Acetate, lactate, propionate, and isobutyrate as electron donors for iron and sulfate reduction in Arctic marine sediments, Svalbard

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Received 25 January 2006; revised 17 July 2006; accepted 10 August 2006.
First published online 27 October 2006.

DOI:10.1111/j.1574-6941.2006.00214.x

Editor: Alfons Stams

Keywords
volatile fatty acids; potential selenate reduction; inhibition; acetate turnover.

Abstract

The contribution of volatile fatty acids (VFA) as e^--donors for anaerobic terminal oxidation of organic carbon through iron and sulfate reduction was studied in Arctic fjord sediment. Dissolved inorganic carbon, Fe^{2+}, VFA concentrations, and sulfate reduction were monitored in slurries from the oxidized (0–2 cm) and the reduced (5–9 cm) zone. In the 0–2 cm layer, 2/3 of the mineralization could be attributed to sulfate reduction and 1/3 to iron reduction. In the 5–9 cm layer, sulfate reduction was the sole mineralization process. Acetate and lactate turnover rates were measured by radiotracer. Inhibition of sulfate reduction with selenate resulted in the accumulation of acetate, propionate, and isobutyrate. The acetate turnover rates determined by radiotracer and accumulation after inhibition were similar. VFA turnover accounted for 21% and 52% of the mineralization through sulfate reduction in the 0–2 and 5–9 cm layer, respectively. Acetate and lactate turnover in the inhibited 0–2 cm slurry was attributed to iron reduction and accounted for 10% and 2% of the iron reduction. Therefore, 88% and 79% of the iron and sulfate reduction in the 0–2 cm layer, respectively, must be fueled by alternative e^--donors. The accumulation of VFA in the selenate–inhibited 0–2 cm slurry did not enhance iron reduction, indicating that iron reducers were not limited by VFA availability.

Introduction

Anaerobic degradation of complex organic material in aquatic systems is a multi-step process involving a large diversity of physiologically specialized microorganisms (e.g. Blackburn, 1987; Capone & Kiene, 1988). The metabolic products of fermentative bacteria serve as electron donors for the terminal oxidizing bacteria that use inorganic electron acceptors for the complete oxidation of the organic matter. In marine sediments, iron reduction and sulfate reduction are generally the most important terminal oxidation processes in the upper anoxic zone (Thamdrup, 2000).

Microorganisms that reduce iron and sulfate may use a broad range of electron donors, yet the list of potential substrates provides little information about the substrates used *in situ* by these organisms. The substrates used by sulfate-reducing bacteria in marine sediments have been determined mainly by two methods: the substrate turnover has been measured using radiolabeled substrates (Christensen & Blackburn, 1982; Shaw *et al.*, 1984; Sansone, 1986; Shaw & McIntosh, 1990; Wellsbury & Parkes, 1995) or the accumulation of substrates has been measured after inhibition of the sulfate reduction by molybdate (Sørensen *et al.*, 1981; Parkes *et al.*, 1989; Shaw & McIntosh, 1990; Fukui *et al.*, 1997). These investigations have shown that volatile fatty acids (VFA), and in particular acetate, together with hydrogen are the major substrates for sulfate reduction. Similar investigations for iron reduction or simultaneous iron and sulfate reduction are lacking for marine sediments. Furthermore, most of these studies were done in temperate sediments and little is known about the substrates for sulfate reducers in permanently cold sediments, which account for $>$ 90% of the ocean floor (Levitus & Boyer, 1994).

Molybdate is a commonly used inhibitor of sulfate reduction. Unfortunately, it complexes VFA (Rosenheim, 1893; Finke, 1999) and thus prevents their determination by the common HPLC technique based on 2-nitrophenyl hydrazine derivatization (Mueller Harvey & Parkes, 1987; Albert & Martens, 1997). As an alternative to molybdate, selenate can be used as a specific inhibitor of sulfate reduction (Oremland & Capone, 1988) and allows subsequent derivatization with 2-nitrophenyl hydrazine (Finke, 1999).

We investigated the relative contributions of iron reduction and sulfate reduction to the terminal oxidation of

organic carbon in permanently cold Arctic sediments. More specifically, we combined VFA turnover measurements using radiotracer incubations with sulfate reduction inhibition studies using selenate to determine the importance of acetate, lactate, propionate, and isobutyrate as electron donors for iron and sulfate reduction. To our knowledge, this is the first study of the contribution of VFA as substrates for iron-reducing bacteria in marine sediments.

Materials and methods

Sediment

Sediment samples were taken with a HAPS corer at Station J (79°42.006N 11°05.199E) in Smeerenburgfjorden on the northwest coast of Svalbard, northern Barents Sea, in August 2004. The *in situ* temperature was 2.3 °C and the water depth 212 m. The sediment and its microbiology are described in further detail in Vandieken *et al.* (2006) and Arnosti *et al.* (2005).

Incubations

Sediment from each of the two depth intervals, 0–2 cm and 5–9 cm, was mixed with an equal amount of anoxic seawater, homogenized under N_2, filled in two glass bottles, and sealed with butyl stoppers. A sodium selenate solution was added to one parallel for each depth ('0–2 cm Se' and '5–9 cm Se') to a final concentration of 5 mM. In our previous experiments with Svalbard sediments, this concentration had proven sufficient to completely inhibit sulfate reduction. The slurries were incubated at 0 °C for 28 days. At 12 time points between 1 h and 28 days after start of the incubation, subsamples were taken for sulfate reduction rate (SRR) measurement and pore water sampling.

Pore water analyses

Pore water for the determination of Fe^{2+}, Mn^{2+}, Ca^{2+}, volatile fatty acids sulfide, sulfate, selenate, selenite, and dissolved inorganic carbon (DIC) was obtained by centrifuging sediment samples in glass centrifuge tubes without headspace for 10 min at 2500 ***g*** at 4 °C. Pore water for volatile fatty acids (VFA) analysis was obtained by centrifugation in Spinex © (Phenomenex) filter units at 2500 ***g*** at 4 °C for 10 min.

