



# Kinetics of microbially mediated reactions: dissimilatory sulfate reduction in saltmarsh sediments (Sapelo Island, Georgia, USA)

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## Abstract

A sediment disk reactor was tested in once flow-through mode to retrieve kinetic parameters for the Monod rate law that describes sulfate reduction. The experimental method was compared with a previously described procedure by the authors where a sediment plug-flow reactor was operated in a recirculation mode. In recirculation mode, accumulation of metabolic byproducts in certain cases may result in negative feedback, thus preventing accurate determination of kinetic information. The method described in this article provides an alternative to the recirculation sediment plug-flow-through reactor technique for retrieving kinetic parameters of microbially mediated reactions in aquatic sediments.

For sulfate reduction in a saltmarsh site, a maximum estimate of the half-saturation concentration,  $K_s$ , of  $204 \pm 26 \mu\text{M}$  and a maximum reaction rate,  $R_m$ , of  $2846 \pm 129 \text{ nmol cm}^3_{(\text{wet sediment})} \text{ d}^{-1}$  was determined. The  $K_s$  value obtained was consistent with the one estimated previously ( $K_s = 240 \pm 20 \mu\text{M}$ ) from a different site within the same saltmarsh mud flat using a recirculating reactor. From the  $R_m$  value and reduction rates determined using  $^{35}\text{SO}_4^{2-}$  incubation experiments, we infer that sulfate reduction is limited in the field. Substrate availability is not the main contributor for the limitation, however. Competition from other microbes, such as iron reducers affects the activity of sulfate reducers in the suboxic to anoxic zones, whereas aerobes compete in the oxic zone. High sulfide concentration in the pore water may also have acted as a toxin to the sulfate reducers in the field.

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**Keywords:** microbial reactions; disk flow-through reactor; sulfate reduction; half saturation constant; saltmarsh; wetlands; monod kinetics; biogeochemical reactions

## 1. Introduction

Sulfate reduction is considered to be one of the major pathways for carbon mineralization in coastal marine and saltmarsh environments (Boudreau & Westrich, 1984; Giblin & Wieder, 1992; Howarth, 1984; Jørgensen, 1977; Luther & Church, 1992; Westrich, 1983). In saltmarsh sediments, sulfate reduction can account for more than 50% of the total integrated rate of organic carbon oxidation (Howarth & Hobbie, 1982; King, 1988). Sulfate reduction also has a number of environ-

mental and ecological consequences. Sulfide produced during sulfate reduction is toxic to biota and may affect their physiological functions profoundly when present in high concentrations (Bagarinao, 1992). Sulfide also reduces Mn- and Fe-(hydro)oxides (Aller & Rude, 1988; Burdige & Nealson, 1986; Peiffer, 1994; Postma, 1985; Thamdrup, Fossing, & Jørgensen, 1994; Yao & Millero, 1996), thereby releasing trace and heavy metals associated with them, potentially causing contamination of the surrounding ground water. On a positive note, the sulfate reduction process has been applied for bioremediation of hydrocarbon, heavy metal, and radioactive contaminants (Farmer, Updegraff, Radehaus, & Bates, 1995; Fortin & Beveridge, 1997; Ludvigsen, Heron, Albrechtsen, & Christensen, 1995; Vile & Wieder,

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1993; Vroblesky, Bradley, & Chapelle, 1996). Treatment of acidity in lakes and mine drainage with sulfate reduction is also widely practiced (Benner, Gould, & Blowes, 2000; Christensen, Laake, & Lien, 1996; Cocos, Zagury, Clément, & Samson, 2002; Elliott, Ragusa, & Catchside, 1998; Foucher, Battaglia-Brunet, Ignatiadis, & Morin, 2001; Tuskamoto & Miller, 1999; Tyrrell, Mulligan, Sly, & Bell, 1997). In spite of its importance in early diagenetic and other environmental processes, little is known about the reaction kinetics of sulfate reduction in natural sediments. Besides their own work, the authors know of only one other study (Boudreau & Westrich, 1984), where the value of the half saturation constant for sulfate utilization in natural marine sediment has been determined.

To quantify sulfate and its movement in surface or subsurface environments, data on the rate of sulfate reduction that are relevant to field situations are required. Various types of reactors, such as sediment plug reactors, batch reactors and stirred tank mixed-flow reactors, are in use for retrieval of kinetic data (Brezonik, 1994; Furrer, Klausen, & Gfeller, 1999; Ganor, Mogolón, & Lasaga, 1999; Lea & Adesina, 1999; Li & Norcross, 1997; de Pablo et al., 1999; Roychoudhury, Viollier, & Van Cappellen., 1998; San, 1994; Van Cappellen & Qiu, 1997). Of these, sediment plug reactors provide an experimental system that mimics the natural soil or sediment system. Furthermore, since most natural systems are open, they are best represented by a continuous flow-through system. Hence, a sediment plug reactor operating in continuous flow-through mode permits precise determination of kinetic parameters at steady state. A plug-flow-through reactor operating in recirculation mode was previously described by the authors to study kinetics of biogeochemical reactions in aquatic sediments (Roychoudhury et al., 1998). The setup combined the advantages of a plug-flow and a

mixed-flow reactor. The recirculating reactor, however, may suffer at times with certain drawbacks similar to batch reactors. The reaction byproducts may cause negative feedback effects on the reaction process in question. In this article we present (a) a newly designed disk reactor and (b) its operation in once flow-through mode to measure Monod reaction constants for dissimilatory sulfate reduction. The method described here can easily be applied to measure kinetic parameters for other microbially mediated reactions.

