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Distribution and fate of sulfur intermediates—sulfite, tetrathionate, thiosulfate, and elemental sulfur—in marine sediments

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ABSTRACT

Most of the sulfide produced in surface marine sediments is eventually oxidized back to sulfate via sulfur compounds of intermediate oxidation state in a complex web of competing chemical and biological reactions. Improved handling, derivatization, and chromatographic techniques allowed us to more closely examine the occurrence and fate of the sulfur intermediates elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$), tetrathionate ($S_4O_6^{2-}$), and sulfite (SO_3^{2-}) in Black Sea and North Sea sediments. Elemental sulfur was the most abundant sulfur intermediate with concentrations ~3 orders of magnitude higher than the dissolved species, which were typically in the low micromolar range or below. Turnover times of the intermediate sulfur compounds were inversely correlated with concentration and followed the order: $SO_3^{2-} \approx S_4O_6^{2-} > S_2O_3^{2-} > S^0$. Experiments with anoxic but non-sulfidic surface sediments from the Black Sea revealed that added sulfide and sulfite disappeared most rapidly, followed by thiosulfate. Competing chemical reactions, including the reaction of sulfite with sedimentary S^0 that led to temporarily increased thiosulfate concentrations, resulted in the rapid disappearance of SO_3^{2-}. Conversely, low thiosulfate concentrations in the Black Sea sediments (<3μM) were attributed to the activity of thiosulfate-consuming bacteria. Experiments with anoxic but non-sulfidic sediments revealed that 1 mol of tetrathionate was rapidly converted to 2 moles of thiosulfate. This tetrathionate reduction was bacterially mediated and occurred generally much faster than thiosulfate consumption. The rapid reduction of tetrathionate back to thiosulfate creates a cul-de-sac in the sulfur cycle, with thiosulfate acting as a bottleneck for the oxidation pathways between sulfide and sulfate.

Keywords: sulfide oxidation, sulfur cycle, diagenesis, tetrathionate, thiosulfate, sulfite.

INTRODUCTION

Sulfur exists in the marine environment predominately in its most oxidized state as sulfate (oxidation state of +VI), and in the reduced form as sulfide and pyrite (oxidation states of −II and −I respectively). In between the oxidized and reduced states, a wide

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variety of sulfur compounds of intermediate oxidation states have been identified. Although they do not form an appreciable quantity of the overall sulfur mass in marine environments, their low concentrations belie their role in a number of biogeochemical reactions and processes within the sulfur cycle. For instance, sulfur intermediates have been shown to influence trace metal solubility and mobility by complexation with polysulfides and thiosulfate (Jacobs and Emerson, 1982; Morse et al., 1987). Polysulfides are suspected to be involved in the formation of pyrite (Luther, 1991), thiols, and organic polysulfides (Vairavamurthy and Mopper, 1989; Kohnen et al., 1989). Sulfonates have been proposed to be formed by the reaction of sulfite or thiosulfate with reactive organic matter (Vairavamurthy et al., 1994). The bacterial disproportionation reactions of sulfite, thiosulfate, and elemental sulfur have been shown to have a strong impact on the fractionation of stable sulfur isotopes (Canfield and Thamdrup, 1994; Cypionka et al., 1998; Habicht et al., 1998) and the interpretation of the sulfur isotope record (Jørgensen, 1990a; Canfield and Teske, 1996).

The formation of sulfur intermediates in marine sediments principally occurs through the oxidation of sulfide produced during bacterial sulfate reduction (Fig. 1, Table 1). Although bacterial sulfate reduction is usually the second most important terminal electron acceptor process for the degradation of organic matter after aerobic respiration in most continental margin sediments, mass balance considerations show that only 10–20% of the produced sulfide is buried in the sediment in its reduced form, principally as pyrite sulfur (Jørgensen, 1982; Ferdelman et al.,

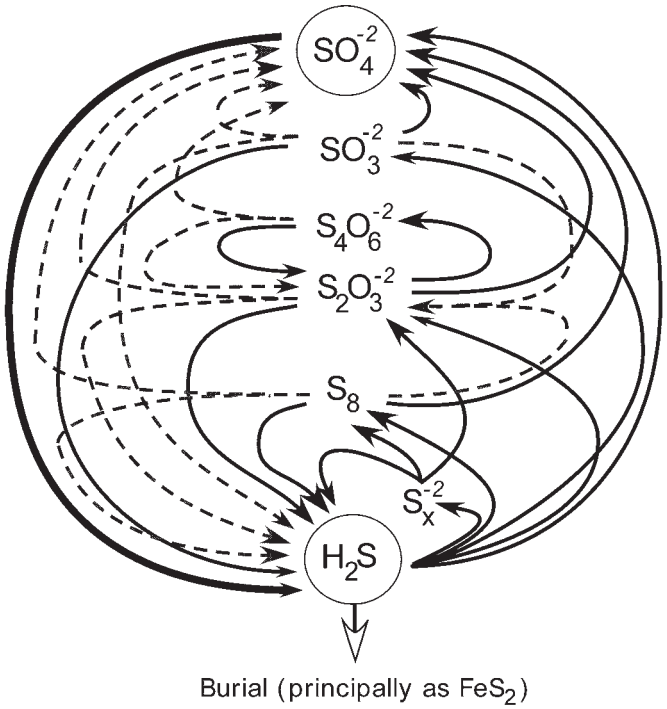

Figure 1. Schematic figure of the sedimentary sulfur cycle where important reductive (left-side, downward arrows) and oxidative (right-side, upward arrows) pathways are shown. Broken lines on the left signify bacterial disproportionation reactions. The cycle is driven by the degradation of organic matter through sulfate-reducing bacteria (thick arrow on the left). Burial of iron-sulfur minerals, mostly FeS_2, represents the dominant sink for reduced sulfur in marine sediments.

TABLE 1. PRODUCTS OF CHEMICAL OR BIOLOGICAL OXIDATION OF MAJOR REDUCED SULFUR COMPOUNDS IN MARINE SEDIMENTS

S-species	Oxidant	Products	Comments[§]	Reference
H_2S	O_2	SO_4^{2-}, $S_2O_3^{2-}$, *SO_3^{2-}*	C	Zhang and Millero, 1993
	O_2	SO_4^{2-} $S_2O_3^{2-}$, *S_n^{2-}*, S^0	C	Chen and Morris, 1972
	O_2	SO_4^{2-}, *$S_2O_3^{2-}$*, *SO_3^{2-}*	M	Kelly, 1989
	O_2	S^0, $S_2O_3^{2-}$, SO_4^{2-}, *$S_nO_6^{2-}$*	M	van den Ende and van Gemerden, 1993
	NO_3^-	S^0, SO_4^{2-}	S	Elsgaard and Jørgensen, 1992
	NO_3^-	S^0, SO_4^{2-}	M	Otte et al., 1999
	Mn_{IV}	S^0, $S_2O_3^{2-}$, SO_4^{2-}, *SO_3^{2-}*	C	Yao and Millero, 1996; Burdige and Nealson, 1986
	Fe_{III}	S^0, $S_2O_3^{2-}$, *SO_3^{2-}*	C	Pyzik and Sommer, 1981
S_n^{2-}	O_2	$S_2O_3^{2-}$, S^0	C	Steudel et al., 1986; Chen and Morris, 1972
FeS	O_2	S^0, $S_nO_6^{2-}$, $S_2O_3^{2-}$, SO_4^{2-}	C	von Rège, 1999
	NO_3^-	SO_4^{2-}	M	Straub et al., 1996
	Mn_{IV}	S^0, SO_4^{2-}	C, S	Schippers and Jørgensen, 2001
	Fe_{III}	*SO_4^{2-}**†	S	Aller and Rude, 1988
FeS_2	O_2	SO_4^{2-}, $S_nO_6^{2-}$, $S_2O_3^{2-}$	C	Moses et al., 1987
	Mn_{IV}	SO_4^{2-}, $S_nO_6^{2-}$, $S_2O_3^{2-}$	C	Schippers and Jørgensen, 2001

Note: The order of products from the left to the right signifies their quantitative importance. Only results from studies conducted at circumneutral pH are included. Intermediates, which are unstable under the experimental conditions or which are only observed in trace quantities are given in italics. For experimental details, we refer to the original literature.

*No sulfur intermediates determined.

†Only weak sulfate production. See also Schippers and Jørgensen (2001) for additional comments.

§Type of study: C—chemical, M—microbiological, S—sediment incubation.

1999). The remaining 80–90% is eventually recycled back to sulfate through sulfur compounds of intermediate oxidation state in a complex web of competing chemical and biological reactions (Fig. 1) (Jørgensen, 1987; Fossing and Jørgensen, 1990; Jørgensen and Bak, 1991). A brief review of some of the important reactions leading to the formation of sulfur intermediates follows.

Review of Sulfide Oxidation Pathways

Oxic Sulfide Oxidation

Where dissolved sulfide (H_2S and HS^-) comes in contact with oxygen, sulfide may be chemically oxidized by dissolved oxygen according to the overall reaction

$$HS^- + 2O_2 \Rightarrow SO_4^{2-} + H^+ \quad (1)$$

However, the chemistry of the reaction is not as simple as the stoichiometry implies, and the exact reaction mechanism still remains to be elucidated (Zhang and Millero, 1993). A number of studies have shown that the oxidation of sulfide does not directly lead to sulfate but passes through several intermediates of different oxidation states (e.g., Avrahami and Golding, 1968; Cline and Richards, 1969; Chen and Morris, 1972; Zhang and Millero, 1993). Among them, sulfite is usually the first product formed (Equation 2).

$$HS^- + 1.5O_2 \Rightarrow HSO_3^- \quad (2)$$

The rapid oxidation of sulfite with oxygen explains the sulfate formation that is commonly observed during sulfide oxidation experiments (Equation 3). Sulfite can also react with HS^- to form thiosulfate ($S_2O_3^{2-}$) (Equation 4).

$$SO_3^{2-} + 0.5O_2 \Rightarrow SO_4^{2-} \quad (3)$$

$$HS^- + SO_3^{2-} + 0.5O_2 \Rightarrow S_2O_3^{2-} + OH^- \quad (4)$$

In most chemical studies, thiosulfate and sulfate were the only stable oxidation products that accumulated during the course of the experiments.

Tetrathionate, $S_4O_6^{2-}$, has been proposed as an intermediate in the incomplete oxidation of thiosulfate to sulfate (Jørgensen, 1990a; Schippers, this volume, Chapter 4). Based on thermodynamic considerations alone, thiosulfate will be oxidized to tetrathionate in the presence of various oxidants, such as O_2, Fe(III), Mn(IV), and I_2. (For instance, the conversion of thiosulfate to tetrathionate in the presence of iodine forms the basis of classic iodometric methods). The reaction between O_2 and $S_2O_3^{2-}$ is kinetically inert, although Xu and Schoonen (1995) have demonstrated that pyrite catalyzes this reaction at pH values of up to 8.6. Thiosulfate, which is the first intermediate product during pyrite oxidation (Moses et al., 1987; Luther 1987), is oxidized by Fe(III) to tetrathionate and eventually through to sulfate in the "thiosulfate-mechanism" of pyrite oxidation (Schippers et al., 1996; Schippers, this volume). MnO_2 will also oxidize thiosulfate to tetrathionate (Schippers and Jørgensen, 2001).

In the presence of trace metals, as is typical for natural environments, the formation of elemental sulfur in the initial step of sulfide oxidation is also possible (Equation 5) (Steudel, 1996; Zhang and Millero, 1993).

$$2HS^- + O_2 \Rightarrow 2S^0 + 2OH^- \quad (5)$$

Elemental sulfur can react with sulfite and sulfide to form thiosulfate (Equation 6) and polysulfides (Equation 7), respectively.

$$S^0 + SO_3^{2-} \Rightarrow S_2O_3^{2-} \quad (6)$$

$$(n-1)S^0 + HS^- \Rightarrow HS_n^- \quad (7)$$

Polysulfides are not stable under oxic conditions and rapidly decompose to thiosulfate and elemental sulfur (Steudel et al., 1986).

