



## Fate of elemental sulfur in an intertidal sediment

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### Abstract

Sediment from a tidal flat at Wedderwarden, near the mouth of the Weser estuary, northern Germany, was amended with elemental sulfur, and concentrations of metabolic end products were monitored. The production of both sulfate and sulfide was consistent with disproportionation as the most important fate of the added elemental sulfur. A population of bacteria conducting active elemental sulfur disproportionation was also enriched from the sediment. In the enrichments, containing both elemental sulfur and Fe oxides as a sulfide 'scrub', sulfide and sulfate were produced in a ratio of 1.5/1, somewhat lower than the predicted ratio of 2/1. The mismatch between predicted and observed production ratios is explained by the channelling of electrons into autotrophic or mixotrophic CO<sub>2</sub> fixation rather than sulfide formation. The production of organic carbon, in the correct amount to explain the observed sulfide to sulfate production ratio, was verified by organic carbon analysis. Finally, rates of sulfate reduction were identical in the elemental sulfur amended sediment, and in control sediment with no added sulfur. Hence, the heterotrophic bacterial community was completely unaffected by an active metabolism conducting elemental sulfur disproportionation.

**Keywords:** Sediment; Elemental sulfur; Disproportionation; Sulfate reduction; Sulfur; Diagenesis

### 1. Introduction

Whereas a single process, dissimilatory sulfate reduction, is by far the most significant primary source of sulfide in sediments, the oxidation of sulfide, and subsequent processing of oxidation intermediates, follows a bewildering array of potential pathways. For example, sulfide can be oxidized abiotically with O<sub>2</sub>, Fe oxides, and Mn oxides producing a combination of oxidation products including S<sup>0</sup>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, SO<sub>3</sub><sup>2-</sup> and SO<sub>4</sub><sup>2-</sup> (Table 1; [1–3]). Simi-

lar products are formed during sulfide oxidation with O<sub>2</sub> or NO<sub>3</sub><sup>-</sup> by colorless sulfur bacteria [4] and even by some sulfate reducers [5].

Once produced, the oxidized intermediates S<sup>0</sup>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup> and SO<sub>3</sub><sup>2-</sup> are quite actively metabolized. For example, both S<sub>2</sub>O<sub>3</sub><sup>2-</sup> and SO<sub>3</sub><sup>2-</sup> are favorable electron acceptors, and hence reduced by many sulfate-reducing bacteria as well as members of other bacterial groups (Table 1; [6,7]). Similarly, the dissimilative reduction of S<sup>0</sup> is carried out by a diverse assembly of bacteria (Table 1; e.g. [8]). The intermediates S<sup>0</sup>, SO<sub>3</sub><sup>2-</sup> and S<sub>2</sub>O<sub>3</sub><sup>2-</sup> may also be oxidized, as for example by *Desulfovibrio desulfuricans*, with O<sub>2</sub> as the electron acceptor [5]. Sulfate-reducing bacteria have also been reported to catalyze S<sup>0</sup> oxidation to SO<sub>4</sub><sup>2-</sup> with manganese oxide [9].

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Table 1  
Examples of sulfur transformations

Sulfide oxidation (unbalanced)	
$\text{H}_2\text{S} + \text{O}_2 \rightarrow \text{S}^\circ, \text{SO}_4^{2-}, \text{S}_2\text{O}_3^{2-}, \text{SO}_3^{2-} + \text{H}_2\text{O}$	
$\text{H}_2\text{S} + \text{MnO}_2 \rightarrow \text{S}^\circ + \text{Mn}^{2+}$	
$\text{H}_2\text{S} + \text{FeOOH} \rightarrow \text{S}^\circ + \text{S}_2\text{O}_3^{2-} (\text{trace}) + \text{Fe}^{2+}$	
Intermediate oxidation (unbalanced)	
$\text{S}^\circ, \text{S}_2\text{O}_3^{2-}, \text{SO}_3^{2-} + \text{O}_2, \text{NO}_3^-, \text{MnO}_x \rightarrow \text{SO}_4^{2-}$	
Intermediate reduction (unbalanced)	
$\text{S}^\circ, \text{S}_2\text{O}_3^{2-}, \text{SO}_3^{2-} + \text{org. carbon} \rightarrow \text{H}_2\text{S}$	
Disproportionation (balanced)	
$\text{S}_2\text{O}_3^{2-} + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + \text{H}_2\text{S}$	
$4\text{SO}_3^{2-} + 2\text{H}^+ \rightarrow 3\text{SO}_4^{2-} + \text{H}_2\text{S}$	
$4\text{S}^\circ + 4\text{H}_2\text{O} \rightarrow 3\text{H}_2\text{S} + \text{SO}_4^{2-} + 2\text{H}^+$	
$3\text{S}^\circ + 2\text{FeOOH} \rightarrow \text{SO}_4^{2-} + 2\text{FeS} + 2\text{H}^+$	
$\text{S}^\circ + 3\text{MnO}_2 + 2\text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 3\text{Mn}^{2+} + 4\text{OH}^-$	