## 2. Materials and methods

### 2.1. Reactor

The reactor consists of a cylindrical disk 2 cm in length and 4.7 cm inside diameter, made from precision bore Plexiglas® (Fig. 1a). Sediments are placed inside the hollow disk and the reactor is closed using Plexiglas® caps kept in place using steel push plates on each end tightened with bolt and wing screw nuts (Fig. 1a). An O-ring pressed between the caps and the reactor cell prevents any leakage of fluid from the pressure joint. Inside each cap is a shallow depression where a 1-mm-thick, 0.45  $\mu\text{m}$  hydrophobic porous plate sits. An additional 0.45  $\mu\text{m}$  PTFE or nylon filter membrane is placed between the sediment plug and the porous plate. Each cap has an input–output channel that opens at the center towards the inner side of the cap. To avoid two-dimensional flow inside the reactor, grooves radiating from the center are constructed inside the caps (Fig. 1b). These grooves help create a radially uniform in-phase flow throughout the cross-section of the sediment plug. The dead volume associated with the grooves represents less than  $5 \times 10^{-3}\%$  of the total reactor volume and it can therefore be neglected when calculating

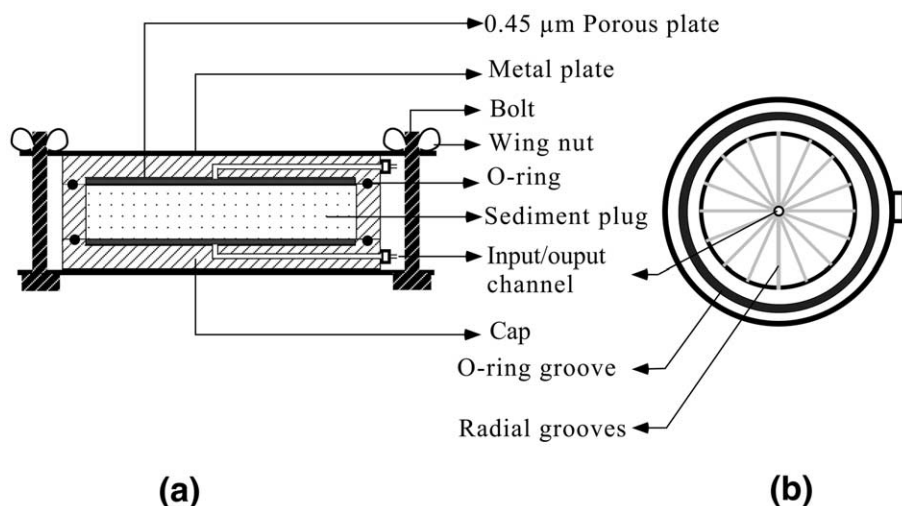


Fig. 1. A schematic figure showing (a) vertical cross-section of the reactor assembly and (b) the top view of inside of the reactor cap.

the interstitial volume of the sediment plug from the measured sediment porosity.

## 2.2. Site description and sediment

Sediment samples were collected from a saltmarsh mud flat at the southern end of Sapelo Island (Georgia, USA). A medium size tidal creek flows through the Southwest end of the marsh, and *Spartina alterniflora* constitutes the dominant vegetation. For the reactor experiments, sediments were collected from the middle of the marsh, 40 m away from the tidal creek bank. Twice every day the site is inundated by tidal water. Piezometers installed at the site show that the water table does not descend below the ground surface even during low tides, suggesting a restrictive movement of groundwater. Fiddler crab burrows, sometimes reaching to a depth of 40–45 cm, are found at the site. *Spartina* roots provide an additional conduit for the diffusion of oxygen below the surface, creating oxic microcosms around the roots that are predominantly surrounded by anaerobic sediments (Dacey & Howes, 1984; Howes, Dacey, & Goehring, 1986; Luther, Giblin, Howarth, & Ryans, 1982).

In order to retrieve data that can be extrapolated to field situations, undisturbed cores constitute an important aspect of the experiments. Hand pack columns or reactors filled with grab samples compromise the integrity of the sediment structure. Mixing of sediment or hand manipulation destroys the porous structure, especially the interconnectivity and the throat size of the pores. Both have a major effect on accurate retrieval of kinetic parameters (Roychoudhury, 2001; Roychoudhury et al., 1998). For reactor experiments, the sediments were collected with a specialized suction corer designed for retrieving undisturbed core sections. Once a core was retrieved, it was transferred to the field laboratory within 5 min and processed in less than half an hour. The reactors were stored in the dark at 4 °C under anaerobic conditions until they were used for experiments the next day. At that time reactors were transferred inside an anaerobic glove box and were kept in dark throughout the experiments. A single reactor with sediments from the depth of 3–5 cm was used for this study.