Although sulfide is basically a waste product of sulfate-reducing bacteria, it still contains a considerable amount of the energy originally stored in the biomass of primary producers. Aerobic lithotrophic bacteria can thrive on the oxidation of sulfide or sulfur intermediates with oxygen. The main product of biological sulfide oxidation is sulfate. Sulfur intermediates are mostly formed transiently under changing environmental conditions and severe oxygen limitation (van den Ende and van Gemerden, 1993). Because chemical sulfide oxidation can be very rapid in the environment, bacteria have had to develop strategies to successfully compete for sulfide. The most important adaptations are high enzyme affinities toward O_2 and sulfide and motility. Motility enables the organisms to position themselves in the oxic/anoxic interface where both oxygen and sulfide are present in low concentrations and are only supplied by diffusion (Jørgensen, 1987). Under such low reactant conditions, chemical sulfide oxidation becomes much slower due to the second order kinetics of the reaction (Zhang and Millero, 1994). Because of the Michaelis-Menthen kinetics of biological oxidation and the very low saturation constants for oxygen and sulfide of 1 µM or below in chemolithotrophic sulfur bacteria (Kuenen and Bos, 1989; van den Ende and van Gemerden, 1993), these organisms can still metabolize at maximal rates and may out-compete the chemical sulfide oxidation (Zopfi et al., 2001a).

Anoxic Sulfide Oxidation

In most marine sediments, sulfide does not diffuse to the sediment surface, but is removed from the pore water below the oxidized surface layer, in the suboxic zone, by oxidation and precipitation. The suboxic zone is characterized by the absence of oxygen and sulfide and increased concentrations of dissolved reduced iron and manganese. For the chemical oxidation of sulfide in marine sediments, only Mn(IV)oxides (Equation 8) and Fe(III)oxides (Equation 9) are of importance, because the reaction with nitrate is kinetically unfavorable. Similar to the

oxic pathways of sulfide oxidation, sulfur intermediates are also formed during anoxic oxidation of sulfide.

$$\delta MnO_2 + HS^- + 3H^+ \Rightarrow Mn^{2+} + S^0 + 2H_2O \quad (8)$$

For instance, elemental sulfur is a main product of the sulfide oxidation with Mn(IV) (Burdige and Nealson, 1986), but with increasing MnO_2/H_2S ratios, thiosulfate and especially sulfate become more important as products (Yao and Millero, 1996). The stoichiometry in Equation 8 is thus an oversimplification and describes only approximately the situation for a 1:1 ratio between sulfide and manganese. Manganese is a powerful oxidant and reacts also with solid phases such as FeS and FeS_2. Tetrathionate and thiosulfate have been reported as intermediates during the oxidation of pyrite with Mn(IV) oxide (Schippers and Jørgensen, 2001).

In most marine sediments, iron is much more abundant than manganese and is responsible for the efficient removal of dissolved sulfide from the interstitial water (Canfield, 1989). Unlike manganese, Fe(III) oxide is a rather poor oxidant for the complete oxidation of sulfide to sulfate (Aller and Rude, 1988; King, 1990; Elsgaard and Jørgensen, 1992). During the reaction of sulfide with Fe(III)oxides, dissolved ferrous iron and elemental sulfur are produced (Equation 9).

$$2FeOOH + HS^- + 5H^+ \Rightarrow 2Fe^{2+} + S^0 + 4H_2O \quad (9)$$

Furthermore, if sulfide is present in excess, dissolved ferrous iron will be precipitated as FeS. However, the formation of polysulfides and small amounts of thiosulfate and sulfite has also been reported (Peiffer et al., 1992; Pyzik and Sommer, 1981; dos Santos and Stumm, 1992).

The sulfur intermediates that are formed during sulfide oxidation may be further transformed by microorganisms. In the presence of an electron donor (i.e., organic matter, hydrogen), all of the sulfur intermediates can be reduced back to sulfide by sulfate-reducing bacteria and others (e.g., *Shewanella* sp., *Dethiosulfovibrio* sp., *Desulfitobacterium* sp., *Clostridium sp.*). Sulfur intermediates are also further oxidized to sulfate when a suitable electron acceptor becomes available. Under anoxic conditions, nitrate and possibly Mn(IV)oxides have been shown to be used by microorganisms as electron acceptors for complete sulfide oxidation (Elsgaard and Jørgensen, 1992; Lovley and Phillips, 1994).

The third type of metabolism responsible for the anaerobic transformation of sulfur intermediates is the so-called disproportionation (Bak and Cypionka, 1987; Thamdrup et al., 1993; Wentzien and Sand, 1999), which is described as a type of inorganic fermentation, where the substrate serves as electron donor as well as electron acceptor (Equations 10–13).

$$4SO_3^{2-} + H^+ \Rightarrow 3SO_4^{2-} + HS^- \quad (10)$$

$$S_2O_3^{2-} + H_2O \Rightarrow SO_4^{2-} + HS^- + H^+ \quad (11)$$

$$4S^0 + 4H_2O \Rightarrow SO_4^{2-} + 3HS^- + 5H^+ \quad (12)$$

$$4S_4O_6^{2-} + 4H_2O \Rightarrow 6S_2O_3^{2-} + S_3O_6^{2-} + SO_4^{2-} + 8H^+ \quad (13)$$

By using radiotracers, it was shown that the disproportionation of thiosulfate is a key process in the sedimentary sulfur cycle (Jørgensen, 1990a).

Scope of this Study

Despite the importance of sulfur intermediates for the biogeochemical cycling of carbon, manganese, iron, and trace metals, comparatively little is known about their occurrence in nature. However, improvements in sample handling and analytical methods now allow us to take another look at the distribution and cycling of sulfur intermediates in marine systems. This study represents a composite of a number of field investigations and experiments made over the past decade using these methods. We provide detailed descriptions of the applied analytical methods and sample processing where necessary, because proper handling and analysis is critical to the determination of these often ephemeral and redox-sensitive compounds. In this report, we present new data on the distribution of sulfur intermediates (mostly S^0, $S_2O_3^{2-}$, and SO_3^{2-}) along a transect extending from the oxygenated shelf to the permanently anoxic waters of the Black Sea. Through a series of amendment experiments, we explore the fate of sulfur intermediate compounds in marine sediments and the extent to which they are regulated by microbial or inorganic reactions. These experiments were performed using Black Sea, estuarine (Weser Estuary, Germany), and continental slope (Skagerrak, Denmark) sediments. Although certainly not all-inclusive, these sites are typical of continental margin sediments where the sulfur cycle plays an important role in the overall cycling of carbon and other elements.

METHODS

Study Sites and Sampling

Black Sea

Sediment for pore-water and solid phase sulfur speciation was collected during a cruise along a transect from the Romanian shelf to the abyssal plain with R/V *Petr Kotsov* in 1997. The sediment surface at Station 2 (77 m deep, 7.2 ºC, 213 µM O_2) was covered with a layer of *Modiolus phaesolinus* shells (Wenzhoefer et al., 2002). The underlying muddy sediment was carbonate-rich and light gray. The total mineralization rate was 1110 nmol C cm^{-2} d^{-1}, and about half of the organic matter in the top centimeter was degraded via Mn reduction (Thamdrup et al., 2000). Sulfate reduction accounted for ~15% of the total mineralization rate (Weber et al., 2001). Station 4 at the shelf break was located at the upper boundary of the chemocline (130 m, 7.8 ºC, <5 µM O_2). The sediment surface was covered with a 1.5 cm thick layer of dead mussel shells followed by homogenous gray sediment

beneath. Between 8 and 17 cm a second, a very porous band of buried mussel shells was observed. Organic matter mineralization was dominated by sulfate reduction (60–80%) and proceeded at a rate of 50–122 nmol C cm^{-2} d^{-1} (Weber et al., 2001). Station 6 was located in the permanently anoxic part of the Black Sea at a depth of 396 m. Sulfide concentration in the bottom water was 75 µM. The sediment was finely laminated, and organic matter was degraded solely by sulfate reduction at a rate of 112 nmol C cm^{-2} d^{-1} (Weber et al., 2001).

Skagerrak

Sediments were obtained from two sites in the Skagerrak basin of the North Sea using a multi-corer from on board the F/S *Victor Hensen*. Station S4 at 190 m was a sandy silt with total carbon oxidation rates of 200–300 nmol cm^{-3} d^{-1} in the upper 5 cm of sediment, with sulfate reduction accounting for ~60% of the total organic carbon degradation (Canfield et al., 1993). Station S9 at 695 m was a clayey-silt with a high concentration of manganese oxide (3–4% by weight). Organic carbon degradation (50–200 nmol cm^{-3} d^{-1}) was dominated by dissimilatory manganese oxide reduction in the upper 5 cm, and sulfate reduction was virtually absent at the same depths (Canfield et al., 1993).

Weser Estuary

The upper 5 cm of sediment from an intertidal mud flat located on the lower Weser Estuary (Weddewarden, 5 km north of Bremerhaven, Germany) was sampled during low tide and stored in buckets with 2–3 cm of overlying water at 4 °C until use in incubation experiments. Due to the relatively high iron contents of the predominately fine-grained silts, free dissolved sulfide is rarely ever present in the uppermost 5 cm of this sediment (Sagemann et al., 1996).

Pore-Water Sampling

Pore water from sediment cores was extracted by pressure filtration (0.45 µm Millipore PTFE filters) at 8 ºC in a N_2-filled glove bag. The pore water was directly led into 1.5 mL reaction tubes containing either a 0.3 mL 20% Zn-acetate dihydrate solution for sulfate and sulfide measurements or the derivatization-mixture (see monobromobimane [MBB] method) for thiosulfate and sulfite determination. Unless the fixed samples were not analyzed within 24 h, they were frozen and stored at −20 ºC.

Sediment and Slurry Incubation Experiments

Time-course studies on the fate of sulfide, thiosulfate, tetrathionate, or sulfite-amended sediments were performed on sediments obtained from the upper three (Black Sea) or upper five (Weser Estuary and Skagerrak) centimeters of sediment. The Black Sea sediment was—after removing mussel shells—homogenized under a N_2 atmosphere and directly poured into gas-tight plastic bags (Canfield et al., 1993). Sediments from the Weser Estuary and Skagerrak were diluted with water (1vol/1vol) from the corresponding site before being poured into the bags. The bags were equipped with glass outlets that were closed with rubber stoppers (sediment incubations) or connected to a three-way Luer stopcock (slurry experiments) to allow for the hermetic removal of sample into a syringe.

Sulfide, thiosulfate, and sulfite amendments were performed with Black Sea sediments. All manipulations of the Black Sea sediments were done in a N_2-filled glove bag at 8 ºC. Amendments of sulfide, thiosulfate, and sulfite were made to a final concentration of ~20–40 µM. The µM concentrations added were not expected to affect the pH of the well-buffered (mM range) marine sediments. At specific times sediment was withdrawn with truncated 1 ml plastic syringes and transferred into 1.5 mL centrifuge tubes for monobromobimane derivatization of sulfide, thiosulfate, and sulfite.

Tetrathionate experiments were performed on Skagerrak and Weser Estuary slurries, which were incubated, unless otherwise indicated, in the dark for 24 h (Skagerrak at 6–7 °C; Weser Estuary at room temperature). After a zero time-point sample was taken, 3–5 mL of 20 mM tetrathionate, freshly prepared in deoxygenated water, was injected into the bag (250–300 cm^{-3}) and mixed thoroughly. Subsamples were taken with 20 mL plastic syringes through the stopcock. Typically, 10 mL of slurry was removed, placed into a centrifuge tube, and spun down. The supernatant was then filtered through 0.4 µm Gelman syringe filters and analyzed by anion-exchange HPLC (high performance liquid chromatography) within one day. Thiosulfate and tetrathionate concentrations in darkened, refrigerated samples were determined to be stable for at least seven days (three days at room temperature). Various pre-treatments or amendments were performed on the Weser Estuary slurries to elucidate the role of bacterial versus inorganic reactions with tetrathionate, and these are described later in this paper. In some experiments, this included the addition of 20 MBq of $^{35}SO_4^{2-}$ (Amersham) in order to follow rates of sulfate reduction in the slurries.