Quite recently a novel mode of anaerobic sulfur transformation has been described where  $\text{S}_2\text{O}_3^{2-}$  and  $\text{SO}_3^{2-}$  are disproportionated to sulfide and  $\text{SO}_4^{2-}$  (Table 1) during the chemolithotrophic growth of pure cultures of *Desulfovibrio sulfodismutans* [10]. This metabolism has also been found in other sulfate reducers [10–12] and has been documented in both marine and freshwater sediments [13,14].

A similar disproportionation of  $\text{S}^\circ$  to sulfide and  $\text{SO}_4^{2-}$  (Table 1) has been reported [15] during the chemolithotrophic growth of anaerobic cultures enriched on  $\text{S}^\circ$  in the absence of any organic substrate. Active growth was found as long as free sulfide was kept to low levels by the addition of Fe oxides or Mn oxides as a sulfide-scrub [15]. With the addition of Mn oxides, sulfide produced during the disproportionation is rapidly reoxidized to  $\text{S}^\circ$ , so the net stoichiometry is the same as with direct  $\text{S}^\circ$  oxidation to  $\text{SO}_4^{2-}$  (Table 1; [15]). More recently, the sulfate-reducing bacterium *Desulfobulbus propionicus* was shown to disproportionately  $\text{S}^\circ$  anaerobically to sulfide and  $\text{SO}_4^{2-}$  in the presence of Fe oxides [9], in the same manner as previously described [15], but only with poor growth.

In this contribution we report on the fate of  $\text{S}^\circ$  amended to sediments from the Weser estuary, northern Germany. We describe the relationship between  $\text{S}^\circ$  disproportionation,  $\text{S}^\circ$  reduction, and sulfate reduction. Our focus here on  $\text{S}^\circ$  is driven in part by its numerous potential turnover pathways (Table

1), the fact that it is a measurable and sometimes significant sulfur species (e.g. [16]), and by the general paucity of information regarding actual turnover mechanisms and rates in natural sediments. This contribution, hence, begins to address the relative significance of  $\text{S}^\circ$  turnover pathways in sediments, and also compliments our previous work on the isotopic consequences of  $\text{S}^\circ$  disproportionation [17].

## 2. Materials and methods

### 2.1. Bag incubations

Our experimental design was to monitor the fate of  $\text{S}^\circ$  added to anoxic natural sediment, and to determine how the amendment influenced other aspects of sediment metabolism. Approximately 6 liters of silty surface sediment from a tidal flat at Wedderwarden near the mouth of the Weser estuary were homogenized under  $\text{N}_2$  and placed into gas-tight plastic bags [18,19]. Two of these bags were further amended with  $\text{S}^\circ$  as flowers of sulfur to a final concentration of approximately  $200 \mu\text{mol cm}^{-3}$  ( $240 \mu\text{mol g}^{-1}$  dry weight). The two remaining bags were unamended and acted as controls. High concentrations of  $\text{S}^\circ$  were necessary in our amended bags, as no reliable radiotracer assay for  $\text{S}^\circ$  metabolism is available, and it was therefore necessary to document  $\text{S}^\circ$  metabolism from chemical changes. Whereas the amounts of added  $\text{S}^\circ$  are high for Wedderwarden sediment, such levels are approached (on a per gram dry weight basis) in many marine environments. For example, concentrations of up to  $70 \mu\text{mol g}^{-1}$  have been measured off the Chilean coast (Thamdrup and Canfield, unpublished),  $350 \mu\text{mol g}^{-1}$  in Mangrove Lake Bermuda (Canfield, unpublished), and approx.  $40 \mu\text{mol g}^{-1}$  in a microbial sulfuretum on the Danish coast [16].

