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Technical Article

An In-lake Reactor to Treat an Acidic Lake: the Effect of Substrate Overdosage

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Abstract. An "in-lake" reactor system was developed to treat acidic mining lakes. The reactor uses the microbial processes of sulfate reduction and iron reduction followed by precipitation of iron sulfides to remove acidity, sulfur, and iron from the lake water. The basic reactor design is a straw-filled tube, which was vertically installed in the water column of an enclosure in the lake. Bottom water was pumped through the reactor, and ethanol was continuously fed as substrate for the microbial processes.

Microbial sulfate reduction and iron reduction took place inside the reactor, even under acidic conditions. Overdosage of substrate led to the accumulation of the potentially toxic intermediates H_2S and acetate. Leakage of ethanol led to anoxic conditions in the entire enclosure, followed by accumulation of H_2S in the water column. Sulfides were not precipitated because the pH was never above 3.8. Mixing of the water column in autumn introduced oxygen into the system and led to reoxidation of the H_2S. Future designs of in situ reactors to treat acidic mine drainage should consider that the limiting step is not the microbial formation of alkalinity but the fixation of the alkalinity gain as pyrite.

Key words: acid lakes, acid mine drainage, alkalinity, sulfate reduction

Introduction

In many mining areas, the oxidation of pyrite leads to the formation of acid mine drainage (AMD). The sudden cessation of mining in eastern Germany and elsewhere (Geller et al. 1998) has led to the formation of many extremely acidic lakes (pH 2 to 4). The lakes cannot be used for fisheries or other purposes and are a significant environmental problem. Many of these lakes have remained in the same state for decades, since natural neutralisation did not take place. Therefore, the search for appropriate remediation strategies has received much attention.

AMD typically contains high amounts of iron and the pH is usually buffered by the hydrolysis reaction of ferric iron with water. A successful remediation strategy must therefore not only remove protons but also iron. Remediation strategies include limestone drains (Evangelou 1998), sawdust dams (Tuttle et al. 1969), ex-situ bioreactors (Elliott et al. 1998) and constructed wetlands (Vile and Wieder 1993; Mitch and Wise 1998). A promising approach to restore these lakes is the stimulation of naturally occurring alkalinity producing microbial processes directly in the lake (Wendt-Potthoff and Neu 1998). The combined action of microbial sulfate and iron reduction leads to the consumption of acidity and the formation of pyrite (Anderson and Schiff 1987):

$$15\ CH_2O + 4\ FeOOH + 8\ SO_4^{2-} + 16\ H^+ \Rightarrow 15\ CO_2 + 4\ FeS_2 + 25\ H_2O \quad (1)$$

This could be done in different ways:

a) Enhance the microbial production of alkalinity by an initial change of environmental conditions directly in the lake;

b) Actively operate an in-situ reactor system to enhance microbial alkalinity production;

c) Support microbial alkalinity production in an actively controlled on-land system.

Strategy a) was tested in situ in enclosures in Mining Lake 111 (ML111) in the Lusatian Mining District in Germany (Frömmichen 2001). The addition of organic material combined with lime initiated the microbial processes in the sediment of the lake. Since neutralisation of the water column in these experiments was very slow, we searched for an alternative strategy to speed up remediation. A land-based process (strategy c) is much more controllable than an in-situ process within the lake. A similar land-based process has been investigated by Glombitza et al. (2001). Thus, it was possible to use data from that project as part of the basic design data for the planned in situ reactor (Table 1).

Table 1. Base line data of an off-site bioreactor (Glombitza 1998)

contact material	Scarified lava
contact time	16 – 20 hours
consumption/kg converted sulfate	
Methanol	~ 1 kg
nutrient (ammonium chloride)	0.5 kg
phosphate (KH2PO4)	0.25 kg
Yeast	~ 0.05 kg
pre-running contact time	30 days

The initial investment and operational costs of actively controlled on-land systems are an impediment to the installation of such systems. To be cost effective and efficient, we investigated the feasibility of using sun and wind as the primary energy supply, simple technical equipment, and cheap consumables. Based on these considerations, we designed and tested an active reactor system that was installed in an enclosure in Mining Lake 111. In this paper, we describe the design of the reactor system and report the results of the initial test run.

