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# Kinetics of microbial sulfate reduction in estuarine sediments

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**Abstract**

Kinetic parameters of microbial sulfate reduction in intertidal sediments from a freshwater, brackish and marine site of the Scheldt estuary (Belgium, the Netherlands) were determined. Sulfate reduction rates (SRR) were measured at 10, 21, and 30 °C, using both flow-through reactors containing intact sediment slices and conventional sediment slurries. At the three sites, and for all depth intervals studied (0–2, 2–4, 4–6 and 6–8 cm), the dependence of potential SRR on the sulfate concentration followed the Michaelis–Menten rate equation. Apparent sulfate half-saturation concentrations, $K_m$, measured in the flow-through reactor experiments were comparable at the freshwater and marine sites (0.1–0.3 mM), but somewhat higher at the brackish site (0.4–0.9 mM). Maximum potential SRR, $R_{max}$, in the 0–4 cm depth interval of the freshwater sediments were similar to those in the 0–6 cm interval of the marine sediments (10–46 nmol $cm^{-3}$ $h^{-1}$ at 21 °C), despite much lower in situ sulfate availability and order-of-magnitude lower densities of sulfate-reducing bacteria (SRB), at the freshwater site. Values of $R_{max}$ in the brackish sediments were lower (3.7–7.6 nmol $cm^{-3}$ $h^{-1}$ at 21 °C), probably due to less labile organic matter, as inferred from higher $C_{org}/N$ ratios. Inflow solutions supplemented with lactate enhanced potential SRR at all three sites. Slurry incubations systematically yielded higher $R_{max}$ values than flow-through reactor experiments for the freshwater and brackish sediments, but similar values for the marine sediments. Transport limitation of potential SRR at the freshwater and brackish sites may be related to the lower sediment porosities and SRB densities compared to the marine site. Multiple rate controls, including sulfate availability, organic matter quality, temperature, and SRB abundance, modulate in situ sulfate-reducing activity along the estuarine salinity gradient.


## 1. Introduction

Dissimilatory sulfate reduction is a key process in the biogeochemical cycles of sulfur, carbon and iron. Sulfate-reducing bacteria anaerobically oxidize organic matter according to the overall simplified reaction, $2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$, where $CH_2O$ represents organic matter. Direct energy substrates for sulfate reducers range from $H_2$ to aromatic compounds (Widdel, 1988), although the most commonly utilized substrates are acetate, lactate, pyruvate and ethanol, i.e., compounds that are prevalent in anaerobic environments.

In coastal marine environments, sulfate reduction may account for a large fraction of benthic respiration (Jørgensen, 1982; Henrichs and Reeburgh, 1987; Canfield, 1989; Chambers et al., 1994; Wellsbury et al., 1996). In general, the relative contribution of sulfate reduction to organic carbon mineralization increases with increasing overall community metabolism. In highly productive saltmarshes, sulfate reduction is often the dominant respiration pathway (Howes et al., 1984; Kostka et al., 2002).

Although sulfur cycling is often neglected in studies of freshwater sediments, the contribution of sulfate-reducing bacteria to organic matter degradation may be significant, as a result of rapid turnover of reduced sulfur to sulfate (Holmer and Storkholm, 2001). Smith and Klug (1981) and Ingvorsen et al. (1981), for example, showed that sulfate reduction rates in sediments of eutrophic lakes can be much higher than would be predicted based on the low sulfate concentrations, even approaching values

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observed in marine sediments. Similar results were obtained by Trimmer et al. (1997) for sediments in the freshwater part of an estuary. The latter authors concluded that sulfate reduction rates are in the same range as found in the brackish/marine part of the same estuary. High sulfate reduction rates in freshwater environments indicate that the resident sulfate-reducing bacteria are able to cope with low sulfate concentrations (Ingvorsen and Jørgensen, 1984).

The $^{35}S$ radiotracer technique has been extensively used for measuring sulfate reduction rates and rate distributions in sediments (e.g., Jørgensen, 1978; Thode-Andersen and Jørgensen, 1989; Li et al., 1996; Hurtgen et al., 1999; Gribsholt and Kristensen, 2003). The large amount of available in situ rate data contrasts with the limited attempts to derive and calibrate predictive mathematical rate models for sulfate reduction. Furthermore, most of the available kinetic parameters describing sulfate reduction, for example maximum rates and half-saturation concentrations, have been obtained with pure cultures, not with natural microbial communities. In part, this reflects the lack of appropriate experimental methods for studying geomicrobial reaction kinetics under environmentally relevant conditions.

Reaction rates in sediments are usually obtained from intact core incubations or slurry experiments (Jørgensen, 1977; Smith and Klug, 1981; Boudreau and Westrich, 1984; Bak and Pfennig, 1991; Koretsky et al., 2003). Whereas the former approach yields depth-integrated in situ rates, it is not well adapted for determining the functional dependencies of rates on environmental variables. Conversely, slurry experiments are well-suited for measuring the response of initial reaction rates to changes in medium composition, but rates may not be representative of field conditions because the original structure of the sediment is destroyed and reaction products accumulate in the medium (e.g., Marxsen and Fiebig, 1993). Experimental conditions in slurries may further select for organisms that are not necessarily the most ecologically relevant components of the in situ microbial community.

In the present study, we use a flow-through reactor approach to calibrate kinetic expressions for sulfate reduction in sediments, under conditions where the physical and microbial structure of the natural porous medium is preserved (Roychoudhury et al., 1998; Pallud and Van Cappellen, 2003). The main advantages of this approach are that: (1) rates are measured under (near) steady-state conditions, (2) dissolved metabolic byproducts do not accumulate in the reactor system, and (3) the solution to solid ratio is identical to that of the natural sediment. The latter is important as subsurface microorganisms tend to be particle-bound.

Kinetic data on sulfate reduction were obtained for a series of temperate estuarine sediments sampled along the salinity gradient. Flow-through reactor experiments were run to determine the vertical distributions of maximum potential sulfate reduction rates and half-saturation concentrations within these sediments, and to investigate which factors control sulfate reduction activity. Traditional slurry incubations were performed with the same samples to evaluate possible methodological artifacts.

## 2. Materials and methods

### 2.1. Study sites

Three intertidal marsh sites were sampled in the Scheldt estuary (Belgium, the Netherlands). Two sites are in the Western Scheldt: Appels is located in the freshwater upper estuary and Waarde in the brackish part of the lower estuary (Fig. 1). Detailed description of these sites can be found in Hyacinthe and Van Cappellen (2004). The third site, Rattekaai, is a saltmarsh in the Eastern Scheldt (Fig. 1). The three sampling locations were in the intertidal, non-vegetated parts of the marshes. Sediment cores were collected at low tide in 2002 and 2003. Each site was sampled once a year. Table 1 summarizes some of the sediments characteristics.

The porosity, dry bulk density ($\rho_d$), organic carbon ($C_{org}$) plus nitrogen (N) concentrations, and median grain size were determined on 1 cm sediment slices. Porosity was calculated from the bulk density and particle density ($\rho_S$) as $1 - (\rho_d/\rho_S)$. The determination consisted of weighing a known volume of wet sediment and then drying it for 2 days at 105 °C, before weighing it again. Organic C and total N contents were determined using a Carlo Erba CN analyzer. Grain-size distributions were determined with a Laser Malvern Mastersizer S, after removal of organic matter (6% $H_2O_2$) and carbonate (1 N HCl), followed by chemical (in 4.5% $Na_4P_2O_7$ + 4.2% $Na_2CO_3$) and ultrasonic dispersion.

### 2.2. Flow-through reactors

The flow-through reactors (Fig. 2A) are designed to measure rates of biogeochemical reactions on undisturbed, water-saturated sediments (Roychoudhury et al., 1998). Each reactor contains a slice of sediment within a Plexiglas ring of 2 cm length and 4.7 cm inside diameter, with 0.2 μm pore size nitrocellulose filters and glass fiber filters at each end. The reactors are closed using Plexiglas caps kept in place using steel plates tightened with screws, whereas O-rings prevent leakage. Input/output channels open at the center of the caps, at the contact with the glass fiber filter. The caps have radial grooves, to spread the input solution and help create a uniform flow throughout the cross-section of the sediment slices.

The sediments are sampled using a hand pushed shuttle corer whose core liner consists of a stacking of reactor cells (Fig. 2B). Undisturbed sediment is thus directly collected in the reactor cells, each of which corresponds to a given depth interval (Fig. 2B). After retrieving a core, reactors containing sediment from different depth intervals are separated, and closed by filters and caps. In this study, the

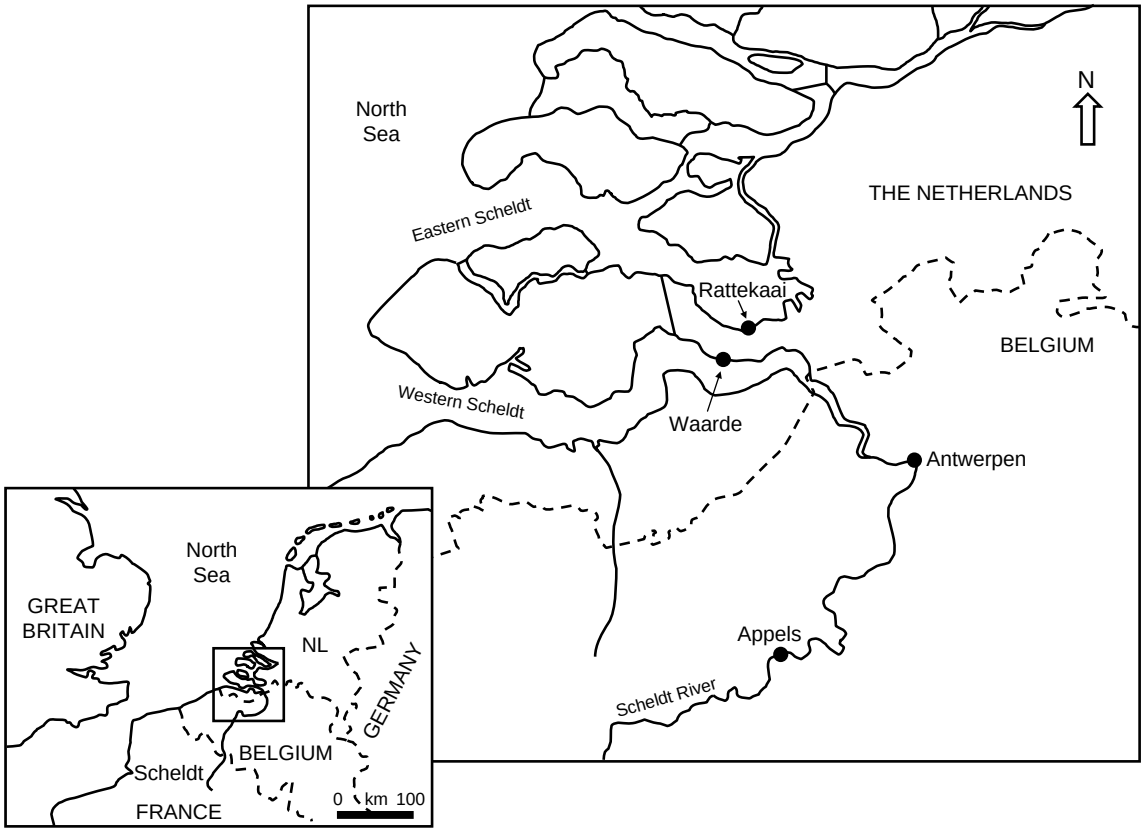



Fig. 1. Map of the Scheldt estuary showing the three study intertidal marshes, Appels (freshwater), Waarde (brackish) and Rattekaai (marine). The first site is situated in Belgium, whereas the two others are located in the Netherlands.