The sediments were characterized for their particle size, porosity and chemical composition. Size analysis of the coarser sediments was done by sieving (ASTM, D 422-63, reapproved, 1990). Finer sediments were suspended in deionized water, sonicated and analyzed by a laser-based particle size analysis system (Brinkman, Model 2010). Porosity was determined by measuring the weight loss of a saturated sediment section packed in a 1 ml syringe after air-drying it. Density was calculated by measuring the weight of sediment in the same syringe used for porosity determination.

Total carbon in the sediment samples was measured by high-temperature (900–1000 °C) combustion, followed by CO<sub>2</sub> titration (Coulometrics, Model 5022). The inorganic (carbonate) carbon content of the sediments was obtained by titrating the CO<sub>2</sub> released after treating the samples with a 2 N H<sub>2</sub>SO<sub>4</sub> solution. The organic carbon concentration was calculated as the difference between total and inorganic carbon. Solid phase sulfides (acid volatile sulfides (AVS) (H<sub>2</sub>S + FeS); and chromium reducible sulfides (CRS) (FeS<sub>2</sub> + S<sup>0</sup>)) were determined by a two-step distillation process modified after Fossing and Jørgensen (1989) and Canfield, Raiswell, Westrich, Reaves, and Berner (1986). Reactive iron was quantified using an ascorbate extraction method described by Kostka and Luther (1995).

## 2.3. Reactor experiments

The experiments were performed inside an anaerobic chamber with nitrogen atmosphere. The usual mixture of N<sub>2</sub>–CO<sub>2</sub>–H<sub>2</sub> was avoided to make sure that the microbial population does not utilize CO<sub>2</sub> or H<sub>2</sub> as electron donor. The reactor assembly was submerged in a water bath to maintain constant temperature of 25 ± 1 °C. The reactor was operated in once flow-through mode to determine rate parameters for sulfate reduction. In once flow-through mode the effluent concentration is measured after the fluid has traversed the length of the reactor once. Input sulfate solution of different concentrations was prepared by mixing reagent grade Na<sub>2</sub>SO<sub>4</sub> in degassed 0.5 M NaCl solution. Sulfate solution of known concentration was forced through the reactor using a programmable syringe pump against gravity. Samples were collected at the outlet at 24-h intervals using a fraction collector. For each sampling period, two test tubes were arranged one after another on the fraction collector. The first one was initially kept empty to collect sample for sulfate and alkalinity analysis. The other tube was pre-charged with sulfide trapping solution (zinc acetate). Towards the end of the experiment, additional tubes pre-charged with ferrozine solution were used to collect samples for the analysis of dissolved iron. The samples were later analyzed using spectrophotometric methods for their alkalinity (Sarazin, Michard, & Prevot, 1999), sulfate (Tabatabai, 1974), total dissolved sulfide (Cline, 1969), and Fe(II) concentration (Stookey, 1970). pH was constantly monitored using a flow-through pH cell attached at the outlet of the reactor. For each experiment, sampling continued until a steady state was achieved. Then the input sulfate concentration was changed to force the system to reach a new steady state. Five different steady states were achieved by using decreasing input sulfate concentrations of 32.2, 24.8, 11.7, 5.4 and 2.1 mM. Between each step where a change in concentration of

input solution was made, the reactor was flushed for 2 d at a flow rate of  $3 \text{ ml h}^{-1}$  and then at a flow rate of  $2.9 \text{ ml d}^{-1}$  for at least 5 d before the start of sampling. Samples were collected for 9 d at a constant flow rate of  $2.9 \pm 0.2 \text{ ml d}^{-1}$ .

#### 2.4. Incubation experiments using $^{35}\text{SO}_4^{2-}$

Within 0.5 m radius of the site used for collecting samples for the reactor, an additional sediment core was collected using a wedge corer. One to four centimeters thick sediment sections were cut under anaerobic conditions and each sediment section was divided into two parts. One part was used to determine sediment porosity and density. The second part was used for preparing duplicate samples for measurement of sulfate reduction rates using  $^{35}\text{SO}_4^{2-}$  radio-tracer technique modified after Jørgensen (1978). Sulfate reduction rates were calculated based on the amount of  $^{35}\text{S}$  incorporated in AVS and CRS fractions.

### 3. Theory

For a flow-through reactor, the steady state reaction rate for utilization of a substrate by microbes can be calculated by a simple expression

$$R = \frac{\Delta C \times Q}{V} \quad (1)$$

where  $\Delta C$  is the change in concentration of the substrate between the inlet and the outlet of the reactor,  $Q$  the volumetric flow rate of the substrate through the reactor, and  $V$  is the volume of the reactor cell.