DIC was analyzed by flow injection with conductivity detection (Hall & Aller, 1992). Fe^{2+} was measured spectrophotometrically (Shimadzu UV 1202) at 562 nm with Ferrozine (1 g L^{-1} in 50 mM HEPES buffer, pH 7) according to Stookey (1970). Mn^{2+} and Ca^{2+} were measured by inductively coupled plasma atomic emission spectrometry (Perkin Elmer Optima 3300 RL). Sulfate was measured by non-suppressed ion chromatography (Waters, column IC-Pak™, 50 × 4.6 mm) (Ferdelman *et al.*, 1997). Sulfide was determined by the methylene blue spectrophotometric method (Shimadzu, UV 1202) at 670 nm (detection limit 1 μM) (Cline, 1969). To determine the selenate reduction rate as a result of selenate addition, selenate and selenite concentrations were analyzed by anion chromatography (Dionex DX500, eluent: 9 mM $NaCO_3$, precolumn: AG9 HC, column: AS9 HC). The detection limit was 0.2 μM for both selenate and selenite. Volatile fatty acids were measured after derivatization with 2-nitrophenyl hydrazine by absorbance at 400 nm on an HPLC (Albert & Martens, 1997), mostly in single samples but with duplicate determinations at selected time points. VFA measured with this method comprise acetate, propionate, lactate, and isobutyrate.

From the mean concentration change over time, production and consumption rates for DIC, iron and selenate were calculated using a regression line. The standard deviation was calculated as deviation from the linear regression over time.

Sulfate reduction rates

At each time point, duplicate subsamples of the slurries were incubated with 50 kBq ^{35}S-sulfate. After 6 h the incubations were stopped with 20% ZnAc and frozen. The ^{35}S-labeled reduced sulfur fraction was extracted using the cold chromium distillation method (Kallmeyer *et al.*, 2004) in single samples for most time points. SRR were calculated as described by Jørgensen (1978).

Volatile fatty acid turnover rates

After 1, 4, 8, and 14 days of incubation, subsamples of the slurries were taken to measure VFA turnover rates. Approximately 10 mL of slurry was filled into N_2 flushed syringes. Tracer solutions were prepared in sterile filtered, anoxic pore water at least 1 h before incubation. $^{14}C_2$-acetate or $^{14}C_u$-lactate 300 kBq was injected into triplicate syringes for each slurry. After 10, 20, 30, 40, 50, and 60 min, *c.* 1.5 mL of the sample was withdrawn into 5 mL 2% NaOH. This served to stop the reaction and fix the ^{14}C-DIC produced. Blank samples were prepared by addition of the tracer to the NaOH before addition of the sediment. ^{14}C-TIC and ^{14}C-acetate/lactate were separated by the shaker method (Joye *et al.*, 2004). In brief, 6 M HCl was added to the sample to drive out the DIC as CO_2, which was trapped in phenylethylamine/NaOH. The trapped ^{14}C-DIC and the remaining ^{14}C in the sample were measured by scintillation counting. The turnover rate constant was determined as the slope of the fraction of tracer turned over per time. Multiplication with the measured concentration yielded the turnover rate for each organic acid.

Acetate, propionate, and isobutyrate oxidation rates coupled to sulfate reduction were calculated for the uninhibited slurries from the accumulation of the fatty acid after inhibition of sulfate reduction with selenate. The accumulation rates in the uninhibited slurries were subtracted from the rates in the inhibited slurries, thus showing the

oxidization rate by sulfate reduction in the uninhibited slurries. The standard deviation was calculated as the deviation from the linear regression over time.

Results

Pore water chemistry

Dissolved inorganic carbon, Ca^{2+}, Fe^{2+}, Mn^{2+}, sulfate, and sulfide pore water concentrations were measured in anoxic sediment slurries from 0–2 and 5–9 cm depths (uninhibited slurries) at 12 sampling points over 28 days of incubation. These parameters were also measured in parallel incubations where 5 mM selenate was added to inhibit sulfate reduction (inhibited slurries). In these incubations, pore water concentrations of selenate and selenite were also determined. Example plots for DIC, Fe^{2+}, and Mn^{2+} are shown in Fig. 1 and for selenate and selenite in Fig. 2. DIC concentrations increased during the incubation in all slurries (Fig. 1a). Pore water Ca^{2+} concentrations were constant in all samples, which indicated that carbonate precipitation did not take place during the experiments (data not shown). Fe^{2+} accumulated in the pore water throughout the incubation (Fig. 1b) with rates of 7 nmol cm^{-3} day^{-1} for the 0–2 cm slurry and 0.5 nmol cm^{-3} day^{-1} for the 5–9 cm slurry. There were no significant differences between the inhibited and uninhibited slurries. Mn^{2+} concentrations stayed constant over the course of the incubation in all slurries (Fig. 1b). Sulfate concentrations did not change over time in all slurries, sulfide concentrations stayed below detection limit throughout the incubation (data not shown). The added selenate in both inhibited slurries decreased in concentration over the course of the incubation (Fig. 2). Selenite, a potential product of microbial selenate reduction (Oremland & Stolz, 2000), was only detectable after 20 days, reaching 4.3 μM at the end in the 5–9 cm inhibited slurry (Fig. 2). No selenite was detected throughout the experiment in the 0–2 cm inhibited slurry.

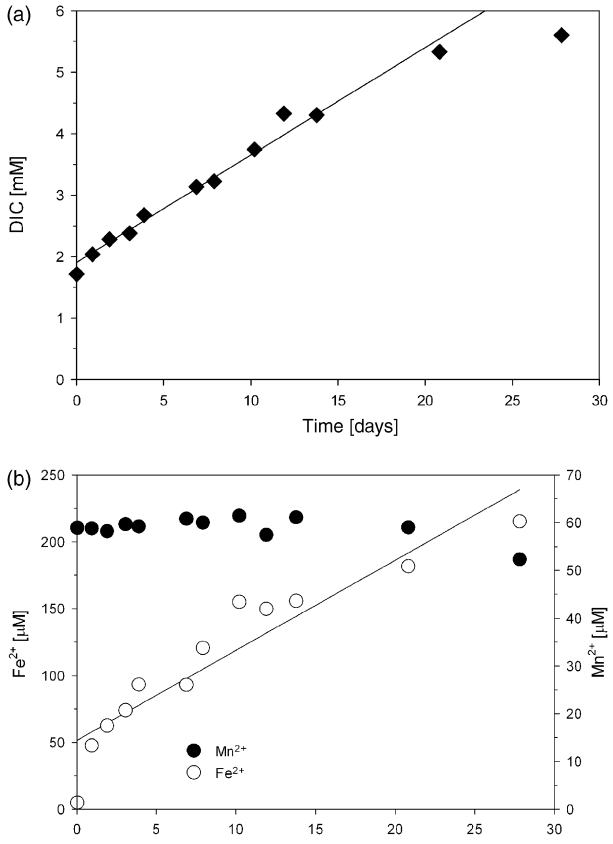

Fig. 1. Time course of DIC, Fe^{2+} and Mn^{2+} concentrations in the pore water of slurry from the 0–2 cm depth interval without selenate inhibition. Production rates were calculated from the time course as indicated by the regression line.