Table 1
Sediment properties

| Site | Depth (cm) | Porosity (%) | Dry bulk density (g $cm^{-3}$) | $C_{org}$ (wt%) | Molar $C_{org}/N$ | Median grain size (μm) | Salinity |
|---|---|---|---|---|---|---|---|
| Appels | 0–2 | 70.0 ± 5.1 | 0.84 ± 0.14 | 1.86 ± 0.12 | 11.82 ± 0.57 | 86.6 | $0.01 \pm 3.10^{-5}$ |
| | 2–4 | 60.0 ± 1.1 | 1.10 ± 0.03 | 2.09 ± 0.67 | 17.92 ± 3.51 | 34.2 | |
| | 4–6 | 59.8 ± 1.7 | 1.13 ± 0.05 | 1.77 ± 0.29 | 18.04 ± 2.33 | 64.3 | |
| | 6–8 | 59.3 ± 1.4 | 1.14 ± 0.04 | 1.92 ± 0.22 | 17.19 ± 2.81 | 78.1 | |
| | 8–10 | 61.3 ± 1.1 | 1.09 ± 0.03 | 12.17 ± 0.38 | 16.38 ± 2.01 | 92.6 | |
| Waarde | 0–2 | 64.3 ± 5.2 | 1.00 ± 0.15 | 0.85 ± 0.41 | 19.10 ± 4.91 | 107.4 | 5.54 ± 0.07 |
| | 2–4 | 61.0 ± 4.1 | 1.09 ± 0.11 | 1.45 ± 0.86 | 22.72 ± 7.29 | 100.9 | |
| | 4–6 | 64.0 ± 2.8 | 1.01 ± 0.08 | 1.71 ± 0.59 | 15.80 ± 2.52 | 67.9 | |
| | 6–8 | 64.8 ± 0.6 | 0.98 ± 0.02 | 2.62 ± 0.14 | 17.54 ± 4.67 | 46.4 | |
| | 8–10 | 63.3 ± 0.7 | 1.02 ± 0.01 | 2.36 ± 0.23 | 17.84 ± 4.15 | 47.3 | |
| Rattekaai | 0–2 | 87.3 ± 1.9 | 0.36 ± 0.05 | 5.23 ± 0.22 | 11.84 ± 1.08 | 31.9 | 27.15 ± 1.00 |
| | 2–4 | 87.0 ± 1.0 | 0.37 ± 0.03 | 5.20 ± 0.28 | 11.97 ± 0.93 | 32.5 | |
| | 4–6 | 86.0 ± 1.3 | 0.38 ± 0.04 | 5.11 ± 0.10 | 12.6 ± 0.98 | 29.1 | |
| | 6–8 | 86.3 ± 0.7 | 0.42 ± 0.03 | 5.01 ± 0.25 | 12.50 ± 0.76 | 41.0 | |
| | 8–10 | 83.3 ± 1.6 | 0.47 ± 0.04 | 4.89 ± 0.32 | 12.19 ± 0.35 | 46.8 | |

Values are averages ± 95% confidence limits obtained from four separate sediment collections throughout the years 2001–2003. Median grain sizes were obtained on sediments sampled in May 2002.

reactors were kept at 4 °C under anaerobic conditions for a few hours, until the flow-through experiments were started.

### 2.3. Potential steady-state sulfate reduction rates

Flow-through reactor experiments were carried out on four depth intervals, 0–2, 2–4, 4–6 and 6–8 cm, at the three sites. The experiments were run inside a glove box under argon atmosphere, with the reactors submerged into a thermostated water bath. Temperatures ranging from 10 to 30 °C were used. An input solution was supplied to the reactors at a constant flow rate ($1.4 \pm 0.15$ or $4.0 \pm 0.20$ $cm^3$ $h^{-1}$) imposed by a peristaltic pump. The input solution contained $SO_4^{2-}$ (from 0.125 to 4 mM) and a

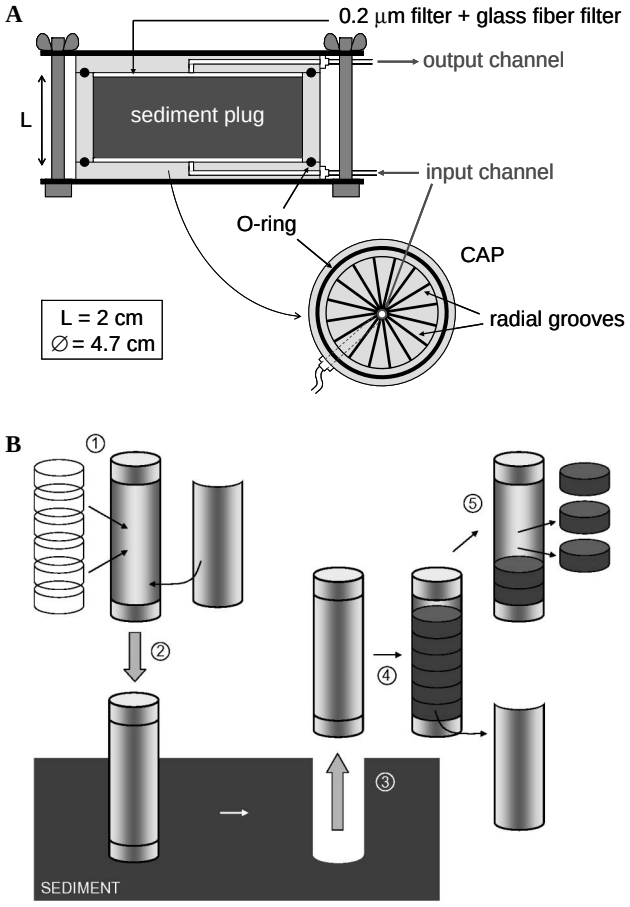



Fig. 2. (A) Side view of a flow-through reactor with a detailed top view of a cap. (B) Use of a shuttle corer to retrieve undisturbed sediment slices corresponding to different depth intervals. (1) Stacked reactor cells form the core liner located within a stainless steel outer sleeve. (2) The shuttle corer is manually pushed into the sediment and closed at both ends. (3) Once retrieved from the sediment, the shuttle corer is opened (4), and the reactors filled with sediment are separated from one another using a teflon knife (5). The reactors are immediately closed by applying filters and filter holders at both ends.

flow tracer ($Br^-$, 2 mM). Salinity was adjusted with NaCl to match that measured in free flowing water at the sites. The reactor outflow was collected by a fraction collector. Collection tubes were pre-filled with 2 mL of sulfide trap solution (1% zinc acetate). Outflow samples were analyzed for $SO_4^{2-}$ and $Br^-$ using ion chromatography (detection limit $\leqslant 5$ μM).

Reactors were supplied with three to five successive input $SO_4^{2-}$ concentrations. Each input concentration was supplied during three to five days, to reach steady-state $SO_4^{2-}$ concentrations in the outflow. In a separate set of experiments, an additional input solution containing lactate (10 mM), as well as $SO_4^{2-}$ (0.52 mM) and $Br^-$ (2 mM), was supplied to reactors with sediment from the three sites. The response to alternative electron acceptors of the bacterial community at the freshwater site was tested using a reactor containing the top 2 cm of Appels sediment collected in November 2002. The composition of the input solution was changed from $SO_4^{2-}$ (2 mM), to $SO_4^{2-}$ (2 mM) plus $NO_3^-$ (2.5 mM), to $SO_4^{2-}$ (2 mM), and finally to $SO_4^{2-}$ (2 mM) plus Fe-citrate (2 mM).

Bromide breakthrough curves (i.e., the $Br^-$ outflow concentration as a function of time) were used to constrain transport parameters, and to check for possible non-ideal flow conditions in the reactors (Roychoudhury et al., 1998; Pallud and Van Cappellen, 2003). Steady-state sulfate reduction rates (SRR) were calculated as follows:

$$\mathrm{SRR} = \frac{(C_{\mathrm{out}} - C_0)Q}{V}, \tag{1}$$

where $C_{\mathrm{out}}$ is the steady-state sulfate concentration in the outflow, $C_0$ is the sulfate input concentration, $Q$ is the volumetric flow rate and $V$ is the volume of the sediment slice in the reactor. The rates obtained are referred to as "potential" rates because they correspond to sulfate reduction activities when the only terminal electron acceptor being supplied to the in situ bacterial community is sulfate.

### 2.4. Slurry experiments

Anaerobic slurries (50% v/v) of 2 cm sediment slices were prepared using depth intervals from 0–2 cm to 12–14 cm. Aliquots of a slurry were dispensed in 100 ml bottles capped with butyl rubber caps and then enriched with sulfate. Initial sulfate concentrations ranged from 0.5 to 2 mM for Appels, 1 to 8 mM for Waarde and 1 to 16 mM for Rattekaai. In a separate series of experiments, duplicate aliquots of slurries were amended with lactate to 10 or 20 mM final concentration. The slurries were incubated under gentle orbital shaking to maintain the sediment in suspension at 10, 21 or 30 °C. Experiments lasted up to 3, 11 and 12 days for Appels, Waarde and Rattekaai, respectively. Slurries were regularly sampled while flushing the bottles vigorously with argon to maintain anaerobic conditions. The samples were immediately centrifuged, the supernatant was removed and filtered through a 0.2 μm pore size syringe filter, before measuring the $SO_4^{2-}$ concentration. Potential SRR were estimated from the initial linear decreases in sulfate concentration with time.

### 2.5. Kinetic parameters

The utilization of a substrate by a microbial population is widely described by the so-called Michaelis–Menten, or Monod, rate equation:

$$R = R_{\max}\frac{C}{K_{\mathrm{m}} + C}, \tag{2}$$

where $R$ is the reaction rate, $C$ the variable concentration of the substrate, $R_{\max}$ the maximum reaction rate and $K_{\mathrm{m}}$ is the half-saturation concentration. A one-dimensional advection-dispersion-reaction model was developed to describe sulfate consumption in the reactor. In this model, sulfate reduction is represented by Eq. (2). The adjustable rate parameters $R_{\max}$ and $K_{\mathrm{m}}$ were constrained by fitting

the model to the measured outflow sulfate concentration versus time. For $R_{max}$, results of experiments run at high sulfate concentrations ($>2K_m$) were used. For $K_m$, experiment results at low output concentrations ($<K_m$) were used. The average sulfate concentration in the reactor at steady-state was calculated from the model-predicted longitudinal concentration profile.