Material and Methods

Mining Lake 111

The study was carried out in Mining Lake 111 (ML111) in the Lusatian lignite mining district in eastern Germany (51°29' north, 13°38' east). The lake has a surface area of 107,000 m^2 and a mean depth of 4.5 m. The maximum depth is 10.5 m (Büttner et al. 1998). The lake has a pH of 2.6 and contains high amounts of SO_4^{2-} (1200 mg L^{-1}) and Fe (150 mg L^{-1}), but low (<1 mg L^{-1}) concentrations of organic and inorganic carbon (Herzsprung et al. 1998). Since the formation of the lake more than 30 years ago, no natural neutralisation of the water has taken place. The geochemistry of the lake (Friese et al. 1998) and the biogeochemistry of iron and sulfur in the lake (Meier 2001) have already been studied in detail.

In November 1997, enclosures of 2.3 x 1.8 m surface area and 6.5 m depth were installed in the lake. They consisted of black landfill liner (polyethylene), which was fixed to the sediment by a stainless steel frame. In July 1998, 80.5 kg Carbokalk were dispersed in water and evenly distributed above the sediment of one enclosure. Carbokalk is a by-product of sugar production and contained (per dry weight) 3.2% organic carbon, 9% inorganic carbon, 0.43% nitrogen, 6.7% phosphorus, and 0.7% sulfate. In May 1998, five small packets (1kg each) of straw were placed on the sediment surface. Similarly, in August 1998, three large packets of straw (10 kg each) of straw contained in PE nets and secured with 25 kg of iron scrap were placed on the sediment surface. After a year of monitoring, the quality of the water in the enclosure had not improved significantly (Frömmichen 2001). At this point, we decided to install an active reactor to speed up the remediation progress.

Design of the Reactor

The reactor is a fixed-bed reactor that integrates two reactor zones (upper and lower) (Figure 1). Flow in the reactor is gravity-driven, though the possibility of recycling the outlet stream exists. The reactor is open to the atmosphere. Straw was used as a carrier material for microorganisms in both reactor zones. In the upper zone, oxygen was consumed by the microorganisms, which produced an anaerobic outflow. The anaerobic state is a prerequisite for sulfate conversion in the lower reactor zone.

Soluble carbon and nutrient sources, e.g. ethanol, can be added at predetermined rates to the water above the upper reactor zone. Sampling of water as well as addition of chemicals at selected levels within the reactor is possible via sampling ports at different depths. Sampling tubes led from the ports to the surface (Figure 1).

A PE-pipe with an inner diameter of 300 mm was integrated into a small enclosure and used as the reactor vessel. This reactor was divided into 3 parts (2 parts that were 2.5 m long and 1 part that was 2.1 m in length). The water circulation system consisted of a pump (SXT 0500, Pumpentechnik Ing. W. Markert, Dittelheim) placed under water at a depth of 1 m and connected to a filter at a depth of 6 m (Figure 1). The pump transported the acid water from a depth of 6 m with a fixed flow into a flow distributor. This was a small tank above the water surface from which 18 L h^{-1} of water were fed into the upper part of the reactor, resulting in a flow velocity of 25 cm h^{-1}. Surplus water from the flow distributor was fed back to the enclosure to a depth of 4 m by a tube. The system was started on July 20, 1999.

To add crystalline iron and provide a sufficient inoculum of sulfate-reducing bacteria, about 1 kg of lake sediment was placed on top of the straw filling. Most Probable Number counts had shown that the sediment contained about 10^3 sulfate-reducing bacteria cm^{-3} (data not shown). The circulation system was successfully operated until November 8, 1999.