Sulfate reduction rates

Sulfate reduction rates in the 0–2 cm slurry varied between 48 and 70 nmol cm^{-3} day^{-1} with rather constant rates after an initial increase (Fig. 3). In the 5–9 cm slurry the sulfate reduction rates were between 18 and 31 nmol cm^{-3} day^{-1} with increased rates of 62–70 nmol cm^{-3} day^{-1} on days 1–4 (Fig. 3). Addition of ~5 mM selenate inhibited sulfate reduction rates to below the detection limit of 2 nmol cm^{-3} day^{-1} in both depth intervals (data not shown). At selected time points the sulfate reduction rates were determined in duplicate samples. The second determination resulted in rates 5–10% different from the original measurement (data not shown).

Volatile fatty acids

Volatile fatty acids concentrations are shown as μM C in Fig. 4. Acetate occurred in the highest concentrations in all

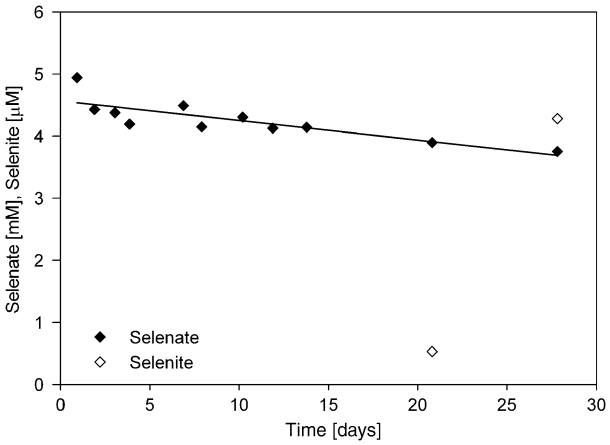

Fig. 2. Time course of selenate and selenite concentrations in the pore water of selenate–amended slurry from the 5–9 cm depth interval. Selenate reduction rates were calculated from the time course as indicated by the regression line.

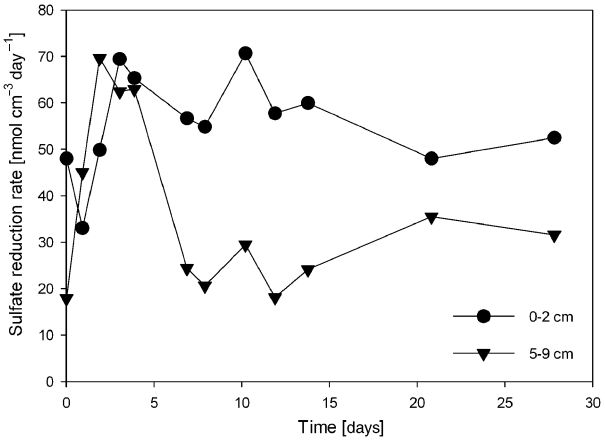

Fig. 3. Sulfate reduction rates measured in the uninhibited slurries from 0–2 cm and 5–9 cm. In the selenate amended slurries the rates were below the detection limit of 2 nmol cm^{-3} d^{-1} (data not shown).

samples, followed by propionate, lactate, and isobutyrate (data not shown). Lactate concentrations remained around 1–2 μM throughout the incubation. In the uninhibited 0–2 cm slurry the acetate concentrations increased from 45 to 78 μM after 2 days and showed a second transient increase with maximum concentrations after 12 days (Fig. 4). The concentrations decreased to 3–8 μM towards the end of the incubation. In the 5–9 cm uninhibited slurry the acetate concentrations increased from 45 to 93 μM after 3 days followed by a decrease to 3–8 μM. Propionate increased from 0.8 to 3 μM after 2 and 3 days in the 0–2 and 5–9 cm slurry, respectively, and decreased again to values around 1 μM. Isobutyrate concentrations remained around 0.5 μM throughout the incubation in both uninhibited slurries (data not shown).

In the 0–2 cm inhibited slurry, the VFA concentrations increased throughout the entire incubation, reaching 680, 60, and 22 μM for acetate, propionate, and isobutyrate, respectively, after 28 days. In the 5–9 cm inhibited slurry, VFA concentrations increased for the first 12 days, reaching 335, 18, and 7.5 μM for acetate, propionate, and isobutyrate, respectively, and decreased towards the end of the incubation. Duplicate measurements at selected time points varied by about 5% to a maximum of 10% (data not shown).

The oxidation of acetate, propionate, and isobutyrate by sulfate reducers was determined from the accumulation in the inhibited slurries. Acetate and propionate accumulated at higher rates in the inhibited vs. the uninhibited slurries at the beginning of the incubation. The difference in the accumulation of acetate and propionate in the inhibited vs. the uninhibited slurry and the isobutyrate accumulation rate in the inhibited slurries was attributed to sulfate reduction (Table 1). VFA accumulation rates were highest for acetate followed by propionate and isobutyrate.

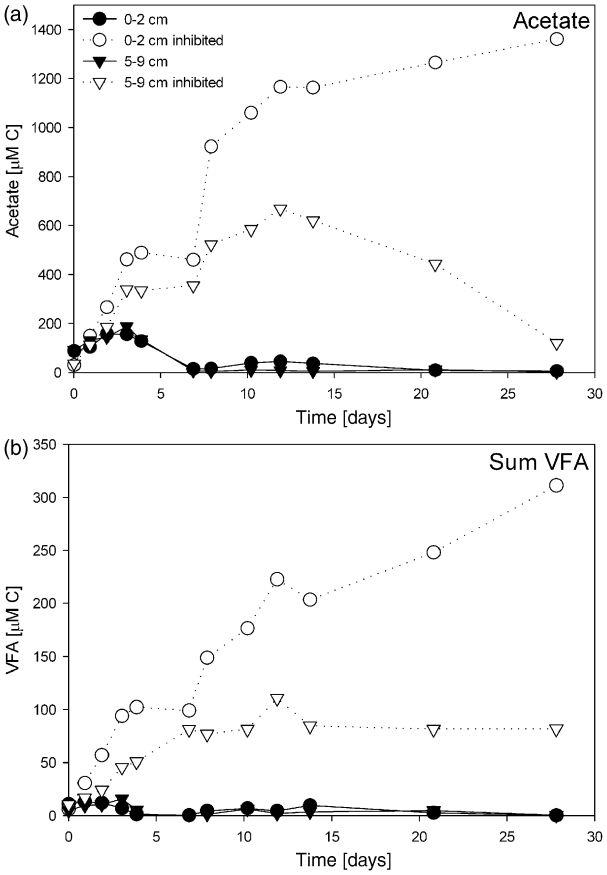

Fig. 4. Volatile fatty acids (VFA) concentrations measured in the pore water of the four slurries. Concentrations are shown in μM C. (a) shows acetate concentrations and (b) the sum of lactate, propionate, isobutyrate.