In the slurry experiments, $R_{max}$ and $K_m$ were obtained after a linearization of the rate versus concentration data using the Hanes plot ($\frac{C}{R} = \frac{K_m}{R_{max}} + \frac{1}{R_{max}}C$), which is preferred over the double reciprocal (Lineweaver–Burk) plot (Henderson, 1993). The slope and $X$-intercept of the Hanes plot yield $\frac{1}{R_{max}}$ and $-K_m$, respectively. The sulfate concentrations, $C$, used in the Hanes plot were the initial concentrations. Both for flow-through reactor and slurry experiments, the kinetic parameters obtained are referred to as "apparent" parameters (as opposed to "true" or "intrinsic" parameters) because they reflect the response of the in situ bacterial community under conditions where factors other than the sulfate concentration may be limiting.

The temperature dependencies of SRR in the top 2 cm of sediment were determined using flow-through reactor experiments (Appels, two replicates) and slurries (Appels, Waarde and Rattekaai, four replicates). The range of temperature considered, 10–30 °C, is representative of conditions encountered in the field between winter and summer. The temperature dependence was expressed both as an Arrhenius activation energy ($E_a$) and as the temperature coefficient, $Q_{10}$, which corresponds to the relative increase of the reaction rate for a 10 °C increase in temperature.

### 2.6. Enumeration of sulfate-reducing bacteria

Sulfate-reducing bacteria (SRB) were enumerated via the most probable number (MPN) method (Woomer, 1994) on fresh sediments, as well as on sediments collected at the end of the flow-through and slurry experiments. Standard protocol and culture media were used, to facilitate comparison with literature data (Wind and Conrad, 1995; Teske et al., 1996; Sass et al., 1997; Sievert et al., 1999; Brandt et al., 2001; Wieringa et al., 2000). Five grams of sediment were suspended in 50 mL sterile saline solution (NaCl, 2%; $MgCl_2$, 0.3%) and shaken thoroughly. Tenfold dilution series (eight replicates) were made directly in culture medium in 96-well microtiter plates. The media used for the cultivation of sulfate-reducing bacteria were adapted from Widdel and Bak (1992) and are presented in Table 2. Lactate and acetate were concomitantly used as electron donors. Sodium sulfate served as the electron acceptor. The medium was supplemented with 1 mL $L^{-1}$ of trace elements solution SL9 (Tschech and Pfennig, 1984) and the pH was adjusted (with NaOH or HCl) to 6.8–7.0 for the freshwater medium, and 7.0–7.3 for the brackish and marine media. The reducing agent in the medium was mercaptoacetic acid and was added shortly before dispensing the medium in the sterile microtiter plates. Microtiter plates were anaerobically incubated at 30 °C in the dark for 6 weeks. The presence of sulfate-reducing bacteria was scored positive upon precipitation of black FeS, indicating sulfide production.

Table 2
Composition (expressed in final concentrations) of the liquid culture media used to enumerate sulfate-reducing bacteria in the sediments from the three sites

| | Freshwater | Brackish | Marine |
|---|---|---|---|
| NaCl | 0 mM | 222 mM | 342 mM |
| $MgCl_2$, $6H_2O$ | 2.5 mM | 10 mM | 15 mM |
| $KH_2PO_4$ | 1.5 mM | 1.5 mM | 1.5 mM |
| $NH_4Cl$ | 5 mM | 5 mM | 5 mM |
| KCl | 4 mM | 4 mM | 4 mM |
| $CaCl_2$, $2H_2O$ | 1 mM | 1 mM | 1 mM |
| $Na_2SO_4$ | 28 mM | 28 mM | 28 mM |
| Yeast extract | 0.1 g $L^{-1}$ | 0.1 g $L^{-1}$ | 0.1 g $L^{-1}$ |
| Resazurine solution 0.1% | 0.5 mg $L^{-1}$ | 0.5 mg $L^{-1}$ | 0.5 mg $L^{-1}$ |
| $NaHCO_3$ | 30 mM | 30 mM | 30 mM |
| Na-lactate | 10 mM | 10 mM | 10 mM |
| Na-acetate | 10 mM | 10 mM | 10 mM |
| Crystalline sodium dithionite | Until the redox indicator resazurin turned colorless | | |
| Mercaptoacetic acid | 0.5 mM | 0.5 mM | 0.5 mM |
| $FeSO_4$ | 0.2 mM | 0.2 mM | 0.2 mM |

## 3. Results

### 3.1. Site and sediment characteristics

At Appels, pore water sulfate was depleted within the upper few centimeters, whereas at Waarde and Rattekaai measurable sulfate concentrations often persisted at depths exceeding 10 cm (Fig. 3A). For the set of profiles displayed, the corresponding sulfate concentrations in the running water were 0.8 mM in Appels, 3.7 mM in Waarde and 22.1 mM in Rattekaai. Sulfate concentrations in the running water were highly variable, however, as indicated by the yearly ranges in Fig. 3A. Porosities were systematically higher at Rattekaai than at Waarde and Appels (Table 1). They were fairly homogeneous over the 0–10 cm depth intervals, except for Appels, which was characterized by a 1–2 cm thick surface deposit of markedly higher porosity. Appels and Waarde sediments contained 1.5–3.1 wt% $C_{org}$, whereas at Rattekaai the concentrations were about twice as high (Table 1). The $C_{org}/N$ ratios were higher at Waarde, compared to the other two sites.

### 3.2. Bacterial densities

At Waarde, MPN counts of SRB showed a higher abundance in the 0–2 cm interval compared to the underlying sediment, whereas no systematic vertical trends were observed at Appels and Rattekaai (Fig. 3B). The SRB densities varied systematically among the sites: they were highest at Rattekaai and lowest at Appels, with average MPN counts of $9 \times 10^8$ and $3 \times 10^4$ cells $cm^{-3}$ wet sediment, respectively (0–8 cm intervals). There were no significant

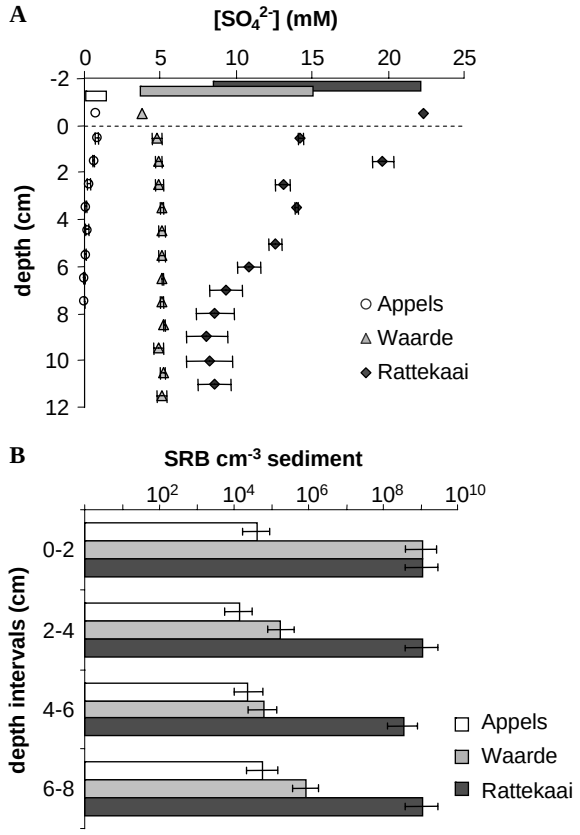



Fig. 3. (A) Pore water sulfate concentrations and (B) Most probable numbers (MPN) of sulfate reducers as a function of depth in the sediments at the three study sites, in February 2003 (Waarde and Rattekaai) and July 2003 (Appels). The dotted line in (A) indicates the water–sediment interface. The bars in (A) correspond to the annual ranges of sulfate concentration in the running water at the three sites. The MPN counts were measured using lactate plus acetate as electron donors. Error bars indicate 0.95 confidence intervals.

changes in MPN counts of SRB between the start and end of the flow-through experiments (data not shown). In contrast, in slurry experiments, except when the initial counts were already on the order of $10^8$ cells $cm^{-3}$ wet sediment, the MPN increased up to three orders of magnitude to reach $10^7$–$10^8$ cells $cm^{-3}$ wet sediment at the end of the experiments (data not shown).

### 3.3. Reactor hydrodynamics

The bromide breakthrough curves measured over a range of flow rates from 0.7 to 8.0 $cm^3\ h^{-1}$ agreed well with simulations using an analytical solution for one-dimensional advective-dispersive flow through a homogeneous porous medium (results not shown). As expected, the dispersion coefficient increased with increasing pore velocity (Pfannkuch, 1963; Bear, 1972). The results were thus consistent with radially homogeneous flow and, therefore, support a one-dimensional reactive transport representation of the flow-through reactor system. The water residence times fell in the ranges 16–22 h (Appels), 14–16 h (Waarde) and 21–25 h (Rattekaai) for the experiments run at a flow rate of 1.4 $cm^3\ h^{-1}$; and 5.7–7.8 h (Appels), 5.5–7.7 h (Waarde) and 5.2–8.5 h (Rattekaai) for a flow rate of 4.0 $cm^3\ h^{-1}$.

### 3.4. Sulfate reduction kinetics: flow-through reactors

The outflow sulfate concentrations reached steady-state after 2–4 pore volumes of solution had been supplied to the reactors. In all flow-through experiments, potential SRR calculated with Eq. (1) increased with increasing pore water sulfate concentration, ultimately approaching a maximum value, $R_{max}$ (Fig. 4). At the three sites, potential SRR generally decreased with depth. The inverse correlation of potential SRR with depth was strongest at Appels (linear correlation coefficient, $r^2 = 0.91$) and Rattekaai ($r^2 = 0.98$), but somewhat weaker at Waarde ($r^2 = 0.50$).