Bags were sampled directly into 10 ml of 20% Zn acetate. The Zn acts as a bactericide and rapidly fixes AVS (acid volatile sulfide including  $\text{H}_2\text{S}$  and  $\text{FeS}$ , but not pyrite) as  $\text{ZnS}$  [20]. Sediment pH was measured on a separate split of sediment. At the beginning of the experiment the control bags were sampled for initial concentrations of various sediment species. Twice during the incubation, rates of

sulfate reduction were measured on splits of control and  $S^{\circ}$  amended bag sediment using  $^{35}\text{SO}_4^{2-}$  with 1 h incubation [21] and the one step  $\text{Cr}^{2+}$  digestion [20].

## 2.2. Enrichments

In a separate experiment, 2% v/v of homogenized sediment was added to a series of 50 ml screw-cap bottles with anoxic saltwater medium (sulfate-free, bicarbonate-buffered, containing no organics other than vitamins; see [15] for details) containing 16 mmol  $S^{\circ}$  flowers and 1.5 mmol ferrihydrite (approx.  $\text{Fe}(\text{OH})_3$  synthesized according to [22]). These bottle experiments will be termed enrichment experiments as they represent the original inoculation of an enrichment series which has, subsequent to this investigation, maintained the same metabolism at comparable rates over 20 subsequent transfers. A further series of bottles without  $S^{\circ}$ , but including  $\text{Fe}(\text{OH})_3$  was inoculated as above. All bags and bottles were incubated at a constant  $24^{\circ}\text{C}$  and sampled regularly over the course of one month where the whole contents of a bottle were added to 20 ml 20% w/v Zn acetate and frozen [21]. A small amount of the bottle solution was used for pH determinations.

## 2.3. Chemical analysis

Zn-fixed samples were thawed and the supernatant solution separated by filtration. The concentration of sulfate was determined on the supernatant fluid gravimetrically by precipitation with excess  $\text{Ba}^{2+}$ ; duplicate determinations were reproducible to  $\pm 4\%$ . The sediment remaining after filtration was dried and subjected to a series of sulfur digestions where first, AVS was digested with 6 N HCl and collected as  $\text{Ag}_2\text{S}$  which was quantified gravimetrically ( $\pm 5\%$ ). The residue from this digestion was Soxhlet extracted for 24 h with acetone to remove  $S^{\circ}$ . In some cases  $S^{\circ}$  was collected by precipitation as CuS with Cu metal, and its concentration determined by further AVS digestion [23]. The concentration of  $S^{\circ}$  was, however, also determined on a split of dried Zn-fixed sediment (before AVS extraction) by double extraction into methanol and measurement by HPLC. There was a good agreement between the two types of determinations, and we estimate the

precision of each determination to  $\pm 7\%$ . Pyrite S was liberated by  $\text{Cr}^{2+}$  distillation of the sediment residue after  $S^{\circ}$  removal [24] with a precision of approximately  $\pm 5\%$ .

Iron oxide and extractable Mn content ( $\pm 3\%$ ) were determined on fresh sediment by extraction with dithionite-citrate-acetic acid solution [25,26]. Iron was determined by complexation with Ferrozine [27] and Mn by flame atomic absorption spectroscopy. Organic carbon was determined on sediment from the enrichment incubations, after AVS digestion, using a Carlo Erba CHNS analyzer with a precision of about  $\pm 8\%$ .

