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A comparison of $^{35}S\text{-}SO_4^{2-}$ radiotracer techniques to determine sulphate reduction rates in laminated sediments

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Abstract

In order to find a simple and efficient method to determine sulphate reduction rates in environmental samples, we tested different $^{35}S\text{-}SO_4^{2-}$ radiotracer techniques. The methods varied in the application of $^{35}S\text{-}SO_4^{2-}$ and subsequent extraction of reduced ^{35}S-sulphur species. Samples were either incubated as sediment slurries mixed with the radiotracer, or as undisturbed sediment cores after core injection of the radiotracer. Reduced ^{35}S-sulphur species were retrieved passively by diffusion or actively by reflux distillation. The methods were applied to surface sediments derived from three aquatic habitats situated in Germany: (1) a tideless brackish water, (2) a mining lake and (3) a natural freshwater lake. The best possible method was expected to yield the highest sulphate reduction rates, which were reproducible with respect to magnitude and depth distribution. At the same time, we aimed to keep the disturbance of samples as well as the expenditure of labour and equipment to a minimum. For all three types of aquatic habitats, the combination of core injection followed by diffusion was the most reliable and efficient method. This combination is therefore recommended for determination of sulphate reduction rates in laminated sediments.

Keywords: Sulphate reduction; ^{35}S/Radiotracer; Core injection; Slurry incubation; Reflux distillation; Diffusion procedure

1. Introduction

Dissimilatory sulphate reduction is a key process in global carbon, sulphur and iron cycling, and its quantification is of major interest (Fauque, 1995; Odom and Singleton, 1993). A wide range of methods exist to measure sulphate reduction rates (overview given in Jørgensen, 1978a,b,c; Skyring, 1987). These include radiotracer techniques, batch-type incubation techniques monitoring the changes in solute concentration, and the mathematical modelling of solute concentration profiles in laminated samples, frequently in combination with microelectrode studies (Kühl and Jørgensen, 1992; Kühl et al., 1998).

Ivanov (1956) introduced the radiotracer technique where tracer amounts of $^{35}S\text{-}SO_4^{2-}$ are added to the sample and, at the end of incubation, the reduced ^{35}S-sulphur is extracted and subsequently quantified. The use of labelled compounds allows direct measurement at extremely high sensitivity under conditions approaching natural conditions

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(Ivanov, 1968). The further development of this technique involved two aspects: (A) the even distribution of $^{35}S\text{-}SO_4^{2-}$ into the sample (Sorokin, 1962; Ivanov, 1968; Jørgensen, 1978a; Jørgensen and Fenchel, 1974) and (B) the complete retrieval of reduced ^{35}S-sulphur (Howarth and Jørgensen, 1984; Canfield et al., 1986). The radiotracer technique has been commonly applied in recent studies, frequently as a reference standard compared to other methods: e.g. pore water SO_4^{2-} consumption (Hordijk et al., 1985; Bak and Pfennig, 1991; Bak et al., 1991), H_2S production (Habicht and Canfield, 1997; Reynolds et al., 1997) and mathematical modelling of SO_4^{2-} concentration profiles (Hordijk et al., 1985; Piker et al., 1998).

In our laboratory, we first applied the radiotracer technique of Rosser and Hamilton (1983), as modified by Babenzien and Sass (1999), which involves the introduction of $^{35}S\text{-}SO_4^{2-}$ into sample slurries, followed by passive extraction of acid volatile ^{35}S-sulphur by diffusion. This rather quick and simple method was replaced by a more advanced technique, which is considered standard and includes core injection of $^{35}S\text{-}SO_4^{2-}$ and the separation of reduced ^{35}S-sulphur into acid volatile and chromium reducible sulphur fractions by reflux distillation (Fossing and Jørgensen, 1989; Piker et al., 1998). The first method allows characterization of different sites as well as distinct sediment horizons according to sulphate reducing activities (Fukui and Takii, 1990; Babenzien et al., 1991). However, a comparison of the two methods revealed that the first method underestimated the rates by a factor of up to 100. The second method had the disadvantage of being time-consuming and requiring more expensive equipment. In order to obtain reliable rates and to optimize the result–performance ratio, we conducted several experiments in which we compared different incubation and extraction procedures. We included the method described by Ulrich et al. (1997), which, in their opinion, has to be verified for other environmental samples to see if the results are generally applicable. Our experiments were performed on sediment samples originating from three quite different aquatic habitats situated in Germany: (1) a tideless brackish water, (2) a mining lake and (3) a natural freshwater lake.

