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

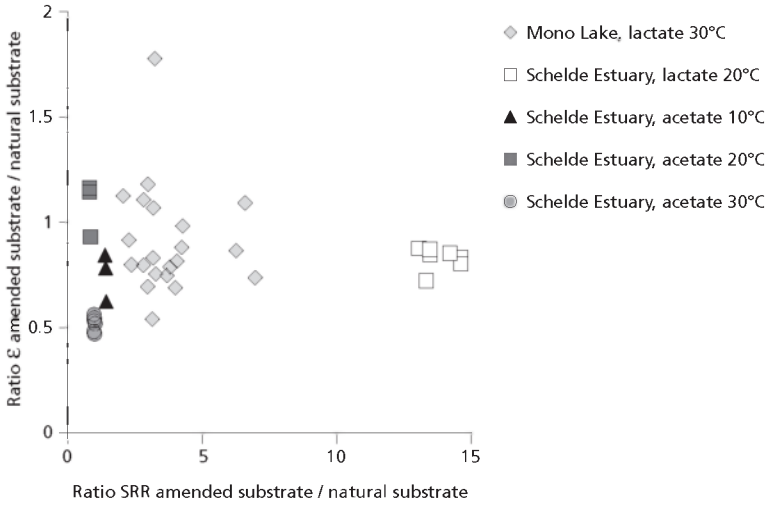

Figure 5.1: Decrease or increase in potential sulfate reduction rate (SRR) or sulfur isotope fractionation (ε) relative to the natural substrate for acetate and lactate. Data obtained from Mono Lake sediments (*Chapter 3*) are also shown.

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

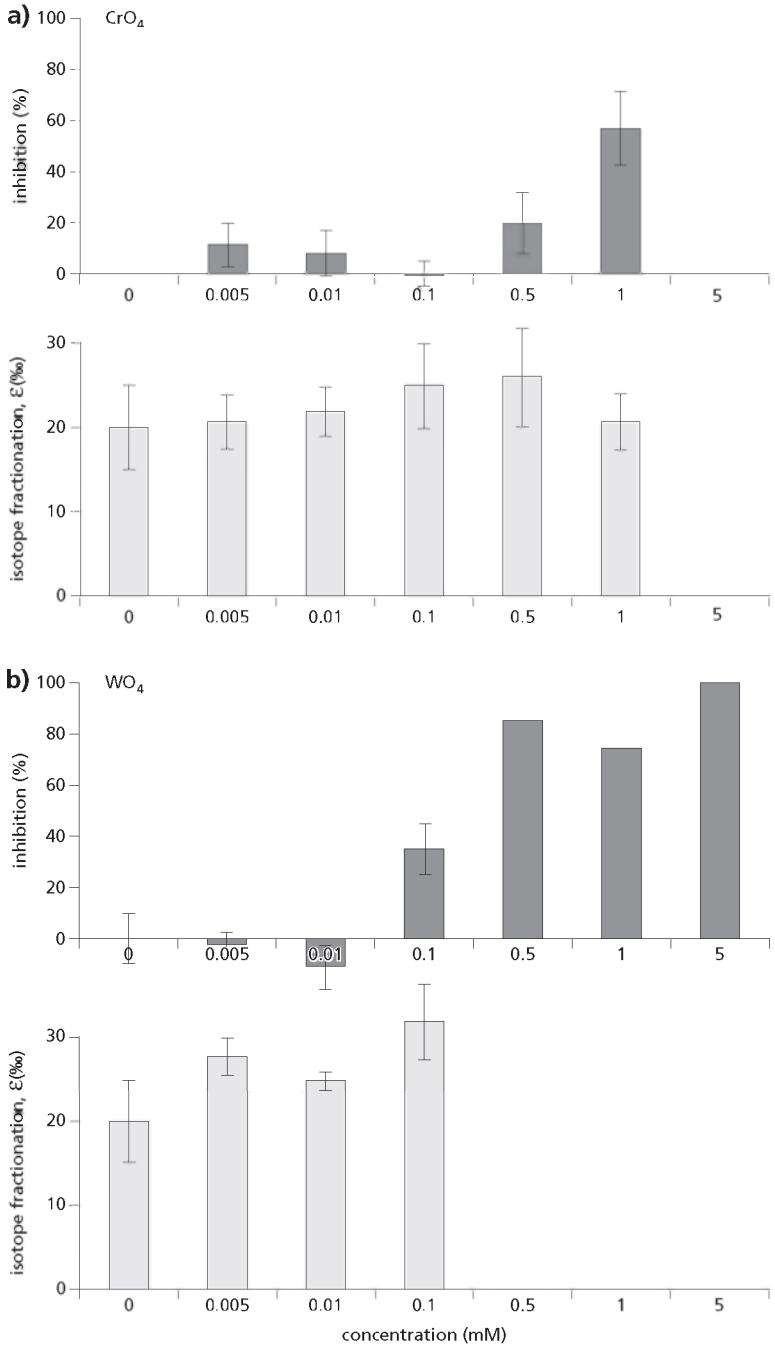

Figure 5.2: Percentage (%) of inhibition of potential sulfate reduction rate (SRR) relative to the control reactor and corresponding sulfur isotope fractionation effects (ε) for CrO_4^{2-} (Panel 5.2a), SeO_4^{2-} (Panel 5.2b), WO_4^{2-} (Panel 5.2c) and MoO_4^{2-} (Panel 2d). Vertical error bars represent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