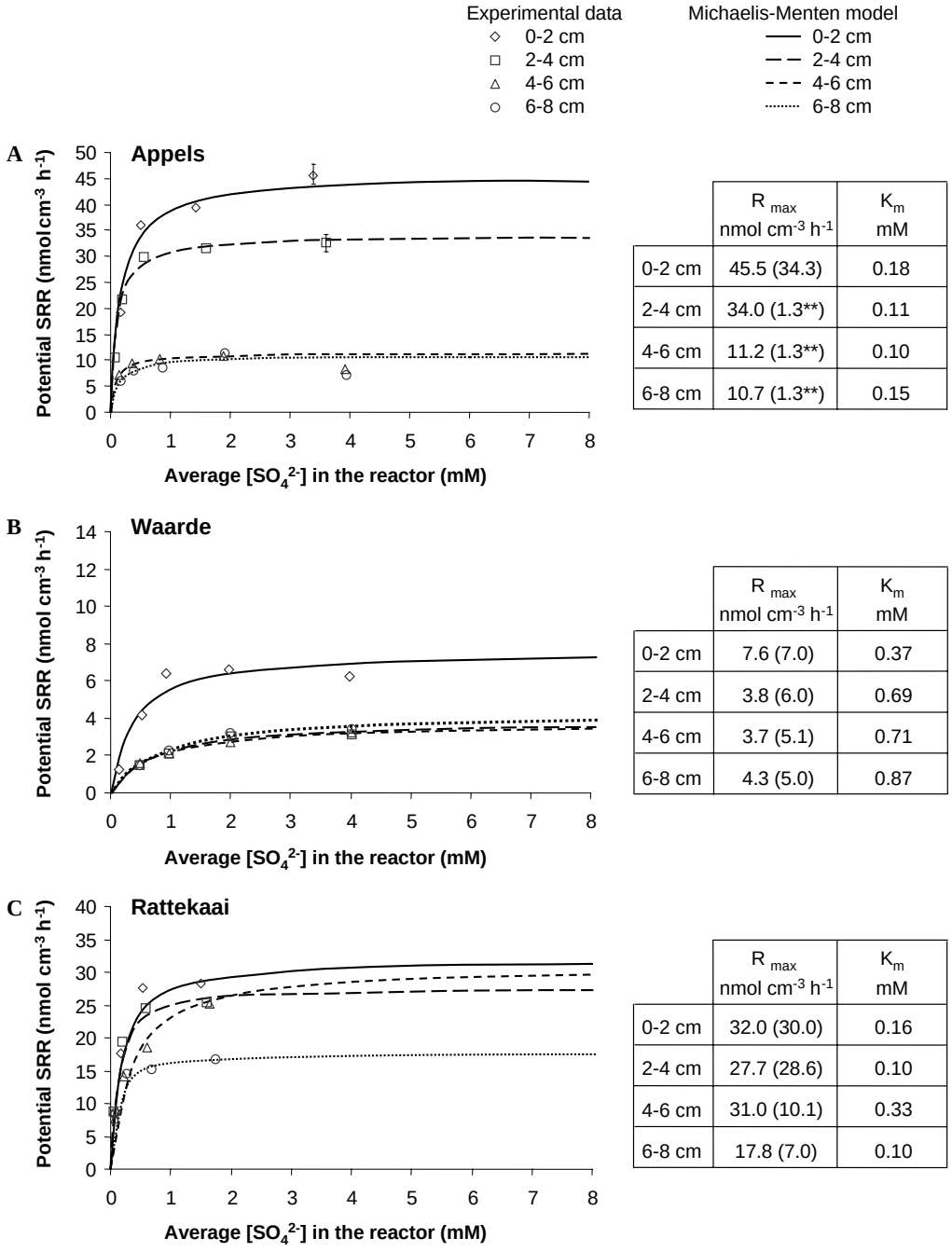
For the 2003 flow-through experiments, the highest value of $R_{max}$ (45.5 nmol $cm^{-3}\ h^{-1}$) was obtained for the 0–2 cm interval at the Appels site (Fig. 4). With increasing depth, $R_{max}$ decreased to values around 11 nmol $cm^{-3}\ h^{-1}$ in the 4–6 and 6–8 cm intervals. In contrast, at Rattekaai, $R_{max}$ values were on the order of 30–35 nmol $cm^{-3}\ h^{-1}$ throughout the upper 6 cm of sediment, and only dropped in the 6–8 cm layer. The Waarde sediments exhibited the lowest $R_{max}$, in the range 4–8 nmol $cm^{-3}\ h^{-1}$. Values of $K_m$ were similar for the Appels and the Rattekaai sediments (0.1–0.3 mM), but were higher at Waarde, with $K_m$ values up to 0.9 mM (Fig. 4).

Values of $R_{max}$ obtained from flow-through experiments varied between sampling times. In the 2–8 cm depth interval of Appels, $R_{max}$ values were about one order of magnitude lower in April 2002 (data not shown), compared to July 2003. In the 0–2 cm depth interval, however, $R_{max}$ was only slightly lower in April 2002 (data not shown). At the other two sites, the general features of $R_{max}$ vertical profiles were similar for both sampling times.

When nitrate was added concomitantly to sulfate to the top 2 cm of the freshwater sediment, potential SRR decreased from 41.6 to 29.0 nmol $SO_4^{2-}$ $cm^{-3}\ h^{-1}$, whereas nitrate was consumed at a rate of 55 nmol $NO_3^-$ $cm^{-3}\ h^{-1}$. Nitrate reduction in this experiment caused a complete consumption of $NO_3^-$ in the reactor, which probably explains the incomplete inhibition of sulfate reduction.

### 3.5. Sulfate reduction kinetics: slurries

For slurries with no lactate addition, the highest $R_{max}$ (127 nmol $cm^{-3}\ h^{-1}$) was measured in the top 2 cm of Appels (Fig. 5). Deeper in the Appels sediment, $R_{max}$ ranged from 31 to 60 nmol $cm^{-3}\ h^{-1}$. At Waarde and Rattekaai, $R_{max}$ ranged from 4 to 16 and from 13 to 40 nmol $cm^{-3}\ h^{-1}$, respectively, and showed decreasing trends with depth (Fig. 5). Apparent $K_m$ values derived from slurries ranged widely (Fig. 5). There were no clear trends in $K_m$ values with depth for any of the sites, whereas




| | $R_{max}$ nmol cm$^{-3}$ h$^{-1}$ | $K_m$ mM |
|---|---|---|
| 0-2 cm | 45.5 (34.3) | 0.18 |
| 2-4 cm | 34.0 (1.3**) | 0.11 |
| 4-6 cm | 11.2 (1.3**) | 0.10 |
| 6-8 cm | 10.7 (1.3**) | 0.15 |

| | $R_{max}$ nmol cm$^{-3}$ h$^{-1}$ | $K_m$ mM |
|---|---|---|
| 0-2 cm | 7.6 (7.0) | 0.37 |
| 2-4 cm | 3.8 (6.0) | 0.69 |
| 4-6 cm | 3.7 (5.1) | 0.71 |
| 6-8 cm | 4.3 (5.0) | 0.87 |

| | $R_{max}$ nmol cm$^{-3}$ h$^{-1}$ | $K_m$ mM |
|---|---|---|
| 0-2 cm | 32.0 (30.0) | 0.16 |
| 2-4 cm | 27.7 (28.6) | 0.10 |
| 4-6 cm | 31.0 (10.1) | 0.33 |
| 6-8 cm | 17.8 (7.0) | 0.10 |

Fig. 4. Steady-state potential sulfate reduction rate (SRR) measured at 21 °C in continuous flow-through reactors ($Q = 1.4 \pm 0.15$ cm$^3$ h$^{-1}$) in the 0–2 (diamonds), 2–4 (squares), 4–6 (triangles) and 6–8 cm (circles) depth intervals of Appels (A), Waarde (B) and Rattekaai (C), as a function of the average sulfate concentration in the reactor, for February 2003 (Waarde and Rattekaai) and July 2003 (Appels). The lines correspond to the Michaelis–Menten rate expression, using $R_{max}$ and $K_m$ summarized in the adjoining tables. $R_{max}$ values in parentheses correspond to values obtained for the alternate sampling times (February, March and April 2002 for Waarde, Rattekaai and Appels, respectively). (**) Indicates that the change between input and output sulfate concentrations was below detection limit, so SRR could not be calculated. Error bars indicate 0.95 confidence intervals. When not visible, the X- and Y-error bars fall within the size of the symbols.

the addition of lactate resulted indifferently in a decrease or increase in $K_m$.

Values of $R_{max}$ obtained with non-lactate-amended slurries of Appels and Waarde sediments were higher by a factor of 2.4–6.5 than values obtained with flow-through experiments (Fig. 6A). In contrast, $R_{max}$ values obtained with the two methods were not significantly different for the Rattekaai sediments (Fig. 6A). For the three sites, $K_m$ values were systematically higher in slurry than in flow-through experiments. Ratios of $K_m$ in slurry versus flow-through experiments ranged from 2.5 to 25 for Appels, 43 for Waarde and 10 for Rattekaai (Fig. 6B).

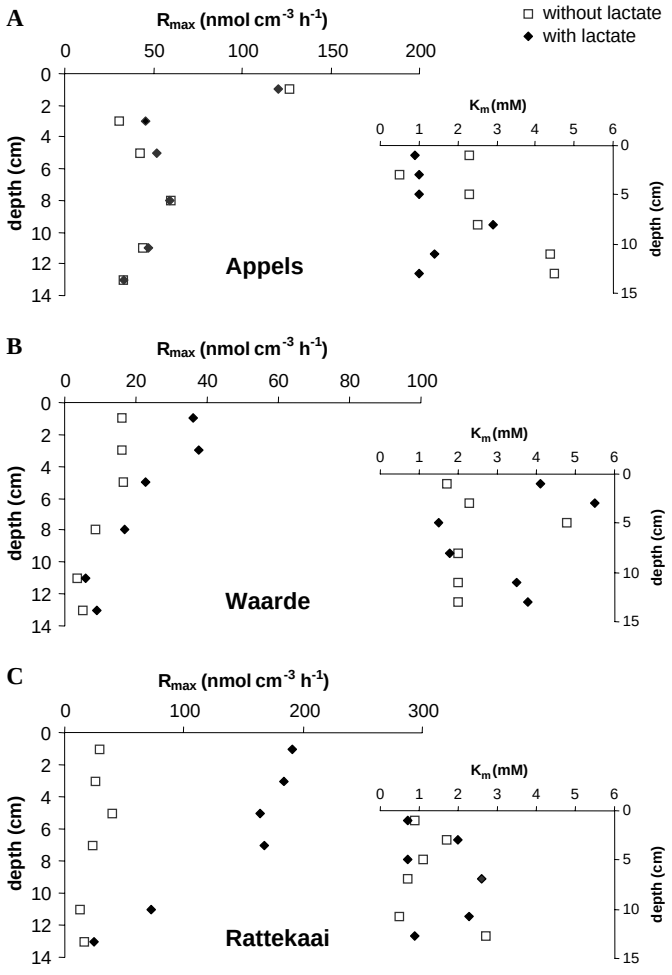



Fig. 5. Depth distributions of apparent maximum reaction rates ($R_{max}$) and half-saturation concentrations ($K_m$) derived from Appels (A), Waarde (B) and Rattekaai (C) rate measurements in slurry experiments, with (black diamonds) or without (white squares) lactate addition at 21 °C. The slurries were performed using homogenized sediment from 2 cm depth intervals. Sampling was carried out in February 2003 (Waarde and Rattekaai) and July 2003 (Appels).