2. Materials and methods

2.1. Site descriptions

Sediment samples were collected from three aquatic environments that have been studied by our laboratory for several years. These habitats were chosen as they differ primarily in their organic carbon supply and ionic composition. The sampling occurred during summer stratification.

Mesotrophic Rassower Strom is a tideless brackish water located in the outer part of the Nordrügensche Bodden at the southern Baltic Coast. Exchange processes between the Baltic Sea and the inner part of the lagoon system (salinity, 8–12‰) has a great impact on the water chemistry. Furthermore, the composition of porewater is strongly influenced by advective transport, induced by wind-driven motion of the water body. In June 1997 and June 1998, sediment samples were taken at a water depth of about 4 m. Above the sediment, the water temperature measured 18.0°C.

Mesotrophic, dimictic Mining Lake B is a small water body that developed 35 years ago in the Lusatian lignite mining district of East Germany. The surface area is about 1 km^2 and the maximum depth is 10.5 m. Unlike many other mining lakes in this region, which suffer from acidification due to pyrite oxidation, the water exhibits a neutral pH that varies between 7.5 and 8. However, elevated ion concentrations (e.g. 4.6 mM SO_4^{2-} in surface water) distinguishes it from natural freshwater lakes. Total dissolved iron is only present in trace amounts in the water column but reaches pore water concentrations of up to 250 μM in reduced sediments. Oxygen concentration decreases in the hypolimnion but is not completely depleted by the end of summer stratification. In June 1996 and June 1998, sediment samples were taken at the deepest site. Above the sediment, the water temperature measured 7.0°C.

Oligotrophic Lake Stechlin belongs to a lake district that was formed during the last glacial epoch and is situated in the north-eastern part of Germany. The lake is dimictic and has a maximum depth of 68 m and a surface area of 4.25 km^2. The hypolimnion stays oxic also throughout the stratification period. In July 1996 and July 1998, sediment samples were taken at a water depth of about 32 m. Above the sediment, the water temperature measured 6.0°C.

2.2. Application of $^{35}S\text{-}SO_4^{2-}$ and sample incubation

The determination of sulphate reduction rates was performed in triplicate (occasionally in duplicate) in six horizons from the upper 10 cm of the sediments: 0–1, 1–2, 2–3, 4–5, 6–7 and 9–10 cm. Undisturbed sediment cores were recovered in Plexiglas tubes by gravity coring. These cores were further subsampled depending on the following procedure

(a) For sediment slurries, the sediment core was extruded stepwise in 1 cm-intervals from the Plexiglas tube and sliced. A 5-ml aliquot of the sediment slice was immediately transferred to a 120-ml glass flask, which contained the same volume of anoxic water from the sampling site (Babenzien and Sass, 1999) and was closed with a butyl rubber stopper by screw cap. Anoxic water was maintained by autoclaving and subsequent flushing with oxygen-free nitrogen. The headspace of the flask was flushed with oxygen-free nitrogen and 25 μl of carrier-free $^{35}S\text{-}SO_4^{2-}$ (3.7 MBq ml^{-1}, Amersham) were added. The samples were incubated for 4 to 6 h in the dark at ambient sediment temperature. Depending on the subsequent passive extraction method, the 120-ml glass flasks were equipped with the appropriate trapping device (see below).