## 3. Results

### 3.1. Sediment amendment experiments

Initial sediment parameters including solid-phase Fe oxides, Mn,  $S^{\circ}$ , AVS, pyrite, pore water sulfate, as well as pH, are presented in Table 2. Sulfate-reducing bacteria were active in the control experiments as evidenced by a decrease in  $\text{SO}_4^{2-}$  and rise in AVS (Fig. 1a), with an average sulfate reduction rate of about  $8.8 \text{ nmol cm}^{-3} \text{ h}^{-1}$  calculated from the sulfate decrease (Table 3). This rate is similar to those measured at both 118 and 287 h with  $^{35}\text{SO}_4^{2-}$  (Table 3). Only about 50% of the sulfide produced by sulfate reduction was recovered as AVS (Fig. 1a), with the rest perhaps forming into pyrite, as  $S^{\circ}$  remained low throughout the incubation (Fig. 1b). The high background levels of pyrite (Table 2), however, would make such a small increase in con-

Table 2  
Initial sediment parameters

Parameter	
porosity	0.67
pH	7.4
$\text{SO}_4^{2-}$	$7.0 \mu\text{mol cm}^{-3}$
AVS <sup>a</sup>	$0.6 \mu\text{mol cm}^{-3}$
$S^{\circ}$	$0.8 \mu\text{mol cm}^{-3}$
$\text{FeS}_2$	$51.0 \mu\text{mol cm}^{-3}$
Fe-oxide	$96.0 \mu\text{mol cm}^{-3}$
extractable Mn	$85.0 \mu\text{mol cm}^{-3}$

<sup>a</sup> Acid volatile sulfide.

centration difficult to confirm (Table 2). During the incubation the pH was steady at about 7.4 (Fig. 1c).

In the  $S^{\circ}$ -amended sediment, rates of sulfate reduction, as measured by radiotracer, were similar to those in the control experiment. These rates also changed little during the course of the incubation (Table 3). By contrast, after a lag period of 50–100 h, sulfate and sulfide (as solid-phase AVS) both accumulated in the incubation bags (Fig. 2a). The AVS from the  $S^{\circ}$ -amended bags caused a blackening

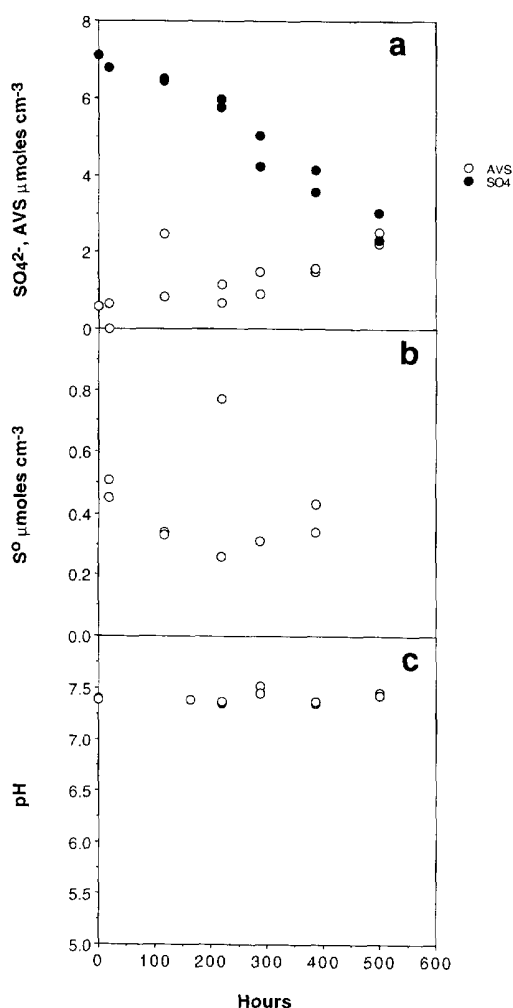


Fig. 1. Results from control incubation with no added elemental sulfur. Incubations were performed in duplicate and results from both are shown together: (a) concentrations of pore water sulfate and AVS (acid volatile sulfide) with time; (b) concentration of elemental sulfur with time; (c) pore water pH.

