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Chemical and isotopic evidence for accelerated bacterial sulphate reduction in acid mining lakes after addition of organic carbon: laboratory batch experiments

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Abstract

Acid mine drainage is a widespread environmental problem in Lusatia in the eastern part of Germany, where extensive brown coal and lignite mining was carried out during the last century. As a result of oxidation of pyrite in strip mine dumps of this region, numerous mine drainage lakes have pH values <3 and are characterized by high concentrations of dissolved sulphate and ferrous iron. Using chemical and isotopic techniques, we tested in laboratory bottle experiments the extent to which the addition of degradable organic carbon to acid lake water and sediments could stimulate bacterial (dissimilatory) sulphate reduction. The overall goal was to evaluate whether bacterial sulphate reduction, and subsequent formation of sedimentary sulphide minerals, could generate enough alkalinity to potentially neutralize the acid lakes in Lusatia.

Pyruvate, glucose, succinic acid, lactate, ethanol, acetate, and various industrial by-products were added in various concentrations to original lake water and sediments in glass bottles and incubated between 4 and 12 weeks in darkness. The extent of bacterial sulphate reduction was determined by analyzing the concentrations and the isotopic compositions of sulphate in the water column, and the contents and δ^{34}S values of various sedimentary sulphur compounds at the beginning and the end of the experiments. Bacterial sulphate reduction was significantly enhanced after addition of pure substances (lactate, pyruvate, acetate, and ethanol) resulting in elevated pH values (4.5 to 6.0) in the bottle water at the end of the experiments. Cheap industrial by-products (whey, molasses, Pfezi-granules, and Carbokalk) as organic carbon source were also shown to be effective in enhancing bacterial sulphate reduction, thereby increasing the pH value of the bottle water. In general, the addition of selected organic carbon compounds enhanced sufficiently bacterial sulphate reduction and the formation of sedimentary sulphur minerals, generating enough alkalinity for raising the pH of the bottle water to near-neutral values. Further research in enclosures and under field conditions is needed to determine whether this technique constitutes a feasible remediation strategy for the acid lakes in Lusatia.

Keywords: Acid mine drainage; Bacterial sulphate reduction; Sulphate; Sulphur isotopes; Oxygen isotopes

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1. Introduction

Acid mine drainage is a widespread environmental problem in parts of eastern Germany (Geller et al., 1998; Steinberg et al., 1999). Lusatia, for example, is a region where extensive brown coal mining has occurred in the past. To facilitate the excavation of lignite from open pits, the groundwater table was lowered over an area of approximately 2500 km^2, forming an enormous drawdown cone with a volume deficit of 13 billion m^3 water (Luckner and Eichhorn, 1996). During mining operations, pyrite-containing bedrock was exposed to oxygen in the overburden and microbial iron and sulphur oxidation produced sulphuric acid. Acid solutions were flushed by surface water runoff, or by the ascending groundwater, into lakes filling the pits after mining activities ceased (e.g. Knöller et al., 2004). A total of 120 acid lakes is anticipated to emerge after a full recovery of the natural groundwater table (Schultze et al., 1999), many of them with pH values <3. In order to make these lakes accessible for future recreational activities it is desirable to increase their pH values to near neutral levels.

Bacterial (dissimilatory) sulphate reduction (BSR) is a naturally occurring process. Under anaerobic conditions, sulphate is used by bacteria as an electron acceptor for oxidation of organic carbon according to the following generalized reaction (e.g. Berner, 1970, 1984; Canfield and Raiswell, 1991):

$$2\langle CH_2O\rangle + SO^{2-}_{4(aq)} + 2H^{+}_{(aq)} \rightarrow 2CO_{2(g)} + H_2S_{(g)} + 2H_2O. \qquad (1)$$

In acid mining lakes, BSR occurs often in combination with microbial (e.g. Canfield and Raiswell, 1991; Vile and Wieder, 1993) and chemical (e.g. Berner, 1970, 1984; Canfield and Raiswell, 1991) iron (III) reduction (Eqs. (2) and (3)),

$$\langle CH_2O\rangle + 4FeOOH_{(s)} + 8H^{+}_{(aq)} \rightarrow CO_{2(g)} + 4Fe^{2+}_{(aq)} + 7H_2O \qquad (2)$$

$$2FeOOH_{(s)} + 3H_2S_{(g)} \rightarrow 2FeS_{(s)} + S^{0}_{(s)} + 4H_2O \qquad (3)$$

resulting in the overall reaction summarized in Eq. (4):

$$30\langle CH_2O\rangle + 12FeOOH + 14SO_4^{2-} + 28H^{+} \rightarrow 30CO_2 + 12FeS + 2S^{(0)} + 50H_2O. \qquad (4)$$

The produced H_2S may precipitate as sedimentary iron sulphide mineral and subsequently convert to pyrite (e.g. Berner, 1970; Berner, 1984). The reduction of iron (III) and sulphate by carbohydrates and the subsequent formation of sedimentary pyrite result in the generation of alkalinity (e.g. Cook et al., 1986; Schindler, 1986), thereby potentially resulting in an effective neutralization of the water column in acid lakes.

If anaerobic conditions are established, BSR and subsequent fixation of H_2S-sulphur in sediments can be theoretically limited by (1) the deficiency of dissolved sulphate, (2) low concentrations of dissolved iron, or (3) a scarcity of degradable organic carbon. The latter is required by sulphur-reducing bacteria as an energy source for their metabolism (e.g. Cook and Kelly, 1992).

Stable isotope techniques constitute a powerful tool to elucidate sulphur transformations in lake systems (e.g. Knöller et al., 2004). BSR is a multistep process that involves kinetic isotope effects for both sulphur and oxygen isotopes. Depending on environmental conditions, sulphur isotope fractionation between sulphate-S and H_2S can be quite variable, with enrichment factors between less than 4 ‰ and more than 46 ‰ (Harrison and Thode, 1958; Rees, 1973; Chambers and Trudinger, 1979; Canfield and Thamdrup, 1994; Canfield et al., 1998). However, Canfield (2001) observed that S isotope fractionation ranged only from 8 ‰ to 21 ‰ in situations where sulphur-reducing bacteria were provided with non-limiting concentrations of amended substrate such as acetate, ethanol, and lactate. Subsequent reaction of the BSR product H_2S with Fe to form sulphide minerals such as pyrite causes negligible sulphur isotope fractionation (Price and Shieh, 1986; Anderson and Pratt, 1995; Wilkin and Barnes, 1996). Hence, sedimentary sulphide minerals are expected to have significantly lower $\delta^{34}S$ values than the reactant dissolved inorganic sulphate. Oxidation of reduced sulphur compounds to dissolved inorganic sulphate is accompanied by relatively small isotope effects (e.g. Kaplan and Rittenberg, 1964; Chambers and Trudinger, 1979).

Since bacteria preferentially rupture ^{32}S- and ^{16}O-containing bonds during BSR, the residual SO_4^{2-} becomes progressively enriched in ^{34}S and ^{18}O as the reaction progresses (e.g. Harrison and Thode, 1958; Mizutani and Rafter, 1973). Hence, increasing $\delta^{34}S$ and $\delta^{18}O$ values and simultaneously decreasing concentrations of dissolved sulphate in the water column are an indicator for the occurrence of BSR. In a closed system, the content of reduced inorganic sulphur compounds with initially low $\delta^{34}S$ values in the sediments should simultaneously increase, provided that Fe is not limited. Subsequently, the isotopic difference between sulphate and sulphide S develops as in a Rayleigh distillation model, with the $\delta^{34}S$ values of the sulphide eventually approaching that of the initial sulphate as the reaction progresses towards completion. Therefore, sulphur isotope analyses constitute an elegant tool for monitoring BSR, provided the isotope ratios of the dissolved SO_4^{2-} and of sedimentary sulphur compounds are monitored.

In lakes, BSR is most prevalent in sediments, but may also occur in the water column (e.g. Trudinger, 1992). Within sediments, the preferred zone of BSR occurrence is usually within the upper few centimeters (Jørgensen, 1982) because of the typically sufficient availability of degradable organic matter. Sulphate-reducing bacteria are obligate anaerobes (Nealson, 1997), but BSR has also been observed in oxic lake sedimentary layers at the water–sediment interface (e.g. Schindler, 1986; Sass et al., 1997). This can be explained by the formation of anoxic micro-niches within the sediments that provide appropriate conditions for sulphate-reducing bacteria. Genera of microorganisms that are known to carry out BSR in lake sediments are *Desulphovibrio*, *Desulphomicrobium* or *Desulphotomaculum* (e.g. Widdel and Hansen, 1992; Nealson, 1997). Most sulphate-reducing bacteria are heterotrophic bacteria, which utilize simple organic carbon compounds as an energy source. They, therefore, depend on fermentative bacteria to supply them with by-products from their own metabolism, such as formate, lactate, acetate, or hydrogen (Chapelle, 1993).

In acid mining lakes of Lusatia, BSR is not frequently observed because reducing conditions are seldom established in the water column. At the water–sediment interface, however, and particularly within the sediments, anaerobic conditions can prevail. Nevertheless, Friese et al. (1998) and Knöller et al. (2004) found that BSR occurred only sporadically in profundal sediments of an acidic lake in Lusatia. Since sulphate concentrations of more than 2000 mg/l (Friese et al., 1998) and average iron concentrations of 160 mg/l (Herzsprung et al., 1998) were observed in the water column of this lake, dissolved sulphate and iron are apparently not the limiting factors for BSR and for subsequent sulphide-S fixation in the sediments. It was, therefore, hypothesized that BSR was limited predominantly by the availability of degradable organic carbon in the lake (e.g. Peine, 1998; Friese et al., 1998).