(b) For incubation of undisturbed samples, sediment cores were subsampled with tipless 5-ml syringes (Piker et al., 1998). The subcores held sediment layers of 5 cm thickness with a total volume of 6 ml. Therefore, to recover the top 10 cm of the sediment, two sets of subsamples were obtained, the first from 0 to 5 cm and the second from 5 to 10 cm sediment depth. The subcores were sealed with Parafilm® and kept in an exsiccator flushed with oxygen-free nitrogen for transport. On three occasions, 8.4 μl of carrier-free $^{35}S\text{-}SO_4^{2-}$ (3.7 MBq ml^{-1}, Amersham, Braunschweig, Germany) were injected vertically into the sediment using a microlitre syringe guided by a micromanipulator. The subcores were sealed with a second layer of Parafilm® and incubated in a second exsiccator flushed with oxygen-free nitrogen for 4 to 6 h at ambient sediment temperature. After incubation, the subcores were sliced into 1-cm-thick segments and fixed in 5 ml of 20% zinc acetate for about 1 h. Then the samples were centrifuged at 1100 *g* for 10 min and washed twice with water. An aliquot of the supernatant was used to measure the remaining $^{35}S\text{-}SO_4^{2-}$.

2.3. Extraction of reduced inorganic ^{35}S-sulphur

The recovery of reduced ^{35}S-sulphur was carried out within two days after incubation. This was done either by passive (diffusion) or by active (reflux distillation) extraction. Samples that had been incubated in subcores and subsequently washed were transferred with 5 ml of 50% ethanol into the extraction vessels.

(a) Passive extraction was carried out in 120-ml glass flasks that were closed with butyl rubber stoppers and screw caps under oxygen-free nitrogen. For sediment slurries, the same vessels that were used during incubation were used for extraction. Two different passive extraction procedures were tested. (i) Applying the method described by Rosser and Hamilton (1983), acid volatile sulphur (AVS: $H_2S + FeS$) was liberated by adding 0.5 ml of 6 M HCl and extracted for a duration of 1–2 h. The $^{35}S\text{-}H_2S$ was trapped in a filter paper soaked with 0.5 ml of 20% zinc acetate. The filter lay in a small cup that was attached to the stopper. (ii) The passive extraction method described by Ulrich et al. (1997) was slightly modified by adding 8 ml of 6 M HCl (instead of 4 ml of conc. HCl) and 8 ml of 1 M $CrCl_2$ (in 0.5 M HCl) to extract total reduced inorganic sulphur (TRIS). The bottles were placed on a rotary shaker (125 rpm) for the duration of the extraction period of 30 h. The $^{35}S\text{-}H_2S$ was trapped in 2.5 ml of 10% zinc acetate within a small reaction tube.

(b) The reflux distillation method followed the protocol of Fossing and Jørgensen (1989). AVS was liberated by adding 7.5 ml of 5 M HCl (in 50% ethanol) and the sample was cold distilled (at room temperature) for 25 min and hot distilled (gentle boiling) for 10 min. Chromium reducible sulphur (CRS: $S^0 + FeS_2$) was extracted by adding 15 ml of 1 M $CrCl_2$ (in 0.5 M HCl) and the sample was hot distilled for 40 min. The evolving ^{35}S-H_2S was carried by an oxygen-free nitrogen stream into 10 ml of 5% zinc acetate.

2.4. Analysis

Reduced ^{35}S-sulphur and the remaining ^{35}S-SO_4^{2-} were determined by liquid scintillation counting (LSC 1600TR, Canberra Packard, Dreieich, Germany; scintillation-cocktail: Lumagel®SB, Mallinckrodt Baker, Griesheim, Germany).

The total of reduced inorganic sulphur (AVS and CRS) in bulk sediments was determined on the same samples obtained for sulphate reduction rates, using the two-step distillation method described above. The mass of reduced sulphur was determined spectrophotometrically by the methylene blue method (Cline, 1969).

Pore water was collected by sediment centrifugation at 27 000 *g* for 10 min at 4°C. The supernatant was filtered (0.2 μm) and stored at −20°C (Rassower Strom and Lake Stechlin) or at 4°C under sterile conditions (Mining Lake B). Concentrations of SO_4^{2-} in the pore water from Rassower Strom and Lake Stechlin were measured by ion chromatography (IC) with conductivity detection (Bak et al., 1991). Concentrations of SO_4^{2-} in the pore water from Mining Lake B were determined turbidometrically (Tabatabai, 1974) instead of by IC because of high concentrations of dissolved iron and the consequent problem of iron oxide precipitation in the column.

Porosity (percentage of pore space volume) was calculated from dry weight and specific gravity values. Dry weight was determined by weight loss at 105°C.