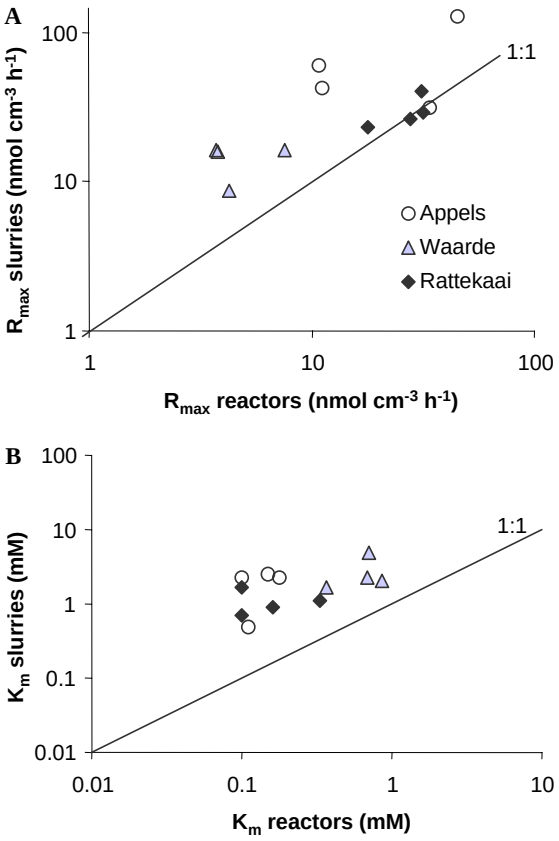



Fig. 6. Comparison of (A) apparent maximum reaction rates ($R_{max}$) and (B) half-saturation concentration ($K_m$) obtained in flow-through reactor and slurry experiments at 21 °C. Sampling was carried out in February 2003 (Waarde and Rattekaai) and July 2003 (Appels).

### 3.6. Effects of lactate and temperature

For slurries amended with lactate, the highest $R_{max}$ was found at Rattekaai, with a value of almost 200 nmol cm$^{-3}$ h$^{-1}$ in the 0–2 cm interval (Fig. 5). In the upper 12 cm, the addition of lactate to the Rattekaai and Waarde slurries increased $R_{max}$ by factors of 4–7 and 2, respectively. An analysis of covariance (ANCOVA, 95% confidence interval) showed that lactate addition and depth together explained around 80% of the variation of $R_{max}$ in Waarde and Rattekaai slurries. The addition of lactate, however, had no clear effect in slurry incubations with Appels sediment: depth and lactate addition only explained 39% of the variation in $R_{max}$. With or without lactate addition, the Waarde site was always characterized by the lowest $R_{max}$ values.

The addition of lactate in flow-through reactor experiments stimulated sulfate reduction activity at all depth of the three sites (Fig. 7). The lowest relative rate increases upon lactate addition were observed for reactors where potential SRR were already high prior to lactate addition. ANCOVA (95% confidence interval) showed that lactate addition and depth explained 74, 72 and 92% of the variation in potential SRR for Appels, Waarde and Rattekaai flow-through reactor experiments, respectively.

The increase in potential SRR after switching to lactate-containing input solutions was immediate for sediment with high $R_{max}$, namely the 0–2 cm interval of the Appels sediment, and the 0–2 and 2–4 cm intervals of Rattekaai (Fig. 8). In all other cases, a lag time between 7 and 37 h was observed before the sulfate-reducing communities started utilizing lactate.

For Appels, SRR measurements at 10, 21 and 30 °C yielded similar values of $E_a$ and $Q_{10}$ in slurry ($E_a = 61.6$ kJ mol$^{-1}$, $Q_{10} = 2.4$) and flow-through experiments ($E_a = 67.6$ kJ mol$^{-1}$, $Q_{10} = 2.6$). The effect of temperature on SRR in Waarde sediment ($E_a = 60.6$ kJ mol$^{-1}$, $Q_{10} = 2.3$) closely followed that observed for Appels. Somewhat lower $E_a$ (43.7 kJ mol$^{-1}$) and $Q_{10}$ (1.9) values were derived for Rattekaai sediment.

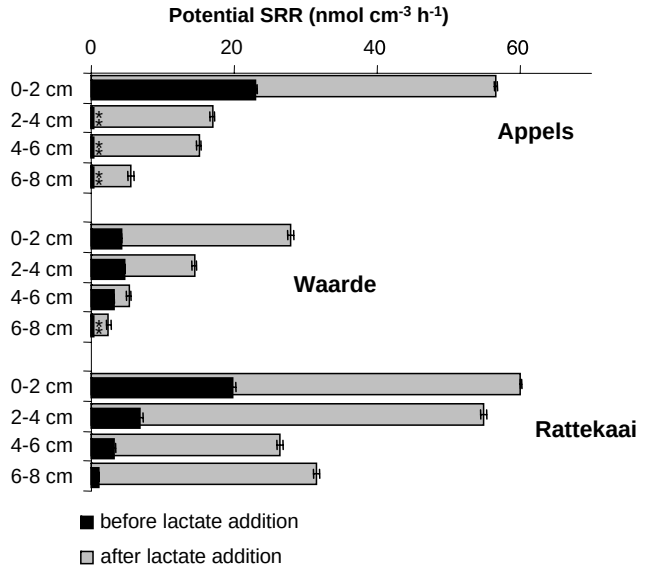



Fig. 7. Vertical distributions of the steady-state potential sulfate reduction rate (SRR) measured before and after lactate addition at 21 °C in continuous flow-through reactors ($Q = 4.0 \pm 0.20\ cm^3\ h^{-1}$) sampled in February, March and April 2002 for Waarde, Rattekaai and Appels, respectively. Sulfate concentration in the input solution was 0.515 mM. (**) Indicates that the change between input and output sulfate concentrations was below detection limit and SRR could not be calculated.

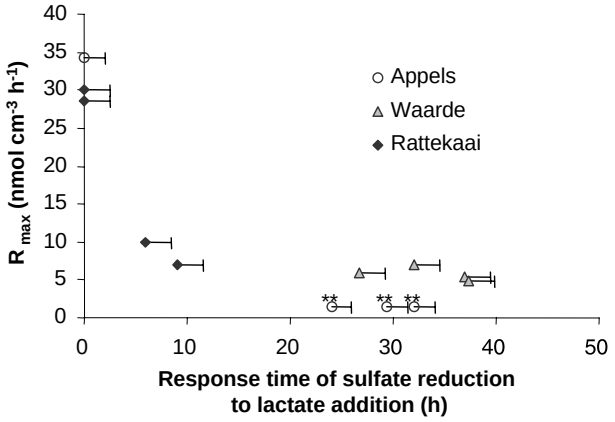



Fig. 8. Response time of sulfate reduction activity to lactate addition versus the maximum sulfate reduction rate ($R_{max}$) prior to lactate addition, in continuous flow-through reactors at 21 °C ($Q = 4.0 \pm 0.20\ cm^3\ h^{-1}$). Sampling was carried out in February, March and April 2002 for Waarde, Rattekaai and Appels, respectively. (**) Indicates that the change between input and output sulfate concentrations was below detection limit and the $R_{max}$ values plotted is a minimum estimates.

## 4. Discussion

The experimental data demonstrate the potential for sulfate reduction at the three sites, for all sampling times and depth intervals investigated. In the flow-through experiments, sulfate reduction started immediately when sulfate was supplied, implying the presence of active in situ SRB communities in the freshwater, brackish and marine sediments. Furthermore, no evidence was found for significant changes in SRB biomass over the duration of the flow-through experiments (up to 600 h). For all sediment slices used, the Michaelis–Menten rate equation adequately described the utilization of sulfate by the resident microbial communities of the estuarine sediments.

### 4.1. Spatial variability and rate controls

The upper Appels sediment exhibits the highest potential for sulfate reduction, although sulfate availability is lowest at this site. This is unexpected given that marine and saltmarsh sediments are thought to be more permanent and significant habitats of SRB due to the high sulfate concentrations (Fauque, 1995). Sulfate availability in the overlying water is thus a poor predicator of the potential sulfate-reducing activity in freshwater sediments (see also,

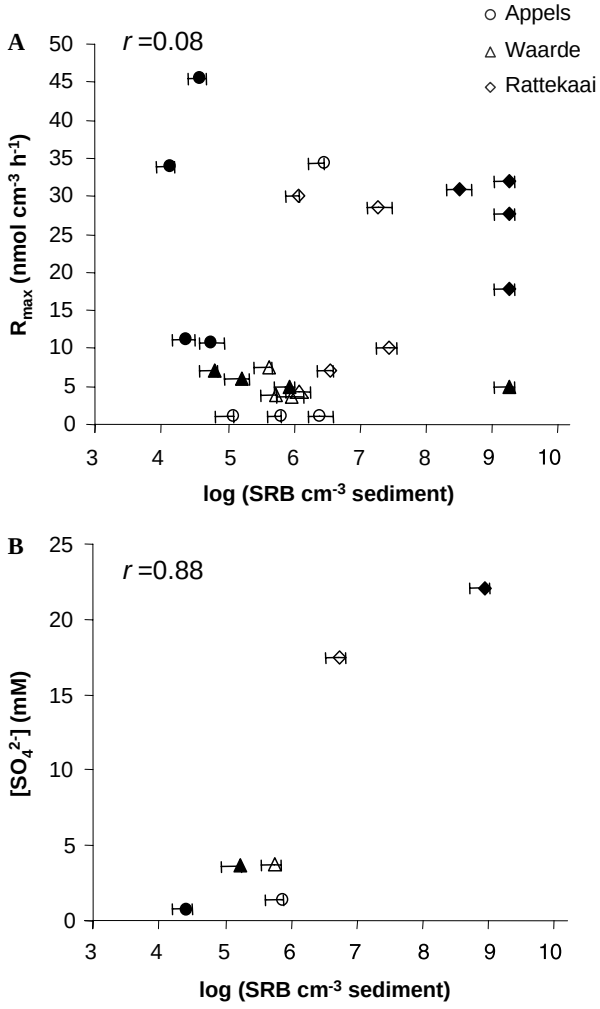



Fig. 9. Estimated population sizes of sulfate-reducing bacteria (SRB) in sediments of Appels, Waarde and Rattekaai, versus (A) the maximum sulfate reduction rate ($R_{max}$) measured at 21 °C in continuous flow-through reactors, and (B) the sulfate concentration in the overlying water. The open symbols correspond to sediments collected in February (Waarde), March (Rattekaai) and April 2002 (Appels); closed symbols to February (Waarde, Rattekaai) and July 2003 (Appels). Error bars for the MPN SRB counts indicate 0.95 confidence intervals.