Microbial reactions are often limited by the availability of nutrients. Sulfate reducers depend on sulfate and organic carbon for their growth. When organic carbon is not limiting, the sulfate reduction rate becomes dependent on the concentration of available sulfate. This functionality can be expressed by an empirical non-linear relationship derived by Monod (1949),

$$R = \frac{R_m [C]}{K_s + [C]} \quad (2)$$

where  $R_m$  denotes the maximum reaction rate,  $K_s$  the half saturation constant, and  $[C]$  is the substrate concentration. The relationship can be linearized by taking the reciprocal of both the sides of Eq. (2),

$$\frac{1}{R} = \frac{K_s}{R_m} \frac{1}{C} + \frac{1}{R_m} \quad (3)$$

This is an equation of a straight line with  $1/R$  and  $1/C$  being the variables. Monod parameters,  $K_s$  and  $R_m$  are determined from the slope and the intercept of this line.

### 4. Results

#### 4.1. Sediments

Physico-chemical characteristics of the sediment used within the reactor are given in Table 1. At the sampling site, sediments were black in color and homogeneous with mostly clay size particles. Occasionally, sand lenses were evident within the sediment. An extensive root zone extending up to the depths of 15–20 cm was observed. Ascorbate extractable iron content was relatively low ( $\sim 80 \mu\text{mol cm}^{-3}$ ) at the surface with a rapid decrease to about  $10 \mu\text{mol cm}^{-3}$  within the first 6 cm. The highest concentration of AVS ( $8 \mu\text{mol cm}^{-3}$ ) was observed in the top 1 cm below which it decreased exponentially. Most of the sulfur was tied in the CRS form ( $125\text{--}395 \mu\text{mol(S) cm}^{-3}$ ) and the concentrations increased with depth. Intermittent peaks between 5 and 8 cm were observed; mostly associated with the *Spartina* roots. Even though the site was vegetated with *Spartina* grass, the organic carbon content of the sediment was not very high ( $4.09 \pm 0.05\%$ ). This value is very

Table 1  
Sulfate reduction experiment results and sediment properties from two separate studies are compared. Both studies were performed on sediments collected from a saltmarsh mud flat in Sapelo Island, GA (USA)

	Previous study <sup>a</sup>	This study
Reactor type	Recirculating plug flow-through reactor	Once flow-through disk reactor
Sediment collection date	October 1996	May 1997
Site location	On the bank of a tidal creek	Middle of the marsh field
Depth from sediment–water interface (cm)	5–9	3–5
In situ temperature (°C)	16	23
Density ( $\text{g cm}^{-3}$ )	1.31	1.25
Porosity (%)	77	87
Mean grain size diameter (cm)	$2 \times 10^{-2}$	$2 \times 10^{-5}$
Inorganic carbon content (%)	0.20	0.15
Organic carbon content (%)	2.81	4.09
Ascorbate extractable reactive iron ( $\mu\text{mol cm}^{-3}$ )	111.77	49.2
Acid volatile sulfides ( $\mu\text{mol cm}^{-3}$ )	3.69	3.54
Chromium reducible sulfides ( $\mu\text{mol cm}^{-3}$ )	159.15	519.48
$R_m$ ( $\text{nmol cm}^{-3} \text{ d}^{-1}$ )	$126 \pm 5$	$2846 \pm 129$
$K_s$ ( $\mu\text{M}$ )	$240 \pm 20$	$204 \pm 26$

<sup>a</sup> See the study by Roychoudhury et al. (1998).

small as compared to those reported in the literature (11–43%) from other east coast saltmarshes (Barnes, Craft, & Windom., 1973). Negligible amount of inorganic carbon was found in the sediment (Table 1).

#### 4.2. Reactor experiments

Laboratory experiments close to field conditions were conducted with sulfate present as the sole electron acceptor in the input solution. During flow-through experiments, the effluent was constantly monitored for pH, alkalinity, dissolved sulfide, iron and sulfate concentration (Fig. 2). Once constant sulfate concentration was observed in the effluent, the system was permitted to change to another steady state by changing the input sulfate concentration. Five different steady states were achieved during a set of experiments (Table 2).

pH was buffered by the sediment between 7 and 7.5 for all the experiments. No further adjustment of pH

was made as the resultant pH of the solution in the reactor was within the range of pH (6.7–7.5) measured in the field. During sulfate reduction, for each mole of sulfate reduced, 2 mol of carbonate alkalinity are produced. This correlation was observed fairly closely for each experiment (Fig. 2). In some cases the measured alkalinity was higher by 0.2–0.5 mM than that expected from the stoichiometry of the reaction. When alkalinity data along with sulfide data are plotted on an extended scale, it is observed that increasing trends in sulfide profile corresponds to alkalinity increase at those time intervals. Calculations confirm that any excess alkalinity is caused by dissolved sulfide. Even though the sulfate reduction rate in each experiment was fairly high (Table 2), only micromolar range of sulfide was measured in the outflow (Fig. 2). The low sulfide concentrations are the result of their reaction with iron oxyhydroxides and subsequent precipitation as iron sulfides within the reactor. No Fe(II) was detected in the outflow except in case of the last two experiments, where dissolved iron