2.5. Rate calculation

Sulphate reduction rates (*SRR*) were calculated (in nmol SO_4^{2-} cm^{-3} d^{-1}) taking into account the porosity values according to Ingvorsen (1998):

$$SRR = \frac{a}{(A + a)\,t} \cdot [SO_4^{2-}] \cdot 1.06$$

where a is the total radioactivity of AVS + CRS, A is the total radioactivity of the sulphate pool after incubation, t is the incubation time, $[SO_4^{2-}]$ is the sulphate concentration in sediment, and 1.06 is the discrimination factor for ^{35}S. For slurry incubation, the dilution factor and SO_4^{2-} concentrations of the additional sample water were taken into account.

As rate determinations were based on a limited number of replicate measurements, we calculated relative errors $(= 100 \cdot \frac{x_i - \bar{x}}{\bar{x}})$ of triplicate (or duplicate) rate determinations. We calculated the relative standard deviation for all triplicate (or duplicate) values of the six sediment horizons sampled at one site to express the variability of *SRR* for each sampling site in combination with the respective method. We assumed that the variance in the relative errors was the same for all sediment depths.

3. Results

The marine influence on the sediments of the tideless brackish water Rassower Strom, and the mining lake origin of Mining Lake B, are recognizable as elevated SO_4^{2-} concentrations in the pore water. In Rassower Strom, maximum values of 6.3 mM SO_4^{2-} were found in the top 2 cm of the sediment. In June 1997, the SO_4^{2-} concentration decreased down to 1.5 mM SO_4^{2-} at a 10-cm sediment depth whereas in June 1998, there was a less pronounced decrease, down to 5.4 mM SO_4^{2-}. The SO_4^{2-} profile over the top 10 cm of sediments in Mining Lake B showed a slight increase with depth from 4.7 to 5.2 mM SO_4^{2-} in June 1996 in contrast to a decrease from 4.2 to 3.0 mM SO_4^{2-} in June 1998. In sediments of the freshwater Lake Stechlin, SO_4^{2-} showed maximum values of 320 μM at the surface and was nearly depleted (20 μM SO_4^{2-}) at a 10-cm sediment depth. The profile from July 1996 was not significantly different from the profile of July 1998, i.e., profiles in Lake Stechlin did not exhibit such dynamics in contrast to the other two sites.

In Rassower Strom, the highest values for total extracted reduced inorganic sulphur (TRIS: here equivalent to the sum of AVS and CRS determined in the two-step distillation procedure) were obtained. TRIS accumulated with depth and ranged from 14 μmol S cm^{-3} at the surface to 63 μmol S cm^{-3} at a 10-cm sediment depth. AVS accounted for a minimum 10% of TRIS at the surface and a maximum 44% of TRIS at 2–5 cm sediment depth. In Mining

Lake B, TRIS ranged from 3 μmol S cm^{-3} at the surface to up to 40 μmol S cm^{-3} at a 10-cm sediment depth. AVS accounted for minimum 44% of TRIS at the surface and maximum 84% of TRIS at 10 cm sediment depth. In Lake Stechlin TRIS reached from 4 μmol S cm^{-3} at the surface to up to 18 μmol S cm^{-3} at 10-cm sediment depth. AVS accounted for a minimum 14% of TRIS at the surface and a maximum 49% of TRIS at 1–2 cm sediment depth.

When we introduced subcore incubations with core injection of the radiotracer and subsequent distillation for extraction of reduced inorganic sulphur, we obtained significantly higher sulphate reduction rates (*SRRs*) than we did previously with slurry incubations combined with the diffusion procedure of Rosser and Hamilton (1983). Furthermore, it yielded a different depth profile of sulphate-reducing activities. In Table 1, the rates for six sediment horizons from the three different habitats obtained by the two techniques are compared. The mean percentage of ^{35}S-sulphur recovered in the AVS fraction with the distillation procedure is given in brackets. For each site and method, the relative standard deviation was calculated. The high relative standard deviation of these results and those in the following reflect the high variability of replicate sediment samples. We did not estimate the experimental error itself as the reproducibility of these methods depends mainly on the homogeneity of the investigated sediment (Jørgensen, 1978a).