The objective of this laboratory batch experiment was to use chemical and isotopic techniques to determine the extent to which the addition of degradable organic carbon to lake water and sediments could stimulate bacterial (dissimilatory) sulphate reduction (BSR). The overall goal was to evaluate whether BSR and subsequent formation of sedimentary sulphide minerals could generate enough alkalinity to potentially neutralize acid lakes in Lusatia.

2. Materials

Bottle experiments were carried out with water and sediments from an acid lake (ML 111) near Plessa in Lusatia, Germany. The hydrogeochemistry of the lake and its vicinity was described by Knöller et al. (2004) and the biogeochemistry of the lake and its sediments was summarized by Friese et al. (1998). These authors reported that lake water had a pH <3 and contained on average 1300 mg/l sulphate and 200 to 300 mg/l total iron. The majority of the lake sediments had a yellowish color and was characterized by total sulphur contents of up to 33.8 mg/g dry weight (dwt), mainly in the form of sulphate- and iron-bearing minerals, such as jarosite ($(K,H_3O,Na)Fe_3(SO_4)_2(OH)_6$) or schwertmannite ($Fe_{16}O_{16}(OH)_{12}(SO_4)_2$) (e.g. Peine, 1998; Göttlicher, 1999). Contents of reduced inorganic sulphur within the yellowish sediments were negligible (0.1 mg/g dwt). In the deepest part of the lake, dark colored sediments with lower total sulphur contents (~ 10 mg/g dwt) and higher contents of reduced inorganic sulphur (~ 1 mg/g dwt) were observed. Both sediment types were used in the batch experiments.

3. Experiments

Batch experiments were conducted in 2l DURAN glass bottles. All bottles were filled with 255 ± 25 g wet sediment with an average water content of 60%, and 2 l of original lake water (Fig. 1). Fifteen grams of straw was added to most bottles as settling media (periphyton) for bacteria, and organic carbon was added in various forms and concentrations. All bottles were subsequently closed airtight and stored in darkness at 21 ± 2 °C for up to 12 weeks. Thereafter, the bottles were opened in a glove box under argon atmosphere and water and sediments were sampled for chemical and isotopic analyses. Three series of experiments were performed.

In the first series, sodium pyruvate, glucose monohydrate, succinic acid, sodium lactate, ethanol, and sodium acetate were added each to two bottles in TOC concentrations between 60 and 180 mmol/l (Table 1). One of the two bottles was sterilized in an autoclave. Two additional bottles with sediment, straw, and water, but without C_{org} additions, represented control variants (Table 1). The initial chemical (e.g. pH, Eh, $[SO_4^{2-}]$) and isotopic ($\delta^{34}S_{sulphate}$ and $\delta^{18}O_{sulphate}$) compositions of the bottle water were determined on samples from two additional bottles, which contained water and straw, but no sediments or added chemicals. These bottles were opened 5 days (0.7 weeks) after the beginning of the experiment (Table 1). For the first series of batch experiments, the dark sediment type from lake ML 111 was used, since it was assumed that it would provide the most favorable conditions for BSR. The bottles were stored in darkness for 4 weeks. The initial chemical and isotopic compositions of sedimentary sulphur compounds ($\delta^{34}S_{AVS}$, $\delta^{34}S_{CRS}$, $\delta^{34}S_{sulphate}$) were determined on sediment material from the control experiment bottles, where no substrate was added.

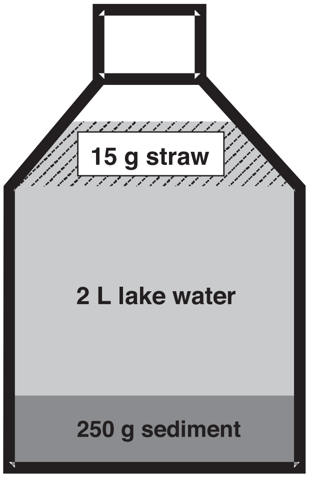

Fig. 1. Set-up of the bottle experiments.

In the second series of batch experiments, industrial by-products such as whey, molasses, Carbokalk (containing both organic C compounds from sugar beets and carbonate generated during clarification of raw sugar beet soap in the sugar beet industry), and Pfezi®-granules (composed of molasses, cement, and sugar beet scraps) were tested as organic carbon sources. Each industrial by-product was added in TOC concentrations between 54 and 468 mmol/l to two bottles with straw and dark sediment (Table 2). One of them was subsequently sterilized. The sealed bottles were stored for 12 weeks in darkness. The initial chemical and isotopic compositions of bottle water and sedimentary sulphur compounds were derived from the control experiment bottles of the first batch series (Table 1).

In the third series of batch experiments, ethanol and Carbokalk were added in TOC concentrations of 60 and 54 mmol/l, respectively, to bottles with and without straw (Table 3). Criteria for selecting these compounds were the availability of these products in large amounts, easy handling, and economic feasibility for field applications. All experiments were conducted with dark sulphidic and yellowish postoxic sediments, respectively. No sterilized variants were examined. Bottles without the addition of organic carbon were used as control variants (Table 3). Two of these bottles were opened after 5 days in order to determine the initial chemical and isotopic parameters for bottle water and sediments. All other bottles were incubated for 8 weeks in darkness.

Table 1

Set-up for the first series of experiments, pH and Eh values, and concentrations and δ^{34}S values of bottle water sulphate at the end of the experiments

Experiment series	Duration (weeks)	Sediment type	Straw	Added substrates	Abbreviation in Figs. 3–8	Chemical formula	TOC-concentration (mmol/l)	Sterilized	pH	Eh (mV)	Sulphate-concentration (mg/l)	$\delta^{34}S_{sulphate}$ (‰)	$\Delta\delta^{34}$S (‰)
1	4	dark	yes	Sodium pyruvate	P	$NaC_3H_3O_3$	90	no	5.5	−40	1160	6.9	3.0
1	4	dark	yes	Sodium pyruvate	P_s	$NaC_3H_3O_3$	90	yes	4.8	250	1650	3.9	≤ ±0.2
1	4	dark	yes	Glucose-D-monohydrate	G	$C_6H_{12}O_6 \cdot H_2O$	180	no	4.2	−90	1300	3.9	≤ ±0.2
1	4	dark	yes	Glucose-D-monohydrate	G_s	$C_6H_{12}O_6 \cdot H_2O$	180	yes	3.3	400	1300	3.8	≤ ±0.2
1	4	dark	yes	Succinic acid	S	$C_4H_6O_4$	120	no	3.1	510	1360	3.3	−0.6
1	4	dark	yes	Succinic acid	S_s	$C_4H_6O_4$	120	yes	3.1	490	1450	4.2	0.3
1	4	dark	yes	Sodium-DL-lactate	L	$NaC_3H_5O_3$	90	no	5.8	−110	360	18.7	14.8
1	4	dark	yes	Sodium-DL-lactate	L_s	$NaC_3H_5O_3$	90	yes	4.2	415	1570	4.3	0.4
1	4	dark	yes	Ethanol	E	C_2H_6O	60	no	4.6	27	1000	4.6	0.7
1	4	dark	yes	Ethanol	E_s	C_2H_6O	60	yes	3.6	540	1460	4.3	0.4
1	4	dark	yes	Sodium acetate	A	$NaC_2H_3O_2$	60	no	5.2	106	1400	5.8	1.9
1	4	dark	yes	Sodium acetate	A_s	$NaC_2H_3O_2$	60	yes	4.8	380	1480	4.3	0.4
1	4	dark	yes	–	Ctr		–	no	4.7	40	1290	5.2	1.3
1	4	dark	yes	–	Ctr_s		–	yes	3.3	500	1450	3.8	≤ ±0.2
1	0.7	–	yes	–	St		–	no	2.8	550	1300	4.0	≤ ±0.2
1	0.7	–	yes	–	St_s		–	yes	2.8	700	1350	3.8	≤ ±0.2

The difference between $\delta^{34}S_{sulphate}$ values at the beginning and the end of the experiments is expressed as $\Delta\delta^{34}$S value.

4. Analytical methods

4.1. Water samples

Immediately after opening the bottles, pH values, redox potentials (Eh), and dissolved oxygen contents were measured. Concentrations of dissolved sulphate were determined by ion chromatography (ICA-5000, GAT) with a precision of typically better than ± 5%.

Immediately after the opening of the bottles, lake water was removed under oxygen-free conditions and ZnAc was added to precipitate dissolved sulphide as ZnS and to prevent re-oxidation of HS^- and H_2S to sulphate. Subsequently, water samples were filtered through 0.45-μm cellulose acetate membranes to remove ZnS, other inorganic precipitates, and particulate organic matter. No isotope analyses were performed on the typically very small dissolved sulphide fraction. Between 20 and 50 ml of a 0.5 M $BaCl_2$ solution was added to ca. 100 ml of filtered water to precipitate dissolved sulphate as $BaSO_4$. After 24 h, the precipitate was filtered, dried at 50 °C, and stored for mass spectrometric analysis.

4.2. Sediment samples

Four sulphur fractions were extracted from the sediment samples for chemical and isotopic analyses. Immediately after the opening of the bottles and removal of water in an oxygen-free environment, sediments were shock-frozen with liquid nitrogen and stored at temperatures of − 36 °C. After thawing the samples, acid volatile sulphur (AVS), HCl-soluble sulphate, elemental sulphur (S°), and chromium reducible sulphur (CRS) were sequentially extracted from the wet sedimentary material using a slightly modified extraction scheme (Fig. 2) of Rice et al. (1993), the latter based on methods described in Zhabina and Volkov (1978) and Canfield et al. (1986).