Analytical Methods

Tetrathionate and Thiosulfate (Ion Chromatography [IC] method)

Initially, tetrathionate and thiosulfate were determined using the anion-exchange HPLC method described by Bak et al. (1993), using a Sykam S2100 pump, with an all–polyether-ether-ketone (PEEK) pumphead, a Rheodyne 9175 PEEK injector (50 or 20 µL sample loop), PEEK tubing, a LCA08 anion-exchange column (a silicon-based, polymer-coated material from Sykam), and a Linear Instruments UV/VIS (Ultraviolet/Visible) detector set for measurement at 216 nm. The eluent consisted of 11.7 g L^{-1} NaCl (Alfa, ultra-pure) dissolved in 64% acetonitrile and 10% methanol. The column was thermostated at 30 °C. With a flow rate of 1 mL min^{-1}, tetrathionate and thiosulfate eluted at 9.1 and 13.6 min, respectively. Due to the relative long-term degradation of the LCA08 column, we switched to a LCA09 (polymer-based, Sykam) anion column part-way through the experiments. Although tetrathionate and thiosulfate could not be measured on

the same isocratic run, retention time stability and peak resolution improved greatly. Tetrathionate was determined using an eluent described above and eluted at 5.81 min. Thiosulfate was determined using an eluent mix of 5.84 g NaCl in 10% methanol (100 mM NaCl) and eluted at 4.82 min. Standard solutions of thiosulfate (from sodium thiosulfate pentahydrate, Merck) and tetrathionate (sodium tetrathionate, 99% pure, Aldrich) were prepared freshly each day of analysis.

Thiosulfate and Sulfite (MBB Method)

Samples for thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}), typically 500 µL, were derivatized at room temperature in the dark with a mixture of 50 µL monobromobimane (Sigma; 45 mM in acetonitrile) and 50 µL HEPES-EDTA buffer (pH 8, 500 mM, 50 mM) (Fahey and Newton, 1987; Vetter et al., 1989). The derivatization reaction was stopped after 30 min by adding 50 µL methanesulfonic acid (324 mM). Samples were frozen at −20 °C until analysis within the next few days. In order to ensure a rapid and complete derivatization reaction, the amount of bimane in the assay was set to be at least twice as high as the total reduced sulfur content (Vetter et al., 1989).

A Sykam gradient controller S2000 (low pressure mixing system) combined with a LiChrosphere 60RP select B column (125 × 4 mm, 5 µm; Merck) and a Waters 470 scanning fluorescence detector (excitation at 380 nm; detection at 480 nm) were used for analysis. Eluent A was 0.25% (v/v) acetic acid pH 3.5 (adjusted with 5N NaOH), eluent B was 100% HPLC-grade methanol, and the flow rate was 1 mL min^{-1}. A modification of the gradient conditions described by Rethmeier et al. (1997) was used: start, 10% B; 7 min, 12% B; 15–19 min, 30% B; 23 min, 50% B; 30 min, 100% B; 33 min, 100% B; 34 min, 10% B; 39 min, 10% B; injection of the next sample. Separate standards for sulfite, thiosulfate, and sulfide were prepared in anoxic Milli-Q water in a N_2-filled glove bag. No difference was observed between calibration curves with standards prepared in seawater or in Milli-Q water. With an injection volume of 100 µL, the detection limits for thiosulfate and sulfite were ~0.05 µM, and the precision for measurements of 10 µM standards was better than ±3% standard deviation. Although some authors reported that MBB derivates were stable at room temperature (Fahey and Newton, 1987), we observed that (for example) thiosulfate values changed with time. We suggest, therefore, using a cooled autosampler (4 °C) and to keep derivatized samples at −70 ºC for long-term storage.

Elemental Sulfur

Sediment samples for elemental sulfur (S^0) were sliced, fixed in zinc acetate dihydrate (20% w/v) solution and stored in 50 mL polyethylene centrifuge tubes at −20 °C. Elemental sulfur in this study is defined as the sulfur extracted with methanol from sediment samples and measured as cyclo-S_8 by Reversed-Phase HPLC. Methanol is as effective as or better than other commonly employed extraction solvents for elemental sulfur, such as acetone or toluene/methanol mixtures or non-polar solvents such as cyclohexane, toluene, and carbon disulfide (Ferdelman, 1994; Ferdelman and Fossing, unpublished data). Elemental sulfur was extracted from a subsample (~0.3 g wet weight) of the fixed sediment for 12–16 h on a rotary shaker with pure methanol. The sample-to-extractant ratio was ~1/10–1/30 (wet weight/vol), depending on the sulfur content. Elemental sulfur in the extracts was determined by reversed-phase chromatography as originally described by Möckel (1984a, 1984b). A Sykam pump (S1100), a UV-VIS Detector (Sykam S3200), a Zorbax octadecylsilane (ODS) column (125 × 4 mm, 5 µm; Knauer, Germany), and 100% methanol (HPLC grade) at a flow rate of 1 mL min^{-1} were employed. S_8 eluted after 3.5 min and was detected at 265 nm; the detection limit was <0.5 µM, and the analytical precision of the method was ±0.5% relative standard deviation. A 2 mM stock solution of S^0 was made by dissolving 16 mg S^0 in 25 mL dichloromethane. After S^0 was completely dissolved, methanol (HPLC-grade) was added to a final volume of 250 mL. Dilutions for secondary standards (1–1000 µM) were prepared in methanol. The stock solution and standards of higher concentrations were stable at 4 ºC for >6 months.

Sulfide

Dissolved sulfide was either determined on Zn-preserved pore-water samples by the colorimetric methylene blue method of Cline (1969) or by using the MBB method. In highly sulfidic sediments, however, the quantification of sulfide with the MBB method was often impaired by neighboring peaks of polysulfide- and thiol-derivates; thus, the Cline (1969) method was used instead

Sulfate Reduction Measurements

Sulfate reduction was determined on the $^{35}SO_4^{2-}$ labeled slurry experiments. At each time point, 10 mL of slurry sample would be injected into 10 mL of 20% (wt/vol) zinc acetate dihydrate solution and frozen. The recovery of radiolabeled reduced sulfur compounds followed the two-step acidic-chromium reduction procedure as described by Fossing and Jørgensen (1989). ^{35}S-radioactivity was determined using a Canberra-Packard Tri-Carb 2400 TR liquid scintillation detector (scintillation fluid: Packard Ultima Gold). Sulfate was determined by non-suppressed ion chromatography and conductivity detection (Ferdelman et al., 1997).

RESULTS AND DISCUSSION

Distribution of the Sulfur Intermediates Sulfite, Thiosulfate, and Elemental Sulfur

Pore-water distributions of sulfur intermediates were determined on both Black Sea and Weser Estuary sediments. No SO_3^{2-} was detected in Weser Estuary sediment and only a few samples showed a small $S_2O_3^{2-}$ peak (data not shown). Since the detection limit was only 0.5 µM at that time, no further conclusion can be made other than thiosulfate was generally ≤0.5 µM. Attempts to measure tetrathionate ($S_4O_6^{2-}$) at the same site with

anion exchange HPLC showed that ambient tetrathionate concentrations were also below the detection limit of 0.5 µM (data not shown). Therefore, further discussion will focus on sulfur distributions in Black Sea sediments.

Black Sea Pore-Water Characteristics

Depth profiles of dissolved and solid phase sulfur species at three stations in the Black Sea are shown in Figure 2. The Black Sea stations selected for study represent sediment sites underlying oxic (Station 2), dysoxic (<5 µM O_2, Station 4), and anoxic, sulfidic (Station 6) waters. The overlying water conditions are partially reflected in the sedimentary sulfide distributions. At the oxic shelf Station 2, sulfide in the pore water was not detected down to 6 cm, and never exceeded 0.7 µM down to 20 cm depth. Despite oxygen concentrations of less than 5 µM (Weber et al., 2001) in the bottom water at Station 4, sulfide concentrations in the top 10 cm were below 0.2 µM. Maximum sulfide concentrations in this core reached ~3 µM and were detected at intermediate depth between 10 and 20 cm. At Station 6, pore-water sulfide concentrations increased steadily with depth

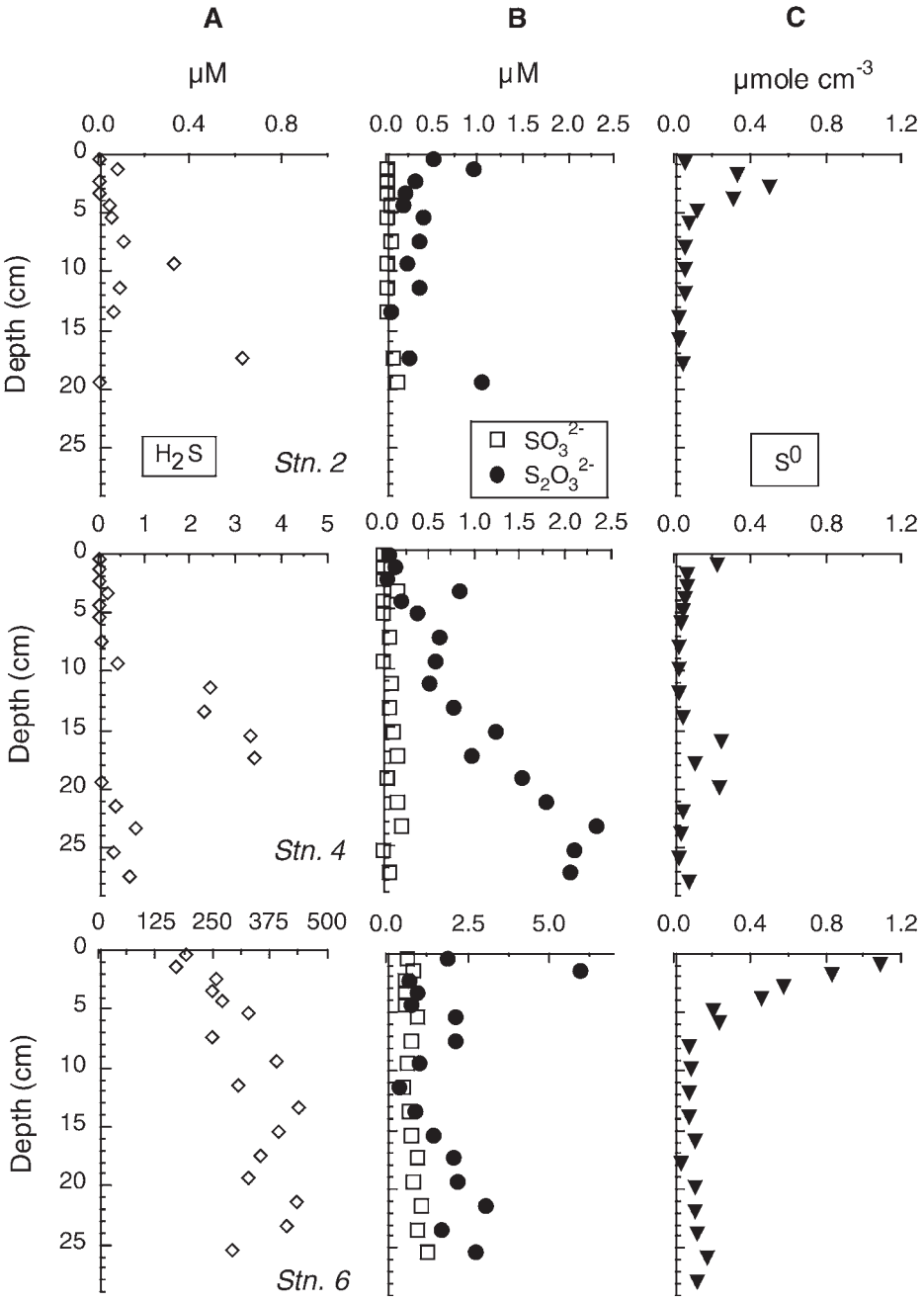

Figure 2. Depth profiles of (A) pore-water sulfide, (B) thiosulfate and sulfite, and (C) solid phase elemental sulfur in Black Sea sediments. Stn—Station. Stn. 2: Oxic bottom water. Stn. 4: Redox transition zone. Stn. 6: anoxic bottom water.

and reached maximum concentrations of 435 µM at 19 cm. A sulfide efflux from the sediment of 27 nmol cm^{-2} d^{-1} was calculated from the concentration profile; however, this value is only half of the sulfide production that was determined by in situ ^{35}S radiotracer incubations at the same station (Weber et al., 2001). In the following, we discuss the distribution of each of the sulfur intermediates (S^0, $S_2O_3^{2-}$, and SO_3^{2-}) in these three distinct Black Sea environments.