Table 3  
Rates of sulfate reduction

Experiment	Time (h)	Rate (nmol cm <sup>-3</sup> h <sup>-1</sup> )
<sup>35</sup> SO <sub>4</sub> <sup>2-</sup>		
S <sup>0</sup> -amended 1	118	8.7
S <sup>0</sup> -amended 2	118	8.5
Control 1	118	9.1
Control 2	118	7.3
S <sup>0</sup> -amended 1	287	8.4
S <sup>0</sup> -amended 2	287	ND <sup>a</sup>
Control 1	287	8.2
Control 2	287	11.6
SO <sub>4</sub> <sup>2-</sup> decrease		
Control 1	0–500	8.7
Control 2	0–500	8.9

<sup>a</sup> Not determined.

of the sediment while the control bags remained brown. Standard linear regression of the combined results from both  $S^{\circ}$ -amended experiments showed AVS and sulfate to accumulate with an average ratio ( $\Delta\text{AVS}/\Delta\text{SO}_4^{2-}$ ) of 2.29/1 ( $R^2 = 0.95$ ). Some AVS was produced, and sulfate reduced, as a result of sulfate reduction. Correcting with measured sulfate reduction rates (Table 3), the ratio of AVS to  $\text{SO}_4^{2-}$  production, in the absence of sulfate reduction, is reduced to 1.75/1. Some sulfide from disproportionation could have also formed into pyrite ( $\text{FeS}_2$ ) as previously described [15]. However, our results show no evidence for any significant pyrite formation in the  $S^{\circ}$ -amended experiments (Fig. 2b).

The concentration of  $S^{\circ}$  decreased in parallel with the production of  $\text{SO}_4^{2-}$  and AVS (Fig. 2a,c). The initial rapid decrease in  $S^{\circ}$  is, however, not matched by changes in any of the other S pools, and is probably an experimental artefact reflecting inhomogeneous  $S^{\circ}$  distribution within the bags in the initial stages of the experiment.

The pH of the pore waters dropped dramatically throughout the incubation as would be expected during the disproportionation of  $S^{\circ}$  (Fig. 2d; Table 1; [15]). Sulfide was first detected in solution (by smell on sediment splits collected from the bags) after about 500 h of incubation. This signalled the consumption, by reaction with sulfide, of the most reactive iron oxide phases [28]. After this time, as sulfide accumulated, the rate of  $S^{\circ}$  disproportionation dra-

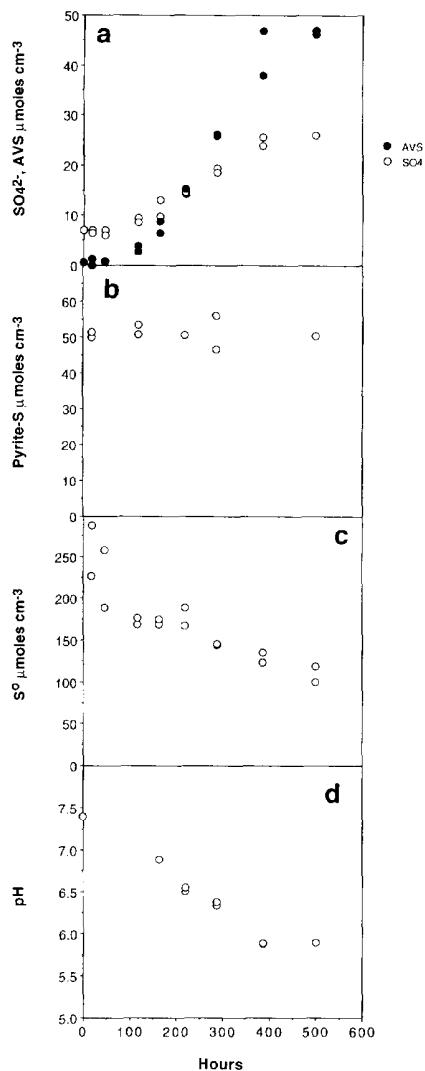


Fig. 2. Results from elemental sulfur amended incubations. Incubations were performed in duplicate and results from both are shown together: (a) concentrations of pore water sulfate and AVS (acid volatile sulfide) with time; (b) concentration of pyrite with time; (c) concentration of elemental sulfur with time; (d) pore water pH.

matically decreased (data not shown), consistent with earlier observations on the inhibiting influence of dissolved sulfide on S<sup>0</sup> disproportionation [15].