The low rates obtained with slurry incubations combined with the diffusion procedure of Rosser and Hamilton (1983) were not only due to the omission of reduced ^{35}S-sulphur in the CRS fraction. To see if the low recovery of reduced ^{35}S-sulphur was due to the passive extraction method, we compared the distillation technique with the diffusion procedure described by Ulrich et al. (1997). Samples were incubated as undisturbed subcores after injection of ^{35}S-SO_4^{2-}. The results for Rassower Strom and Mining Lake B are shown in Figs. 1 and 2, respectively. For rate determination in sediments of Rassower Strom, the relative standard deviation was ±29% for subcore incubation combined with the distillation technique and ±50% for subcore incubation combined with the diffusion procedure of Ulrich et al. (1997) (rates were not determined in sediments from a depth of 1–2 cm). For rate determination in sediments of Mining Lake B, the standard deviations were ±32 and ±37%, respectively. The two methods show good agreement with respect to absolute values and depth distribution of SRRs. Only for the sediment horizon of 2–3 cm in Mining Lake B were the rates obtained by the diffusion method higher than those obtained by distillation. The highest rates were

Table 1
Sulphate reduction rates (SRRs) determined for six sediment horizons of Rassower Strom (June 1997), Mining Lake B (June 1998) and Lake Stechlin (July 1996) applying (a) slurry incubations followed by the diffusion procedure of Rosser and Hamilton (1983) and (b) incubation of subcores followed by the distillation procedure[a]

	Rassower Strom June 1997 *SRR* (nmol SO_4^{2-} cm^{-3} d^{-1})		Mining Lake B June 1998 *SRR* (nmol SO_4^{2-} cm^{-3} d^{-1})		Lake Stechlin July 1996 *SRR* (nmol SO_4^{2-} cm^{-3} d^{-1})	
	(a) Slurry incubation diffusion (Rosser and Hamilton (1983))	(b) Subcore incubation distillation procedure	(a) Slurry incubation diffusion (Rosser and Hamilton (1983))	(b) Subcore incubation distillation procedure	(a) Slurry incubation diffusion (Rosser and Hamilton (1983))	(b)[b] Subcore incubation distillation procedure
0–1	7.0	77.4 (16%)	5.0	59.3 (27%)	0.2	78.1 (31%)
1–2	7.5	168.5 (38%)	5.4	94.5 (63%)	2.5	66.8 (43%)
2–3	6.4	178.1 (59%)	3.7	171.3 (85%)	2.7	78.6 (58%)
4–5	7.3	85.6 (61%)	4.3	79.9 (83%)	1.9	72.0 (46%)
6–7	13.6	38.0 (48%)	1.1	23.9 (77%)	1.7	134.4 (58%)
9–10	8.6	23.6 (56%)	1.4	5.8 (51%)	1.2	12.3 (44%)
R.S.D.	±11%	±32%	±39%	±32%	±35%	±23%

[a] Rates are expressed as the mean value of three replicates. The percentages of ^{35}S-sulphur recovered in the AVS fraction for (b) are given in brackets. For each site and method, the relative standard deviation (R.S.D.) is given.

[b] In duplicate.

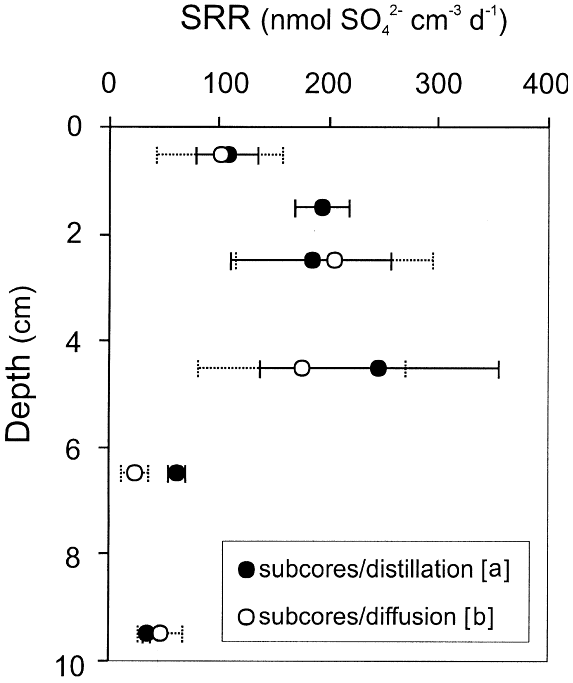

Fig. 1. Comparison of sulphate reduction rates (*SRRs*) obtained in subcore incubations followed by (a) distillation procedure and (b) diffusion procedure of Ulrich et al. (1997) (rates were not determined at a sediment depth of 1–2 cm). Sediment was sampled from six horizons of Rassower Strom (June 1998). Rates are expressed as the mean value of duplicates; bars indicate values of the two replicates.