AVS, widely believed to represent monosulphide minerals, was liberated as H_2S by the addition of 6 M HCl. Between 20 and 100 g of wet sediment was placed in a reaction vessel and attached to a distillation apparatus modified after Tuttle et al. (1986). The reaction system was de-oxygenated by a stream of N_2 for 30 min to establish anaerobic conditions. AVS was then extracted by introducing 40–80 ml 6 M HCl under a continuous stream of N_2. The released H_2S

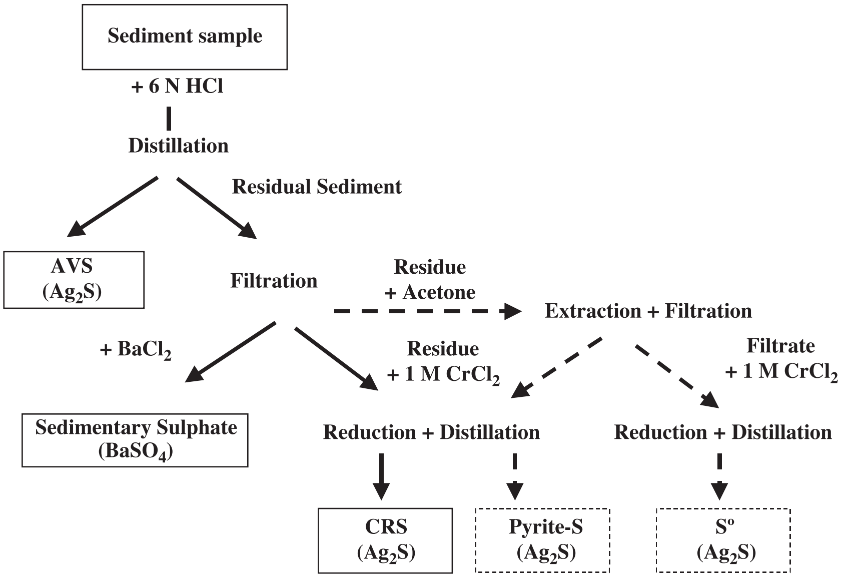

Fig. 2. Scheme of sequential extraction of various sedimentary sulphur compounds for content and isotope analyses.

was swept by the carrier gas into a trap with 0.1 M zinc acetate solution, where ZnS precipitated. After 25 min at room temperature, the reaction vessel was heated to 70 °C for 15 min. Thereafter, the acetate trap was removed from the distillation apparatus and ZnS was converted to Ag_2S by adding ~ 20 ml 0.1 M $AgNO_3$ solution. The Ag_2S precipitate was subsequently filtered, air-dried, weighed, and stored for isotope analysis.

The residual sample material was removed from the reaction vessel, filtered, and thoroughly rinsed with hot deionized water in order to remove soluble sulphate. Approximately 100 ml of a 0.5 M $BaCl_2$ solution was added to the filtrate to precipitate $BaSO_4$, which was further processed as described above. The $BaSO_4$ was weighed and subsequently used to determine the isotopic composition of inorganic sedimentary sulphate.

The residual sample was subjected to chromium reduction in order to extract elemental sulphur and disulphide (pyrite) sulphur. CRS was extracted in the same distillation apparatus that was used for the AVS determination. Sediment samples were boiled under a continuous stream of nitrogen with 40–60 ml 1 M $CrCl_2$ solution and 20 ml 6 M HCl (Canfield et al., 1986; Tuttle et al., 1986). Reduced inorganic sedimentary sulphur compounds such as FeS_2 and S° were released concomitantly as H_2S and carried by the nitrogen stream into a ZnAc trap, where ZnS precipitated. After 90 min, the ZnAc trap was removed from the distillation apparatus and ZnS was converted to Ag_2S as described above. The Ag_2S precipitate was dried, weighed, and stored for subsequent isotope analysis.

For sediment samples from the third experimental series, elemental sulphur and pyritic sulphur were extracted separately. After the removal of AVS and acid soluble sulphate, elemental sulphur was extracted by adding 70–150 ml acetone to the residual sediments and shaking for 16 h (Wieder et al., 1985; Rice et al., 1993). The acetone solution, which contained dissolved elemental sulphur, was subsequently filtered through 45-μm PTFE membranes. The acetone solution was then transferred to the reaction vessel of the distillation apparatus and elemental sulphur was reduced to H_2S by chromium reduction as described above. Pyrite sulphur was extracted from the residual sediment by $CrCl_2$ reduction. The obtained Ag_2S was dried, weighed, and stored for subsequent isotope analysis.

The contents of various sulphur fractions in the sediments (noted in mg S per gram dry sediment) were determined gravimetrically, utilizing the quantities of recovered Ag_2S or $BaSO_4$ and the known sample weights. Analytical uncertainty for gravimetric sulphur determinations was usually better than ± 8%. The isotopic compositions of dissolved sulphate, AVS, HCl soluble sulphate, elemental sulphur (S^o), and CRS were determined by converting $BaSO_4$ or Ag_2S into SO_2, which was then introduced into a dual inlet isotope ratio mass spectrometer (Finnigan MAT 251) (Yanagisawa and Sakai, 1983; Ueda and Krouse, 1986). Samples of less than 5 mg $BaSO_4$ or Ag_2S were analyzed by isotope ratio monitoring elemental analyzer mass spectrometry (irm-EAMS) using a Carlo Erba 1500 connected to a VG Prism (cf. Giesemann et al., 1994). For oxygen isotope analyses of dissolved sulphate, $BaSO_4$ was mixed with graphite and converted to CO and CO_2 at 1000 °C followed by a high voltage conversion of CO to CO_2 in a discharge chamber (Rees and Holt, 1991). Oxygen isotope ratios were determined by conventional dual inlet isotope ratio mass spectrometry using a VG Sira 10.

Isotope compositions are reported on the usual δ-scale in parts per thousand deviation relative to internationally accepted standards, CDT (troilite from the Canyon Diabolo meteorite) for sulphur, and V-SMOW (Vienna Standard Mean Ocean Water) for oxygen isotope measurements. The reproducibility of sulphur isotope measurements on aqueous sulphate was better than ± 0.25‰. The overall reproducibilities of extractions, gas preparations, and mass spectrometric analyses for sulphur isotope determinations on sedimentary AVS, inorganic sulphate, S°, and CRS, determined by duplicate analyses, were usually better than ± 0.5‰. The analytical reproducibility of oxygen isotope measurements on sulphate was ± 0.9‰.

5. Results

5.1. Water

The pH and Eh values of the bottle water at the beginning and at the termination of the experiments are summarized in Tables 1–3 and Fig. 3a–c. The initial lake water that was used for the first two experimental series had pH values of 2.8 and Eh values between 550 mV (not sterilized) and 700 mV (sterilized). In the third experimental series, pH values of the initial water varied between 2.5 (yellowish sediment) and 2.9 (dark sediment) and Eh values were 650 mV (dark sediment) and 690 mV (yellowish sediment), respectively.

At the end of the first experimental series with addition of C_{org} containing compounds, slightly increased pH (up to 4.8) and Eh values generally higher than 200 mV were observed in all sterilized bottles (Fig. 3a). The nonsterilized bottle water was, with one exception, characterized by pH values between 4.2 and 5.8 and Eh values ranging between − 120 and 110 mV. Only in the bottle with succinic acid addition were the pH and Eh values unchanged.

At the end of the second experimental series featuring the addition of industrial by-products, pH values ranged between 6.3 and 7.0 and Eh values varied between − 80 and + 80 mV in the nonsterilized bottles (Fig. 3b). In the sterilized bottles, which received molasses and whey additions, Eh values of 320 ± 20 mV and pH values of 4.2 were observed. Sterilized bottle water with Carbokalk and granules addition had Eh values between 140 and 220 mV and pH values between 7.0 and 10.8.

At the end of the third experimental series, the pH and Eh values of water from the control experiments without straw additions (Fig. 3c) were similar to those of the initial lake water (pH ~ 2.6, Eh >600 mV). Control experiments with straw addition resulted in increased pH values of 4.4 (yellowish sediment) and 4.8 (dark sediment) and Eh values of less than 120 mV. At the end of the experiment with straw and Carbokalk addition, pH values >6.5 and Eh values < − 90 mV were observed in the bottle water independent of sediment type. For the same experimental setup without straw additions, pH values of 6.3 and 6.5 and Eh values of + 230 and + 210 mV were recorded. For bottles that received ethanol and straw, the pH values were 5.8 and 5.9 and the Eh values ranged between + 20 and + 60 mV for both sediment types. Significant differences in the pH and Eh values of the water with differing sediment types were observed only for bottles with ethanol addition without straw (Fig. 3c). Whereas the pH and Eh values were similar to those of the initial water for the experiment with the yellowish sediment, a significantly increased pH value (6.0) and a decreased Eh value (+ 150 mV) were observed in the experiment with the dark sediment.

Concentrations of SO_4^{2-} in the bottle water were determined at the beginning and the end of all experiments (Tables 1–3). For the first and second experimental series, sulphate concentrations in the initial water were 1350 (sterilized) and 1300 mg/l (nonsterilized). In the third experimental series, the initial SO_4^{2-} concentrations were 1300 mg/l (dark sediment) and 1220 mg/l (yellowish sediment).