In the ^{14}C-tracer incubations the rate constants for the turnover of the acetate and lactate pools were determined (Table 2). The constants for lactate were generally 3–15-fold higher than for acetate, but due to higher concentrations of acetate compared to lactate the turnover rates of acetate were 1.5–9-fold higher in the uninhibited slurries and 3–120-fold in the inhibited slurries than for lactate (Fig. 5). The acetate turnover rates were higher in the uninhibited than in the inhibited slurries during the first 1- and 4-day incubations but were similar or highest in the inhibited slurries during the following 8- and 14-day incubations. The lactate turnover rates were 2–10-fold higher in the uninhibited compared to the inhibited slurries throughout the experiment.

Contribution of different electron acceptors to dissolved inorganic carbon production

The anaerobic carbon mineralization during the incubations was determined by measurement of DIC production. The DIC production rates in the inhibited slurries were

Table 1. Acetate, lactate, propionate, and isobutyrate as substrates for sulfate reduction. Turnover rates as measured at the beginning of the incubation

	Acetate		Lactate	Propionate	Isobutyrate	
	Tracer*	Inhibition	Tracer*	Inhibition	Inhibition	Sum†
VFA turnover ($nmol\,cm^{-3}\,day^{-1}$)						
0–2 cm	6.1 ± 2.7	6.9 ± 1.1	3.4 ± 0.6	1.1 ± 0.2	0.42 ± 0.02	
5–9 cm	15.1 ± 1.9	18.9 ± 1.9	1.1 ± 0.3	1.7 ± 0.1	0.19 ± 0.02	
Contribution to sulfate reduction (%)‡						
0–2 cm	10 ± 4	12 ± 2	5.8 ± 0.9	3.3 ± 0.5	1.8 ± 0.1	20.9 ± 5.5
5–9 cm	40 ± 5	50 ± 5	2.9 ± 0.8	7.9 ± 0.3	1.3 ± 0.1	52.2 ± 6.2

*Turnover attributed to sulfate reduction calculated as difference in rates measured in inhibited and uninhibited slurries.

†Acetate contribution taken from tracer incubation.

‡Contribution of turnover of the acids to sulfate reduction based on a stoichiometry of organic acid : sulfate of 1 : 1 for acetate, 1 : 1.25 for lactate, 1 : 1.75 for propionate, and 1 : 2.5 for isobutyrate assuming complete oxidation of the acid to DIC. Sulfate reduction rates were taken from Table 3.

Rates were measured with radiotracer for acetate and lactate and from accumulation after selenate inhibition for acetate, propionate and isobutyrate. Contribution to sulfate reduction was calculated based on the stoichiometry given above. Mean and standard deviation of triplicate determinations for the tracer incubations. Standard deviation of the inhibition experiment based on the deviation of the concentration time course from a straight regression line.

approximately half those in the uninhibited slurries (Fig. 6; Table 3). Their contribution to the oxidation of organic carbon and, thus, to DIC production were calculated from the mean sulfate reduction rate and selenate reduction rate according to the reactions given in Table 4. Sulfate reduction accounted for 76% and 96% of the DIC production in the 0–2 and 5–9 cm slurries, respectively.

Sulfate reduction was inhibited by selenate addition and part of the DIC production was attributed to microbial carbon oxidation coupled to selenate reduction. In sediments, selenate is usually reduced to selenite or elemental selenium (Se°) (Majers *et al.*, 1988; Steinberg & Oremland, 1990; Oremland *et al.*, 1994a; Stolz & Oremland, 1999; Herbel *et al.*, 2000; Lucas & Hollibaugh, 2001; Knight *et al.*, 2002). As selenite accumulated only towards the end of the incubation at very low rates, reduction to Se° was assumed for the calculation of the contribution of selenate reduction to DIC production (Table 3). In the 0–2 cm inhibited slurry, selenate reduction accounted for 50% of the DIC production. In the 5–9 cm inhibited slurry, the selenate reduction exceeded the DIC production (Table 3), but the difference was not larger than the uncertainty of the rates.

The DIC production not accounted for by sulfate or selenate reduction was attributed to microbial iron reduction according to the stoichiometry given in Table 4 (Canfield *et al.*, 1993). The uncertainty of the iron reduction was calculated as the sum of the uncertainties of the DIC production and sulfate or selenate reduction. In the 0–2 cm uninhibited slurry the calculated microbial iron reduction rate was $60\,nmol\,C\,cm^{-3}\,day^{-1}$ and iron reduction accounted for 34% of the DIC production. In the 0–2 cm inhibited slurry the rate of DIC production attributed to iron reduction was similar to the uninhibited slurry, $41\,nmol\,C\,cm^{-3}\,day^{-1}$ (Table 3). The uncertainty was larger than the difference between the rates in the two slurries. Thus, the inhibition of sulfate reduction did not change the

Table 2. Turnover rate constants (h^{-1}) for acetate and lactate as measured in the radiotracer incubations

	Day 1	Day 4	Day 8	Day 14
Acetate				
0–2 cm	0.009 ± 0.002	0.015 ± 0.002	0.015 ± 0.005	0.015 ± 0.003
0–2 cm inhibited	0.0013 ± 0.0006	0.0013 ± 0.0005	0.0010 ± 0.0001	0.0013 ± 0.0012
5–9 cm	0.012 ± 0.001	0.0093 ± 0.0019	0.030 ± 0.003	0.063 ± 0.016
5–9 cm inhibited	0.00085 ± 0.00007	0.0017 ± 0.001	0.00077 ± 0.00026	0.0013 ± 0.0005
Lactate				
0–2 cm	0.25 ± 0.04	0.17 ± 0.020	0.21 ± 0.040	0.32 ± 0.15
0–2 cm inhibited	0.028 ± 0.004	0.051 ± 0.009	0.031 ± 0.012	0.031 ± 0.001
5–9 cm	0.072 ± 0.005	0.12 ± 0.03	0.073 ± 0.015	0.22 ± 0.15
5–9 cm inhibited	0.020 ± 0.008	0.038 ± 0.017	0.012 ± 0.004	0.038 ± 0.016