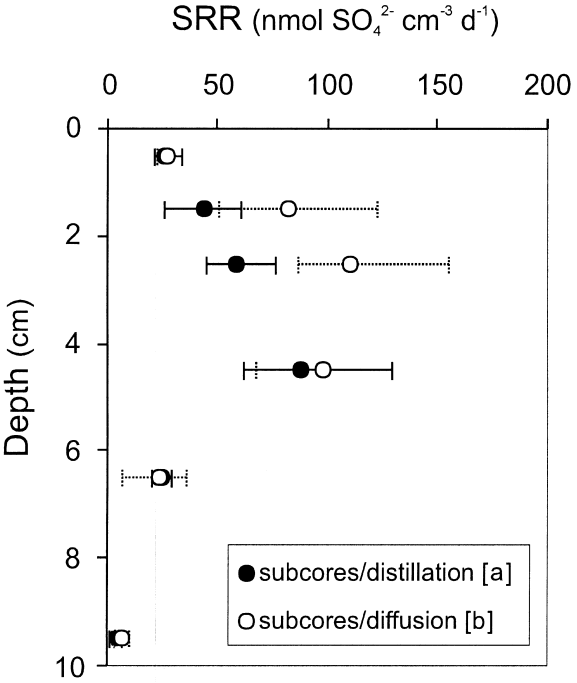

Fig. 2. Comparison of sulphate reduction rates (*SRRs*) obtained in subcore incubations followed by (a) distillation procedure and (b) diffusion procedure of Ulrich et al. (1997). Sediment was sampled from six horizons of Mining Lake B (June 1998). Rates are expressed as the mean value of triplicates; bars indicate the minimum and maximum values.

observed in Rassower Strom, with a maximum of 355 nmol SO_4^{2-} cm^{-3} d^{-1} at a sediment depth of 4–5 cm. In Mining Lake B, we found maximum rates of 155 nmol SO_4^{2-} cm^{-3} d^{-1} at a sediment depth of 2–3 cm. Also, for Lake Stechlin, the rates obtained in subcores with the subsequent diffusion procedure of Ulrich et al. (1997) (see Fig. 3, samples from July 1998, relative standard deviation of ±21%) are comparable to those determined in subcores with subsequent distillation (see Table 1, samples from July 1996). The rates obtained in samples from July 1998 are lower in the top 4 cm (20–42 nmol SO_4^{2-} cm^{-3} d^{-1}) than those obtained in July 1996 (67–79 nmol SO_4^{2-} cm^{-3} d^{-1}) but, for both years, we found a maximum rate of 130 nmol SO_4^{2-} cm^{-3} d^{-1} at a sediment depth of 6–7 cm.

The diffusion procedure of Rosser and Hamilton (1983) was also successful when applied to samples incubated as subcores after core-injection of the radiotracer (relative standard deviation ±54%). This is illustrated for sediment samples from Mining Lake B in Fig. 4, where rates obtained by this method are compared to rates calculated from the amount of reduced ^{35}S-sulphur recovered in the AVS fraction during distillation.

In order to improve the recovery of reduced ^{35}S-sulphur from an experimental set-up using slurry incubations, we applied the diffusion procedure of Ulrich et al. (1997). The results for Lake Stechlin are given in Fig. 3. For rate determination, the relative standard deviation was ±50% for slurry incubation and ±21% for subcore incubation, both combined with the diffusion procedure of Ulrich et al. (1997). From Figs. 3 and 4, it is clear that the rates obtained with sediment slurries stay far below those obtained from intact sediment cores irrespective of the subsequent extraction procedure.