At the end of the first experimental series, SO_4^{2-} concentrations in the control variants without the addition of organic carbon were unchanged (nonsteri-

Table 2
Set-up for the second series of experiments, pH and Eh values, and concentrations and $\delta^{34}S$ values of bottle water sulphate at the end of the experiments

Experiment Series	Duration (weeks)	Sediment type	Straw	Added substrates	Abbreviation in Figs. 3–8	TOC-concentration (mmol/l)	Sterilized	pH	Eh (mV)	Sulphate-concentration (mg/l)	$\delta^{34}S_{sulphate}$ (‰)	$\Delta\delta^{34}S$ (‰)
2	12	dark	yes	Whey	W	82	no	6.3	80	< 1	N.D.	N.D.
2	12	dark	yes	Whey	W_s	82	yes	4.2	340	1420	4.1	≤ ± 0.2
2	12	dark	yes	Molasses	M	97	no	6.3	30	< 1	N.D.	N.D.
2	12	dark	yes	Molasses	M_s	97	yes	4.2	320	1380	4.5	0.6
2	12	dark	yes	Carbokalk	C	54	no	6.6	− 80	360	48.8	44.9
2	12	dark	yes	Carbokalk	C_s	54	yes	7.0	140	1740	4.2	0.3
2	12	dark	yes	Granules	G	468	no	7.0	50	< 1	N.D.	N.D.
2	12	dark	yes	Granules	G_s	468	yes	10.8	220	1640	6.0	2.1

The difference between $\delta^{34}S_{sulphate}$ values at the beginning and the end of the experiments is expressed as $\Delta\delta^{34}S$ value.

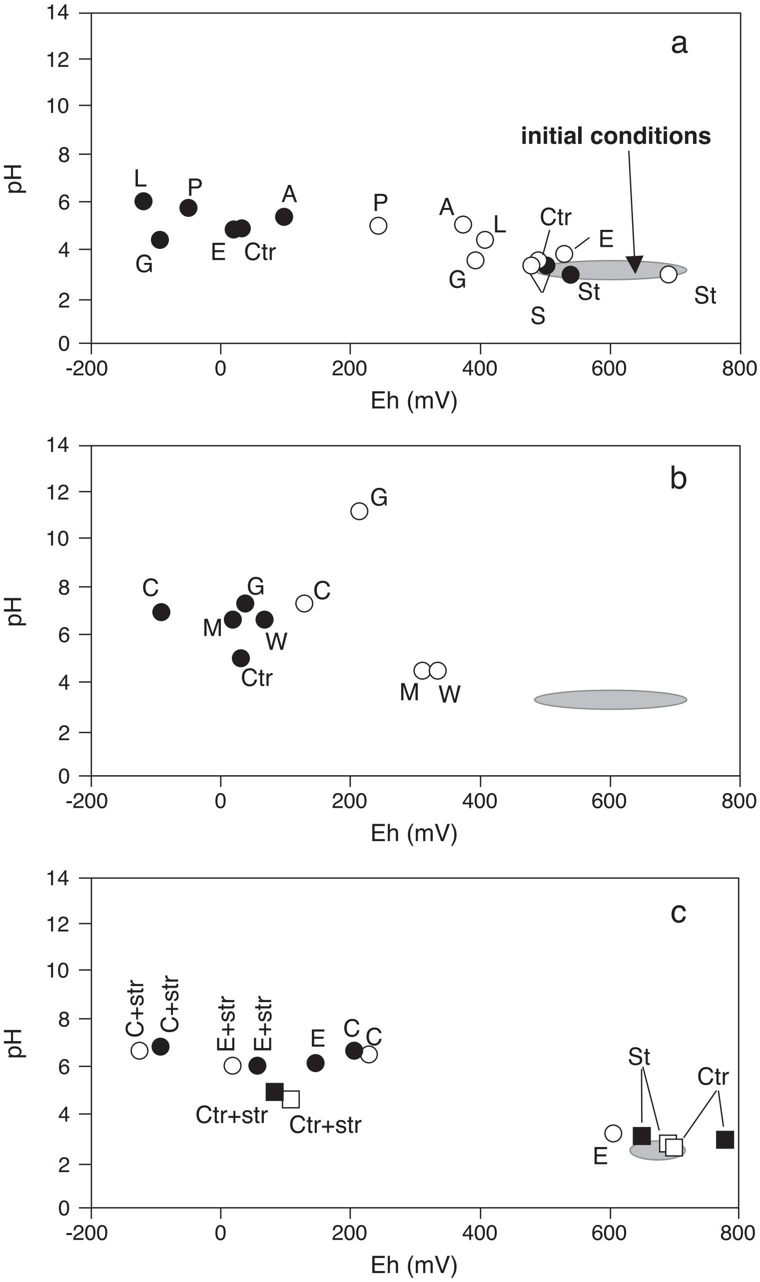

Fig. 3. Eh and pH values of the bottle water at the beginning (shaded oval) and at the termination of all three series of bottle experiments. Solid circles in (a) and (b) represent experiments with nonsterilized bottles, open circles indicate experiments with sterilized bottles. In (c), solid circles represent experiments with dark sediments and open circles indicate those with yellowish sediments. For abbreviations of the different experimental treatments, see Tables 1–3.

lized) or slightly increased (sterilized) compared to those of the initial water (Table 1). The same was true for SO_4^{2-} concentrations in all sterilized bottles with additions of organic carbon. In the nonsterilized bottles, glucose, succinic acid, and acetate additions caused no significant changes in sulphate concentrations, but SO_4^{2-} concentrations had decreased significantly at the termination of experiments with lactate (360 mg/l), ethanol (1000 mg/l), and pyruvate addition (1160 mg/l). For the second experimental series, SO_4^{2-} concentrations in the sterilized bottles remained either constant (molasses) or increased (whey, granules, Carbokalk) after 12 weeks. In contrast, a complete removal of SO_4^{2-} from the lake water was observed in nonsterilized bottles with additions of whey, molasses, and granules (Table 2). In the Carbokalk experiment, SO_4^{2-} concentrations also decreased significantly (360 mg/l). At the end of the third experimental series, markedly lower SO_4^{2-} concentrations in the bottle water were observed for three variants with ethanol additions (Table 3). The combination of ethanol, straw, and dark sediment caused an almost complete removal of SO_4^{2-} from the lake water. The same experimental variant without straw resulted in SO_4^{2-} concentrations of 110 mg/l. A sulphate concentration of 210 mg/l was observed after ethanol and straw were added to the bottle with yellow sediments. In contrast, ethanol addition to bottles without straw and with yellowish sediments, as well as all other experimental variants, including those with Carbokalk, resulted in increased SO_4^{2-} concentrations compared to those at the beginning of the experiment.

Sulphur isotope ratios for dissolved sulphate in the bottle water were also determined at the beginning and at the end of the experiments (Tables 1–3). The $\delta^{34}S$ value of SO_4^{2-} in the initial bottle water was $+3.9 \pm 0.2$ ‰ ($n=4$). At the end of the experiments, the $\delta^{34}S_{sulphate}$ values in the bottle water had increased significantly for several variants. The differences between $\delta^{34}S_{sulphate}$ values at the beginning and the end of the experiments, expressed as $\Delta\delta^{34}S$, are summarized in Tables 1–3.

At the end of the first experimental series, $\delta^{34}S$ values for SO_4^{2-} after lactate addition increased by 14.8 ‰ in the nonsterilized bottle (Table 1). The addition of pyruvate, acetate, and ethanol resulted in increases of 3.0 ‰, 1.9 ‰, and 0.7 ‰, respectively. Interestingly, a $\Delta\delta^{34}S$ value of 1.3 ‰ was also observed in the nonsterilized control experiment without the addition of organic carbon. In contrast, no significant change in the sulphur isotope ratios of SO_4^{2-} was observed at the end of the experiment with glucose addition. The addition of succinic acid

Table 3

Set-up for the third series of experiments, pH and Eh values, and concentrations and isotopic compositions of bottle water sulphate at the end of the experiment

Experiment series	Duration (weeks)	Sediment type	Straw	Added substrates	Abbreviation in Figs. 3–8	TOC-concentration (mmol/l)	Sterilized	pH	Eh (mV)	Sulphate-concentration (mg/l)	$\delta^{34}S_{sulphate}$ (‰)	$\Delta\delta^{34}S$ (‰)	$\delta^{18}O_{sulphate}$ (‰)
3	0.7	dark	no	–	St_d	–	no	2.9	650	1300	4.1	$\leq \pm 0.2$	N.D.
3	0.7	yellowish	no	–	St_y	–	no	2.5	690	1220	3.8	$\leq \pm 0.2$	N.D.
3	8	dark	no	–	Ctr_d	–	no	2.7	700	1470	4.2	0.3	− 0.1
3	8	yellowish	no	–	Ctr_y	–	no	2.4	780	1500	4.0	$\leq \pm 0.2$	− 0.4
3	8	dark	yes	–	$Ctr_d + Str$	–	no	4.8	90	1570	5.1	1.2	3.3
3	8	yellowish	yes	–	$Ctr_y + Str$	–	no	4.4	110	1530	4.7	0.8	1.4
3	8	dark	yes	Carbokalk	$C_d + Str$	54	no	6.6	− 90	1460	8.6	4.7	3.6
3	8	yellowish	yes	Carbokalk	$C_y + Str$	54	no	6.5	− 120	1600	8.3	4.4	2.9
3	8	dark	no	Carbokalk	C_d	54	no	6.5	210	1930	4.5	0.6	N.D.
3	8	yellowish	no	Carbokalk	C_y	54	no	6.3	230	2020	4.6	0.7	− 0.6
3	8	dark	yes	Ethanol	$E_d + Str$	60	no	5.8	60	2	N.D.	N.D.	N.D.
3	8	yellowish	yes	Ethanol	$E_y + Str$	60	no	5.9	20	210	19.6	16.2	6.1
3	8	dark	no	Ethanol	E_d	60	no	6.0	150	110	N.D.	N.D.	N.D.
3	8	yellowish	no	Ethanol	E_y	60	no	3.0	610	2070	4.8	0.9	N.D.