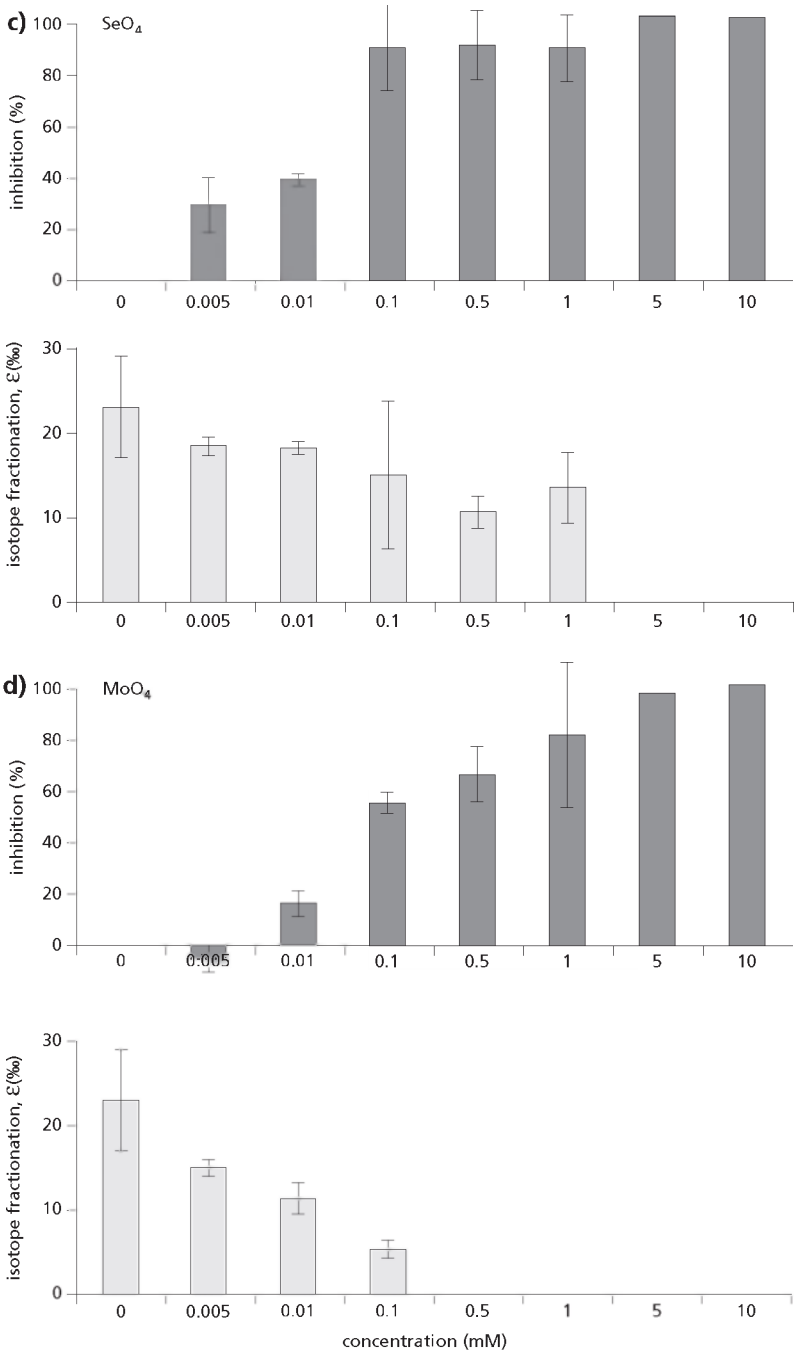

Figure 5.1: Continued

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).

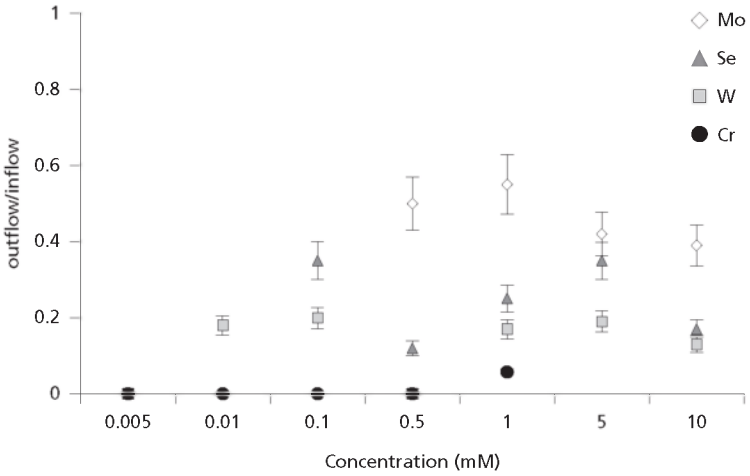

Figure 5.3: Ratios of concentrations of Cr (filled circles), W (gray square), Se (gray triangle), Mo (open diamonds) detected in the outflow solution relative to the inflow solution. External measurement precision was 14 % calculated at the 1 standard deviation level.

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.