Distribution of Elemental Sulfur (S^0)

Elemental sulfur is the main reaction product of sulfide oxidation by Mn(IV)oxides and Fe(III)oxides (e.g., Yao and Millero, 1993, 1996; Pyzik and Sommer, 1981). Sulfur is also formed during oxic and anoxic FeS oxidation (Moses et al., 1987; Schippers and Jørgensen, 2001), and microorganisms produce S^0 as an intermediate or final product during bacterial oxidation of sulfide and thiosulfate (Taylor and Wirsen, 1997; Kelly, 1989). In contrast to sulfide, polysulfides, and sulfite, cyclic elemental sulfur is almost insoluble and can best be described as a Lewis acid. It is much less reactive and accumulates in the sediment to higher concentrations (Table 2) than other sulfur intermediates (Table 3). This greatly facilitates quantification, which is either done by cyanolysis and subsequent spectrophotometry (Bartlett and Skoog, 1954; Troelsen and Jørgensen, 1982), sulfitolysis and subsequent thiosulfate measurement (Luther et al., 1985; Ferdelman et al., 1991), or by reversed phase liquid chromatography and UV-detection (Möckel, 1984a, 1984b). During the last few years, the HPLC method has been applied to a variety of samples and has proved to be very sensitive and robust (e.g., Ramsing et al., 1996; Ferdelman et al., 1997; Henneke et al., 1997; Zopfi et al., 2001a, 2001b). The ease by which elemental sulfur is extracted by a relatively polar organic solvent such as methanol suggests that elemental sulfur in marine sediment (extracellular and intracellular) exists principally in the form of colloidal sols (Steudel, 1989), rather than as highly insoluble, crystalline elemental sulfur.

Peak concentrations of S^0 in the three Black Sea stations were between 0.22 and 1.08 µmol cm^{-3}. This is at the lower end of what has been reported previously (Table 2), but in the same range that Wijsman et al. (2001) found along the northwestern margin of the Black Sea. Although there are some exceptions, it appears that S^0 concentrations are higher in environments with increased sulfate reduction rates. The S^0 content in the three Black Sea stations fits this hypothesis because the sulfate reduction rates (0.5–0.8 mmol m^{-2} d^{-1}) are comparatively low (Skyring, 1987). Similarly, Moeslund et al. (1994) found during a seasonal study of bioturbated coastal sediment that S^0 concentrations increased from spring to late fall as sulfate reduction rates and bioturbation activities increased. In wintertime, S^0-consuming processes outweigh S^0 production until settling detritus from the spring bloom refuels higher benthic sulfate reduction rates (Moeslund et al., 1994). Schimmelmann and Kastner (1993) observed in the Santa Barbara Basin that sediments deposited during periods of decreased productivity and more oxygenated conditions in the water column were depleted in total organic carbon and S^0. Exceptionally high concentrations (>10 µmol cm^{-3}) are only found in very active and dynamic environments such as sulfureta, salt marshes, and organic-rich sediments from upwelling areas (see Table 2).

Although the concentrations are fairly comparable between the three Black Sea stations, the distribution of S^0 is different. Station 2, for example, exhibits a subsurface maximum of S^0 as is frequently found in bioturbated coastal marine sediments (e.g., Troelsen and Jørgensen, 1982; Sørensen and Jørgensen, 1987; Thode-Andersen and Jørgensen, 1989; Moeslund et al., 1994; Thamdrup et al., 1994a, 1994b; Zopfi, 2000). The balance between producing and consuming processes determines the concentration of S^0 in the sediment. Assuming that all pore-water sulfide is first oxidized to S^0 and after that to sulfate, the turnover time for S^0 can be calculated by dividing the S^0 pool (µmol cm^{-3}) by the sulfate reduction rate (µmol cm^{-3} d^{-1}) in the same depth interval. The average turnover time of S^0 in the top 2 cm at Station 2 is only 10 days, but rapidly increases to 66 days (3–4 cm) and falls again to ~27 d below 5 cm depth. Thus, the S^0 peak at 3 cm rather represents a turnover minimum than a production maximum. Above the S^0 peak, S^0 is rapidly produced, but also rapidly oxidized further to sulfate. The required oxidants, O_2, NO_3^- and Mn(IV), may be supplied by bioturbation (Aller and Rude, 1988) or advection (Huettel et al., 1998). At 3–4 cm depth, the supply of oxidants may be sufficient to remove sulfide from the pore water, but not for the complete oxidation of the produced S^0 to sulfate. Below that depth, S^0-consuming processes, such as dissimilative S^0 reduction, S^0 disproportionation, and pyrite formation dominate and lead to decreasing concentrations with depth. Whether a subsurface S^0 peak indeed indicates bioturbation activity and whether the location of the maximum may be a measure for the average bioturbation depth needs to be established by more detailed studies that should include combined S^0 and ^{234}Th and ^{210}Pb measurements.

At Stations 4 and 6, maximum S^0 concentrations were determined at the sediment-water interface. Similar distributions have been observed in sulfidic sediments and sediments overlain by anoxic bottom water (Thode-Andersen and Jørgensen, 1989; Troelsen and Jørgensen, 1982; Zopfi, 2000). Since elemental sulfur is only produced during oxidative pathways in the sulfur cycle (Fig. 1), the distribution of S^0 at Station 6 suggests that a part of the pore-water sulfide in the uppermost centimeters of the core is oxidized to S^0. At this depth, oxygen and nitrate can be excluded as oxidants. Although in the sulfidic water column of the Black Sea most settling iron reaches the sediment surface as FeS or FeS_2, some Fe(III)oxides or Mn(IV)oxides with a lower reactivity toward sulfide must become deposited and buried as well. They will finally react with pore-water sulfide. The produced S^0 then reacts further with sulfide and forms a range of polysulfides, depending on the pH in the sediment (Jacobs and Emerson, 1982; Morse et al., 1987). Polysulfides are more reactive nucleophiles than sulfide and are expected to play an important role in formation of organosulfur compounds and pyrite (Vairavamurthy and Mopper, 1989; Luther, 1991)

TABLE 2. SOLID PHASE AND PORE-WATER CONCENTRATIONS OF ZEROVALENT SULFUR (S^0) IN BRACKISH AND MARINE SEDIMENTS

Ecosystem/Site	Concentration S^0		Method	Reference
	Solid phase μmol cm^{-3}	Pore water μM		
Marshes				
Spartina-Salt Marshes	up to 500	up to 555	RP-HPLC/Sulfitosis	Ferdelman, 1994
Brackish Water Marshes	0.22–95		RP-HPLC	Ferdelman, 1994
Sulfureta				
Texel, Netherlands	6.2		UV-spectroscopy	Visscher and van Gemerden, 1993
Kalø Lagoon, Denmark	6.8		Cyanolysis	Thode-Andersen and Jørgensen, 1989
Aarhus Bay, Denmark	10–17		Cyanolysis	Troelsen and Jørgensen, 1982
Marine sediments				
Weser Estuary, Germany	0.8		RP-HPLC	Canfield and Thamdrup, 1996
Aarhus Bay, Denmark	<1–4.6		Cyanolysis	Thode-Andersen and Jørgensen, 1989; Thamdrup et al., 1994b; Moeslund et al.,
North Sea, Denmark	2.2		Cyanolysis	Sørensen and Jørgensen, 1987
Kattegat, Denmark	0.5		Cyanolysis	Sørensen and Jørgensen, 1987
Skagerrak, Denmark	0.2		Cyanolysis	Sørensen and Jørgensen, 1987
Saguenay Fjord, Canada		5.5–21	UV-spectroscopy	Gagnon et al, 1996
Central Peru, upwelling area	0–1.6		Cyanolysis	Fossing, 1990
Central Chile, upwelling area	2–15 (76)	4–59	RP-HPLC	Ferdelman et al. 1997; Thamdrup and Canfield, 1996; Zopfi, 2000
Black Sea Shelf, Romania	0.22–0.67		RP-HPLC	This study
Mid-Atlantic Bight, USA	0.04–13.7	<1–19.0	RP-HPLC/Sulfitosis	Ferdelman, 1994
Euxinic sediment				
Gotland Deep, Baltic	6.4		Cyanolysis	Podgorsek, 1998
Arkona Deep, Baltic	0.34		Cyanolysis	Podgorsek, 1998
Black Sea	1.1		RP-HPLC	This study
Tyro Basin, Mediterranean	2.5*		RP-HPLC	Hennecke et al., 1997
Bannock Basin, Mediterranean	4.8*		RP-HPLC	Hennecke et al., 1997

*Recalculated from μmol g^{-1} dry weight (d.w.) by using a water content of 75% and a sediment density of 1.1 g cm^{-3}. (Original data: 11 μmol g^{-1} d.w. Tyro and 21 μmol g^{-1} d.w. Bannock). UV—ultraviolet. RP-HPLC—reverse phase high performance liquid chromatography.

TABLE 3. CONCENTRATION RANGES OF SULFITE (SO_3^{2-}) AND THIOSULFATE ($S_2O_3^{2-}$) DETERMINED IN MARINE SEDIMENTS

Ecosystem/Site	Concentration (μM)		Method	Reference
	$S_2O_3^{2-}$	SO_3^{2-}		
Spartina-Salt Marshes				
Great Marsh, Delaware, USA	130–530	<0.1–177	Hg^{2+}-titration	Boulègue et al., 1982
Sippewissett, Massachusetts, USA	<15–340	n.a.	Cyanolysis	Howarth et al., 1983
Sippewissett, Massachusetts, USA; Great Marsh, Delaware, USA	<0.2–1000	0–7.3	Voltametry	Luther et al., 1985, 1986, 1991; Luther and Church, 1988
Mission Bay, California, USA	0–45	0–6	Bimane HPLC	Vetter et al., 1989
Unvegetated Marshes				
Mission Bay, California, USA	2–84	3–56	Bimane HPLC	Vetter et al., 1989
New York, New York, USA	3–50	n.a.	Cyanolysis	Swider and Mackin, 1989
Skallingen, Denmark	<0.05–0.6	0.1–1.1	DTNP HPLC	Thamdrup et al., 1994b
Sulfureta				
Orkneys, UK	0–2500	n.a.	Cyanolysis	van Gemerden et al., 1989
Texel, Netherlands	0–35	n.a.	Cyanolysis	Visscher et al. 1992
Marine Sediments				
Walvis Bay, Namibia	15–145	n.a.	Hg^{2+}-titration	Boulègue and Denis, 1983
Kysing Fjord, Denmark	<1–10	n.a.	Cyanolysis	Troelsen and Jørgensen, 1982
Gulf of California	0-500	n.a.	?	Lein, 1984
Orleans, Massachusetts, USA	400–1300	6.8–7.9	Voltametry	Luther et al., 1985
Mid-Atlantic Bight, USA	<1–89	n.a.	Voltametry	Ferdelman, 1994
Aarhus; Skagerrak, Denmark	<0.05–0.45	0.1–0.8	DTNP HPLC	Thamdrup et al., 1994b
Chesapeake Bay, Maryland, USA	0.1–7.1	n.a.	IP RP-HPLC	MacCrehan and Shea, 1995
Saguenay Fjord, Canada	0–15		Hg^{2+}-titration	Gagnon et al., 1996
Venice Lagoon, Italy	0–35	n.a.	Voltametry	Bertolin et al., 1997

Note: Table modified and extended from Thamdrup et al., 1993. n.a.—not analyzed. DTNP—2,2′-dithiobis(5-nitropyridine). IP-RP-HPLC—ion pair reverse phase high performance liquid chromatography.