The difference between $\delta^{34}S_{sulphate}$ values at the beginning and the end of the experiments is expressed as $\Delta\delta^{34}S$ value.

resulted even in a slight decrease of the $\delta^{34}S_{sulphate}$ value at the end of the experiment. In the sterilized bottles, the $\delta^{34}S$ values of SO_4^{2-} deviated by less than 0.5‰ from the initial sulphur isotope ratios.

At the end of the second experimental series, the $\delta^{34}S$ value of SO_4^{2-} in most sterilized bottles remained essentially unchanged compared to that of the initial sulphate ($\Delta\delta^{34}S \leq 0.6$‰). Only the addition of granules resulted in a slight increase in the sulphur isotope ratio of SO_4^{2-} ($\Delta\delta^{34}S = 2.1$‰). Three of the four nonsterilized bottles did not contain enough SO_4^{2-} for isotope analysis. The $\delta^{34}S$ value of SO_4^{2-} at the end of the experiment with the Carbokalk was +48.8‰, almost 45‰ higher than that of the initial sulphate.

At the end of the third experimental series, the $\delta^{34}S$ values of SO_4^{2-} from control experiments without straw were essentially identical to those of the initial bottle water (Table 3). The control experiment without organic carbon addition but with straw resulted in slightly increased $\delta^{34}S_{sulphate}$ values in the bottles with yellowish ($\Delta\delta^{34}S = 0.8$‰) and dark sediment ($\Delta\delta^{34}S = 1.2$‰). The addition of Carbokalk to bottles with straw caused an increase in $\delta^{34}S_{sulphate}$ values of 4.5 ± 0.2‰ for both sediment types, whereas the addition of the same organic carbon compound to bottles without straw resulted only in small $\Delta\delta^{34}S$ values (0.7 ± 0.2). In the experimental variants with ethanol, bottles with dark sediment did not contain enough SO_4^{2-} for isotope analyses. For bottles with the yellowish sediment, the $\delta^{34}S_{sulphate}$ values increased by 16.2‰ with straw, but only by 0.9‰ without straw, compared to those of SO_4^{2-} in the initial bottle water.

In the third experimental series, the $\delta^{18}O$ values of SO_4^{2-} were also determined (Table 3). The initial $\delta^{18}O_{sulphate}$ values were 0.0‰ (dark sediment) and 1.1‰ (yellowish sediment), respectively. At the end of the experiment, the $\delta^{18}O_{sulphate}$ values from the control experiments without straw were −0.1‰ (dark sediment) and −0.4‰ (yellowish sediment), similar to those of the initial SO_4^{2-}. In control experiments with straw, $\delta^{18}O_{sulphate}$ was 3.3‰ (dark sediment) and 1.4‰ (yellowish sediment). Carbokalk experiments with straw resulted in $\delta^{18}O_{sulphate}$ values of 3.6‰ (dark sediment) and 2.9‰ (yellowish sediment). In the ethanol experiment with yellowish sediment and straw, a $\delta^{18}O$ value of 6.1‰ was observed for SO_4^{2-} at the end of the experiment.

5.2. Sediments

The contents of inorganic sulphate, AVS, CRS, and elemental S (only for the third experimental series) in the sediments of all experiments are shown in Fig. 4a–c. For the first two experimental series, the initial contents of AVS, CRS, and inorganic sulphate in the dark lake sediments were determined on material from the sterilized control experiment opened after 4 weeks (Ctr in Fig. 4a,b). In the initial sediments, the AVS content was less than 0.1 mg/g, the CRS content was 1.1 mg/g, and that of sedimentary sulphate-S was 3.9 mg/g. For the third experimental series, the initial contents of the sedimentary sulphur fractions were determined on material from the standard experiments that were opened after 5 days (Fig. 4c). The sulphate-S contents were 16.6 mg/g in the yellowish sediments and 10.2 mg/g in the dark sediments. Less than 0.2 mg/g elemental sulphur and pyrite sulphur, respectively, and no AVS was detected in either sediment type.

At the end of the first experimental series, no significant amounts of AVS were detected in the sediments of the sterilized bottles (Fig. 4a). CRS contents were 1.5 + 0.4 mg/g, similar to those of the initial sediments. Sedimentary sulphate contents in the sterilized bottles decreased from the initial level (3.9 mg S/g) to values between 3.3 and 1.4 mg S/g at the end of the experiments. It is interesting to note that sulphate concentrations in the bottle water of most sterilized variants had increased at the end of the experiment (Table 1).

In the nonsterilized bottles with succinic acid and glucose additions, AVS and CRS contents were similar to and sulphate contents slightly lower than those in the initial sediments. After acetate, ethanol, pyruvate, and lactate additions to the nonsterilized bottles, AVS and CRS contents increased, whereas those of sedimentary sulphate (< 1.5 mg S/g) decreased compared to those in the initial sediments. AVS contents increased from 0.1 mg/g to values between 1.1 (ethanol) and 5.3 mg/g (pyruvate). CRS contents increased from 1.1 mg/g to values between 2.1 (acetate) and 5.7 mg/g (lactate). It is interesting to note that the total sulphur content in the sediments

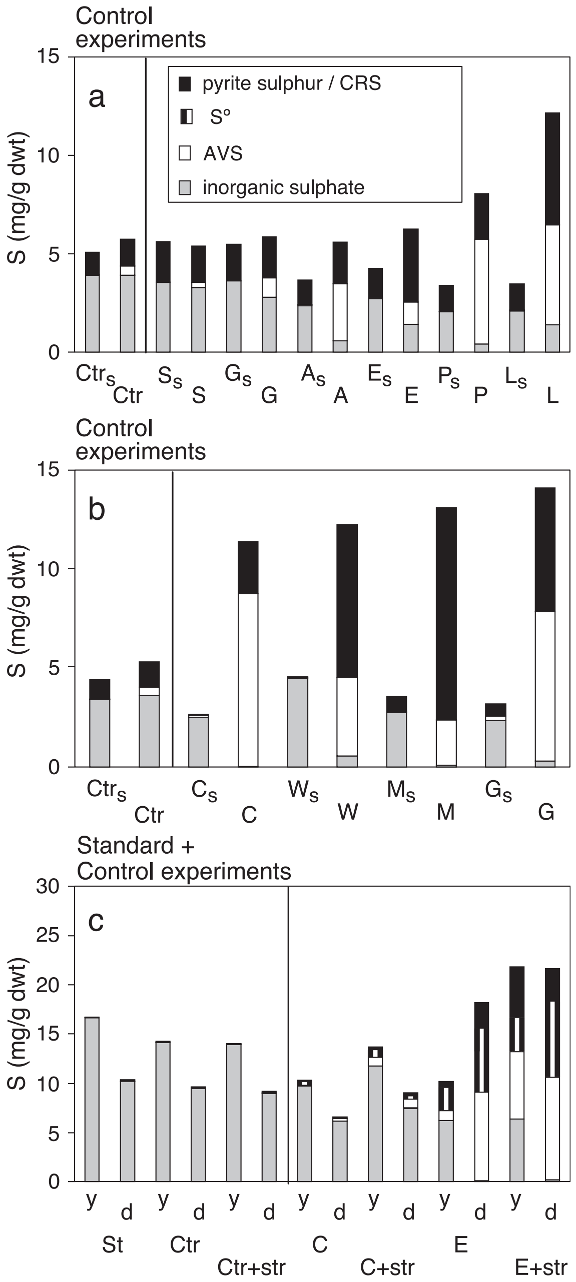

Fig. 4. Contents of various sedimentary sulphur compounds determined at the end of the first (a), second (b), and third experimental series (c). The entire bar indicates total sulphur contents, comprised of inorganic sulphate, acid volatile S, chromium-reducible S, and elemental S (see legend). For abbreviations of the different experimental treatments, see Tables 1–3.

increased significantly in the variants with pyruvate and lactate additions (Fig. 4a), whereas the bottle water showed decreased SO_4^{2-} concentrations (Table 1). In the remaining nonsterilized bottles, total sedimentary sulphur contents at the end of the experiment did not deviate significantly from that of the initial sediments.

At the end of the second experimental series, AVS contents <0.3 mg/g and CRS contents <1.0 mg/g were detected in the sediments of the sterilized variants (Fig. 4b). Sedimentary sulphate-S decreased from the initial level (3.9 mg/g) to values of 2.9 ± 0.8 mg/g. It is noteworthy that the SO_4^{2-} concentrations in the bottle water of all sterilized variants had increased (Table 2). In the four experiments with nonsterilized bottles, reduced inorganic sulphur contents (AVS and CRS) increased markedly from the initial level (1.2 mg/g) to values between 11.4 (Carbokalk) and 13.8 mg/g (granules). In the variants with Carbokalk and granules, reduced inorganic sulphur was detected predominantly in the form of AVS (8.7 and 7.6 mg/g, respectively). CRS was dominant in sediments from the experiments with whey (7.7 mg/g) and molasses (10.7 mg/g). The contents of sedimentary sulphate were less than 0.6 mg/g, but total sedimentary S had increased markedly at termination of all four experimental variants (Fig. 4b).