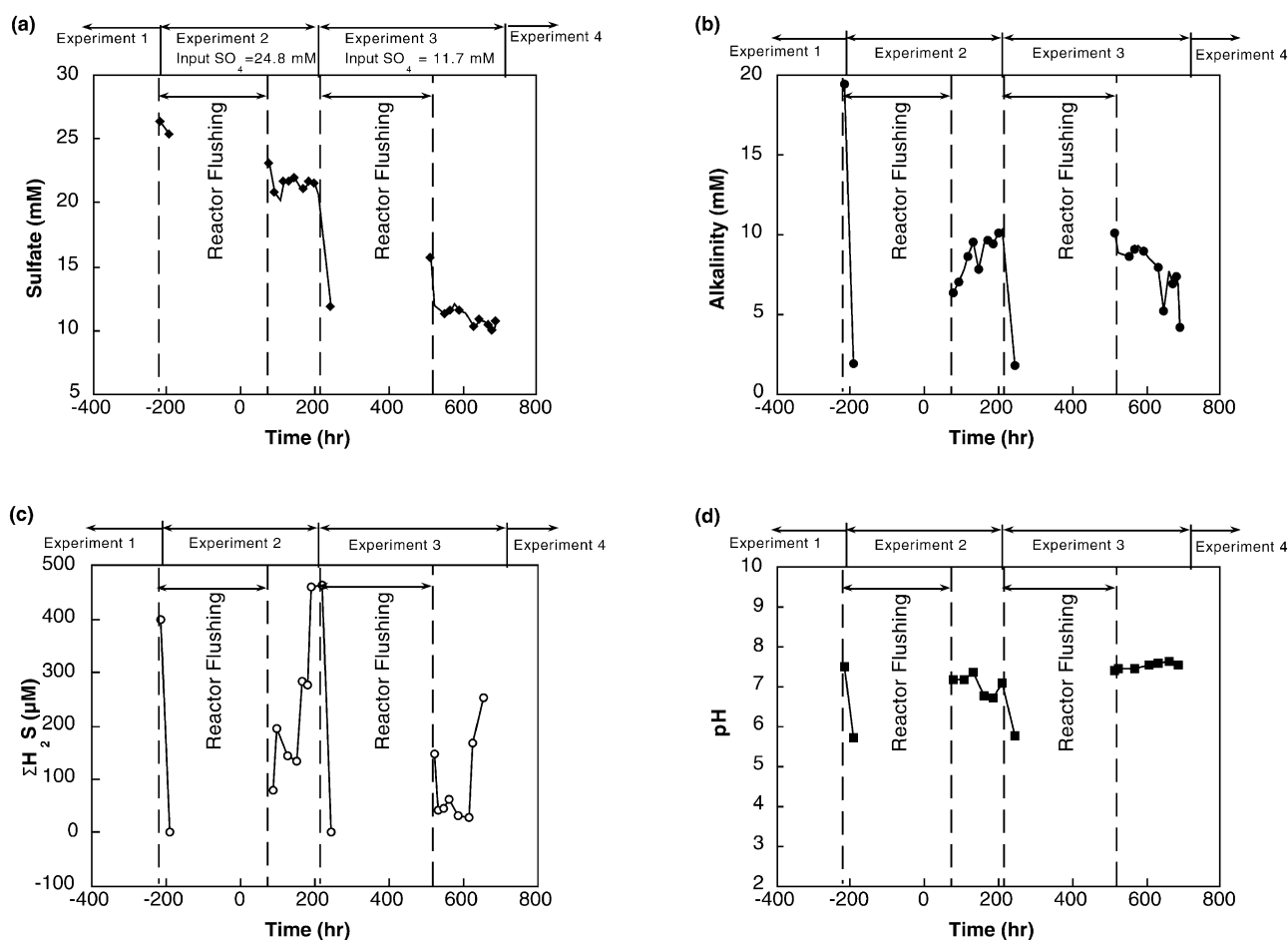


Fig. 2. Time course analysis of (a) sulfate, (b) alkalinity, (c) total sulfide, and (d) pH in the effluent from two of the experiments (2 and 3) performed in series using the disk flow-through reactor (Table 2). The reactor was flushed each time a change in input solution was made after reaching a steady state and before sampling started. Zero on the time axis denotes the time when flow rate for the second experiment was set for sampling. The change in concentration of sulfate at steady state between the inlet and the outlet of the reactor was used to calculate the rate of sulfate reduction.

Table 2

Calculated steady state sulfate reduction rates from disk flow-through reactor experiments. A constant temperature ( $25 \pm 1^\circ\text{C}$ ) and flow rate ( $2.9 \pm 0.2\text{ cm}^3\text{ d}^{-1}$ ) was maintained during the experiments

Experiment no.	$\text{SO}_4^{2-}$ (mM)		Rate ( $\mu\text{mol cm}^{-3}\text{ d}^{-1}$ )
	Input	Output	
1	32.6	$26.4 \pm 0.10$	$0.519 \pm 0.008$
2	24.8	$21.4 \pm 0.10$	$0.304 \pm 0.008$
3	11.7	$10.4 \pm 0.25$	$0.116 \pm 0.022$
4	5.4	$4.6 \pm 0.03$	$0.072 \pm 0.003$
5	2.1	$1.8 \pm 0.05$	$0.027 \pm 0.005$

concentrations between 10 and  $40\ \mu\text{M}$  were measured (data not shown). This further indicates the formation of iron sulfides within the reactor.