Polysulfides are not easy to quantify in environmental samples since they decompose to ZnS and S^0 as soon as the sediment is fixed with Zn-acetate. Thus, S^0 concentration determined in sulfidic sediments always includes the sulfane sulfur from polysulfides. Under the simplified assumption that all S^0 is transformed into polysulfides if sulfide is present in excess, S^0 concentrations can be used as an upper estimate for the total polysulfide concentration. For Station 6 at 7 cm and below, a polysulfide concentration of 115 µM is calculated by using the average porosity and S^0 values from the same depths (0.1 µmol S^0 cm^{-3}/0.87 ml cm^{-3} = 0.115 µmol mL^{-1} = 115 µM).

Distribution of Thiosulfate ($S_2O_3^{2-}$) and Sulfite (SO_3^{2-})

Table 3 summarizes the results from previous determinations of thiosulfate and SO_3^{2-} in marine sediments and illustrates the large variability in the measured concentrations, ranging from low nM to mM. As already pointed out by Thamdrup et al. (1994b), a variety of different methods have been used for quantification, and it is thus unclear to what extent the variability in the data is due to environmental conditions, sample treatment, or method applied. Since thiosulfate and SO_3^{2-} concentrations in the Black Sea sediments (Fig. 2), an intertidal mud flat in the Weser Estuary, eutrophic sediments off the coast of Central Chile, and a hypersaline cyanobacterial mat (Table 4) were all determined by the MBB derivatization method, a comparison between different systems is now possible. Together with earlier MBB data from salt marsh sediments (Table 3; Vetter et al., 1989), it appears that thiosulfate and SO_3^{2-} concentrations in normal marine sediments are typically in the low micromolar range or below. The low concentrations indicate a high turnover and suggest a tight coupling between sulfur intermediate producing and consuming processes. As for S^0, increased concentrations were mostly observed in highly active and/or dynamic environments, where non–steady-state conditions lead to transient accumulation of sulfur intermediates. For instance, high thiosulfate concentrations in salt marsh sediment are likely caused by intense pyrite oxidation (Luther et al., 1991). In microbial mats, thiosulfate and SO_3^{2-} may be produced in large amounts during the incomplete oxidation of sulfide by cyanobacteria or anoxygenic phototrophic microorganisms (Rabenstein et al., 1995, Wieland et al., 2004).

The values for thiosulfate and SO_3^{2-} presented in this study are in the same range as Thamdrup et al. (1994b) found by 2,2′-dithiobis(5-nitropyridine) (DTNP) derivatization. Despite the report by Witter and Jones (1998) that derivatization with DTNP perturbs coupled equilibria between reactive sulfur species and may lead to a 33% overestimation of thiosulfate, the derivatization methods tend to result in lower concentrations than other methods (Table 3). This suggests that the history of a sample (e.g., exposure to O_2, manipulations and additions, temperature and pH changes) can affect the sulfur speciation even more significantly. Also, the time between sampling and analysis is critical because sulfur speciation can change within minutes if the conditions are unfavorable. The advantage of derivatization methods is therefore that labile sulfur species like sulfite, sulfide, and thiols are rapidly fixed, and reactions between the compounds or with oxygen are excluded. The risk of typical oxidation artifacts, such as the loss of sulfite and increased thiosulfate concentrations, is thereby minimized.

Whereas in some environments maximum thiosulfate concentrations were detected close to the sediment-water interface (Station 2, Fig. 2; Zopfi, 2000; Troelsen and Jørgensen, 1982) where sulfide oxidation is most intense, a similar distribution was not observed at Station 4. There, thiosulfate concentrations increased steadily with depth but did not correlate with pore-water sulfide, thus making an oxidation artifact unlikely. In contrast to S^0, thiosulfate can also be a product of reductive processes (Fitz and Cypionka, 1990). The formation of extracellular thiosulfate has been observed in sulfate-reducing cultures growing under substrate limiting conditions (Vainshtein et al., 1980; Sass et al., 1992). The mineralization rates at Station 4 were very low, and the quality of organic matter decreases typically with sediment depth. Thus, the distribution of thiosulfate could be explained by the incomplete reduction of sulfate under starvation conditions.

TABLE 4. SUMMARY OF SO_3^{2-} AND $S_2O_3^{2-}$ MEASUREMENTS WITH THE MONOBROMOBIMANE DERIVATIZATION METHOD

Site	Concentration (µM)		Comments
	$S_2O_3^{2-}$	SO_3^{2-}	
Weser Estuary, Germany	≤0.5	n.d.	Mud flat, no free sulfide
Black Sea Stn. 2	0.1–1.0	0–0.1	77 m depth, 213 µM O_2*
Black Sea Stn. 4	0.07–2.3	0–0.15	130 m depth, <5 µM O_2* , RTZ†
Black Sea Stn. 6	0.7–3.0	0.5–1.2	396 m depth, 75 µM H_2S*
Chile (Concepción Bay)	1.3–5.7	0.4–2.6	Sulfidic sediment, >1mM H_2S§
Chile (shelf)	0.2–2.2	0.1–0.5	H_2S in sediment <5µM, *Thioploca*
Cyanobacterial mat (Camargue, France)	360	45	Hypersaline, *Microcoleus* sp. dominated

Note: Data from this study, Zopfi (2000), and Wieland et al. (2003).
*Bottom water concentration.
†Redox transition zone.
§Pore-water concentration

This hypothesis could be tested by stimulating sulfate reduction through the addition of organic substrates to intact sediment cores and monitoring changes in thiosulfate concentrations.

Pore-water sulfite concentrations at the three Black Sea stations were typically lower than 1.2 µM. Although SO_3^{2-} is observed in many sulfide oxidation reactions (Table 1), it does not reach high concentrations in the environment, most likely due to its high chemical reactivity.

Sulfide, Thiosulfate, and Sulfite Transformations

Surface sediment (0–3 cm) from Station 2 in the Black Sea was amended with sulfide, thiosulfate, and sulfite in incubation experiments designed to provide insight into the observed thiosulfate and sulfite pore-water distributions. The experiments were performed in duplicates, but as all of them showed qualitatively identical results, only data from one bag of each amendment experiment is shown in Figure 3.

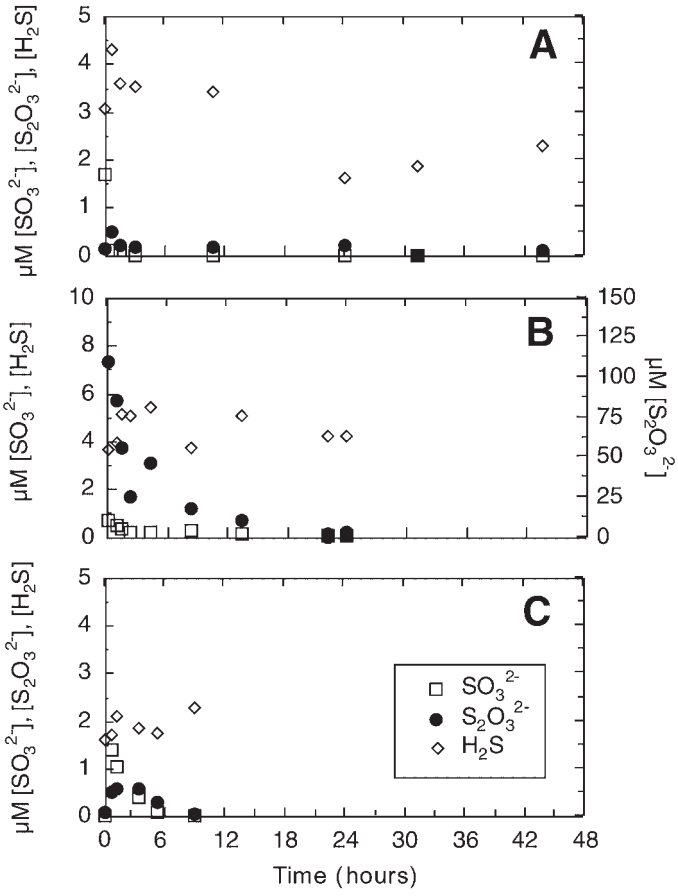

Figure 3. Sulfide, thiosulfate, and sulfite concentrations during a time series experiment with surface sediment from Station 2 in the Black Sea and different amendments: (A) sulfide, (B) thiosulfate, and (C) sulfite addition.

Sulfide Amendment

Sulfide was added to the bag from a freshly prepared stock (2 mM) to obtain a final concentration of ~30–40 µM. The sulfide concentration in the bag was initially 3 µM, but was only slightly higher (4.3 µM) 40 min after the addition. Sulfide then slowly decreased to a minimum concentration of 1.6 µM at 24 h, but increased again toward the end of the experiment, probably due to bacterial sulfate reduction. The sediment in the first 1.5 cm was particularly rich in particulate manganese (125 µmol cm^{-3}) and contained up to 45 µmol cm^{-3} Fe(III)oxides (Thamdrup et al., 2000). Most likely, sulfide was rapidly removed from the pore water by oxidation and precipitation by reactive metal oxides. The concentration of thiosulfate before the addition was 0.14 µM, slightly lower than observed in the pore-water depth profiles, but reached a transient maximum of 0.5 µM immediately after the amendment. Thereafter, the concentrations fell to a rather constant value of 0.2 µM, which is comparable to the pore-water concentration. Sulfite was only measurable immediately after the addition, and concentrations did not exceed 0.08 µM.

Thiosulfate Amendment

By mistake, thiosulfate was added to a much higher concentration than in the other incubations. However, this allowed us to observe the strong rate dependence of the thiosulfate concentration. The disappearance rate was 42 µM h^{-1} at 82 µM $S_2O_3^{2-}$, 8.5 µM h^{-1} at 21 µM $S_2O_3^{2-}$, and only 1.1 µM h^{-1} at a concentration of 6 µM. Despite the addition of 120 µM thiosulfate, the sulfide concentration increased only transiently from 3.6 µM to 5.4 µM. Sulfite immediately rose to 0.7 µM and then fell rapidly to 0.18 µM after 2 h. (In the duplicate bag where the thiosulfate concentration reached only 40 µM, sulfide production was also stimulated, but no dynamics in pore-water sulfite were observed.)

Interestingly, a transient sulfite accumulation accompanied the addition of relatively high concentrations of thiosulfate. This demonstrates a tight coupling between the two species, although the reason for sulfite formation is not yet clear. Sulfite may be produced from thiosulfate by enzymatic reduction according to Equation 14:

$$S_2O_3^{2-} + 2\,[H] \rightarrow HSO_3^- + HS^- \qquad (14)$$

where [H] represents a reducing equivalent delivered by the thiosulfate reductase (Barrett and Clark, 1987). The ability to reduce thiosulfate (and tetrathionate; see below) is widely spread in the domains of Bacteria and Archaea. Most sulfate-reducing bacteria reduce thiosulfate to sulfide by soluble enzymes located within the cytoplasm. In contrast, other microorganisms reduce thiosulfate by a periplasm facing membrane-enzyme. Since many of them are unable to use the formed sulfite as an additional electron acceptor (Barrett and Clark, 1987), it is released to the environment. The increase in extracellular sulfite during the incubation experiment is therefore consistent with a partial reduction of thiosulfate by non–sulfate-reducing bacteria. The sulfite released

may then react further with extracellular S_8 to form more thiosulfate. Such a "sulfur clearing" mechanism has been proposed for the growth of *Salmonella enterica* (Hinsley and Berks, 2002). Since sulfite is also an intermediate of the bacterial thiosulfate disproportionation (Cypionka et al., 1998), a contribution by this process cannot be excluded; however, thiosulfate disproportionation is a cytoplasmatic process and the appearance of extracellular sulfite is probably less likely.