Mean and standard deviations of three parallel determinations

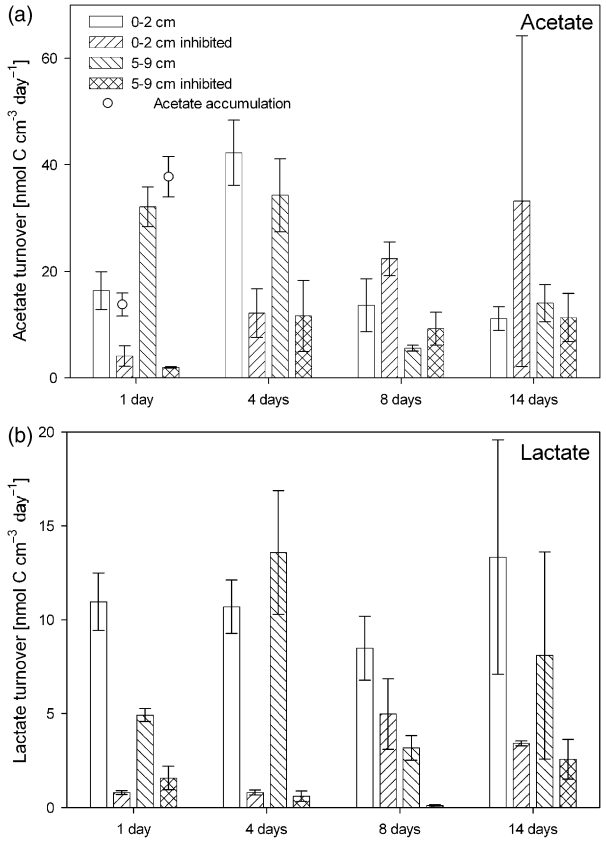

Fig. 5. ^{14}C-acetate and ^{14}C-lactate turnover rates measured in the 0–2 cm, 0–2 cm Se, 5–9 cm, and 5–9 cm Se slurries. The rates are given in mol C produced during substrate oxidation. The open circles in the acetate turnover graph represent the rates calculated from the difference in the acetate accumulation in the inhibited compared to the uninhibited slurries. Mean and standard deviations from triplicate incubations.

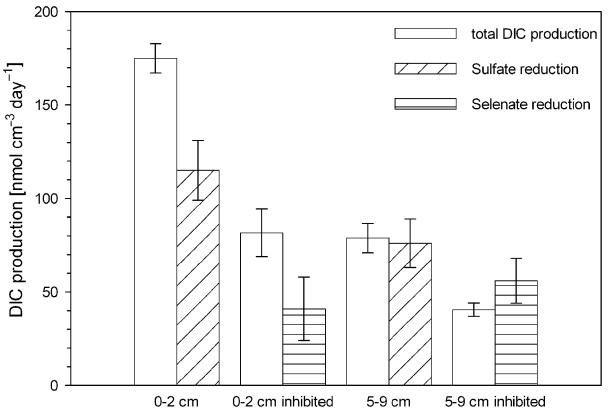

Fig. 6. Dissolved inorganic carbon (DIC) production in the 0–2 cm, 0–2 cm Se, 5–9 cm, and 5–9 cm Se slurries. Actual dissolved inorganic carbon production as calculated from dissolved inorganic carbon accumulation and theoretical production as calculated from sulfate reduction and selenate reduction according to the reactions shown in Table 4. Standard deviations are based on the deviation of the concentration time course from a linear regression.

iron reduction within the accuracy of our method. This was supported by similar rates of Fe^{2+} accumulation in the pore water in both incubations. In the uninhibited 5–9 cm slurry the calculated iron reduction rates of 3 nmol cm^{-3} day^{-1} were less than the sum of the uncertainties of the sulfate reduction and DIC production. In agreement with the fact that no HCl-extractable Fe(III) was available in this sediment horizon (Vandieken *et al.*, 2006), we concluded that sulfate reduction was the sole electron accepting process.

Discussion

Mineralization of organic matter

Anaerobic bacteria performing the terminal oxidation of organic carbon to CO_2 in marine sediments rely on hydrolytic and fermentative bacteria to degrade complex polymers into small organic molecules. The most important electron acceptors below the oxic zone in coastal sediments are iron oxides and sulfate (Thamdrup, 2000). In a range of marine environments from temperate to permanently cold sediments, microbial iron reduction accounted for 0–75% of anaerobic carbon oxidation (Sørensen, 1982; Canfield & Marais, 1993; Thamdrup & Canfield, 1996; Kostka *et al.*, 1999, 2002; Glud *et al.*, 2000; Kristensen *et al.*, 2000; Thamdrup, 2000; Jensen *et al.*, 2003). Comparison of rates measured at different *in situ* temperatures indicates that temperature does not control the relative importance of iron and sulfate reduction (Thamdrup, 2000). The concentration of poorly crystalline iron oxides seems to be important for the competition between iron and sulfate reducers and, thus, determines the relative importance of these two processes (Thamdrup, 2000; Jensen *et al.*, 2003; Vandieken, 2005). As a result, the vertical separation between these oxidation processes is usually not complete and microbial iron reduction often co-occurs with sulfate reduction (Vandieken *et al.*, 2006; Sørensen, 1982; Canfield *et al.*, 1993; Thamdrup & Canfield, 1996; Kristensen *et al.*, 2000; Kostka *et al.*, 2002). HCl-extractions of Smeerenburgfjorden sediment showed reactive Fe(III) in the top 2 cm (Vandieken *et al.*, 2006), resulting in concurrent iron and sulfate reduction in the 0–2 cm layer (Table 3). In the 5–9 cm layer sulfate reduction was the sole detectable terminal oxidation step. The rates of microbial iron reduction and sulfate reduction in this study (Table 3) were similar to rates determined previously for this sediment (Vandieken *et al.*, 2006).