At the end of the third experimental series, sediments of the control variants had AVS, CRS, and elemental S contents of less than 0.2 mg/g, respectively, and inorganic sulphate contents similar to those of the initial sedimentary material (Fig. 4c). In the experiment with Carbokalk and without straw, the reduced sedimentary inorganic sulphur contents (AVS, CRS, and elemental S) were less than 0.5 mg/g, respectively. The sulphate-S contents of the yellowish (9.7 mg/g) and dark (6.2 mg/g) sediments were significantly lower than those of the control variants (16.6 and 10.2 mg/g, respectively). In experiments with the addition of Carbokalk and straw, between 1.5 mg/g (dark sediment) and 1.9 mg/g (yellowish sediment) were detected in the reduced inorganic sulphur fractions. AVS contents were 1.1 mg/g (yellowish sediment) and 0.9 mg/g (dark sediment) and elemental sulphur and pyrite-S contents varied between 0.2 and 0.6 mg/g in both sediment types. After 8 weeks, 11.8 mg/g (yellowish sediment) and 7.5 mg/g (dark sediment) sulphate-S were determined in the sediments. The addition of ethanol resulted in all variants in significant increases in sedimentary sulphide contents (AVS, CRS, S°), with 4.0 mg/g (without straw) to 15.5 mg/g (with straw) in experiments with yellowish sediments, and 18.1 mg/g (no straw) to 21.4 mg/g (with straw) for the dark ones (Fig. 4c). On average, 50% of the reduced inorganic sulphur in the dark sediments occurred as AVS, 36% as elemental sulphur, and 14% as CRS. The yellowish sediments contained 6.3 ± 0.1 mg/g inorganic sulphate, while the dark sediments were characterized by < 0.2 mg/g sulphate-S at the end of the experiment.

Isotope ratios of the various sedimentary sulphur compounds varied from − 38.8 to + 16.9 ‰, depending on the sulphur fraction and experiment (Fig. 5a–c). AVS and CRS from the sediments of the sterilized control experiment of the first and second batch experiment series had δ^{34}S values of − 38.6 (AVS) and − 35.3 ‰ (CRS). The δ^{34}S value of sedimentary sulphate was 2.1 ‰.

At the end of the first experimental series (Fig. 5a), the δ^{34}S values of AVS and CRS in the sediments of the sterilized bottles were − 34.5 ± 5.4 ‰ and − 35.3 ± 0.8 ‰, respectively, similar to those in the control experiments. Similar values were observed for sediments from bottles where glucose and succinic acid were added. In contrast, nonsterilized sediments from bottles with lactate, pyruvate, ethanol, and acetate addition had $\delta^{34}S_{AVS}$ values between − 9.3 ‰ and − 3.4 ‰, and $\delta^{34}S_{CRS}$ values between − 11.5 ‰ and − 7.4 ‰, markedly higher than those in the control experiments. The average δ^{34}S value of sedimentary sulphate in the sterilized bottles was 2.0 ± 0.8 ‰. The δ^{34}S values of sedimentary sulphate from nonsterilized bottles with glucose, lactate, ethanol, and acetate additions varied between 2.2 ‰ and 4.8 ‰, whereas pyruvate addition resulted in the highest δ^{34}S value for sedimentary sulphate (9.9 ‰).

At the end of the second experimental series, the δ^{34}S values of reduced inorganic sulphur in the sterilized sediments were similar to those of the control experiment (Fig. 5b). The δ^{34}S values of reduced inorganic sulphur in sediments from the nonsterilized bottles were significantly higher, ranging between − 10.7 ‰ and 2.2 ‰. Addition of Carbokalk resulted in $\delta^{34}S_{AVS}$ and $\delta^{34}S_{CRS}$ values of − 10.7 ‰ and − 10.1 ‰, respectively. In contrast, the δ^{34}S values of reduced inorganic sulphur com-

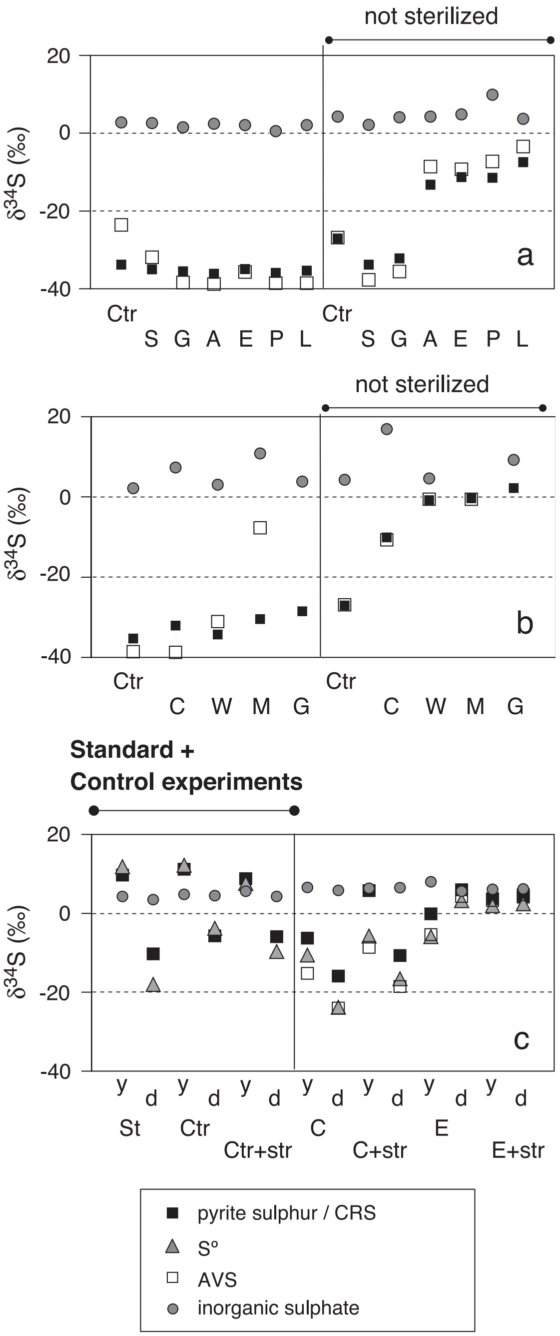

Fig. 5. Isotope ratios of various reduced sedimentary sulphur compounds at the end of the first (a), second (b), and third experimental series (c). For abbreviations of the different experimental treatments, see Tables 1–3.

pounds from sediments of bottles with whey, molasses, or granules additions had δ^{34}S values between − 0.8 ‰ and 2.2 ‰, similar to those of the initial sulphate in the bottle water. The δ^{34}S values of sedimentary sulphate were 6.2 ± 3.6 ‰ for the sterilized bottles and ranged between 6.2 ‰ and 10.2 ‰ for the nonsterilized sediments.

Reduced inorganic sulphur compounds in the sediments of the standard experiments (0.7 weeks) from the third batch experiment series had δ^{34}S values between − 18.0 ‰ and − 10.3 ‰ in the dark sediments, and varied between + 9.7 (pyrite-S) and + 11.8 ‰ (S^0) in the yellowish sediments (Fig. 5c). Sulphur isotope ratios of sedimentary sulphate were with + 3.4 ‰ (dark) and + 4.3 ‰ (yellowish) similar for both sediment types. At the end of the third experimental series, chromium-reducible sulphur and elemental sulphur extracted from sediments of the control experiments (8 weeks) with and without straw addition were characterized by δ^{34}S values between − 9.7 ‰ and − 3.8 ‰ for the dark sediment, and ranged between 7.6 ‰ and 12.2 ‰ for the yellowish one. The δ^{34}S values of sedimentary sulphate varied between 4.3 ‰ and 5.6 ‰ for both sediment types. For the experiments with Carbokalk additions without straw, the δ^{34}S values for reduced inorganic sulphur in the yellowish sediment varied between − 6.3 ‰ and − 15.2 ‰ and for the dark one between − 15.9 ‰ and − 24.0 ‰ (Fig. 5c). δ^{34}S values of 6.6 ‰ (yellowish sediment) and 5.8 ‰ (dark sediment) were determined for sedimentary sulphate. Addition of straw and Carbokalk resulted in δ^{34}S of reduced inorganic sulphur between − 8.6 ‰ and + 5.8 ‰ in the yellowish sediments, and between − 18.6 ‰ and − 10.7 ‰ in the dark ones. The δ^{34}S values of sedimentary sulphate had increased slightly to an average of 6.4 ± 0.1 ‰ from their initial values of 4.3 ‰ (yellowish) and 3.5 ‰ (dark). In the experiments with ethanol addition but without straw, the δ^{34}S values of reduced inorganic sulphur ranged from − 5.9 ‰ to − 0.1 ‰ in the yellowish sediments, and from 3.2 ‰ to 6.0 ‰ in the dark sediments. After ethanol addition with straw, δ^{34}S values of reduced inorganic sulphur varied between 1.9 ‰ and 4.7 ‰ for both sediment types. δ^{34}S values between 5.6 ‰ and 8.0 ‰ were observed for sedimentary sulphate in all experiments with ethanol addition.

6. Discussion

During bacterial (dissimilatory) sulphate reduction (BSR), sulphate is used as the terminal electron acceptor in the oxidation of organic carbon (or hydrogen). Hydrogen sulphide is produced and can react with reduced forms of metals, such as iron (Fe II), to form sulphide minerals. The formation of monosulphide minerals may generate acidity (provided that aqueous iron is involved), but the formation of pyrite generates alkalinity (Berner, 1970; Berner, 1984). Hence, permanent fixation of H_2S-sulphur from BSR as sedimentary pyrite produces alkalinity and may result in increased pH values (Anderson and Schiff, 1987). The gain in alkalinity produced by reduction of ferric iron and sulphate is not sustainable if sulphide and ferrous iron are re-oxidized. Oxidation must be therefore prevented to achieve a permanent increase in alkalinity. Dissolved and solid sulphide compounds can be re-oxidized both chemically (Pankow and Morgan, 1980) and biologically (Roy and Trudinger, 1970). In acidic mining lakes in Lusatia, the process of chemical oxidation of hydrogen sulphide by FeIII (e.g. from FeIII-bearing minerals) appears to be the predominant re-oxidation process (Peine, 1998).