Sulfite Amendment

Added SO_3^{2-} disappeared very rapidly and reached similar concentrations as found in the pore water of an undisturbed core. Sulfite was not detected in the bag pore water before the amendment and the concentration only increased to 1.4 µM 40 min after the addition. A fraction of the sulfite was transformed into thiosulfate, which rapidly built up to 0.6 µM and decreased again to the same concentration as at the beginning of the experiment (0.07 µM). This may reflect a reaction with S^0 or sulfide to form thiosulfate as observed in laboratory experiments (Atterer, 1960; Chen and Morris, 1972; Heunisch, 1977). As in the thiosulfate experiment, sulfite led to increased sulfide concentrations in the bag. A sample taken after 21 h in the duplicate bag indicated that this sulfide increase was only transient and concentrations decreased again later. Whether this sulfide production was due to disproportionation or dissimilatory reduction of sulfite by sulfate-reducing bacteria cannot be deduced from this experiment. Pure culture studies with sulfate-reducing bacteria, however, showed that sulfite (and thiosulfate) is preferred over sulfate as an electron acceptor, because sulfite reduction precludes the highly energy demanding step of sulfate activation (Widdel, 1988). In recent years, an increasing number of non–sulfate-reducing bacteria have been found to use SO_3^{2-} as an electron acceptor, including members of the genera *Desulfitobacter* sp. (Lie et al., 1999) and *Shewanella* sp. (Perry et al., 1993).

Most of the SO_3^{2-} added to the surface sediment was not recovered in any measured sulfur pool. It is possible that SO_3^{2-} was oxidized to sulfate by reacting with Fe(III)oxides or Mn(IV)oxides. Because sulfite is a strong nucleophile, it could also have reacted with organic molecules to form sulfonates ($R\text{-}SO_3^-$), which have been recognized as a major class of organic sulfur compounds in marine sediments (Vairavamurthy et al., 1994; Vairavamurthy et al., 1995). A reactant half-life of ~5 min has been reported, indicating that the reaction between SO_3^{2-} and organic molecules can be very fast (Vairavamurthy et al., 1994).

Thamdrup et al. (1994b) observed similar variations of SO_3^{2-} and thiosulfate with sediment depth, which was explained either by an oxidative production at a fixed ratio or by coupled transformations as described in Equation 6. In the Black Sea sediments, a covariation of the two sulfur intermediates was not observed, and thiosulfate concentrations were, as is also found in other environments (Tables 3 and 4), typically higher than SO_3^{2-}. Although both compounds can be oxidized, reduced, or disproportionated by bacteria, there are clear differences in terms of their chemical reactivity. Thiosulfate is chemically stable in absence of microorganisms under pH neutral conditions (Millero, 1991) and is less reactive toward organic compounds (Vairavamurthy et al., 1994). Thus, while competing chemical reactions contribute to the rapid disappearance of SO_3^{2-}, the low thiosulfate concentrations in the Black Sea sediments (<3 µM) are mostly due to the activity of thiosulfate-consuming bacteria.

Measurements of Tetrathionate in Natural Environments

Polythionates such as tetrathionate appear as products of the chemical oxidation of H_2S, FeS, and FeS_2 (Table 1). Tetrathionate also forms as an intermediate during the aerobic microbial oxidation of sulfide or thiosulfate to sulfate (e.g., Kelly, 1989; Kelly et al., 1997; van den Ende and van Gemerden, 1993; Podgorsek and Imhoff, 1999). Chemoorganoheterotrophic bacteria oxidizing sulfide and S^0 to tetrathionate as the sole product have been described recently by Sorokin (1996). Under anoxic conditions, tetrathionate is abiotically formed from thiosulfate by oxidation with Mn(IV)oxide (Schippers and Jørgensen, 2001). The anaerobic formation of tetrathionate from thiosulfate with NO_3^- as oxidant, however, is bacterially mediated (Sorokin et al., 1999).

In contrast to the results from laboratory experiments, measurements of tetrathionate in natural environments are few. This is partially due to the lack of simple and sensitive analytical methods, but probably more importantly to the fact that tetrathionate is not a major constituent of dissolved sulfur pools in marine sediment pore waters. It is presently also not possible to directly fix and store tetrathionate with compounds such as monobromobimane or other additives. With a few exceptions, such as salt marsh sediments (300 µM, Luther et al., 1986), concentrations fall below detection limits of ~0.01 µM in Kysing Fjord, Denmark (Bak et al., 1993); 0.5 µM in sediments of intertidal Weser Estuary and Chilean continental shelf (Ferdelman and Fossing, unpublished); and 1 µM in the chemocline of Mariager Fjord (Ramsing et al., 1996). Podgorsek and Imhoff (1999) report finding detectable concentrations of tetrathionate (up to 21.6 µM) in Baltic Sea sediments that were anoxic and contained relatively high concentrations of dissolved hydrogen sulfide. As sulfide readily reacts with tetrathionate to form elemental sulfur and thiosulfate (Atterer, 1960; Steudel, 1989), according to Equation 15

$$S_4O_6^{2-} + H_2S \rightarrow 2\ S_2O_3^{2-} + 2\ H^+ + S^0 \qquad (15)$$

they suggested that the rate of tetrathionate formation must therefore be exceeding its consumption. They proposed a model of sulfide oxidation whereby sulfide is oxidized to zero-valent sulfur in the presence of catalytic amounts of tetrathionate, which in turn is regenerated through the oxidation of thiosulfate (Podgorsek and Imhoff, 1999); however, no possible oxidants for thiosulfate under such reducing conditions were named. Conversely, tetrathionate was not detected in sediment depths that contained low concentrations of hydrogen sulfide (Podgorsek and Imhoff, 1999).

Transformations of Tetrathionate Added to Marine Sediments

Oxidized versus Reduced Sediment

Any tetrathionate that may be formed through either biological or chemical reactions is readily removed from pore-water solution to concentrations below 1 µM. Figure 4 shows the typical course of tetrathionate addition to both oxidized and reduced (but not sulfidic) sediment slurries. In this particular experiment, the effects of sediment reduced substances and oxidation state of the sediment on tetrathionate dynamics were examined by comparing an artificially oxidized sediment with a minimally altered sediment (i.e., reduced). Two slurries were prepared. One of the slurries was vigorously bubbled with air until the normally black sediment had taken on a browner, oxidized appearance. After two hours had elapsed, tetrathionate was added to both slurries, and the tetrathionate and thiosulfate concentrations were measured over time. Additionally, 20 MBq of carrier-free $^{35}SO_4^{2-}$ (Amersham) was added to the anoxic bag (giving an approximate activity of 80 kBq cm^{-3}) in order to track sulfate reduction.

In the reduced slurry (Fig. 4A), tetrathionate disappeared within several hours, at a rate of 31.8 µM h^{-1}, and thiosulfate concentrations increased with a 2:1 $S_2O_3^{2-}$:$S_4O_6^{2-}$ ratio at a rate of 64.7 µM h^{-1}. After the tetrathionate sank to concentrations below 10 µM, the thiosulfate concentrations peaked and began decreasing, albeit at a substantially slower rate (5.9 µM h^{-1}). The oxidized sediments (Fig. 4B) exhibited a somewhat decreased rate of tetrathionate consumption by 25%. Correspondingly, the rate of thiosulfate increase in the oxidized sediment slurry was also slightly lower than in the untreated, reduced slurry, hence the 2:1 stoichiometry between tetrathionate consumption and thiosulfate remained constant. In contrast, the rate of thiosulfate concentration decrease, after the build-up of thiosulfate, was similar for both the reduced and oxidized slurries (5.9 and 6.4 µM h^{-1}, respectively). In neither slurry was dissolved sulfide measurable at any time point. Interestingly, the oxidized sediment exhibited a small lag of one hour before the onset of tetrathionate consumption in the oxidized slurry, and repeated additions of tetrathionate had the effect of increasing tetrathionate consumption (data not shown). These and numerous following incubation experiments confirm the initial observations of Bak et al. (1993) that demonstrate a complete consumption of tetrathionate in anoxic sediments with a concomitant and stoichiometric release of thiosulfate

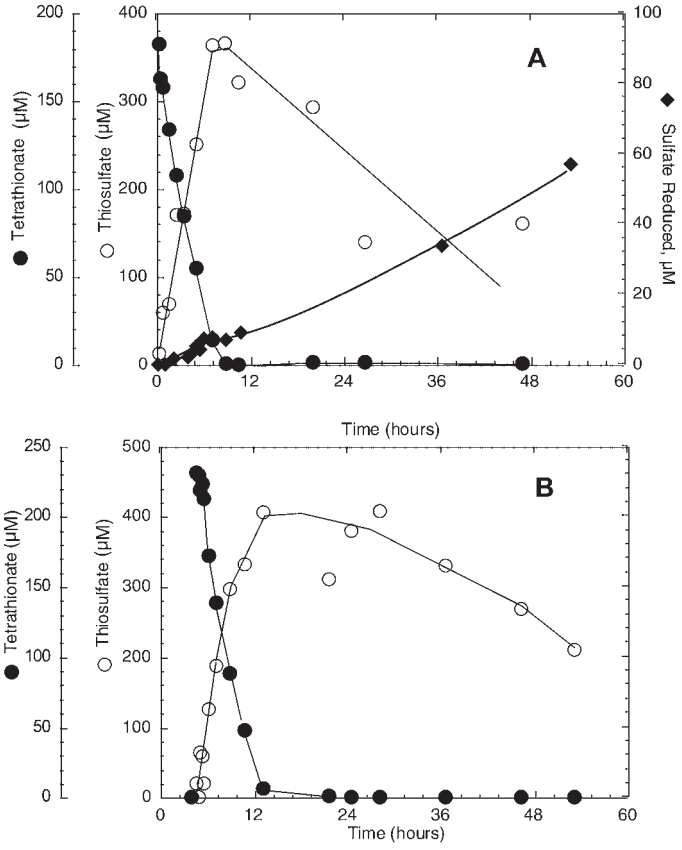

Figure 4. Tetrathionate and thiosulfate concentrations during a time series experiment with (A) reduced and (B) oxidized Weser Estuary sediments. The amount of sulfate reduced in the reduced slurry as measured by ^{35}S-sulfate labeling is also depicted in A.

Inhibition of Microbial Activity

Bak et al. (1993) suggested that the reduction of tetrathionate to thiosulfate is a microbially mediated process. Our experiments with Weser Estuary sediment also show that this conversion is principally a microbial process. We inhibited microbial activity in the sediments either by formaldehyde poisoning (final concentration of 0.1%; Tuominen et al., 1994) or heat sterilization (tyndallization). Formaldehyde treatment and heat sterilization strongly inhibited the rate of tetrathionate reduction relative to the control experiment (85% and 94% inhibition, respectively; data not shown). These inhibition experiments and the temperature response (see below) of tetrathionate consumption clearly indicate a role for bacteria in the reduction of tetrathionate to thiosulfate.

Role of Temperature

Figure 5 shows the rate of tetrathionate degradation in seawater and in Weser Estuary sediment slurries as a function of temperature. Five mL of slurry was added to each of 148 10 mL glass test tubes, fitted with rubber stoppers. The overlying headspace was purged with N_2 and stored at 11 °C overnight (in situ temperature). The filled test tubes were placed in ~2 °C intervals between 10–60 °C in a temperature-gradient block. After the slurry samples were allowed to equilibrate within the temperature gradient block (~1 hr), an exact amount of tetrathionate (170 µM) was then injected into each of the test tubes through the stopper. The test tubes were briefly shaken to equally distribute sediment and tetrathionate and placed back into the temperature gradient block. For each temperature, incubations were stopped at four time points, generally between 10 and 150 min. The incubations were stopped by immediately plunging the test tube into an ice bath until the slurry could be filtered through a 0.4 µm cellulose acetate (Millipore) filter using a pneumatic pore-water squeezer.