At steady state, rate of reduction was calculated using Eq. (1) (Table 2). Monod parameters for sulfate reduction were derived by plotting reduction rate data on a Lineweaver–Burk plot (Roels, 1983). From the y-intercept of the best-fit line ( $R^2 = 0.99$ ), a  $R_m$  value of  $2846 \pm 129\ \text{nmol cm}^{-3}_{(\text{wet sediment})}\text{ d}^{-1}$  was obtained. From the slope of the line a  $K_s$  value of  $204 \pm 26\ \mu\text{M}$  was calculated (Fig. 3).

#### 4.3. Incubation experiments

Depth profile of the sulfate reduction rate measured during incubation experiments is shown in Fig. 4(a). Sulfate reduction was confined up to a depth of 20 cm from the sediment–water interface. A reduction rate of  $785 \pm 21\ \text{nmol cm}^{-3}\text{ d}^{-1}$  was measured in the surficial sediments (0–1 cm). Below 1 cm, rates increased rapidly and a peak was observed at a depth of 5 cm (Fig. 4(a)). Exponential decrease in the reduction rates was observed below 5 cm and no sulfate reduction was observed below a depth of 20 cm.

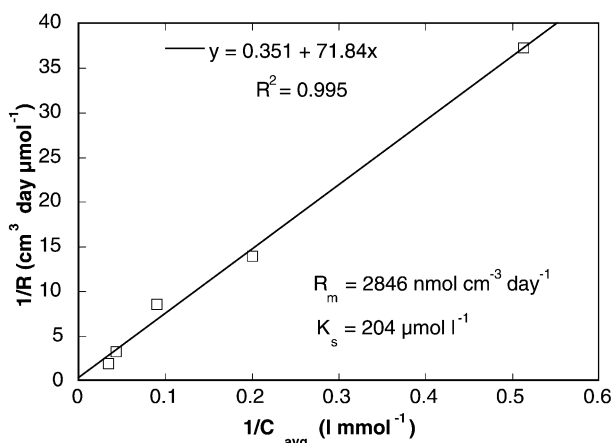


Fig. 3. Reciprocal of the average steady state concentration of sulfate inside the reactor is plotted versus the inverse of the measured sulfate reduction rate. A least square fit of the experimental data is used to retrieve  $K_s$  and  $R_m$  parameters in the Monod expression (Eq. (2)).

## 5. Discussion

Continuous flow-through reactors can be classified as column, recirculation, or disk reactor based on their physical shape and mode of operation (Brezonik, 1994). In column reactors, length of the reactor cell is much larger than its diameter whereas in disk reactor the cell length is negligibly small. A column or disk reactor showing ideal behavior is known as a plug-flow reactor. A volume of fluid entering the inlet in such a case remains a discrete entity as it flows through the reactor. Recirculation reactors are completely mixed at all times such that reactant concentrations are the same throughout their volume. Perfect mixing has two important consequences for kinetic studies of biological systems. Rates are constant throughout the reactor and are equal to the rates at the outlet. The mass balance derivation of kinetic equation is simplified. Reaction rate can be determined directly from the influent and effluent concentrations, reactor volume and flow rate (Eq. (1)). Therefore, recirculation reactors are preferred over plug-flow reactors to study biological systems. However, in recirculation reactors, since most of the fluid is circulated back into the reactor, in certain cases metabolic byproducts may accumulate over time causing inhibitory effects on the microbial process within the reactor. In such cases the once flow-through system is advantageous.

In once flow-through systems, however, the concentration of the reactants constantly changes as the fluid traverses the length of the reactor. Correspondingly, rates at each point within the plug-flow reactor also change. For this reason it is much more complicated to model a plug-flow system. If kinetic information is to be retrieved using numerical models, a priori knowledge of dispersion, advection and a function defining reaction rate law is a must. This poses a major obstacle to study microbially mediated reactions as rate laws for most biogeochemical reactions are not known.

The newly designed disk reactor can be operated in once flow-through mode to obtain useful kinetic information without a priori knowledge of rate laws, same as any recirculating reactor. Certain assumptions during data analysis are necessary, though. For equal extents of reaction, the rates throughout the recirculation reactor are equal to the lowest rate, i.e. rate measured at the outlet of a plug-flow reactor. Hence, Eq. (1) also defines the overall rate for a disk reactor. Because of a concentration gradient inside the plug-flow reactor, unlike in the recirculation reactor, the rate data cannot be plotted as a function of concentration measured at the outlet to retrieve Monod parameters. However, in a disk reactor the distance between the inlet and outlet is very short. Short length of the reactor has two advantages. A linear concentration gradient between the inlet and the outlet