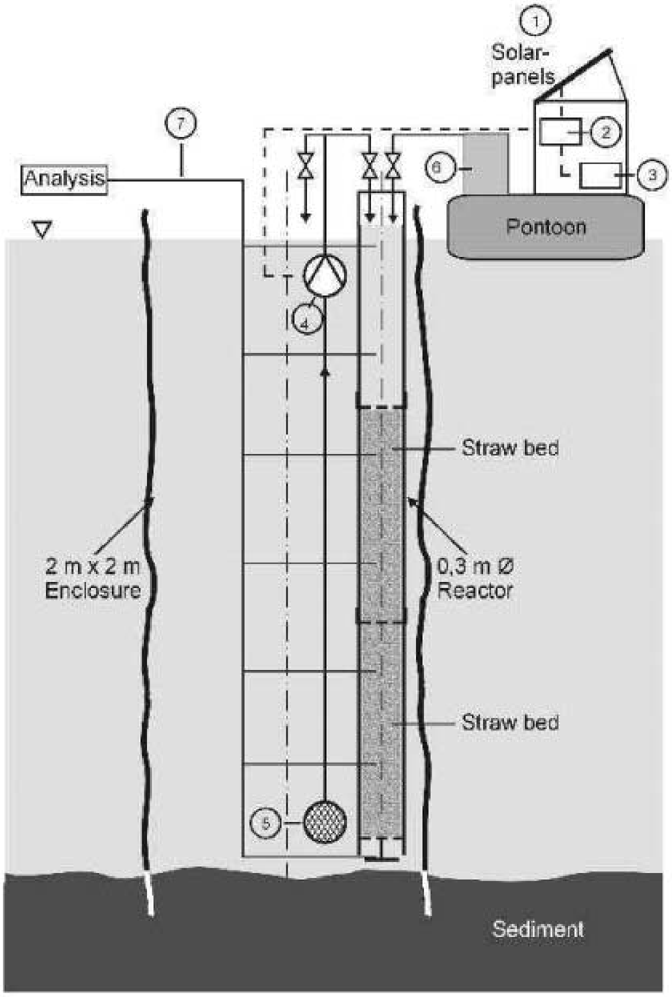


Figure 1. Vertical in-situ reactor. (1) Solar panels: AEG, type MQ 36/530, (2) Controller (3) Solar battery: Hoppeke Energy, type 85505, 70 Ah, (4) solar pump: Pumpentechnik Ing. W. Markert, type SXT 0500, (5) filter, (6) dosage for nutrient, (7) sampling hoses.

Nutrient was fed into the reactor by gravity using a drip cock attached to a 15 L reservoir containing an ethanol (33 % v/v) water mixture. The dosage was reduced several times and stopped in August 1999. The design of the experimental system is summarised in Table 2.

Electricity was provided by two solar panels (AEG-TST MQ 36/530) with a maximum voltage of 17.2 V. The electrical energy was stored in an accumulator (70 Ah, Hoppeke 85505). Special electronic devices controlled charging of the accumulator and the supply to the system components. No troubleshooting was necessary during its operation in 1999.

Table 2. Operational data of the vertical reactor in RL 111 (design data)

Flow of acidic water	up to 20 L h^{-1}
Dosage of ethanol	up to 10 g h^{-1}
Retention time	approx. 20 h
Height of fixed bed (straw)	approx. 5 m

Sampling the reactor

A teflon tube (4 mm inner diameter) was inserted through the sampling hoses to obtain water samples from inside the reactor. In the tip of the tubing, an oxygen microprobe (Presens, Neuburg, Germany) was installed. A 10 m long optical fiber (1 mm diameter) inside of the teflon tube led the signal to the measuring instrument (Microx I, Presens). Water was extracted through the teflon tube by means of a 60 ml syringe connected to the tube by a three-way stopcock. The oxygen probe was calibrated using the surface water of the lake (100% saturation) and water from the anoxic hypolimnion (0% saturation). The oxygen concentration in the surface water was checked with a standard oxygen sensor (WTW, Weinheim). With this set-up, it was possible to measure the oxygen saturation inside of the reactor while taking water samples.

Values of pH and redox potential (E) were immediately measured in the samples using standard electrodes (WTW, Weinheim, Germany). E values were normalised to the standard hydrogen electrode and pH 7: $Eh = E + E_{ref} - 56\ (7 - pH)$ [mV], where E_{ref} = 217 mV was the potential of the Ag/AgCl reference electrode.

Ethanol was determined on site by a colorimetric field test (Dr. Lange, Düsseldorf, Germany). Samples for further analysis were filtered (0.2 µm) and stored on ice.

We sampled only 1 of the 3 sampling ports at each depth interval except on July 27th, when all 3 ports at each depth were sampled to check for homogeneous flow inside of the reactor.

Measurements in the water column

Vertical profiles of physical and chemical parameters in the enclosure were measured using an IDRONAUT multi-parameter probe. Beyond standard CTD characteristics, this probe can also measure oxygen saturation, pH values, and redox potential. The vertical resolution of these profiles was 0.1 m. The redox potential was corrected for the standard hydrogen electrode and pH 7 as described above. H_2S

was measured using a commercially available amperometric H_2S-electrode in combination with a multi-parameter probe (AMD).

Chemical analysis

All samples were filtered through a 0.45 µm cellulose acetate filter. Samples for total dissolved iron analysis were stabilised by adding nitric acid and were stored in 50 ml centrifuge bottles. Samples for sulfate, ferrous iron, and soluble reactive phosphorus (SRP) analyses were filled in brown glass flasks with obliquely cut glass stoppers. Ferrous iron and SRP were measured photometrically by segmented-flow analysis. The procedure for ferrous iron analysis using dialysis as an automatic dilution procedure has been published elsewhere (Herzsprung et al. 1998). SRP was analysed using ammonium molybdate (Boltz and Mellon 1948). Sulfate was analysed by ion chromatography using the single column technique (Herzsprung et al. 2000). The total dissolved iron concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP-OES). Acid soluble reactive iron in sediment samples was determined according to Lovley and Phillips (1987).