Ingvorsen et al., 1981; Smith and Klug, 1981; Trimmer et al., 1997).

Half-saturation constants, $K_m$, derived from the flow-through experiments are comparable to sulfate concentrations observed in the Scheldt river water at Appels, but are much lower than sulfate concentrations encountered in the water flooding the Rattekaai and Waarde sites. Consequently, at the freshwater site, in situ sulfate reduction is limited by the low availability of sulfate, especially below 4 cm, whereas at the two other sites severe sulfate limitation is not expected within the 0–8 cm depth range (Fig. 3A).

The size of the sulfate-reducing microbial community does not correlate in a simple way with potential sulfate reduction rates either (Fig. 9A). For Appels and Rattekaai, the estimates of SRB numbers are homogeneous over the entire depth interval investigated (Fig. 3B), whereas the potential SRR show decreasing trends with depth (Fig. 4). This observation is in line with the results of recent experimental and modeling studies, which imply a lack of correspondence between the vertical microbial community structure and the vertical distribution of metabolic activities in sediments exhibiting high rates of microbial respiration (Koretsky et al., 2005; Thullner et al., 2005). Furthermore, the highest potential SRR is found at Appels, where the MPN counts of sulfate reducers are one to three orders of magnitude lower than at Waarde and Rattekaai.

The SRB densities in the sediments roughly correlate with the ambient sulfate availability at the sites (Fig. 9B). Thus, for the freshwater sediments, limitation of sulfate reduction by low pore water sulfate levels could be exacerbated by the low abundance of SRB. The flow-through experiment in which the input was switched from a $SO_4^{2-}$ only to a $SO_4^{2-}$ plus $NO_3^-$ solution (Section 3.4) provides evidence for limitation of potential sulfate reduction by the SRB population density at Appels. Assuming a 1:2 $SO_4^{2-}$ to organic carbon molar ratio for sulfate reduction, the rate of organic carbon oxidation before switching the input solution equals $41.6 \times 2 = 83$ nmol C cm$^{-3}$ h$^{-1}$. Using a 5:4 ratio of $NO_3^-$ to carbon for denitrification yields a carbon oxidation rate of $(29 \times 2) + (55 \times 1.25) = 127$ nmol C cm$^{-3}$ h$^{-1}$ after switching to the $SO_4^{2-}$ plus $NO_3^-$ input solution. The increase in carbon oxidation rate indicates that the resident denitrifying community has a greater capacity to utilize the available organic carbon substrates than the SRB community. In a separate study on the Appels sediments, the denitrifiers have been shown to be far more abundant than SRB, with MPN counts on the order of $10^8$ cells cm$^{-3}$ wet sediment, while $R_{max}$ values for nitrate reduction are also systematically higher than those for sulfate reduction (A. Laverman, Utrecht University, personal communication). These observations point to a limitation of potential SRR by the small SRB community size at the freshwater site.

The observation that the potential SRR measured with the flow-through reactors are highest in the uppermost centimeters of the three sediments is consistent with the reported high in situ SRR in the upper, oxidizing portions of lake and estuarine sediments (Ingvorsen et al., 1981; Jones and Simon, 1981; Bak and Pfennig, 1991; Wellsbury et al., 1996; Li et al., 1999), as well as marine and saltmarsh sediments (King, 1988; Nedwell et al., 1993; Wieringa et al., 2000; Koretsky et al., 2003). As shown by Koretsky et al. (2005), a high supply of labile organic matter and intense mixing of the topmost sediment by bioturbation results in a lack of a clear vertical separation of functional groups of microorganisms. A high metabolic versatility of the microbial communities in the upper sediment layers presumably confers an ecological advantage in the highly dynamic estuarine environment.

Despite limitation by low sulfate levels and SRB abundance, the highest potential SRR is observed in the surface layer of the freshwater sediment. This is attributed to the input of highly degradable organic matter. Previous work has demonstrated that the variability in benthic carbon mineralization rates along the Western Scheldt is primarily due to spatial differences in lability of organic matter (Middelburg et al., 1996). The relatively low molar $C_{org}/N$ ratios in the 0–2 cm interval at Appels (10–12) suggest an input of fresh phytodetritus or anthropogenic organic matter. Both sources probably contribute organic matter to the Appels site, as the upper Scheldt estuary receives a very large influx of sewage-derived detritus (Wollast, 1988), whereas abundant algal and diatom remains are observed visually in the sediments.

The importance of organic matter lability in controlling potential SRR is evident from the inverse correlation between $R_{max}$ and the $C_{org}/N$ ratio (Fig. 10). At Appels, the sharp drop in $R_{max}$ with depth is accompanied by an increase in the $C_{org}/N$ ratio to values on the order of 13–18, indicating a decrease in the relative abundance of fresh organic matter in the deeper sediment layers. At Rattekaai, the $C_{org}/N$ ratios are fairly uniform in depth interval 0–6 cm, as are the potential SRR, with values of $R_{max}$ and

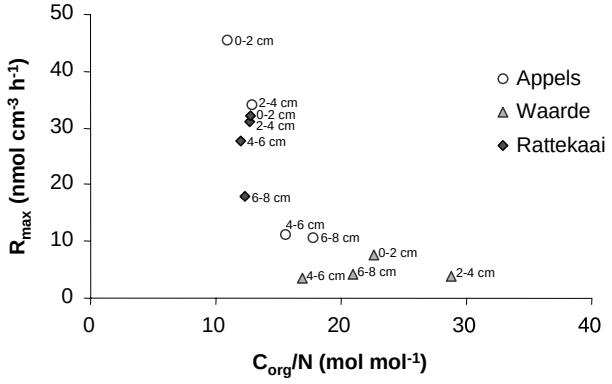



Fig. 10. Maximum sulfate reduction rates obtained at 21 °C in continuous flow-through reactors for sediments of Appels, Waarde and Rattekaai, as a function of the $C_{org}/N$ ratio of the sediments measured after termination of the reactor experiments. Sampling was carried out in February 2003 (Waarde and Rattekaai) and July 2003 (Appels).

Table 3
Literature review of maximum rates ($R_{max}$), sulfate half-saturation concentrations ($K_m$), activation energies ($E_a$) and temperature coefficients ($Q_{10}$) for sulfate reduction

| $R_{max}$ (nmol $cm^{-3}$ $h^{-1}$) | $K_m$ (mM) | $E_a$ (kJ $mol^{-1}$) | $Q_{10}$ | Material | Reference |
|---|---|---|---|---|---|
| | | | | Slurry experiments, hydrothermal springs, Yellowstone Park | |
| 3.9 | 1.24 | 38 | 1.53 | Black sediment pool | Roychoudhury (2004) |
| 0.96 | 3.17 | 119 | 3.79 | Mushroom spring | |
| | | 17 | 1.21 | Obsidian pool | |
| 27–83 | 0.25–0.37 | | | Plug flow-through reactors, freshwater sediment, lake Aydat | Viollier (personal communication) |
| | 0.003 | | | Pure culture of *Thermodesulfobacterium* sp. strain JSP and of *Thermodesulfovibrio* sp. strain R1Ha3 isolated from a microbial mat downstream of a hotspring, Iceland | Sonne-Hansen et al. (1999) |
| 5.2 | 0.24 | | | Plug flow-through reactor used in a recirculation mode, saltmarsh sediment | Roychoudhury et al., 1998 |
| | | 39–88 | | Core incubation, surface sediments (0–2 cm), Chesapeake Bay | Marvin-DiPasquale and Capone (1998) |
| | 0.012 | | | Core incubation, freshwater surface sediment, Lake Kizaki | Li et al. (1996) |
| | 0.244 | | | Free-living cells of *Desulfovibrio desulfuricans* | Fukui and Takii (1994) |
| | 0.009 | | | Anion exchange resin associated cells of *D. desulfuricans* | |
| | 0.008 | | | FeS precipitate associated cells of *D. desulfuricans* | |
| | 0.005 | | | Pure culture of *D. desulfuricans* isolated from a rice paddy soil | Dalsgaard and Bak (1994) |
| | | 114 | 3.3 | Enrichment from sediment of a hydrothermal vent, freshwater Lake Tanganyika | Elsgaard et al. (1994) |
| | 0.01–0.02 | | | Pure culture of *D. desulfuricans* | Okabe et al. (1992) |
| | | 54.1 | 2.25 | Slurry experiments, freshwater littoral sediment (0–5 cm), Lake Constance | Bak and Pfennig (1991) |
| | | | | Core incubation, marine sediment, Long Island Sound | |
| | | 51–68 | 2.1–2.6 | Surface sediment (2–6 cm) | Westrich and Berner (1988) |
| | | 105 | 4.5 | Deep sediment (70–120 cm) | |
| | 0.2 | | | Pure culture of a marine sp. of *Desulfobacter postgatei* | Ingvorsen et al. (1984) |
| 4.3 | 1.62 | | | Core incubation, marine sediment (depth 50–100 cm), Long Island Sound | Boudreau and Westrich (1984) |
| | 0.005 | | | Pure culture of a freshwater sp. of *Desulfovibrio vulgaris* | Ingvorsen and Jørgensen (1984) |
| | 0.077 | | | Pure culture of a marine sp. of *Desulfovibrio salexigens* | |
| | | | 2.9 | Core incubation, eutrophic freshwater lake surface sediment (depth 2–3 cm), Madison, USA | Ingvorsen et al. (1981) |
| 13.2 | 0.068 | | | Core incubation, eutrophic lake basin surface sediment, Michigan | Smith and Klug (1981) |
| | | 85.4 | 3.5 | Core incubation, saltmarsh surface sediment | Abdollahi and Nedwell (1979) |
| | | | 3.4 | Core incubation, coastal marine sediment (depth 0–10 cm), Limfjorden, Denmark | Jørgensen (1977) |

$C_{org}$/N ratios similar to those in the uppermost sediment at Appels.

The uniformly low $R_{max}$ at Waarde correlate with consistently higher $C_{org}$/N ratios, in the range 17–34. These high $C_{org}$/N values imply more degraded organic matter, possibly reflecting a contribution from terrestrial vegetation (Meyers, 1994). Gribsholt and Kristensen (2003) also observed low sulfate reduction activities in the non-vegetated part of the Waarde marsh. They reported much higher activity levels in vegetated areas characterized by high root biomass and elevated pore water dissolved organic carbon (DOC) concentrations.

The enhancement of potential SRR in flow-through experiments upon switching to lactate-amended input solutions confirms that organic carbon availability is a limiting factor for sulfate reduction at all three sites. Furthermore, the relative increases in potential SRR were generally highest in those sediments characterized by organic matter with high $C_{org}$/N ratios and low $R_{max}$ values (Section 3.6), that is, in the sediments where SRB are most limited by organic carbon availability. This is consistent with the delayed response of potential SRR to lactate in these sediments (Fig. 8), which indicates that the resident sulfate-reducing communities need time to adapt to the influx of the labile organic substrate.