Selenate reduction

Selenate reduction potential was previously detected in sediments ranging from polluted to pristine sites by

Table 3. Dissolved inorganic carbon production in the 0–2 cm, 0–2 cm inhibited (with selenate addition), 5–9 cm, and 5–9 cm inhibited (with selenate addition) slurries

	Total DIC production	Sulfate reduction	Selenate reduction	Calculated iron reduction		Iron accumulation
0–2 cm	175 ± 8	115 ± 16	–	60 ± 24	34%*	1.7 ± 0.2
0–2 cm inhibited	82 ± 13	< 2	41 ± 17	41 ± 30	50%*	1.9 ± 0.1
5–9 cm	79 ± 8	76 ± 13	–	3 ± 21	0% †	0.16 ± 0.03
5–9 cm inhibited	41 ± 4	< 2	56 ± 12	0	0%	0.11 ± 0.09

*Relative contribution of iron reduction to DIC production.

†Contribution set to 0% as the uncertainty is larger than the calculated rate.

DIC, dissolved inorganic carbon.

Contribution of the different electron accepting processes to the production of dissolved inorganic matter was calculated on a C-molar basis (nmol C cm^{-3} day^{-1}) according to the reactions shown in Table 4. The values represent a mean of the 28 days of incubation. Standard deviations are based on the deviation of the concentration time course from a linear regression line

Table 4. Terminal oxidation reactions of organic carbon and acetate using different electron acceptors

Electron donor	Electron acceptor	Reaction	Stoichiometry Electron donor:acceptor
Organic carbon	Sulfate	$2CH_2O+SO_4^{2-} \rightarrow 2HCO_3^-+HS^-+H^+$	2 : 1
Organic carbon	Iron(III)	$CH_2O+4Fe(OH)_3 \rightarrow HCO_3^-+4Fe^{2+}+3H_2O+7OH^-$	1 : 4
Organic carbon	Selenate	$3CH_2O+2SeO_4^{2-} \rightarrow 3HCO_3^-+2Se^\circ+H^++2OH^-$	3 : 2
Acetate	Sulfate	$CH_3COO^-+SO_4^{2-} \rightarrow 2HCO_3^-+HS^-$	1 : 1
Acetate	Iron	$CH_3COO^-+8Fe(OH)_3 \rightarrow 2HCO_3^-+8Fe^{2+}+5H_2O+15OH^-$	1 : 8

Organic carbon is represented as CH_2O with an oxidation state of 0 for carbon

following the reduction of added selenate (Steinberg & Oremland, 1990; Lucas & Hollibaugh, 2001). The solid phase selenium concentrations in Smeerenburgfjorden sediments were below our detection limit of 16 ppb (M. Isenbeck-Schröter, unpublished data). Selenate can be microbially reduced to selenite, Se°, or selenide (Stolz & Oremland, 1999; Lloyd *et al.*, 2001). In bacterial cultures and in aquatic sediments selenate is usually reduced to selenite or Se° (Majers *et al.*, 1988; Steinberg & Oremland, 1990; Oremland *et al.*, 1994a; Stolz & Oremland, 1999; Herbel *et al.*, 2000; Lucas & Hollibaugh, 2001; Knight *et al.*, 2002). Selenite accumulated only towards the end of our incubation in the 5–9 cm slurry (Fig. 2). Thus, we assume that organic matter was mainly coupled to the reduction of selenate to Se° in both inhibited slurries according to the reaction given in Table 4. The potential selenate reduction rates of 27 and 37 nmol cm^{-3} day^{-1} in the 0–2 and 5–9 cm layer, respectively, lie within the reported range of potential selenate reduction rates in pristine and contaminated sediments of 1.7 to 530 nmol cm^{-3} day^{-1} (Steinberg & Oremland, 1990; Lucas & Hollibaugh, 2001).

By comparing the DIC production in the uninhibited and inhibited slurries, the major effects of selenate addition seemed to be inhibition of sulfate reduction and initiation of selenate reduction. The time course of Fe^{2+} and Mn^{2+} concentrations were the same in the inhibited and uninhibited slurries. Therefore, a general toxic effect of the added high selenate concentrations to the microbial community was not evident.

Volatile fatty acids

Acetate turnover measurements

The importance of acetate as substrate for sulfate-reducing bacteria in marine sediments has been investigated previously using radiotracer and molybdate inhibition experiments (Ansbaek & Blackburn, 1980; Sansone & Martens, 1981; Christensen & Blackburn, 1982; Winfrey & Ward, 1983; Christensen, 1984; Shaw *et al.*, 1984; Shaw & McIntosh, 1990). To evaluate the contribution of acetate as an electron donor for sulfate reduction, radiotracer incubations were performed in sediments with sulfate being the sole terminal oxidation process. These measurements often lead to a discrepancy between sulfate reduction and acetate turnover rates with measured acetate turnover rates exceeding sulfate reduction rates or total mineralization based on DIC or NH_4^+ production (Ansbaek & Blackburn, 1980; Sansone & Martens, 1981; Christensen & Blackburn, 1982; Shaw *et al.*, 1984; Wu & Scranton, 1994). In contrast, molybdate inhibition experiments gave acetate turnover rates (calculated from the accumulation of acetate) that were lower than sulfate reduction rates or total mineralization (Sørensen *et al.*, 1981; Winfrey & Ward, 1983;

Christensen, 1984; Shaw & McIntosh, 1990). The higher acetate turnover rates measured in the radiotracer studies probably were a result of an overestimation of the bioavailable acetate pool. It was suggest that some of the pore water acetate was complexed and less bioavailable, whereas the tracer was added in a free form and thus was readily taken up by the microorganisms (e.g. Shaw & McIntosh, 1990; Kristensen *et al.*, 1994). Christensen & Blackburn (1982) reported decreasing acetate turnover rate constants for incubation times of more than 10 min, probably due to partitioning of the tracer in the different acetate pools. The rates determined with shorter incubation times, however, exceeded total mineralization based on NH_4^+-production by five-fold.

In the present study, the tracer solution was prepared in sterile-filtered, anoxic pore water at least 1 h before injection. Thus, we assume that the tracer should be complexed just as the acetate in the pore water, and the turnover rate constants measured should reflect the turnover of the total acetate pool. From the oxidation rates determined with this method, acetate oxidation coupled to sulfate reduction can be calculated from the difference of the tracer turnover in the inhibited and uninhibited slurry (Fig. 5). The acetate turnover rates measured with the radiotracer were not significantly different from the accumulation rates in the inhibited slurries (Table 1) and accounted for 10% and 40% of the sulfate reduction in the 0–2 cm and 5–9 cm layer, respectively, based on the reactions shown in Table 4. This supports the suggestion that complexation of acetate was responsible for the overestimation of turnover rates in previous studies.