In a separate experiment, a series of test tubes containing tetrathionate-amended seawater (no sediment) were run to examine the inorganic decomposition of tetrathionate between 11 and 78 °C. In tetrathionate-amended slurries, tetrathionate consumption increased with rising temperature and peaked at temperatures between 35 °C and 41 °C before decreasing. Without sediment, tetrathionate exhibited only very low rates of chemical degradation at temperatures below 50 °C in seawater. Only at temperatures >50 °C did the rates increase considerably. The peak in tetrathionate reduction at temperatures between 30 and 40 °C (Fig. 5) suggests the role of an enzymatic or biologically catalyzed reaction typical of a mesophilic bacterial population.

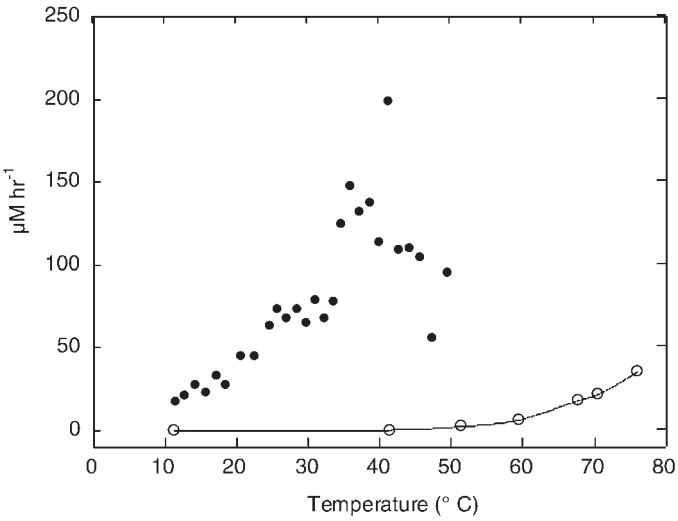

Figure 5. Response of the rate of tetrathionate reduction in Weser Estuary sediments (February 1994) to temperature (closed circles). Open circles indicate the disappearance rate of tetrathionate dissolved in seawater.

Role of Reduced Inorganic Compounds

These experiments do not provide conclusive proof that bacteria directly participate in tetrathionate reduction in these sediments. As shown in Equation 15, dissolved sulfide readily reduces tetrathionate to form thiosulfate and zero-valent sulfur. However, sulfide or other reduced substances do not appear to be chemically reducing tetrathionate in these experiments. In both the Weser Estuary and Skagerrak sediments, dissolved sulfide was not detectable (<1 µM). Oxidizing the sediments to remove sulfides, either free in solution, adsorbed to surfaces, or present as iron sulfides, had little impact on the rate of tetrathionate consumption (Fig. 4). The addition of another reduced compound, Fe(II), to a concentration of 500 µM increased the rate of tetrathionate consumption only slightly over that of the control (16% increase), and concentrations of dissolved iron remained constant throughout the experiment as measured using the Ferrozine method (Stookey, 1970).

Another source of sulfide for the reduction of the tetrathionate could be the continuous production of hydrogen sulfide due to sulfate reduction. Sorokin et al. (1996) propose such a mechanism as a means of regenerating thiosulfate from tetrathionate for further oxidation of thiosulfate and subsequent energy gain in *Catenococcus thiocycli*. Podgorsek and Imhoff (1999) propose a similar mechanism to explain observed tetrathionate concentrations in sulfidic Baltic Sea sediments. We measured the production of sulfide via the turnover of ^{35}S-labeled sulfate in the experiment with the reduced slurry. Sulfide was continually produced from sulfate reduction in the reduced sediment slurry (Fig. 4A); however, the rate of sulfate reduction was much lower than the disappearance rate of tetrathionate.

We sought to exclude sulfide reduction of tetrathionate by blocking sulfate reduction with the addition of molybdate, which is a well-known inhibitor of sulfate reduction. Sodium molybdate was added to slurry to give a final concentration of 20 mM MoO_4^{2-} (approximately equivalent to the sulfate concentration). A second slurry was not treated with molybdate. Within 30 min, tetrathionate was added to both slurries and sampling commenced for the determination of thiosulfate and tetrathionate concentrations. Sulfate reduction was also measured in these slurries. Twenty hours prior to molybdate addition, $^{35}SO_4^{2-}$ was added to both bags, and samples were taken for sulfate reduction rate measurements during, before, and after the molybdate-tetrathionate additions.

In the molybdate-untreated slurry, sulfate reduction proceeded in the first 20 h before addition of tetrathionate at a rate of 4.5 µM h^{-1} (Fig. 6A). Addition of tetrathionate to a concentration of 180 µM had no immediate effect on the sulfate reduction rate. The tetrathionate concentration decreased at a rate of 36.6 µM h^{-1} with a concurrent rise in thiosulfate concentration of 87.2 µM h^{-1}. At maximum thiosulfate concentration and when tetrathionate was fully consumed, a break in the rate of sulfate reduction was observed and the sulfate reduction rate decreased to 2.0 µM h^{-1}, until thiosulfate concentrations fell below 50 µM, at which point sulfate reduction rates increased to 3.3 µM h^{-1}. Thiosulfate decreased in the untreated slurry at a rate of 13.5 µM h^{-1}.

In the slurry that had been treated with molybdate, sulfate reduction initially proceeded at a rate of 3.6 µM h^{-1} until molybdate was added, at which point sulfate reduction ceased for the remainder of the experiment (Fig. 6B). Tetrathionate, added after the molybdate addition, decreased in concentration at a rate of 26.4 µM h^{-1} (72.1% of the rate in the untreated slurry). As with the molybdate-free slurry, stoichiometric increases in thiosulfate matching the decrease in tetrathionate were observed (at a rate of 62.6 µM h^{-1}). Thiosulfate consumption, however, was significantly lower than the molybdate-free slurry (at 1.0 µM h^{-1} or 7.5% of the rate of thiosulfate consumption in the untreated slurry). The experiments demonstrate that although sulfate reduction was fully inhibited by molybdate (and thiosulfate reduction was significantly inhibited), tetrathionate reduction was only partially affected (by ~25–26%). Moreover, rates of tetrathionate reduction significantly exceeded those for sulfate reduction (between 7.5- and 27-fold higher). Thus, sulfide from sulfate reduction could not be titrating the tetrathionate added to the slurries. We therefore conclude that a direct microbial reduction must be responsible for the rapid rates of tetrathionate reduction that were observed.

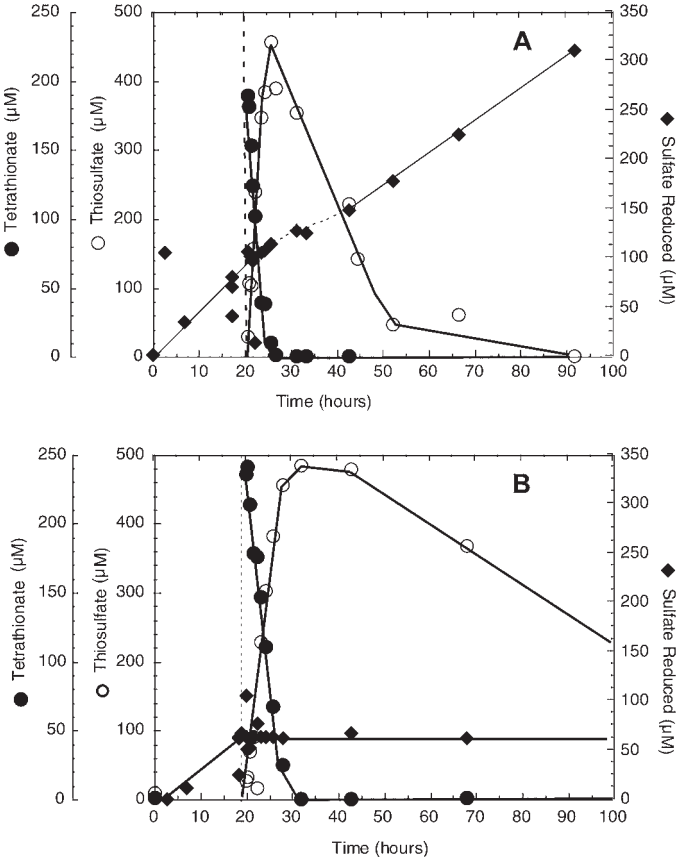

Figure 6. Tetrathionate and thiosulfate concentrations during a time series experiment with (A) untreated and (B) molybdate treated Weser Estuary sediments. Sulfate reduction was also measured in both experiments (^{35}S-sulfate labeling). The vertical dashed line indicates the time the tetrathionate was added to the slurry.

Possible Ecological Role of Tetrathionate Reduction in Marine Sediment

In a review of tetrathionate reduction by non–sulfate-reducing bacteria, Barrett and Clark (1987) suggested that the ability to reduce tetrathionate using the enzyme tetrathionate reductase is more common among anaerobes than the ability to reduce sulfite, the latter being a distinguishing feature of sulfate-reducing bacteria. Tetrathionate reductase catalyzes the following reaction:

$$S_4O_6^{2-} + 2\,[H] \rightarrow 2\,S_2O_3^{2-} + 2\,H^+, \qquad (16)$$

where [H] represents tetrathionate reductase containing reducing equivalents. Tetrathionate reductase is membrane bound, functions best at a pH >7, is regulated by the presence of oxygen and nitrate, and may be part of a reversible enzyme system that catalyzes both the oxidation of thiosulfate and the reduction of tetrathionate (Tuttle and Jannasch, 1973; Tuttle, 1980; Barrett and Clark, 1987). The redox couple of $S_4O_6^{2-}/S_2O_3^{2-}$ lies at a relatively high potential of +170 mV (Barrett and Clark, 1987). The free energies of reaction for the oxidation of organic matter (CH_2O) under standard biochemical conditions (pH = 7.0), via sulfate and tetrathionate reduction, respectively, are shown below (as calculated from compiled $\Delta G'_0$ values in Thauer, 1989).

$$SO_4^{2-} + 2\,CH_2O \rightarrow 2\,HCO_3^- + HS^- + H^+ \quad -195.5\ \text{kJ/reaction} \qquad (17)$$

$$2\,S_4O_6^{2-} + CH_2O + 2\,H_2O \rightarrow HCO_3^- + 5\,H^+ + 4\,S_2O_3^{2-} \quad -190.8\ \text{kJ/reaction} \qquad (18)$$

Per mole of reduced carbon or H_2 tetrathionate reduction is more energetically favorable than sulfate reduction (−190.8 kJ/mol versus −97.8 kJ/mol, respectively). Thus, tetrathionate reduction may become favorable when the electron donating substrate is limiting, which is the typical situation in most sediments.

Substrate Amendment

Our experiments indicate that tetrathionate reduction, unlike dissimilatory sulfate or thiosulfate reduction, is not directly coupled as a terminal electron acceptor to the oxidation of organic matter. We base this conclusion on the observation that tetrathionate reduction takes place at substantially higher rates than observed for either sulfate reduction or even thiosulfate consumption. Assuming that the slurries are substrate (organic carbon) limited, the rate of tetrathionate reduction should be only fourfold that of sulfate reduction, based on the stoichiometries in Equations 17 and 18; however, they fell between 7.5 and 27 times the sulfate reduction rate in all experiments where both sulfate reduction and tetrathionate reduction were measured.

The effect of organic matter availability on tetrathionate reduction was studied in a substrate addition experiment (data not shown). Four different slurries were prepared: (a) no substrate, no molybdate, (b) no substrate plus molybdate (ca. 20 mM), (c) substrate, no molybdate, and (d) substrate plus molybdate. The substrate additions consisted of a cocktail containing formate, acetate, propionate, butyrate, and lactate that yielded a 1 mM concentration of each fatty acid in the slurry. These fermentation products are typical substrates for sulfate-reducing bacteria. Molybdate was added to block indirect tetrathionate reduction via sulfide production from dissimilatory sulfate reduction. Addition of substrate yielded only a slight increase in the rate of tetrathionate reduction (221 and 168 μM h^{-1} with and without substrate, respectively). The slurries where sulfate reduction was inhibited showed a similar pattern, albeit at slightly lower rates (142 and 124 μM h^{-1} with and without substrate, respectively). These results suggest that tetrathionate reduction is not necessarily linked to the terminal oxidation of substrate to CO_2 and that, more specifically, sulfate reducing bacteria are only minimally involved in tetrathionate reduction in marine sediments.