The observed activation energies of sulfate reduction fall in the range of previous estimates (Table 3). Although the difference in activation energy for Rattekaai, compared to Appels and Waarde, may reflect physiological differences of the microbial assemblages present in the sediments, further data are needed to confirm this. Nonetheless, the relatively high $E_a$ and $Q_{10}$ values imply that seasonal temperature variations represent a major environmental forcing on benthic sulfate-reducing activity in the estuarine environment (see also Koretsky et al., 2003).

### 4.2. Transport limitation of sulfate reduction

For Appels and Waarde sediments, maximum potential sulfate reduction rates, $R_{max}$, are systematically higher in slurry experiments, compared to flow-through experiments (Fig. 6A). Thus, SRR in the flow-through reactors may be partly limited by transport processes. A similar effect is not observed at Rattekaai, however. Flow-through experiments with intact sediments from Appels and Waarde may experience a higher degree of what Park et al. (2001) refer to as reactive heterogeneity: all flow paths do not necessarily sample the same biomass and therefore do not experience the same reaction history. The lack of mass transfer limitation in Rattekaai sediment may be due to the much higher SRB densities and higher sediment porosities, as these would reduce the transfer distances of dissolved substrates from the pore water medium to particle-bound SRB cells.

Studies on soils show that the micro-scale distribution of microorganisms influences their activities measured at the macroscopic scale (Dechesne et al., 2003; Pallud et al., 2004). Although no information on the spatial distribution of SRB at the micro-habitat level is available for the sediments studied here, one may expect that the probability of dissolved substrates exiting the reactors without encountering particle-bound SRB increases as the cell density decreases. In well-mixed slurry experiments, mass transfer limitations are removed and encounter probabilities between substrates and cells are maximized. Consequently, substrate accessibility for bacteria is artificially increased in slurries, compared to undisturbed sediments, and rates are enhanced (Marxsen and Fiebig, 1993).

Transport limitation due to low SRB community densities at the freshwater site apparently affects both the activity and growth of these organisms in the intact sediment slices. Rapid increases of the SRB community density, by several orders of magnitude, are observed in the slurries. In contrast, measurable SRB biomass growth does not occur during the flow-through experiments, despite the continuous supply of sulfate via the inflow. Because of the absence of stimulated growth, rate parameters measured in the flow-through experiments are therefore more likely to reflect in situ conditions than values obtained from slurry experiments.

### 4.3. Half-saturation concentrations for sulfate

Flow-through experiments and slurry incubations not only yield differences in $R_{max}$ values, but also in sulfate half-saturation concentrations (Fig. 6B). Values of $K_m$ derived from the flow-through reactor experiments more closely approach in situ values, because rate determinations are carried out under conditions of mass transfer limitation, and for spatial distributions of microorganisms and solid-bound substrates, that are similar to those encountered in the field. In addition, the increases in MPN counts of SRB observed in slurry experiments suggest that slurries can also induce significant changes in the microbial community. This may explain in part why $K_m$ values obtained in the slurry experiments are highly variable, even for sediments from the same site (Fig. 5). In contrast, flow-through experiments yield $K_m$ values that fall in much narrower ranges.

The results in Fig. 6B highlight the ambiguities associated with comparing kinetic parameters obtained using different experimental approaches (Park et al., 2001). In sediment slurry experiments, microbial aggregates as well as particle-bound biofilms are disrupted (Marxsen and Fiebig, 1993), which results in an increase of free-living cells over particle-bound cells. The latter may significantly affect apparent $K_m$ values. Fukui and Takii (1994), for instance, showed that $K_m$ for sulfate are 30 times lower for cells of *Desulfovibrio desulfuricans* bound to resin or FeS, compared to free-living cells (Table 3). A similar effect could explain the systematically larger apparent $K_m$ values obtained in the slurry experiments (Fig. 6B).

Notwithstanding potential experimental artifacts, sulfate half-saturation concentrations reported for pure cul-

tures are typically one or two orders of magnitude lower than those inferred for natural SRB communities (Table 3). Pure cultures are typically selected for their high affinity for sulfate, whereas natural SRB communities may comprise organisms exhibiting a range of sulfate uptake efficiencies. Hence, apparent $K_m$ values for natural communities are weighted averages of the $K_m$ values of all the organisms capable of utilizing sulfate as terminal electron acceptor. It is thus not entirely unexpected that the whole sediment $K_m$ values obtained here deviate from those of pure cultures.

It has been proposed that freshwater SRB exhibit a greater affinity for sulfate than their marine counterparts, based on the order of magnitude lower $K_m$ values observed for freshwater culture enrichments (Ingvorsen and Jørgensen, 1984; Ingvorsen et al., 1984; Dalsgaard and Bak, 1994; Table 3). Half-saturation constants derived from core incubation experiments have provided further support for this hypothesis (Smith and Klug, 1981; Boudreau and Westrich, 1984; Li et al., 1996). Presumably, the development of a high affinity uptake system for sulfate by SRB in freshwater environments allows them to cope with the low in situ sulfate concentrations (Purdy et al., 2001).

In contrast, the $K_m$ values obtained in the flow-through experiments in this study are similar for the freshwater and marine sediments (Fig. 5). Our $K_m$ values, however, are consistent with those obtained on intact sediments by other researchers. For instance, Roychoudhury et al. (1998) report a $K_m$ value of 240 μM for a saltmarsh sediment, whereas, for sediments of a freshwater lake, values in the range 250–370 μM have been measured (E. Viollier, Univ. Paris VI, personal communication). Although the available data set is limited, it appears that $K_m$ values for natural SRB communities mostly fall in the range 0.1–1.0 mM, with no systematic dependence on salinity (Table 3). Possibly, $K_m$ values largely reflect the diversity of the resident SRB community. High $K_m$ values ($\geqslant$0.1 mM) indicate a diverse community that includes organisms with both high and low uptake affinities for sulfate, whereas low $K_m$ values (<0.1 mM) correspond to a more specialized SRB community exhibiting a high affinity for sulfate.

## 5. Conclusions

Flow-through experiments with intact sediment slices provide a means to measure kinetic parameters describing the activity of natural microbial communities under near-in situ conditions. Conventional slurry experiments yield $R_{max}$ and $K_m$ values for sulfate reduction that may deviate significantly from those obtained with flow-through reactors. These deviations reflect artifacts induced by the disruption of the sediment and microbial community structure in the slurries.

The Michaelis–Menten rate equation reproduces the observed saturation behavior of the steady-state potential SRR measured in the flow-through experiments, with respect to sulfate. The sulfate half-saturation constants are similar for the freshwater and marine sediments (0.1–0.3 μM), but somewhat higher for the brackish sediments (0.4–0.9 μM). Despite the low sulfate availability and small SRB community density, the freshwater sediments exhibit $R_{max}$ values that are of the same order of magnitude or higher than those obtained for the sediments from the brackish and marine sites. The high potential sulfate-reducing activities at the freshwater site are attributed to a high input of labile organic matter.

In addition to limitation by organic matter availability, transport of dissolved substrates to particle-bound SRB represents a limiting factor for sulfate reduction in the freshwater and brackish sediments. Mass transfer limitation at the Appels and Waarde sites is apparently enhanced by the low sediment porosities and small SRB community sizes, and causes a sub-optimal utilization of available electron donors. Because of the artificially enhanced delivery of substrates to SRB, slurry experiments overestimate in situ potential sulfate reduction rates in these sediments.

Variations in the dissolved sulfate concentration, SRB community size, organic matter availability and temperature all contribute to the highly variable in situ sulfate-reducing activity in sediments along the estuarine gradient. Kinetic experiments such as those presented here can help to quantitatively interpret and predict the spatial variations and temporal variations in SRR in these environments.

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## References

Abdollahi, H., Nedwell, D.B., 1979. Seasonal temperature as a factor influencing bacterial sulfate reduction in a saltmarsh sediment. *Microb. Ecol.* **5**, 73–79.

Bak, F., Pfennig, N., 1991. Microbial sulfate reduction in littoral sediment of Lake Constance. *FEMS Microbiol. Ecol.* **85**, 31–42.

Bear, J., 1972. *Dynamics of Fluids in Porous Media*. Elsevier, New York.

Boudreau, B.P., Westrich, J.T., 1984. The dependence of bacterial sulfate reduction on sulfate concentration in marine sediments. *Geochim. Cosmochim. Acta* **48**, 2503–2516.

Brandt, K.K., Vester, F., Jensen, A.N., Ingvorsen, K., 2001. Sulfate reduction dynamics and enumeration of sulfate-reducing bacteria in hypersaline sediments of the Great Salt Lake (Utah, USA). *Microb. Ecol.* **41**, 1–11.

Canfield, D.E., 1989. Sulfate reduction and oxic respiration in marine sediments: implications for organic carbon preservation in euxinic environments. *Deep-Sea Res. Part I* **36**, 121–138.

Chambers, R.M., Hollibaugh, J.T., Vink, S.M., 1994. Sulfate reduction and sediment metabolism in Tomales bay, California. *Biogeochemistry* **25**, 1–18.

Dalsgaard, T., Bak, F., 1994. Nitrate reduction in a sulfate-bacterium, *Desulfovibrio desulfuricans*, isolated from a rice paddy soil: sulfide inhibition, kinetics and regulation. *Appl. Environ. Microbiol.* **60**, 291–297.

Dechesne, A., Pallud, C., Debouzie, D., Flandrois, J.P., Gaudet, J.P., Grundmann, G.L., 2003. A method to study the microscale 3-D spatial distribution of bacteria in soil. *Soil Biol. Biochem.* **35**, 1537–1546.

Elsgaard, L., Prieur, D., Mukwaya, G.M., Jørgensen, B.B., 1994. Thermophilic sulfate reduction in hydrothermal sediment of Lake Tanganyika, East Africa. *Appl. Environ. Microbiol.* **60**, 1473–1480.

Fauque, G.D., 1995. Ecology of sulfate-reducing bacteria. In: Barton, L.L. (Ed.), *Sulfate-Reducing Bacteria*. Plenum Press, New York, London, pp. 217–242, Chapter 8.

Fukui, M., Takii, S., 1994. Kinetics of sulfate respiration by free living and particle associated sulfate-reducing bacteria. *FEMS Microbiol. Ecol.* **13**, 241–247.

Gribsholt, B., Kristensen, E., 2003. Benthic metabolism and sulfur cycling along an inundation gradient in a tidal *Spartina anglica* salt marsh. *Limnol. Oceanogr.* **48**, 2151–2162.

Henderson, J.F., 1993. Statistical analysis of enzyme kinetic data. In: Eisenthal, R., Danson, M.J. (Eds.), *Enzyme Assays: A Practical Approach*. Oxford University Press, Oxford.

Henrichs, S.M., Reeburgh, W.S., 1987. Anaerobic mineralization of marine organic matter: rates and the role of anaerobic processes in the oceanic carbon economy. *Geomicrobiol. J.* **5**, 191–237.