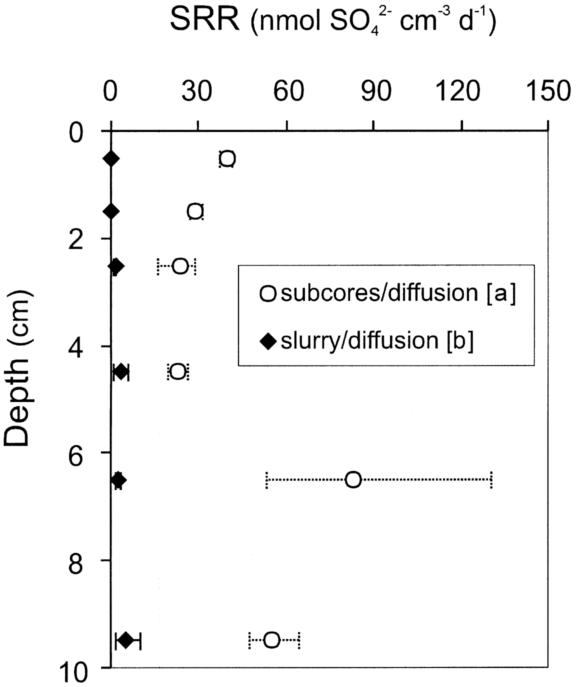

Fig. 3. Comparison of sulphate reduction rates (*SRRs*) obtained in (a) subcore incubations and (b) slurry incubations, both followed by the diffusion procedure of Ulrich et al. (1997). Sediment was sampled from six horizons of Lake Stechlin (July 1998). Rates are expressed as the mean value of triplicates; bars indicate the minimum and maximum values.

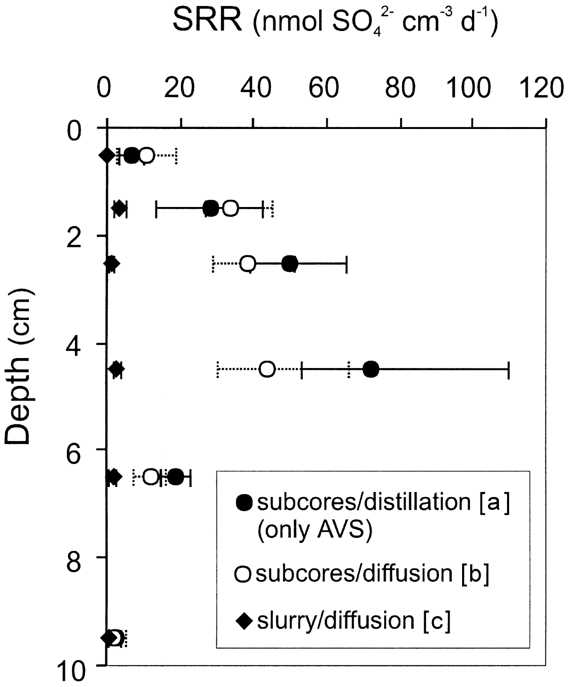

Fig. 4. Comparison of sulphate reduction rates (*SRRs*) obtained in (a) subcore incubations followed by distillation (only AVS), (b) subcore incubations followed by the diffusion procedure of Rosser and Hamilton (1983) and (c) slurry incubations followed by the same extraction procedure as (b). Sediment was sampled from six horizons of Mining Lake B (June 1998). Rates are expressed as the mean value of triplicates; bars indicate the minimum and maximum values.

4. Discussion

The radiotracer technique is the method of choice for comparing sulphate reduction rates in different environments. Its reliability is less dependent on the prevailing conditions and, for a successful application, fewer prerequisites have to be fulfilled in comparison with other methods, e.g. the mathematical modelling of solute gradients (Kühl and Jørgensen, 1992; Piker et al., 1998; Ingvorsen, 1998). The modelling approach based on SO_4^{2-} concentration profiles is not applicable to sediments of Rassower Strom and Mining Lake B as some of the prerequisites are not fulfilled (e.g. steady-state conditions, diffusion-controlled transport of solutes, exponential decrease of sulfate with depth).