### 3.2. Enrichment experiments

As above, after a lag period of approximately 100 h, both SO<sub>4</sub><sup>2-</sup> and AVS accumulated in the S<sup>0</sup> plus

Fe-oxide enrichment bottles (Fig. 3a), and the precipitate turned from reddish brown to black. A small amount of sulfur also formed into pyrite (Fig. 3b; about 5% of the total reduced S), though to a lesser

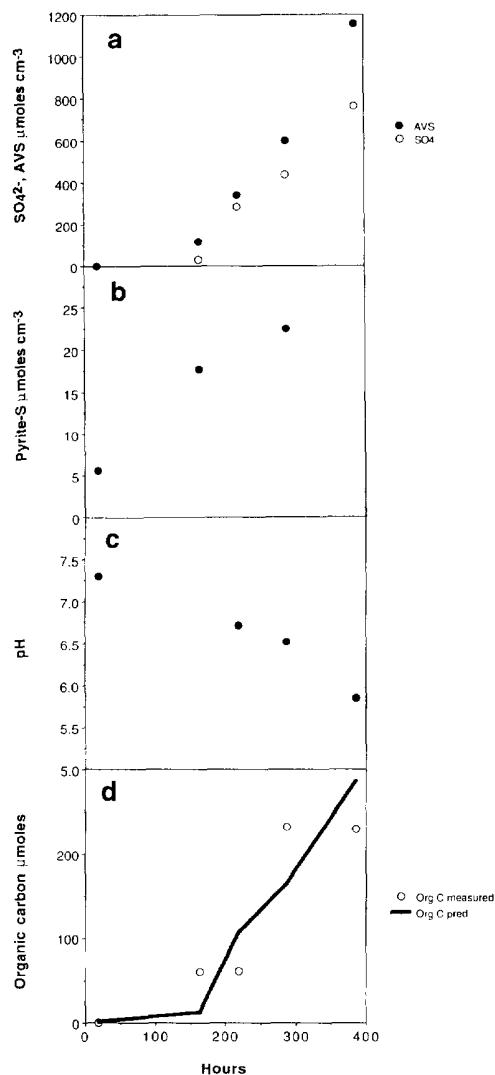


Fig. 3. Results from enrichment incubations; (a) quantity of pore water sulfate and AVS (acid volatile sulfide) in incubation bottles with time; (b) amount of pyrite with time; (c) pore water pH; (d) amount of organic carbon formed during the incubation. This parameter was calculated from measured organic carbon concentrations, and sediment mass, after correcting for the amount of organic carbon added with the inoculum. The solid line is the amount of organic carbon predicted from Eq. 5 and the measured amounts of sulfate formed during the incubation.

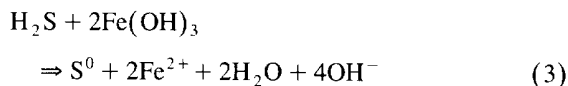
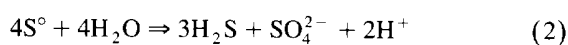
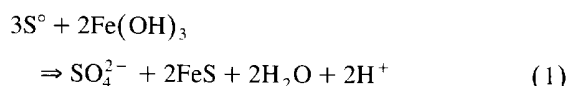
extent than reported previously [15]. Overall, the ratio of sulfide to sulfate produced was 1.5/1 after adding the sulfide formed into pyrite. The organic content of the enrichment bottles increased concomitantly with the production of sulfide and sulfate (Fig. 3d).

As in the  $S^{\circ}$ -amended sediment incubations, a sharp drop in pH was observed during the incubation, consistent with  $S^{\circ}$  disproportionation. Finally, very low levels of sulfate and AVS were measured (approximately 2  $\mu\text{mol}$  per bottle, each) in the Fe oxide-only amended bottles, and these concentrations did not change during the incubation. Thus, no major sulfur transformations occurred in the absence of added  $S^{\circ}$ . Also, pH in these incubations stayed relatively constant at around 7.5 (data not shown).