Volatile fatty acids as substrate for terminal oxidation

The inhibition experiment showed that acetate was the most important of the VFA as a substrate for sulfate reducers, as seen by the highest accumulation rate (Table 1). The only other VFA accumulating after inhibition were propionate and isobutyrate but at lower rates than acetate.

The turnover rates calculated from VFA accumulation are only appropriate if the selenate reducers did not oxidize these acids at similar rates. Isolated selenate-reducing organisms used acetate and lactate as electron donors in pure culture studies (Oremland *et al.*, 1994b, 1989; Stolz & Oremland, 1999; Oremland & Stolz, 2000). Addition of acetate to sediment slurries enhanced selenate reduction (Oremland *et al.*, 1989). In our experiment the lactate and acetate turnover was initially strongly reduced in the inhibited slurries (Fig. 5), indicating that selenate-reducing bacteria did not use acetate and lactate as their major substrates at the beginning of the incubation. The accumulation rates of propionate and isobutyrate were 7–10 and 17–90 times lower than for acetate, a similar ratio to that found in previous investigations (Table 5). However, due to the high variability in reported turnover rates it is difficult to evaluate whether the selenate reducers oxidized these acids.

Many sulfate reducers are known to utilize lactate (Rabus *et al.*, 2000). Surprisingly, lactate did not accumulate in the inhibited slurries, even though tracer incubations showed that the oxidation was strongly inhibited (Fig. 5). The fact that there is an almost complete inhibition of lactate turnover in the first tracer incubations in the inhibited slurries suggests that lactate served as substrate for sulfate reducers. Apparently, its production is strongly reduced in the inhibited slurry. Thus, we attribute the lactate turnover measured in the tracer incubation to sulfate reduction. Together, acetate, lactate, propionate, and isobutyrate accounted for 21% and 52 % of the sulfate reduction in the 0–2 cm and 5–9 cm slurries, respectively (Table 1).

Christensen (1984) reported a contribution of 65% for acetate and 5% and 8% for propionate and isobutyrate to sulfate reduction in Danish coastal sediments. (Table 5). Parkes & Jorck-Ramberg (1984) reported that VFA accounted for $>$ 75% of the substrates for sulfate reduction in temperate marine and estuarine sediments. In Smeerenburgfjorden sediment, acetate was the most important electron donor of the investigated VFA, followed by lactate and propionate, and finally isobutyrate (Table 1). In the 5–9 cm layer, which was dominated by sulfate reduction, the 42% contribution of acetate to sulfate reduction was similar to previous investigations using molybdate inhibition (Table 5). In the 0–2 cm zone, where sulfate reduction and iron reduction occurred simultaneously, acetate was much less important as an electron donor. Based on the stoichiometry given in Table 4, only 10% of the sulfate reduction could be attributed to acetate oxidation (Table 1).

Iron-reducing bacteria are very diverse and may use a wide range of electron donors (Coates *et al.*, 1996, 1998; Anderson *et al.*, 1998; Kashefi *et al.*, 2003). To date there are no investigations of substrates for iron reducers in marine sediments. In a previous study of substrates for iron reducers in a freshwater sediment, addition of ^{14}C-labeled glucose showed that acetate is the most important intermediate in glucose degradation (Lovley & Phillips, 1989). Roden & Wetzel (2003) identified acetate as important substrate for iron reducers in freshwater sediments.

The maximum contribution of acetate and lactate turnover to iron reduction can be calculated from the acid turnover not attributed to sulfate reduction. Acetate accumulation in the inhibited vs. the uninhibited slurry was the same as the turnover rates determined with radiotracer incubations (Table 1). This indicates that the selenate reducers were not important for acetate oxidation at the beginning of the incubation. The acetate turnover

Table 5. Compilation of acetate (Ac), lactate (La), propionate (Pro), and isobutyrate (iB) concentration and turnover measurements in sulfate-reducing marine sediments

Location	VFA concentration (μM)	Acetate turnover rate constants (h^{-1})	VFA oxidation rate (nmol cm^{-3} day^{-1})	Sulfate reduction (nmol cm^{-3} day^{-1})	Temperature (°C)	Technique	Reference
Mangrove, Thailand	Ac 0.5–31	0.96–5.8	33–1200	5–130	28/33	^{3}H tracer	Kristensen *et al*. (1994)
Cape Lookout Bight, USA	Ac 54–700* La 4–32* Pro 1–24* iB 0.2–6*	0.5–6.2	423–13000 65–1200 17–170	480–1200	6–28	^{14}C tracer	Sansone & Martens (1981, 1982), Sansone (1986), SRR: Martens & Klump (1980)
Loch Eil, Loch Etive, and Tay Estuary, Scotland	Ac 14.3–35.5 La 3.5–24 Pro 1.3–2.4 iB 0–0.02	0.006–0.22†	2.3–272‡ 0–155‡ 0–34‡ 2.9–8.8‡	1.2–1560	25	MoO_4^{2-} inhibition	Parkes *et al*. (1989)
Tamar Estuary, UK	Ac 2–20	0.27–0.69	20–250	15–40	25	^{14}C tracer	Wellsbury & Parkes (1995)
Coastal lagoon	Ac 5 Pro 1 iB 1	4.3†	480–650§ 72–170§ 29–58§	980–1300§	20	MoO_4^{2-} inhibition	Sørensen *et al*. (1981)
Ise Bay, Japan	Ac 30–140 Pro < 1–43 iB < 1–8.1	0.069–0.17†	200–363 13–16 0–9.7	50–210	~24¶	MoO_4^{2-} inhibition	Fukui *et al*. (1997)
Danish coastal waters	Ac 2–70	1–13	500–8500	(16% of VFA oxidation rate)	3.5/12	^{14}C tracer	Christensen & Blackburn (1982)
Skan Bay, Alaska Skan Bay Alaska	Ac 3.1–10.8 Ac 1.1–13.8	0.77–1.7† 1	57.6–400‡ 26–36	12–72‡ 50	4 4	^{14}C tracer ^{14}C tracer, MoO_4^{2-} inhibition	(Shaw *et al*. (1984) Shaw & McIntosh (1990)
Limfjorden, Denmark	Ac 0.1–6 Pro 0.04–0.19	1–4.8	24–288	(33% of VFA oxidation rate)	2/7	^{14}C tracer	Ansbaek & Blackburn (1980)
Coastal lagoon, Denmark	Ac 15–40	0.095–0.15†	30–77 Pro 3–8 iB 0.5–2	50–120	0	MoO_4^{2-} inhibition	Christensen (1984)
Smeerenburgfjorden, Svalbard (0–2 cm / 5–9 cm)	Ac 15–40 La 0.8–2.7 P 0.5–1.1 iB 0.11–0.24	0.009–0.012	8.2/16 1.6/3.7 1.1/1.7 0.42/0.19	58/38	0	^{14}C tracer, selenate inhibition	This study