Sulphate reduction rates obtained in undisturbed sediment cores after direct injection of the radiotracer are reproducible irrespective of the subsequent passive or active extraction method for retrieval of reduced ^{35}S-sulphur (Figs. 1 and 2). A comparison of the two extraction methods demonstrated a good agreement of sulphate reduction rates in order of magnitude as well as depth profile. This has been observed in sediments from all three aquatic environments we have investigated. Our results corroborate the findings of Ulrich et al. (1997) and the applicability of their diffusion method can be extended to laminated systems.

Despite the omission of a solvent for elemental sulphur and a shorter extraction time of 30 h in this study in comparison to those of Ulrich et al. (1997) and Hsieh and Yang (1989), the diffusion procedure gave rates that were equal to those of the distillation method. Therefore, the extraction conditions chosen were believed to be appropriate.

Since the formation of pyrite as a short-term end-product of sulphate reduction was first reported by Howarth (1979), retrieval of reduced ^{35}S-sulphur from the CRS fraction has been included in many studies to determine sulphate reduction rates in environmental samples. Transformations of $^{35}S\text{-}H_2S$ in situ and during extraction procedures (Howarth and Jørgensen, 1984; Thode-Andersen and Jørgensen, 1989; Rice et al., 1993; Duan et al., 1997) as well as isotope exchange reactions between reduced sulphur species (Fossing and Jørgensen, 1990) lead to other forms of reduced ^{35}S-sulphur. We also found only partial recovery of reduced ^{35}S-sulphur in the AVS fraction for sediments at all three sites (Table 1). The advantage of the diffusion procedure described by Ulrich et al. (1997) towards the distillation procedure is that the extraction of CRS does not involve extra time or effort and can be easily included in routine programmes.

Comparing the extraction methods in this study as well as looking at the descriptions in the original papers, it appears that different amounts of reagents were used to liberate and to trap AVS and/or CRS. Although the duration of extraction is too short according to Ulrich et al. (1997), and the amount of zinc acetate is insufficient according to Fukui and Takii (1990), the extraction procedure for AVS of Rosser and Hamilton (1983) yielded similar results to those found using the distillation procedure (Fig. 4). The near doubling of volume of 1 M $CrCl_2$ from 8 ml (diffusion method of Ulrich et al. (1997)) to 15 ml (distillation method) did not lead to an increase of recovered labelled sulphur (Figs. 1 and 2). This indicates that the amounts of these reactants could be reduced.

The low recoveries of reduced ^{35}S-sulphur obtained from slurry incubations might, in part, be due to dilution effects and the subsequent deficiency of volatilizing and reducing reactants. However, as discussed above, some reactants were added in excess and recoveries were not improved by increasing the amount of reactants (Figs. 3 and 4). The low rates are more likely to be due to the effects of sediment handling during slurry incubation. Our findings correspond to the results of Jørgensen (1978a), who demonstrated that the difference between mixed and undisturbed samples depended on dilution factor, length of incubation time, incubation temperature and the type of sediment. Bak and coworkers (1991, 1991, 1988) applied the same methods as used by Jørgensen (1978a) and us, but they obtained generally higher rates with slurry incubation.

The discrepancy between the incubation of disturbed and undisturbed samples appears to be unpredictable. Mixing destroys previously existing microenvironments and the addition of water leads to dilution or enrichment of organisms and/or substrates and consequently to enhancement or inhibition of certain processes. As these changes are difficult to control, mixing and dilution should be kept to a minimum, especially with laminated samples. The recovery of undisturbed subcores by means of syringes is practical and enables easy handling during transport, incubation and transfer of sediments into reaction vessels. From this point of view, this method should be favoured in addition to the above-mentioned advantages of the core injection method.

5. Conclusions

For the determination of sulphate reduction rates in laminated sediments, we recommend core injection of $^{35}S\text{-}SO_4^{2-}$ into intact sediment cores followed by a diffusion procedure to retrieve ^{35}S-labelled TRIS. Subcores allow the incubation of undisturbed sediments and require only small amounts of sediments and, subsequently, small amounts of reactants. The diffusion procedure is a quick and simple method for extracting TRIS, with good efficiency that is comparable to that of the distillation procedure.

6. Abbreviations

AVS	Acid volatile sulphur
CRS	Chromium reducible sulphur
SRR	Sulphate reduction rate
TRIS	Total reduced inorganic sulphur

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