Moreover, tetrathionate had no effect on the sulfate reduction rate, unlike the subsequent appearance of thiosulfate, which significantly depressed the sulfate reduction rate. Thiosulfate

consumption also exhibits an immediate and strong response to the addition of molybdate, whereas tetrathionate reduction decreases by less than one-fourth (see Figs. 4 and 6). This effect of thiosulfate on the sulfate reduction rate has been attributed to the greater energy gain due to thiosulfate reduction over sulfate reduction (Widdel, 1988; Jørgensen, 1990b). In pure cultures of some fermenting heterotrophs (e.g., *Salmonella enterica* [Hinsley and Berks, 2002] and *S. typhimurium* [Hensel et al., 1999]), tetrathionate is also the preferred electron acceptor over thiosulfate. In marine sediments, however, tetrathionate apparently plays no such similar role as preferred electron acceptor, because the concentration of tetrathionate appears to have no direct impact on either the rate of sulfate or thiosulfate reduction.

Alternatives to Dissimilatory Tetrathionate Reduction

If it is not being used as a terminal electron acceptor for sulfate-reducing bacteria, what possible role could tetrathionate reduction have in the microbial community? Anaerobic disproportionation of 4 moles of tetrathionate (Equation 13) to form 6 moles of thiosulfate, 1 mol of trithionate, and 1 mol of sulfate (1.5:1 $S_2O_3^{2-}$:$S_4O_6^{2-}$ ratio) has been shown for the facultative heterotroph *Thiomonas intermedia* K12 (Wentzien and Sand, 1999) at circumneutral pH. Disproportionation of other intermediate sulfur compounds in marine sediments has been demonstrated (Jørgensen, 1990a; Jørgensen and Bak, 1991; Canfield and Thamdrup, 1994, 1996), and there is no reason to think that tetrathionate disproportionation may not occur as well. The major argument, however, that tetrathionate disproportionation is not the principal pathway of tetrathionate consumption, is that the stoichiometry of thiosulfate formation to tetrathionate disappearance is closer to the 2:1 stoichiometry of tetrathionate reduction (Equation 16) than to that of disproportionation (Equation 13). Furthermore, we observed no trithionate formation, which should have appeared during the chromatographic runs.

Tetrathionate reduction as expressed in Equation 16 may also be linked to fermentation, which conforms well to our earlier observation that sulfate- and tetrathionate-reducing bacteria do not have the same substrate spectrum. Fermenting bacteria have a problem getting rid of excess reducing power they generate in form of NADH or NADPH in the oxidative branches of fermentation pathways. Many of them have developed means of releasing electrons to syntrophic partner organisms or external electron acceptors. Such an external electron sink allows fermenters to regenerate NAD(P), and thus to oxidize organic matter further, which results in more ATP production per substrate. Moreover, Barrett and Clark (1987) suggested that tetrathionate reduction may even be coupled with the production of ATP through oxidative phosphorylation. Fermentative bacteria have been shown to dump electrons onto, for example, elemental sulfur, humic substances, and iron oxide and other metal oxides (e.g., Jones et al., 1984; Stal and Moezelaar, 1997; Benz et al., 1998). We speculate that, in sediment where the sulfur cycle is active and tetrathionate may arise through sudden oxidation events, the ability to channel electrons through a membrane-bound tetrathionate reductase may be widespread among facultative and strictly anaerobic bacteria and not just among those involved in sulfate reduction or thiosulfate consumption (reduction or disproportionation).

Tetrathionate Dynamics in the Presence of Oxidants

Although this study has focused principally on the fate of tetrathionate added to sediment slurries under anaerobic conditions, there are indications that the thiosulfate-tetrathionate system is altered in the presence of oxidants such as oxygen, nitrate, and manganese oxides. Where air was continually bubbled through the slurry, tetrathionate consumption decreased to 41.8% of the untreated control (data not shown). In the two experiments where nitrate was added to a final concentration of 200 µM, the rates of tetrathionate consumption decreased to 89% and 55% of the unamended rates. Nitrate addition tended to flatten out the thiosulfate response (Fig. 7). The initial increase in thiosulfate was only 36.4% of the unamended rate, and the decrease was also lower (27.9%). Both of these experiments conform to the observation from pure culture studies that tetrathionate reductase is repressed by higher redox potential electron acceptors such as oxygen and nitrate (Barrett and Clark, 1987).

Manganese oxides may also inhibit tetrathionate reduction, as shown by the results from the two Skagerrak sites (Fig. 8). At Station S4, where sulfate reduction rates vary between 8 and 12 µM h^{-1} (Canfield et al., 1993), tetrathionate disappeared at a rate of 35.7 µM h^{-1} and exhibited a nearly stoichiometric increase in thiosulfate concentration (60.9 µM h^{-1}). At this typical continental margin site, tetrathionate decreased to below detection limits within 8 h, and thiosulfate, after its initial build-up, decreased to near 10 µM within 32 h. In contrast, the behavior of tetrathionate and thiosulfate in the manganese oxide-rich sediments of Station S9 was strikingly different. A lag time of 8 h was required before any tetrathionate reduction occurred. At

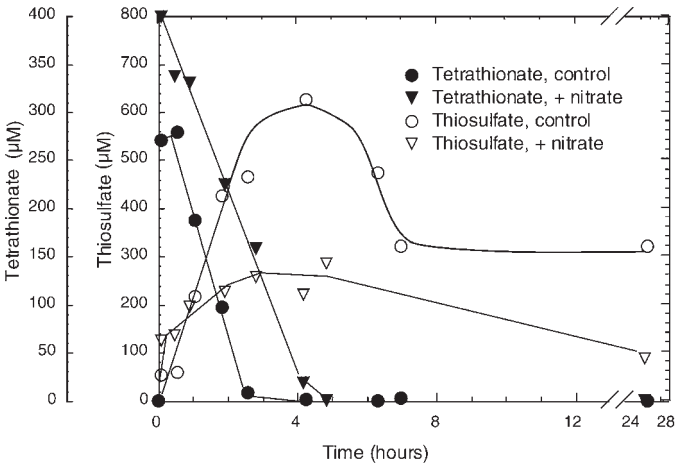

Figure 7. Tetrathionate and thiosulfate concentrations during a time series experiment with untreated and nitrate amended Weser Estuary sediments.

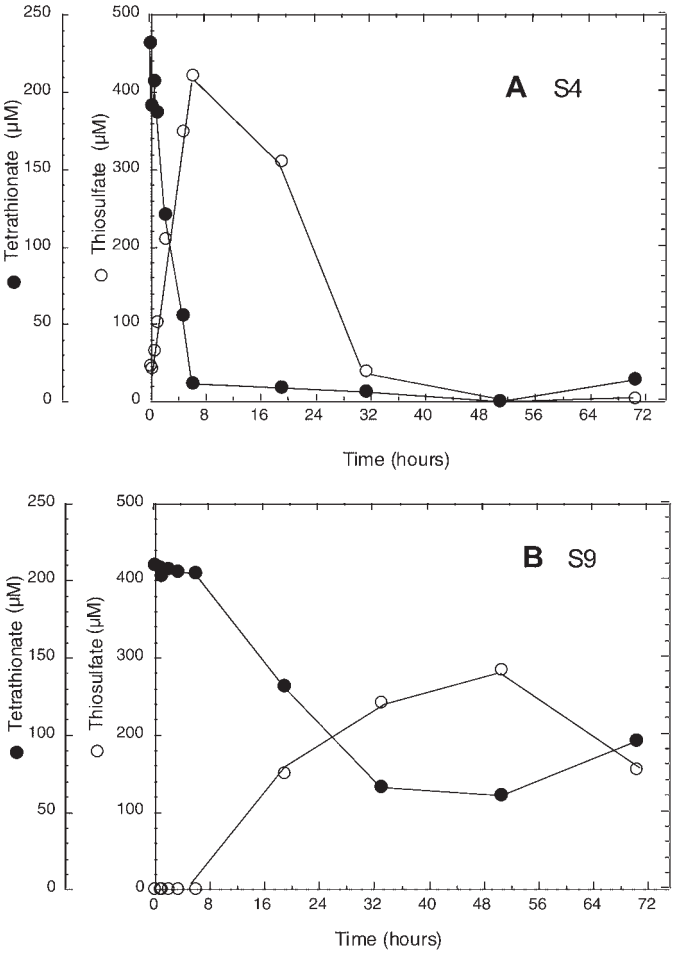

Figure 8. Tetrathionate and thiosulfate concentrations during time series experiments with sediment from (A) Station S4 (190 m water depth) and (B) Station S9 (695 m water depth) from the continental slope of the Skagerrak region of the North Sea.

this point, tetrathionate consumption commenced, but at a much lower rate of 5.1 μM h^{-1}, with a corresponding increase in thiosulfate of 8.9 μM h^{-1}. Furthermore, tetrathionate concentrations never went to zero. Rather, they remained constant at near 60 μM or even slightly increased over the remaining 36 h of the experiment, which may reflect the concurrent reoxidation of thiosulfate to tetrathionate by MnO_2 (Schippers and Jørgensen, 2001). The increase in thiosulfate also exhibited the characteristically flat response, as seen in the experiments with aerated and nitrate amended sediments.

In oxidized sediments, tetrathionate typically disappeared only after a time lag of up to several hours, which suggests that the capacity to reduce tetrathionate must first be induced. However, in most marine coastal sediments, the response to tetrathionate additions is immediate, suggesting that the bacteria are primed and waiting for tetrathionate arising from various sulfide oxidation events.

CONCLUSIONS

This work demonstrates that in most marine sediments the concentrations of SO_3^{2-}, and $S_2O_3^{2-}$, and $S_4O_6^{2-}$ are in the sub-micromolar range with maximum values not exceeding a few micromoles per liter. Elemental sulfur is the most abundant sulfur intermediate in coastal marine sediments. In sediments deposited under oxic conditions, a distinct subsurface maximum of S^0 is often observed, possibly associated with the depth of the bioturbation zone, whereas in anoxic environments (e.g., in the Black Sea), the highest values of S^0 are found at the sediment-water interface.

The low concentrations of the dissolved intermediates reflect equilibrium conditions where the rates of production and consumption are tightly coupled. Disequilibrium conditions due to bioturbation events or rapid temperature changes, for example, may lead to sudden and high concentration excursions in one or more of the intermediate sulfur compounds, but they will rapidly return to low equilibrium concentrations.

Both chemical and biochemical pathways are operating to maintain such low concentrations. Sulfite disappeared rapidly and was, most likely, chemically oxidized to sulfate or reacted with other sulfur compounds, such as elemental sulfur or sulfide. Tetrathionate is readily reduced in the presence of excess sulfide to give thiosulfate and polysulfides. However, in non-sulfidic sediments, which comprise the majority of surface marine sediments, tetrathionate and thiosulfate are chemically stable. Under such conditions, both tetrathionate and thiosulfate are consumed directly in bacterially mediated processes that drive the concentrations of both tetrathionate and thiosulfate to low equilibrium concentrations.

The rates at which the concentrations of sulfur intermediates return to equilibrium decrease in the order: $SO_3^{2-} \approx S_4O_6^{2-} > S_2O_3^{2-} > S^0$. Elemental sulfur and thiosulfate are the key intermediates in sulfide oxidation, based both on their concentration and on their lower rates of turnover. For example, thiosulfate is consumed much more slowly than tetrathionate is reduced to thiosulfate. If tetrathionate is formed during any of the various sulfide oxidation pathways, it will primarily be reduced back to thiosulfate, and thus, sulfur cycling through tetrathionate acts mostly as a closed-loop under anoxic conditions. Therefore, the processes regulating thiosulfate consumption are rate-determining steps, or bottlenecks, in the oxidative half of the sulfur cycle.

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