Short-chain organic acids were analysed by HPLC (Thermo Separation Products) using an organic acid resin column (300x8 mm, eluent 0.05 M H_2SO_4, flow rate 0.6 ml/min, column temperature 30°C). A diode array detector (Spectra System UV6000LP) was used as an UV detector at 206 nm.

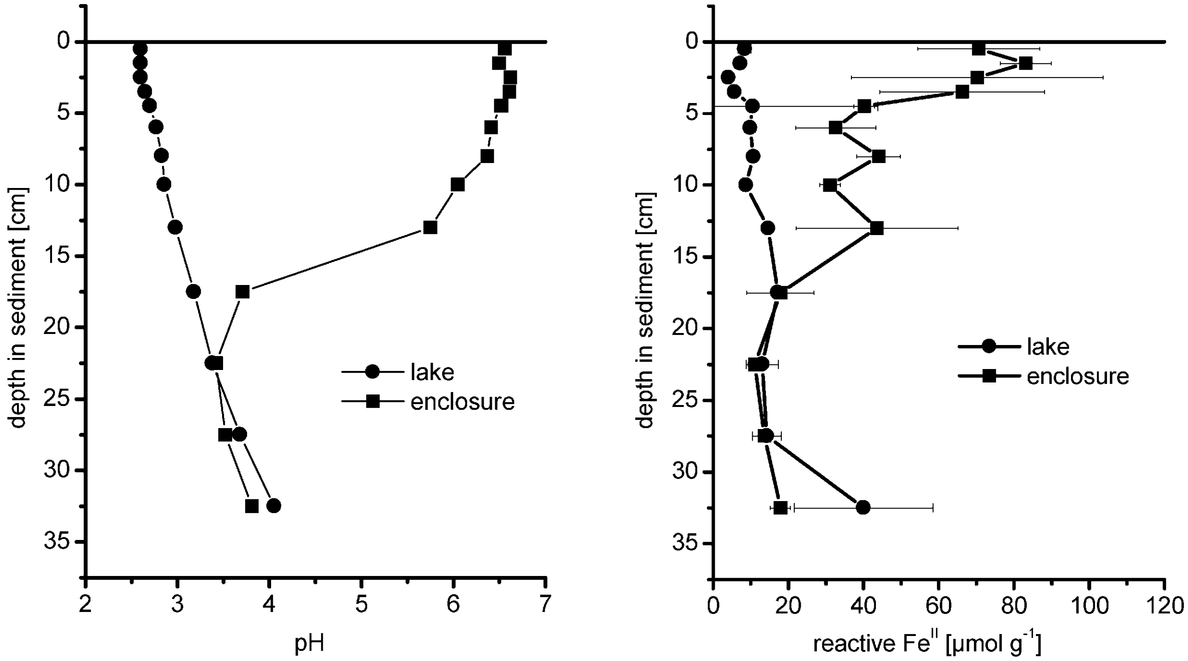
Chemical equilibrium calculations

The program PHREEQC-2.4 (Parkhurst and Appelo 1999) was used for hydrogeochemical modelling. For the calculations, we utilised the thermodynamic database of the PHREEQC program, modified slightly with complementary data on the hydrogeochemistry of acidic mining lakes (Bigham et al. 1996; Baron and Palmer 1996).

Results

Situation before the installation of the reactor

Between the addition of substrates to the sediment of the enclosures in 1997 and the installation of the reactor in 1999, no significant increase in the pH of the water column in the enclosure occurred. Although the profundal water became temporarily anoxic, no significant accumulation of H_2S in the water column was observed (Frömmichen 2001). On the day before the reactor was installed, the sediment of the enclosure was sampled. The pH in the uppermost 10 cm of the sediment was above pH 6 and high amounts of reactive iron were present (Figure 2).



a

b

Figure 2. pH (a) and reactive ferrous iron (b) in the sediment of the enclosure before installation of the reactor