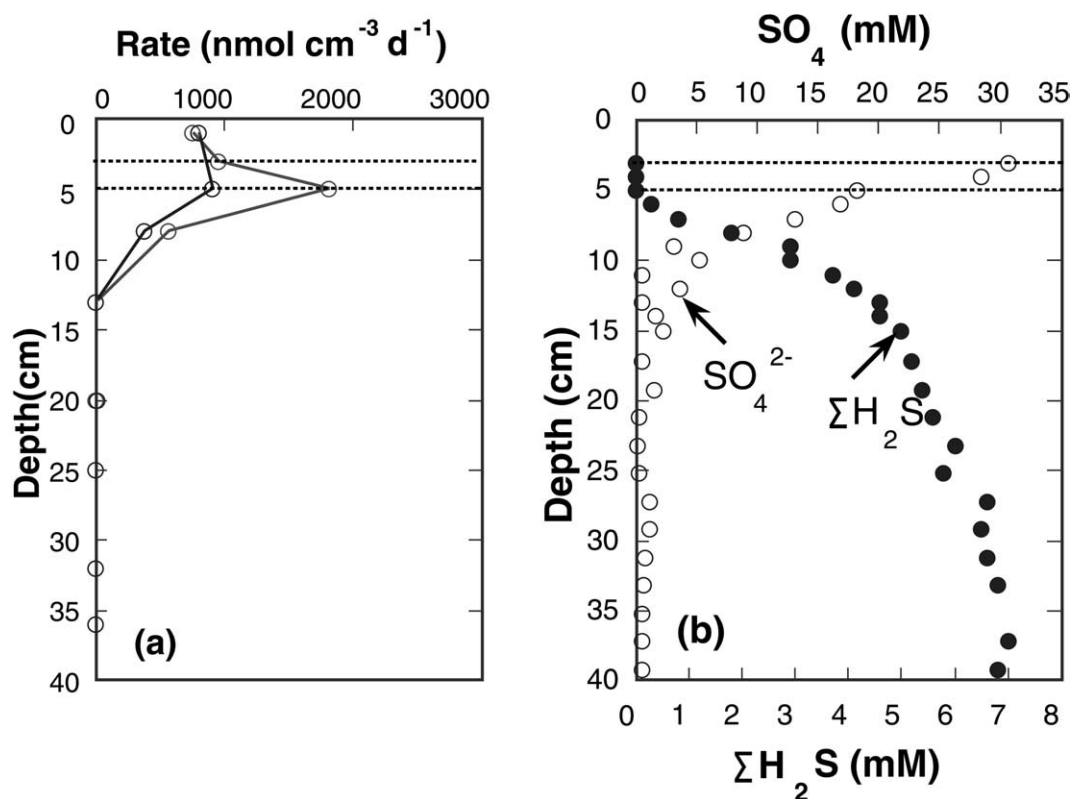


Fig. 4. Depth distribution profiles of (a) duplicate sulfate reduction rate measurements using  $^{35}\text{SO}_4^{2-}$  radio-tracer technique (b) pore water sulfate and total dissolved sulfide concentration. Samples were collected in May 1997 at the time of collection of sediments for the reactor. The horizontal broken lines denote the depth from which sediment for reactor experiments were used.

can be assumed. Also, the reaction time is small and if the rate of reaction is not very high, a substantial change in concentration of the substrate between the inlet and outlet does not occur. Therefore, concentration inside the reactor can be approximated as the average of the inlet and outlet concentrations. For disk reactor then, the rate data are plotted as a function of the average concentration inside the reactor and relevant Monod parameters retrieved (Fig. 3).

The disk reactor was designed by modifying a previously described plug-flow reactor (Roychoudhury et al., 1998). The length of the reactor was reduced to 2 cm for the reasons mentioned before. Ideally the thickness of the disk reactor should be negligibly small. The length was constrained by the fact that the residence time of fluid inside the reactor should be long enough to produce measurable change in substrate concentration. Also, our intention was to use a system that mimics the natural solid to solution ratio. The inside diameter of the reactor was increased to 4.7 cm, both to increase the diameter to length ratio and to accommodate commercially available standard size filter membranes (4.7 cm diameter) for convenience. The cap closing mechanism was modified to avoid leakage of fluid when excess back-pressure develops inside the reactor. A case often observed while using fine grain sediments.

In order to illustrate a successful application of the disk reactor, we discuss a case study of dissimilatory sulfate reduction in saltmarsh sediments. We chose sulfate reduction because its product, sulfide, is known to be toxic and inhibits microbial growth of various anaerobes (Koster, Rinzema, de Vegt, & Lettinga, 1986; McCartney & Oleskiewicz, 1991; Oleskiewicz, Marsteller, & McCartney, 1989; Widdel, 1988). Near circumneutral pH, even low levels of sulfide can have drastic effect on microbial growth of sulfate reducing bacteria (O'Flaherty, Mahony, O'Kennedy, & Colleran, 1998; Okabe, Nielson, & Characklis, 1992; Reis, Almeida, Lemos, & Carrondo, 1992). For kinetic studies, thus, accretion of sulfide inside the reactor is undesirable. Previously we had studied sulfate reduction in Sapelo Island saltmarsh sediments using a recirculating reactor (Roychoudhury et al., 1998). Sulfide toxicity effects were not evident because of the high iron content of the sediment (Table 1). Any free sulfide produced was immediately consumed by reaction with solid phase iron oxyhydroxides. Choice of a different sampling site was necessary. Based on measured high sulfate reduction rates and low iron content of the sediment, another site 40 m away from the previous one was chosen (Table 1, Fig. 4a). These conditions assured generation of excess sulfide inside the reactor.