Microbially catalyzed redox processes are characterized by typical redox-potential (Eh) values. These can be regarded as indicators of the dominant process in the sequence of redox reactions during degradation of organic matter, with Eh values decreasing from oxygen reduction (< 500 mV, at pH 7; Sigg and Stumm, 1996) to methane fermentation (< − 100 mV, at pH 7). If BSR is the dominant process, Eh values of generally less than 0 mV at pH 7 are typical (Hütter, 1994; Sigg and Stumm, 1996). Therefore, the Eh and pH values in the bottle water at the end of the experiments provided an initial qualitative indicator of the reductive processes and their intensity. Additionally, the following criteria were used as evidence for BSR: (1) decreasing concentrations of dissolved sulphate in the water column with progressively increasing δ^{34}S values in the remaining sulphate; (2) formation of new reduced sedimentary sulphur compounds, such as acid volatile and chromium-reducible sulphur.

In most of the control and sterilized experiments, pH remained at less than 4 and Eh was > + 200 mV

(Fig. 3). Concentrations and sulphur isotope ratios of dissolved sulphate in the bottle water were similar to those of the initial lake water (Tables 1 and 2). Contents and isotope ratios of reduced inorganic S compounds in the sediments corresponded to values measured in the original lake sediments (Figs. 4 and 5). This is evidence that BSR did not occur in most control and sterilized experiments.

Nonsterilized bottles from the first experimental series featuring the addition of pure substrates yielded quite contrasting results. After the addition of glucose and succinic acid, concentrations and sulphur isotope ratios of the bottle water sulphate remained essentially unchanged (Table 1, Fig. 6a). The contents and isotope ratios of sedimentary sulphur compounds at the end of these experiments were also similar to those of the initial sediments (Figs. 4a and 5a) indicating no significant BSR. In contrast, there is evidence that BSR occurred after the addition of acetate, pyruvate, ethanol, and lactate additions. Sulphate concentrations in the bottle water decreased slightly (ethanol, pyruvate) to markedly (lactate), whereas δ^{34}S values of the remaining sulphate increased by up to 3‰ in the ethanol, pyruvate, and acetate experiments, and by almost 15‰ in the lactate variant (Table 1, Fig. 6a). At the end of the experiments, the contents of sedimentary sulphate had decreased and that of reduced inorganic sulphur compounds (including pyrite) in the sediments increased in all four variants (Fig. 4a). The content of total sedimentary S at the end of the experiment with ethanol addition had more than doubled compared to that of the initial sediments, suggesting the highest extent of BSR in the ethanol treatment. The pH at the end of the ethanol experiment was 5.8.

Nonsterilized bottle water of the second experimental series featuring whey, molasses, granules, and Carbokalk additions was characterized by Eh values of less than +100 mV and pH values above 6 at the end of the incubation period (Fig. 3b). Sulphate was completely removed from the bottle water after whey, molasses, and granules addition (Table 2). At the end of the experiment with Carbokalk addition, 360 mg/l sulphate remained in solution with a δ^{34}S value of +49 ‰ (Fig. 6b). In all four treatments, significant amounts of sedimentary sulphur were found at the end of the experiments mainly as AVS and CRS, whereas sedimentary sulphate had almost disappeared (Fig. 4b). Total sedimentary S contents had more than doubled compared to that of the initial sediments (Fig. 4b). This indicates that BSR had resulted in significant (after Carbokalk addition) or almost complete (after whey, molasses, and granules addition) conversion of dissolved and sedimentary sulphate into reduced inorganic sulphur compounds such as pyrite. Apparently, this caused an increase in the bottle water pH from less than 4 to more than 6.

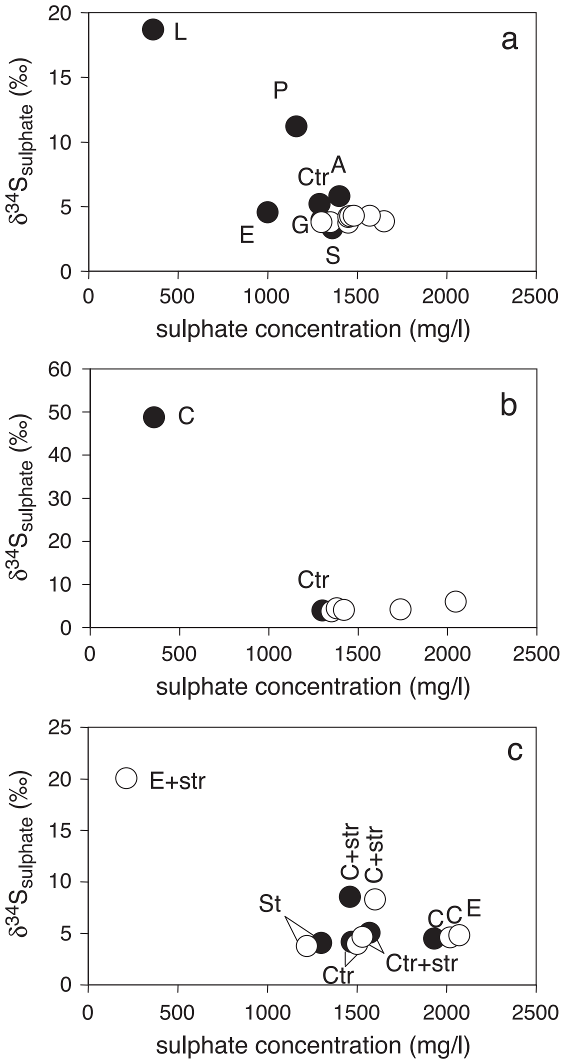

Fig. 6. Concentration and isotopic composition of residual sulphate at the end of the first (a), second (b), and third experimental series (c). For abbreviations of the different experimental treatments, see Tables 1–3.

In the third experimental series, ethanol and Carbokalk were tested as organic carbon source using two different sediments including the yellowish type that is representative for most of the acid lakes in Lusatia. Three distinct patterns were observed. Ethanol addition to bottles with dark sediment (with and without straw) and to the bottle with yellowish sediment in combination with straw resulted in markedly decreased sulphate concentrations in the bottle water (Table 3). Where measurements were possible, the δ^{34}S values of the remaining sulphate had increased by 16 ‰ (Fig. 6c). Total sedimentary S contents at the end of the experiments were significantly higher than those of the initial sediments (Fig. 4c). Most of the sedimentary S was detected as AVS, CRS, or elemental S, whereas sedimentary sulphate contents had markedly decreased (Fig. 4c). This is evidence that BSR had occurred during the experiments and that some of the generated H_2S had been fixed as pyrite, possibly via the reaction of iron monosulphides with elemental S (Berner, 1970). This authigenic pyrite formation contributed to the observed increase of pH in the bottle water to approximately 6.

The addition of Carbokalk and straw to both sediment types and the ethanol addition to bottles with yellowish sediment without straw resulted in the following pattern: sulphate concentrations in the bottle water remained constant or increased and the δ^{34}S values of dissolved sulphate increased between 1 ‰ and 5 ‰ (Table 3, Fig. 6c). The contents of sedimentary sulphate decreased slightly compared to those in the initial sediments, whereas the contents of reduced inorganic S compounds increased between 1 and 4 mg/g (Fig. 4c). This is evidence that some BSR had occurred in the sediments. The addition of Carbokalk without straw to both sediment types resulted in the following pattern: sulphate concentrations in the bottle water increased and the δ^{34}S values of dissolved sulphate remained unchanged (Table 3, Fig. 6c). Sedimentary sulphate contents had decreased at the end of the experiments compared to those of the initial sediments, but reduced inorganic sulphur compounds comprised less than 0.5 mg/g (Fig. 4c) indicating that BSR was less effective. The pH values of the bottle water at the end of all experiments with Carbokalk addition were above 6. We observed that the addition of Carbokalk induced a rapid pH increase in the water and the sediments. The increased pH values caused the dissolution of minerals such as jarosite ($(K,H_3O,Na)Fe_3(SO_4)_2(OH)_6$) or schwertmannite ($Fe_{16}O_{16}(OH)_{12}(SO_4)_2$) (e.g. Peine, 1998; Göttlicher, 1999), resulting in markedly increased sulphate concentrations in the bottle water. Subsequently, BSR commenced resulting in increasing δ^{34}S values of dissolved sulphate in bottles with Carbokalk and straw additions. A comparison of experiments with straw and without straw reveals that straw appeared to be a catalyst for BSR after ethanol and Carbokalk addition, since the amount of newly formed reduced inorganic S compounds was higher in the sediments of the experimental variants with straw addition (Fig. 4c).

The above considerations suggest that sulphur isotope analyses on aqueous sulphate can be used to monitor the occurrence of BSR, since δ^{34}S values increase progressively in the remaining sulphate. Fig. 7 shows that δ^{18}O values for dissolved sulphate had also increased at the end of experiments of the third series, which were characterized by moderate (Carbokalk + straw) to intense (ethanol + straw) BSR. However, the question arises whether elevated δ^{34}S (and δ^{18}O) values in the remaining bottle water sulphate can be used for quantifying the extent of BSR.