Holmer, M., Storkholm, P., 2001. Sulphate reduction and sulphur cycling in lake sediments: a review. *Freshwater Biol.* **46**, 431.

Howes, B.L., Dacey, J.W.H., King, G.M., 1984. Carbon flow through oxygen and sulphate reduction pathways in salt marsh sediments. *Limnol. Oceanogr.* **29**, 1037–1051.

Hurtgen, M.T., Lyons, T.W., Ingall, E.D., Cruse, A.M., 1999. Anomalous enrichments of iron monosulfide in euxinic marine sediments and the role of $H_2S$ in iron sulfide transformations: examples from Effingham Inlet, Orca Basin, and the Black Sea. *Am. J. Sci.* **299**, 556–588.

Hyacinthe, C., Van Cappellen, P., 2004. An authigenic iron phosphate phase in estuarine sediments: composition, formation and chemical reactivity. *Mar. Chem.* **91**, 227–251.

Ingvorsen, K., Jørgensen, B.B., 1984. Kinetics of sulfate uptake by freshwater and marine species of *Desulfovibrio*. *Arch. Microbiol.* **139**, 61–66.

Ingvorsen, K., Zehnder, A., Jørgensen, B.B., 1984. Kinetics of sulfate and acetate uptake by *Desulfovibrio postgatei*. *Appl. Environ. Microbiol.* **47**, 403–408.

Ingvorsen, K., Zeikus, J.G., Brock, T.D., 1981. Dynamics of bacterial sulfate reduction in a eutrophic lake. *Appl. Environ. Microbiol.* **42**, 1029–1036.

Jones, J.G., Simon, B.M., 1981. Differences in microbial decomposition processes in profundal and littoral lake sediments, with particular reference to the nitrogen cycle. *J. Gen. Microbiol.* **123**, 297–312.

Jørgensen, B.B., 1978. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments I. Measurement with radiotracer techniques. *Geomicrobiol. J.* **1**, 11–27.

Jørgensen, B.B., 1977. The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). *Limnol. Oceanogr.* **22**, 814–932.

Jørgensen, B.B., 1982. Mineralization of organic matter in the sea bed—the role of sulphate reduction. *Nature* **296**, 643–645.

King, G.M., 1988. Patterns of sulfate reduction and the sulfur cycle in a South Carolina salt marsh. *Limnol. Oceanogr.* **33**, 376–390.

Koretsky, C., Moore, C., Lowe, K., Meile, C., DiChristina, T., Van Cappellen, P., 2003. Seasonal oscillation of microbial iron and sulfate reduction in saltmarsh sediments. *Biogeochemistry* **64**, 179–203.

Koretsky, C.M., Van Cappellen, P., DiChristina, T.J., Kostka, J.E., Lowe, K.L., Moore, C.M., Roychoudhury, A.N., Viollier, E., 2005. Salt marsh pore water geochemistry does not correlate with microbial community structure. *Estuar. Coast. Shelf Sci.* **62**, 233–251.

Kostka, J.E., Roychoudhury, A., Van Cappellen, P., 2002. Rates and controls of anaerobic microbial respiration across spatial and temporal gradients in saltmarsh sediments. *Biogeochemistry* **60**, 49–76.

Li, J.-H., Purdy, K.J., Takii, S., Hayashi, H., 1999. Seasonal changes in ribosomal RNA of sulfate-reducing bacteria and sulfate-reducing activity in a freshwater lake sediment. *FEMS Microbiol. Ecol.* **28**, 31–39.

Li, J.-H., Takii, S., Kotakemori, R., Hayashi, H., 1996. Sulfate reduction in profundal sediments in Lake Kizaki, Japan. *Hydrobiologia* **333**, 201–208.

Marvin-DiPasquale, M.C., Capone, D.G., 1998. Benthic sulfate reduction along the Chesapeake Bay central channel. I. Spatial trends and controls. *Mar. Ecol. Prog. Ser.* **168**, 213–228.

Marxsen, J., Fiebig, D.M., 1993. Use of perfused cores for evaluating extracellular enzyme activity in stream-bed sediments. *FEMS Microbiol. Ecol.* **13**, 1–12.

Meyers, P.A., 1994. Preservation of elemental and isotopic source identification of sedimentary organic matter. *Chem. Geol.* **114**, 289–302.

Middelburg, J.J., Klaver, G., Nieuwenhuize, J., Wielemaker, A., de Haas, W., Vlug, T., van der Nat, J.F.W.A., 1996. Organic matter mineralization in intertidal sediments along an estuarine gradient. *Mar. Ecol. Prog. Ser.* **132**, 157–168.

Nedwell, D.B., Walker, T.R., Ellis-Evans, J.C., Clarke, A., 1993. Measurements of seasonal rates and annual budgets of organic carbon fluxes in Antarctic coastal environment at Signy Island, South Orkney Islands, suggest a broad balance between production and decomposition. *Appl. Environ. Microbiol.* **59**, 3989–3995.

Okabe, S., Nielsen, P.H., Characklis, W.G., 1992. Factors affecting microbial sulfate reduction by *Desulfovibrio desulfuricans* in continuous culture: limiting nutrients and sulfide concentration. *Biotechnol. Bioeng.* **40**, 725–734.

Pallud, C., Van Cappellen, P., 2003. Kinetic study of sulfate reduction in estuarine sediments. *Geophysical Research Abstracts* **5**, EGS-AGU–EUG Joint Assembly, Nice, France, April 6–11.

Pallud, C., Dechesne, A., Gaudet, J.P., Debouzie, D., Grundmann, G.L., 2004. Modification of the spatial distribution of the 2,4-Dichlorophenoxyacetic acid degrader micro-habitats during growth in soil columns. *Appl. Environ. Microbiol.* **70**, 2709–2716.

Park, J.-H., Zhao, X., Voice, T.C., 2001. Comparison of biodegradation kinetic parameters for naphthalene in batch and sand column systems by *Pseudomonas putida*. *Environ. Prog.* **20**, 93–102.

Pfannkuch, H.O., 1963. Contribution à l'étude des déplacements de fluides miscibles dans un milieu poreux. *Rev. Inst. Fr. Petrol.* **18**, 215–270.

Purdy, K.J., Nedwell, D.B., Embley, T.M., Takii, S., 2001. Use of 16S rRNA-targeted oligonucleotide probes to investigate the distribution of sulphate-reducing bacteria in estuarine sediments. *FEMS Microbiol. Ecol.* **36**, 165–168.

Roychoudhury, A., 2004. Sulfate respiration in extreme environments: a kinetic study. *Geomicrobiol. J.* **21**, 33–43.

Roychoudhury, A.N., Viollier, E., Van Cappellen, P., 1998. A flow-through reactor for studying biogeochemical reactions in undisturbed aquatic sediments. *Appl. Geochem.* **13**, 269–280.

Sass, H., Cypionka, H., Babenzien, H.-D., 1997. Vertical distribution of sulfate-reducing bacteria at the oxic–anoxic interface in sediments of the oligotrophic Lake Stechlin. *FEMS Microbiol. Ecol.* **22**, 245–255.

Sievert, S.M., Brinkhoff, T., Muyzer, G., Ziebis, W., Kuever, J., 1999. Spatial heterogeneity of bacterial populations along an environmental gradient at a shallow submarine hydrothermal vent near Milos Island (Greece). *Appl. Environ. Microbiol.* **65**, 3834–3842.

Smith, R.L., Klug, M.J., 1981. Reduction of sulfur compounds in the sediments of a eutrophic lake basin. *Appl. Environ. Microbiol.* **41**, 1230–1237.

Sonne-Hansen, J., Westermann, P., Ahring, B.K., 1999. Kinetics of sulfate and hydrogen uptake by the thermophilic sulfate-reducing bacteria *Thermodesulfobacterium* sp. strain JSP and *Thermodesulfovibrio* sp. strain R1Ha3. *Appl. Environ. Microbiol.* **65**, 1304–1307.

Teske, A., Wawer, C., Muyzer, G., Ramsing, N.B., 1996. Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments. *Appl. Environ. Microbiol.* **62**, 1405–1415.

Thode-Andersen, S., Jørgensen, B.B., 1989. Sulphate reduction and the formation of $^{35}$S-labeled FeS, $FeS_2$, and S in coastal marine sediments. *Limnol. Oceanogr.* **34**, 793–806.

Thullner, M., Van Cappellen, P., Regnier, P., 2005. Modeling the impact of microbial activity on redox dynamics in porous media. *Geochim. Cosmochim. Acta* **69**, 5005–5019.

Trimmer, M., Purdy, K.J., Nedwell, D.B., 1997. Process measurement and phylogenetic analysis of the sulphate reducing bacterial communities of two contrasting benthic sites in the upper estuary of the Great Ouse, Norfolk, UK. *FEMS Microbiol. Ecol.* **24**, 333–342.

Tschech, A., Pfennig, N., 1984. Growth yield increase linked to caffeate reduction in *Acetobacterium woodii*. *Arch. Microbiol.* **137**, 163–167.

Wellsbury, P., Herbert, R.A., Parkes, R.J., 1996. Bacterial activity and production in near-surface estuarine and freshwater sediments. *FEMS Microbiol. Ecol.* **19**, 203–214.

Westrich, J.T., Berner, R.A., 1988. The effect of temperature on rates of sulfate reduction in marine sediments. *Geomicrobiol. J.* **6**, 99–117.

Widdel, F., 1988. Microbiology and ecology of sulfate- and sulfur-reducing bacteria. In: Zehnder, A.Jb. (Ed.), *Biology of Anaerobic Organisms*. Wiley, New York, pp. 469–586, Chapter 10.

Widdel, F., Bak, F., 1992. Gram-negative mesophilic sulfate-reducing bacteria. In: Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H. (Eds.), *The Procaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*. Springer Verlag, New York, pp. 3352–3378.

Wieringa, E.A., Overmann, J., Cypionka, H., 2000. Detection of abundant sulphate-reducing bacteria in marine oxic sediment layers by a combined cultivation and molecular approach. *Environ. Microbiol.* **2**, 417–427.

Wind, T., Conrad, R., 1995. Sulfur compounds, potential turnover of sulfate and thiosulfate, and numbers of sulfate-reducing bacteria in planted and unplanted paddy soil. *FEMS Microbiol. Ecol.* **18**, 257–266.

Wollast, R., 1988. The Scheldt estuary. In: Salomons, W., Blayne, B., Duursma, E., Forstmer, U. (Eds.), *Pollution of the North Sea: An Assessment*. Springer Verlag, Berlin, pp. 183–193.

Woomer, P.A., 1994. Most probable number counts. In: Mickelson, S.H., Bigham, J.M. (Eds.), *Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties*. SSSA Book Series, No. 5. Madison, WI, pp. 59–79.