From the reactor experiments, a  $K_s$  value of  $204 \pm 26 \mu\text{M}$  and a  $R_m$  value of  $2846 \pm 129 \text{ nmol cm}_{(\text{wet sediment})}^{-3} \text{ d}^{-1}$  was determined. These values of  $R_m$  and  $K_s$  are valid only with the assumption that the concentration of organic carbon inside the reactor remains constant during the course of the experiment. Calculations based on the measured sulfate reduction rates show that on an average,  $\sim 500 \mu\text{mol C}$  is mineralized over the course of the experiment. This is negligible compared to the organic carbon concentration ( $147,831 \mu\text{mol C}$ ) calculated to be present inside the reactor (Table 1). Thus, our assumption of constant carbon concentration inside the reactor is valid.

The retrieved  $K_s$  value ( $204 \pm 26 \mu\text{M}$ ) was consistent with the one previously determined from the same saltmarsh ( $240 \pm 20 \mu\text{M}$ ) (Table 1). A similarity in microbial population and organic detritus quality for the two sites may be inferred. This is not surprising, given that the two sites are only 40 m apart. It is further implied that excess sulfide generated inside the reactor did not affect the experimental results. The previously estimated  $R_m$  value  $126 \pm 5 \text{ nmol cm}_{(\text{wet sediment})}^{-3} \text{ d}^{-1}$ , though, was considerably lower than the one retrieved during this study. The cause may be the dominance of iron reduction due to readily available iron oxyhydroxides and low organic carbon content ( $\sim 31\%$  less than that found at the current site) of the sediment (Table 1).

The actual rates measured in the field between 3 and 5 cm depth using  $^{35}\text{S}$  incubation experiments in May 1997 were considerably lower than the calculated  $R_m$  values (Fig. 4a). Based on the  $K_s$  value ( $\sim 204 \mu\text{M}$ ), it is evident that pore water sulfate concentrations (28.3–30.7 mM, Fig. 4b) at a depth of 3–5 cm cannot be the limiting factor for lower in situ sulfate reduction rates (Fig. 4a). Both laboratory and field measurements were made on sediment collected in the month of May from the same depth and in cores collected within a 0.5 m radius. Therefore, the difference between field measured rates and the  $R_m$  value cannot be explained by the difference in organic carbon content or quality. Thus, substrate limitation can be ruled out as the cause of inhibition.

The sulfate reduction rate profile (Fig. 4a) exhibits a peak at 5 cm and the rates decrease both, above and below that depth. SRB385-positive nucleic acid signals indicate the presence of sulfate reducing bacteria at all the sampled depths (DiChristina et al., unpublished results), although, their activity remains at a minimum below a depth of 13 cm (Fig. 4a). In oxic and sub-oxic zones (0–5 cm), iron reducers or aerobic bacteria outcompete sulfate reducers for the mineralization of organic matter. Sulfide toxicity due to high sulfide concentrations in pore water (1.8–7.7 mM, Fig. 4b) at greater depths may have resulted in inhibition of microbial activity. During laboratory

experiments, sulfide produced inside the reactor did not have the same toxic effect because the experiments were conducted in once flow-through mode. Any sulfide that was produced during sulfate reduction was constantly removed with the outflow or was precipitated as iron sulfide within the reactor. At the field site, however, subsurface water movement was restrictive resulting in accumulation of sulfide over time.

## 6. Conclusions

1. If the rate law is not known, it is questionable to use a plug-flow reactor operating in once flow-through mode for kinetic studies. In this article we show that we can overcome this dilemma by modifying the reactor design. In future, disk reactors may prove especially useful to gather accurate kinetic parameters for reactions with unknown rate laws, a case almost ubiquitous to microbially mediated reactions.
2. The  $K_s$  value obtained with the newly designed disk reactor was consistent with the one determined previously using a recirculating plug-flow-through reactor technique. Thus, disk reactors are a viable option to gather kinetic information.
3. The disk reactor was applied to estimate half saturation concentration and maximum rate of sulfate reduction, when substrate concentration is not limiting. At  $25^\circ\text{C}$ , the values obtained were  $204 \pm 26 \mu\text{M}$  and  $2567 \pm 129 \mu\text{mol cm}_{(\text{wet sediment})}^{-3} \text{ d}^{-1}$ , respectively.
4. The  $K_s$  and  $R_m$  values suggest that sulfate reduction in Sapelo Island saltmarsh sediments is limited. The inhibition is not caused by substrate availability, though. Possible reasons are competition from other microbial populations to mineralize organic carbon and sulfide toxicity.

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