6.2. Open square (freshwater site), gray squares (brackish estuary), black triangles (hypersaline 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.

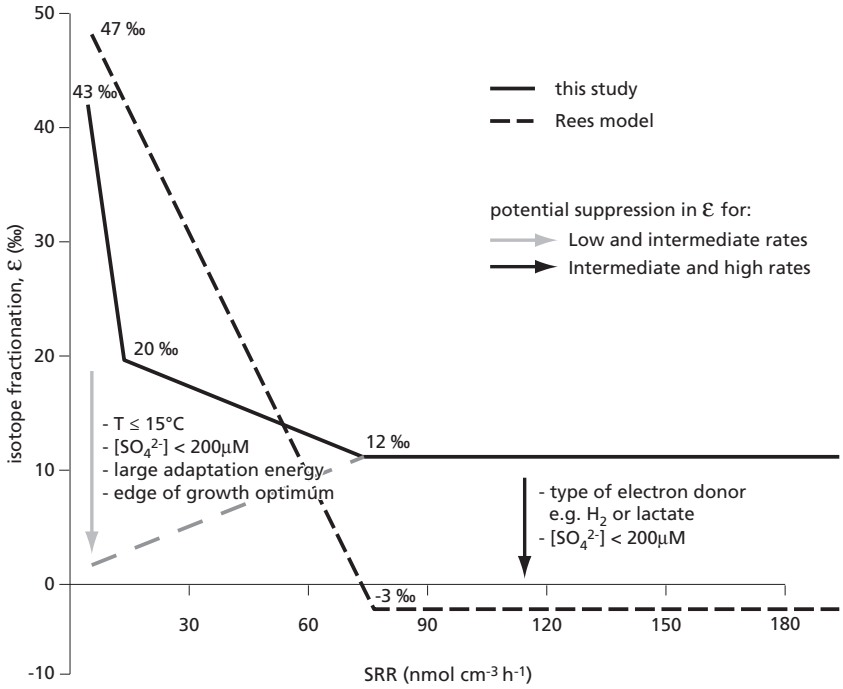


Figure 6.3: The range in sulfur isotope fractionation effects (ε) and type of ε-SRR relationship predicted from the Rees (1973) Model and as observed in this study. Errors indicate possible environmental conditions that could result in deviations from the standard model, at low and intermediate rates (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**

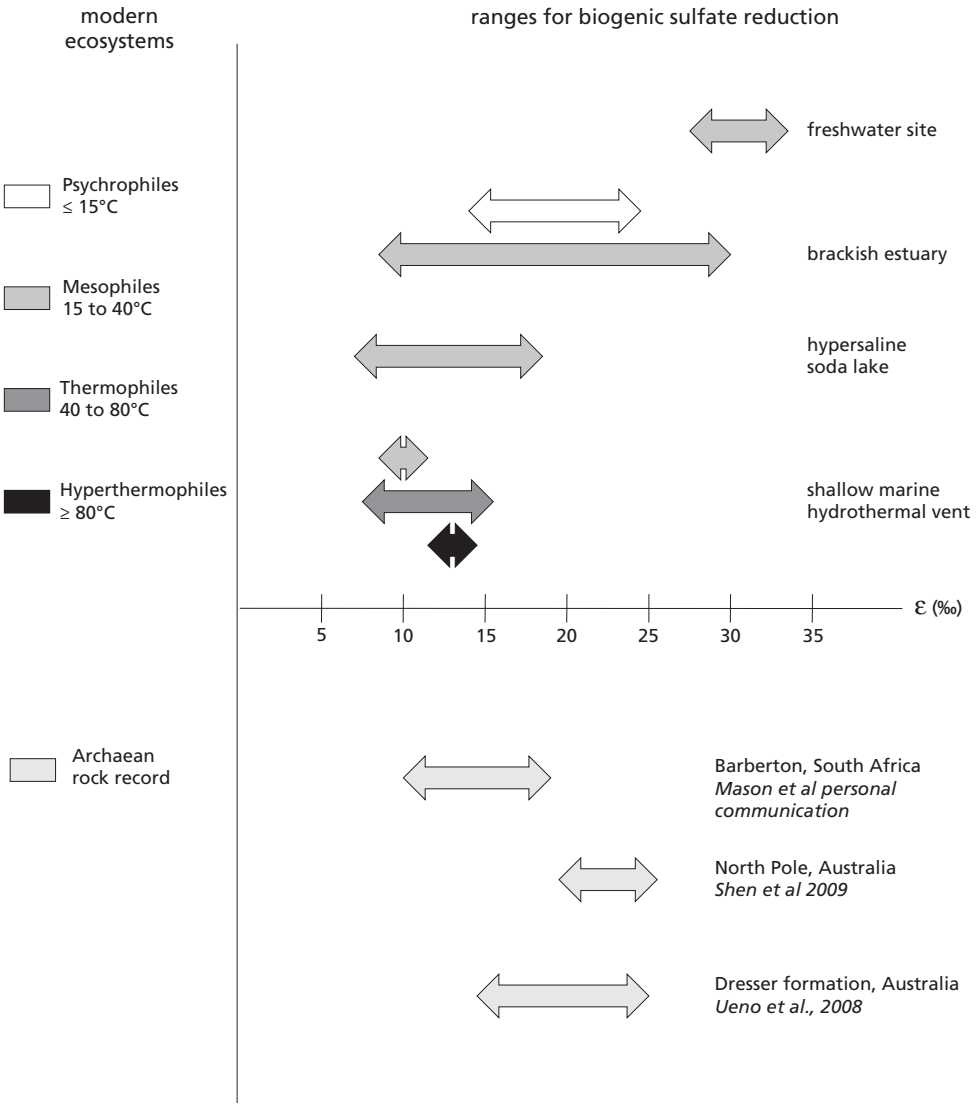


Figure 6.4: Ranges in isotope fractionation effects (ε) observed for the different sampling sites presented in this study compared to fractionation effects related to biogenic sulfate reduction preserved in Archean Rocks. Data are shown for the Barberton Greenstone Belt in South Africa (Mason et al. 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