In a closed system, the evolution of the δ^{34}S values of sulphate and sulphide (H_2S) and the accumulation of the product (sedimentary sulphide) due to BSR can be modeled by the Rayleigh equation:

$$R_t = R_0 f^{(\alpha-1)} \quad (5)$$

where f is the sulphate conversion rate, R the ^{34}S/^{32}S ratios of initial (0) and subsequently remaining (t)

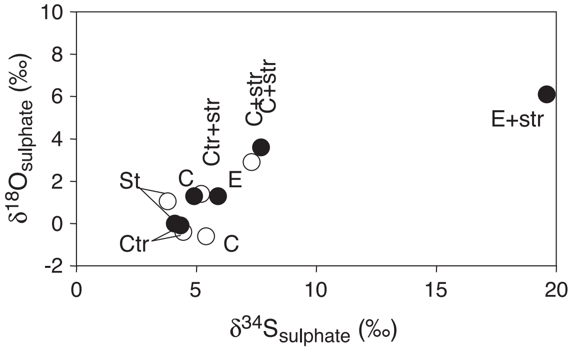

Fig. 7. Sulphur and oxygen isotope ratios of dissolved sulphate at the end of the third experimental series. See Table 3 for abbreviations of the different experimental treatments.

dissolved sulphate, and α the sulphur isotope fractionation factor during BSR (e.g. Nakai and Jensen, 1964; Hoefs, 1997; Clark and Fritz, 1997). The initially produced sulphide will be depleted in ^{32}S compared to the initial sulphate, depending on the extent of isotope fractionation. As BSR progresses towards completion, the $\delta^{34}S$ value of sedimentary sulphide asymptotically approaches that of the initial sulphate. The fractionation factor α can be precisely determined if the sulphur isotope ratios of the remaining dissolved sulphate, the newly formed sedimentary sulphide, and the conversion rate f are known. There are, however, several problems in adapting this approach to our experiments. Firstly, some of the initial sediments contained reduced inorganic sulphur compounds and it can be challenging to delineate the exact contents and sulphur isotope ratios of newly formed sulphides from the initial ones. Secondly, it had been shown that in some experiments re-mobilization of sedimentary sulphate replenished the dissolved sulphate pool, thus complicating the determination of conversion rates f (= remaining sulphate/initial sulphate). Nevertheless, we have plotted the $\delta^{34}S$ values of the remaining dissolved sulphate and of sedimentary sulphides versus approximate conversion rates (f) in Fig. 8. Also shown are hypothetical trend lines for the $\delta^{34}S$ values of residual sulphate, instantaneous sulphide, and accumulated sulphide assuming a fractionation factor $\alpha = 1.015$. Despite the above-mentioned limitations, a number of interesting observations can be made from this diagram and Fig. 5.

The extent of sulphur isotope fractionation during BSR depends on the specific sulphate reduction rate (rate/cell/time; e.g. Habicht and Canfield, 1997). Sulphur isotope ratios of reduced inorganic S compounds in the initial sediments used for the first and second experimental series were generally below − 30‰ (Fig. 5) indicating significant sulphur isotope fractionation during BSR under natural conditions. Such a high sulphur isotope fractionation ($\alpha > 1.030$) typically occurs at low specific rates of sulphate reduction (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Canfield, 2001). For the original lake sediments of ML 111, specific sulphate reduction rates of less than 10 nmol/cm^3/day were reported (Packroff et al., 2000). It appears likely that slow bacterial metabolism was caused by a lack of degradable organic carbon in the acid lake environment resulting in large sulphur isotope fractionation during BSR.

If sulphate-reducing bacteria metabolize at optimal temperatures and with abundant organic substrate, increased specific rates of sulphate reduction are

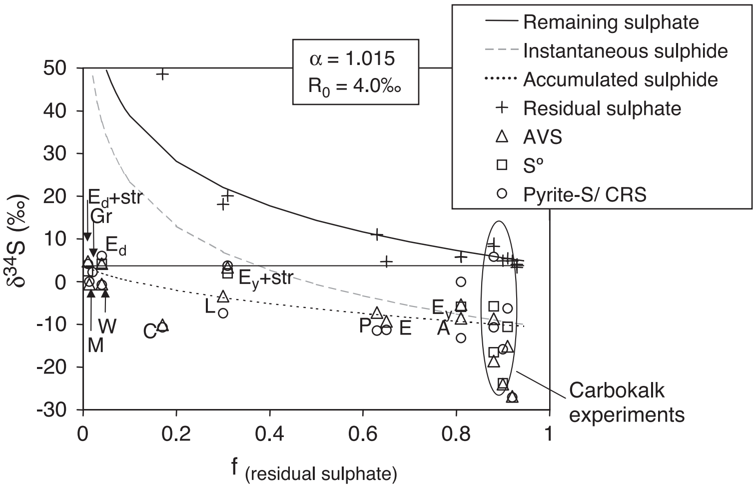

Fig. 8. Rayleigh plot showing the $\delta^{34}S$ values of residual sulphate and those of reduced sedimentary sulphur versus the fraction of residual sulphate (f) at the end of the first, second, and third experimental series. Theoretical $\delta^{34}S$ values for the residual sulphate (solid line), H_2S (dashed line) and for accumulated sedimentary sulphide (dotted line) were calculated using the Rayleigh equation, a sulphur isotope fractionation factor (α) of 1.015, and an initial $\delta^{34}S$ value of dissolved sulphate (R_0) of + 4‰. For abbreviations of the different experimental treatments, see Tables 1–3.

typically encountered. Under such conditions, sulphur isotope fractionation during BSR has been reported to vary between 10‰ and 26‰ (Kaplan and Rittenberg, 1964; Chambers and Trudinger, 1979; Habicht and Canfield, 1997; Canfield, 2001). For our experiments with organic carbon additions, absolute sulphate reduction rates were estimated based on the amount of total sulphate (dissolved + sedimentary sulphate) that was converted (in nmol), the duration of the experiment (in days), and the sediment volume (in cm^3). The calculated values represent minimum sulphate reduction rates (SRR), since several days might have passed before the commencement of bacterial activity. Also, in some cases reaction might have been completed prior to opening the bottles. No specific sulphate reduction rates were determined because cell density data were not available. The highest absolute sulphate reduction rates were observed in experiments with ethanol plus straw additions (third series: 3900 $nmol/cm^3/day$) and after lactate addition (first series: 3600 $nmol/cm^3/day$). Data from most other experiments yielded minimal SRR between 200 and 1000 $nmol/cm^3/day$. This indicates that sulphate reduction rates during the experimental conditions were significantly higher than under natural conditions in the acid lake. Hence, it is likely that the extent of sulphur isotope fractionation during BSR changed after the addition of organic carbon to the lake water in our experiments.

For experiments in which more than 90% of the available sulphate was converted during BSR, the $\delta^{34}S$ values of the accumulated sulphides were similar to that of the initial sulphate (Figs. 5 and 8). In these cases no information about the extent of sulphur isotope fractionation was discernible. For experiments of the first series with sulphate conversions ranging between 10% and 90% (nonsterilized bottles with acetate, ethanol, pyruvate, and lactate addition), the $\delta^{34}S$ values of the residual sulphate and those of the reduced sedimentary sulphur compounds plot near the calculated trend lines shown in Fig. 8 assuming a sulphur isotope fractionation factor $\alpha = 1.015$. Sulphur isotope data for bottles from the third experimental series after ethanol addition suggest a lower sulphur isotope fractionation during BSR ($\alpha \approx 1.010$) than for all other experiments (Fig. 8). This is consistent with results from Kemp and Thode (1968) and Canfield (2001), who also observed lower sulphur isotope fractionation during BSR with ethanol compared to experiments with other organic C sources. In contrast, sulphur isotope data from experiments of the second and third experimental series with Carbokalk addition appear to suggest a somewhat higher sulphur isotope fractionation during BSR than $\alpha = 1.015$. This suggests that not only the specific sulphate reduction rate will cause variations in the extent of sulphur isotope fractionation during bacterial sulphate reduction, but also the type of available organic carbon.

7. Conclusions

The extent of sulphur isotope fractionation during bacterial sulphate reduction may vary significantly depending on specific reduction rates and organic carbon substrate. Monitoring of the progressively increasing $\delta^{34}S$ values in the remaining dissolved sulphate in the bottle water indicated the occurrence of BSR, but cannot be used to precisely quantify the extent of this process. This is particularly true, since redissolution of sedimentary sulphate may also occur, thereby influencing concentrations and isotope ratios of dissolved sulphate. In contrast, the contents and isotope ratios of AVS, CRS, and sedimentary sulphate provide a means for differentiation between initial and newly formed sedimentary sulphur compounds. This enables a more detailed assessment of the extent of BSR in the investigated systems. Using this approach, we were able to show that addition of organic carbon compounds to acid lake water significantly increased the rates of BSR. Addition of pure substances such as lactate, pyruvate, acetate, and ethanol resulted in elevated pH values (4.5 to 6.0) in the bottle water at the end of the experiments. Cheap industrial by-products such as whey, molasses, Pfezi-granules, and Carbokalk were also shown to be effective organic carbon sources enhancing BSR thereby increasing the bottle water pH. It was also shown that the addition of straw to the investigated systems further stimulated BSR, suggesting that sulphate-reducing bacteria used straw as an additional carbon source.

Our experiments provided evidence that additions of selected organic carbon compounds to acid lake water and sediments enhance the reduction of iron and sulfate and result in the formation of significant amounts of sedimentary sulphide minerals including pyrite. These processes generated enough alkalinity to increase the pH of the bottle water to near-neutral values. This technique appears to be in theory a feasible remediation strategy for acid lakes in Lusatia. However, it is essential that re-oxidation of the newly formed sulphide minerals such as pyrite is prevented in order to sustain the gain of alkalinity by reduction of sulphate and ferric iron. Further enclosure and field experiments are necessary to test the practicability of this in situ bioremediation technique under natural conditions.

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