## 4. Discussion

### 4.1. Fate of elemental sulfur

The ratios of AVS to  $\text{SO}_4^{2-}$  production in the  $S^{\circ}$ -amended sediment and enrichment cultures (1.75/1 and 1.5/1 respectively; see above) are similar to, though a bit lower than, the 2/1 ratio expected for  $S^{\circ}$  disproportionation with FeS (AVS) formation in the presence of Fe oxides (Table 1; [15]). The overall 2/1 stoichiometry (Eq. 1) arises from disproportionation (Eq. 2) followed by oxidation of ferric oxide to the reduced ferrous state (Eq. 3), and subsequent precipitation as FeS (Eq. 4, [15]).

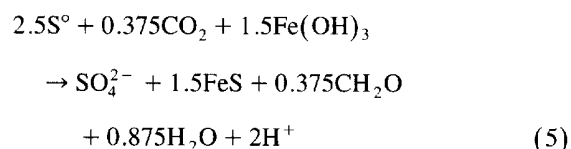


The high concentrations of ferric iron initially present in the sediment (96  $\mu\text{mol cm}^{-3}$ ; Table 2) and enrichment cultures (1.5 mmol per bottle) are sufficient to provide an effective reactant for sulfide [25].

High concentrations of extractable Mn were also present (Table 2) in the  $S^{\circ}$ -amended bag incubations,

providing a potential additional reactant for sulfide [15]. An important difference compared to ferric iron, however, is that sulfide is completely oxidized by Mn oxides to  $S^{\circ}$  or  $\text{SO}_4^{2-}$ , with no accumulation of AVS [2,29]. Hence, our somewhat low ratio of AVS to  $\text{SO}_4^{2-}$  accumulation of 1.75/1 could conceivably result from reaction between sulfide and Mn oxides. We note, however, that sulfide and sulfate began to accumulate at the same time (Fig. 2a). Thus, no significant early reaction between Mn oxides and sulfide could have occurred, suggesting that the extractable Mn in our experiment was initially present in the reduced form and not available as an oxidant. Unfortunately, we did not save pore waters for Fe and Mn analysis which may have been used as additional evidence for the significance of Mn oxides in sulfide oxidation.

From the measured production of organic C in the enrichment cultures  $\text{CO}_2$  fixation is demonstrated, requiring possibly autotrophic growth, but not precluding the mixotrophic incorporation of organic substrate during  $S^{\circ}$ -disproportionation (Fig. 3d). The fact that enrichment cultures maintain active disproportionation after numerous transfers, diluting out completely any original organic substrate from the sediment inoculum, provides the argument for an autotrophic metabolism. The autotrophic or mixotrophic nature of  $S^{\circ}$  disproportionating organisms, however, requires further studies from pure cultures. It is clear, though, that transfer of electrons to  $\text{CO}_2$  instead of sulfide could explain the difference between the 1.5/1 production ratio of AVS (FeS) to  $\text{SO}_4^{2-}$  observed in our enrichment cultures, and the ratio of 2/1 predicted by Eq. 1. Using an oxidation level of zero for the fixed C, the following equation predicts the amount of organic C production necessary to explain the observed AVS to sulfate production ratio:



In Fig. 3d the production of organic carbon is predicted from the sulfate accumulation (Fig. 3a) data and Eq. 5, and is compared to the measured values. The good agreement shows that the fixation of  $\text{CO}_2$

to cell biomass has altered the strictly inorganic reaction stoichiometry of Eq. 1 by a predictable amount. Also, the production of organic C as shown in Eq. 5, when calculated per mole S disproportionated, compares with the growth yields reported for the disproportionation of  $S_2O_3^{2-}$  and  $SO_3^{2-}$  by *Desulfovibrio sulfodismutans* [10]. Due to the rather high background of organic C in the sediment, a similar C-fixation would not be detectable in the bag incubations, though, the rather low ratio of AVS to sulfate accumulation could be explained by a similar autotrophic or mixotrophic production of cell biomass.