VFA, volatile fatty acids.
*Concentrations given as nmol cm^{-3}.
†Calculated from turnover rate and concentration given.
‡Turnover given as μM day^{-1}.
§Turnover given as nmol g^{-1} day^{-1}.
¶Water temperature.

in the inhibited slurry was attributed to iron reduction. Lactate did not accumulate in the inhibited slurries, making it more difficult to evaluate the effect of the selenate addition on lactate turnover. However, attributing all lactate turnover in these slurries to iron reduction gave a maximal contribution of lactate to iron reduction. Altogether, only 10% and 2% of the iron reduction in the 0–2 cm layer could be attributed to acetate and lactate oxidation, respectively. If part of the acetate and lactate was oxidized by selenate reducers, the contribution would be even lower.

The increased acetate concentrations in the selenate inhibited slurry did not enhance the iron reduction (Table 3). If acetate was the dominant electron donor for the iron reducers and the iron reduction was electron donor limited, increasing the acetate concentration should stimulate iron reduction. The absence of a stimulation of the iron reduction after increase in VFA concentrations shows that the iron reducers used other electron donors or were not electron donor limited.

In conclusion, 88% and 79% of the iron and sulfate reduction, respectively, in the 0–2 cm layer must be driven

by electron donors other than the investigated VFA. Potential substrates for iron and sulfate reducers range from small to larger molecules, such as hydrogen, short alcohols and VFA, longer alcohols, and fatty acids (Rabus *et al.*, 2000; Lovley *et al.*, 2004), sugars (Coates *et al.*, 1998; Sass *et al.*, 2002; Kashefi *et al.*, 2003), amino acids (Stams *et al.*, 1985; Kashefi *et al.*, 2003), and aromatic (Widdel, 1980; Anderson *et al.*, 1998) and aliphatic hydrocarbons (Aeckersberg *et al.*, 1991). Parkes *et al.* (1989) found that amino acids accounted for up to 10% of the sulfate reduction in marine sediments. Investigations on other potential substrates for iron and sulfate reducers are not available. To our knowledge our study is the first investigation of substrates for iron reduction in marine sediments and the first on substrates for sulfate reducers with co-occurring iron reduction. It remains unclear, whether the low contribution of VFA to terminal oxidation is related to the co-occurrence of the two processes.

Effect of temperature

Investigations on organic matter degradation in temperate sediments reported decreasing overall rates with decreasing temperatures in temperate sediments (Jørgensen & Sørensen, 1985; Crill & Martens, 1987; Westrich & Berner, 1988). In contrast, permanently cold sites did not show generally lower rates than temperate sediments at higher temperatures (Vandieken *et al.*, 2006; Sagemann *et al.*, 1998; Thamdrup & Fleischer, 1998; Knoblauch & Jorgensen, 1999). Several investigations on bacteria in seawater have shown a decreased substrate affinity at low temperatures and, thus, a requirement for higher concentrations of organic substrates (Wiebe *et al.*, 1992, 1993; Nedwell, 1999). Weston & Joye (2005) examined the effect of changing temperatures on the coupling of fermentation and terminal oxidation. At temperatures below 25 °C, potential fermentation rates exceeded potential terminal oxidation rates, resulting in increased VFA concentrations. A seasonal investigation on VFA concentration in Cape Lookout Bight sediments showed low VFA concentrations in winter (Sansone & Martens, 1982). Investigations of VFA concentrations from Svalbard and the German Wadden Sea showed a close coupling of fermentation and sulfate reduction, resulting in constantly low VFA concentrations between 0° and 25–30 °C (Finke, 2002). Thus, in marine sediments not amended with complex organic matter, a decoupling of the fermentation and terminal oxidation yielding higher *in situ* concentrations at low temperatures does not seem to occur.

The acetate turnover rates measured in this study at 0 °C (Table 1) were similar to rates reported in previous studies using the molybdate inhibition technique, being 4–7 times higher than the lowest reported rates from Scottish costal sediment at 25 °C (Parkes *et al.*, 1989) and half the rates found in Arctic and cold temperate sediments (Christensen, 1984; Shaw & McIntosh, 1990). The highest acetate turnover rates were measured with the ^{14}C-tracer technique in organic rich Cape Lookout Bight sediments at 28 °C (Sansone & Martens, 1982) and in Danish coastal waters at 8 °C (Christensen & Blackburn, 1982). However, these rates were probably too high due to overestimation of the acetate pool.

Measured turnover rates of VFA might not reflect the actual biogeochemistry of the sediments due to uncertainty of the VFA pool size. The turnover rate constant is a more robust parameter to compare different investigations. Wu *et al.* (1997) found a close coupling of the turnover rate constant to *in situ* temperature in Long Island Sound sediments. In contrast, similar maximum rate constants in late winter and late summer were found in Cape Lookout Bight sediments (Sansone & Martens, 1982). The highest turnover rate constants were found in Danish coastal sediments at temperatures around 8 °C (Christensen & Blackburn, 1982), followed by Cape Lookout Bight at 25 °C (Sansone, 1986). The rate constants found in this study (Table 2) were similar to the lowest reported rates found at Loch Etive at 25 °C (Parkes *et al.*, 1989) but were 10 times lower than found in Danish lagoon sediments at the same temperature (Christensen, 1984) and 100 times lower than in the permanently cold Skan Bay sediments (Shaw *et al.*, 1984; Shaw & McIntosh, 1990). Thus, parameters other than *in situ* temperature seem to be more important in determining the VFA turnover rate and turnover rate constant.

Acknowledgements

We thank Maren Nickel and Jacqueline Schmidt for help with the sampling and Kristina Burkert for help with ^{14}C analysis. Thanks to Martina Alisch for measuring samples with the Dionex and Silvana Hessler at the University of Bremen for measuring samples with the ICP-AES. We thank Margot Isenbeck-Schröter from the University Heidelberg for determining the solid phase selenate. We thank the skipper, Stig Henningsen, and the first mate, John Mortensen, for help on board the MS FARM. We thank the University Centre on Svalbard (UNIS) for providing laboratory space. This study was funded by the Max Planck Society.

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