Results from enrichment experiments demonstrated the potential for  $S^0$  disproportionation in Wedderwarden sediment, while the  $S^0$ -amended sediment bag incubations showed  $S^0$  disproportionation in the presence of a 'normal' diverse and active anaerobic sediment bacterial community. Further, the ratio of AVS to  $SO_4^{2-}$  produced in the  $S^0$ -amended bags (1.75/1; see above), indicates that  $S^0$  disproportionation was the main  $S^0$  metabolism. It is significant that  $S^0$  reduction was apparently not an important pathway of  $S^0$  metabolism since the potential for this metabolism in marine sediments has been demonstrated [8]. One could argue that our experimental design would not optimize for the metabolism of  $S^0$ -reducing bacteria as natural rates of organic matter hydrolysis and fermentation may not deliver enough organic substrate to fuel significant reduction of the large amount of added  $S^0$ . It is also clear, however, that  $S^0$ -reducing organisms did not take any obvious advantage of the added  $S^0$ . This argument is made from the observation that sulfate reduction rates in the  $S^0$ -amended sediment remained the same over time, even as rates of  $S^0$  metabolism accelerated, and from the fact that rates of sulfate reduction were the same in the control and  $S^0$ -amended sediments (Table 2). This means that sulfate-reducing bacteria experienced no enhanced competition for organic substrate in the presence of the active  $S^0$  metabolism.

A partial explanation of the dominance of  $S^0$ -disproportionation is that, from pure cultures studies,  $S^0$ -reducing bacteria are dependent on the solubilization of  $S^0$  as polysulfides and, hence, on the presence of free  $H_2S$  [30,31]. This dependency may be a significant hindrance for the growth of these bacteria

in environments rich in  $H_2S$ -scavenging Fe, such as the sediment studied here, where it should give the  $S^0$ -disproportionating bacteria an advantage in the competition for  $S^0$ .

#### 4.2. Formation of pyrite

In an earlier report [15] considerable formation of pyrite was observed during the disproportionation of  $S^0$ ; in some cases pyrite was the main Fe-sulfide end-product. A bacterial mediation of pyrite formation is a potentially very significant finding since pyrite is the main authigenic mineral found in marine sediments and its formation mechanisms are not well known (e.g. [32]). Our results corroborate the earlier findings [15] in that we observed pyrite formation directly in our enrichment incubations (Fig. 3b). Some pyrite may also have formed in our control sediment incubations (see above), but we observed no quantitatively important formation of pyrite in our  $S^0$ -amended sediment incubations (Fig. 2b), and pyrite was not a demonstrably significant end product in any of our incubations (Figs. 1–3). After further transfers of the enrichment culture, however, a dense grey precipitate similar to the pyrite previously described [15] did form during the incubation. This suggests an inhibitory effect on pyrite formation of the sediment matrix present in the initial inoculum and points to some pyrite formation pathway, perhaps microbial, that has yet to be elucidated.

### 5. Conclusions

The large number of inorganic and biologically mediated oxidative processes that give rise to  $S^0$ , indicate a significant role for  $S^0$  in sediments (Table 1). Of interest, then, is the fate of  $S^0$  once formed, and the possibilities include oxidation, reduction, and disproportionation (Table 1). We suggest that the fate of  $S^0$  will likely depend on where it is found in a sediment. In a speculative assessment, it seems that in environments rich in  $O_2$ ,  $NO_3^-$ , or maybe  $MnO_2$ , oxidation may be most likely. When  $S^0$  contacts high concentrations of sulfide, such as daily in a microbial mat [33] reduction may dominate.

Maximum concentrations of  $S^0$ , are, however, normally observed in the sediment region between

the thin oxic surface layer and the depth of H<sub>2</sub>S accumulation [16,34]. In coastal sediments NO<sub>3</sub><sup>-</sup> and Mn oxides are also frequently excluded from this zone [19] and it is these sediment circumstances that our experimental system was designed to reproduce. Hence, abundant iron oxides (Table 1) were present to buffer sulfide to low levels. Oxygen was excluded by enclosing the sediment in gas impermeable bags, and nitrate, if originally present, would have been quickly depleted at the beginning of the incubation. Under these conditions S<sup>0</sup> disproportionation was the main metabolism of S<sup>0</sup>. Also, the disproportionation was accompanied by CO<sub>2</sub>-fixation, and did not apparently interfere with the activity of the heterotrophic sediment bacterial community.

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