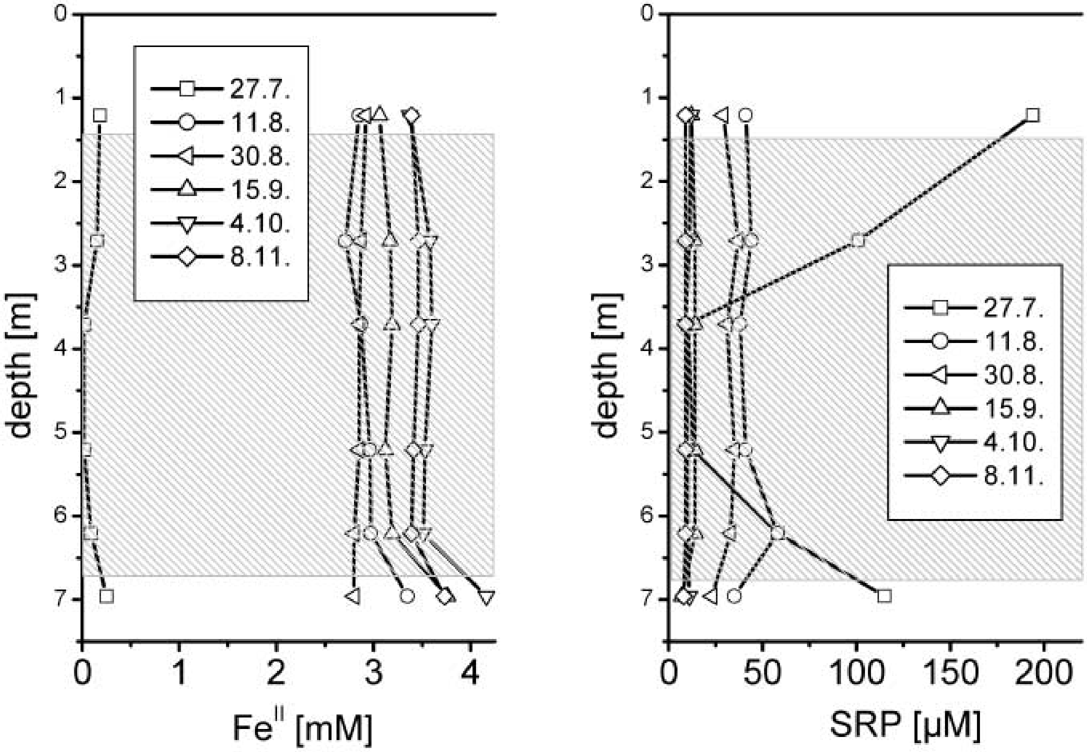


Figure 3. Vertical profiles of Fe^{II} and SRP inside the reactor

^{35}S tracer measurements showed that microbial sulfate reduction took place in the uppermost 3 cm of the sediment with a depth-integrated rate of 0.5 mmol m^{-2} d^{-1}. Measurements in an untreated control enclosure confirmed that the enclosure itself was not affecting the biogeochemistry of the sediment (Frömmichen 2001).

Performance of the reactor

The reactor was installed on July 19th and sampled for the first time the following day. At that time, the pH inside the reactor was above 4, probably due to buffering by organic substances eluted from the straw. At the same time, high concentrations of phosphate (SRP) were observed (Figure 3), presumably also leached from the straw. After a week, the pH had dropped to 2.6, the pH of the lake. Phosphate also returned to low values. Presumably, substances initially eluted from the straw were washed out from the reactor and diluted in the enclosure. The upper part of the reactor became anoxic and a gradient of ethanol inside the reactor indicated consumption of ethanol. Since the concentration at the outflow of the reactor was above 2.8 mM, and ethanol was leaking into the enclosure, we reduced the flow of ethanol from 2.8 to 0.54 ml min^{-1}. The redox potential started to decrease and the reactor gradually became completely anoxic. Ferric iron was reduced to ferrous iron.

No vertical gradient of iron concentration was observed in the reactor. The ethanol gradient vanished and a homogeneous ethanol concentration of about 8 mM was measured. This was approximately the concentration of the inflow, which means that ethanol consumption in the reactor had stopped. On August 11th, the ethanol inflow was further reduced to 0.33 ml min^{-1}. Since the ethanol concentration in the reactor continued to be high and ethanol leaked to the enclosure, we stopped the ethanol addition on August 30th. At that time, water was circulating without any substrate addition and ethanol in the reactor decreased. However, even on October 4th, the ethanol concentration was still above 100 µM. Despite the changes in ethanol dosage, the redox potential inside of the reactor continued to decrease, to values below 100 mV on October 4th (Figure 4b). The pH in the reactor increased until October 4th and was highest in the straw layer. The highest pH value measured was 3.8 on September 20th (Figure 4a). Because we were afraid of freezing, which might have destroyed the pump system, the experiment was terminated on November 8th.

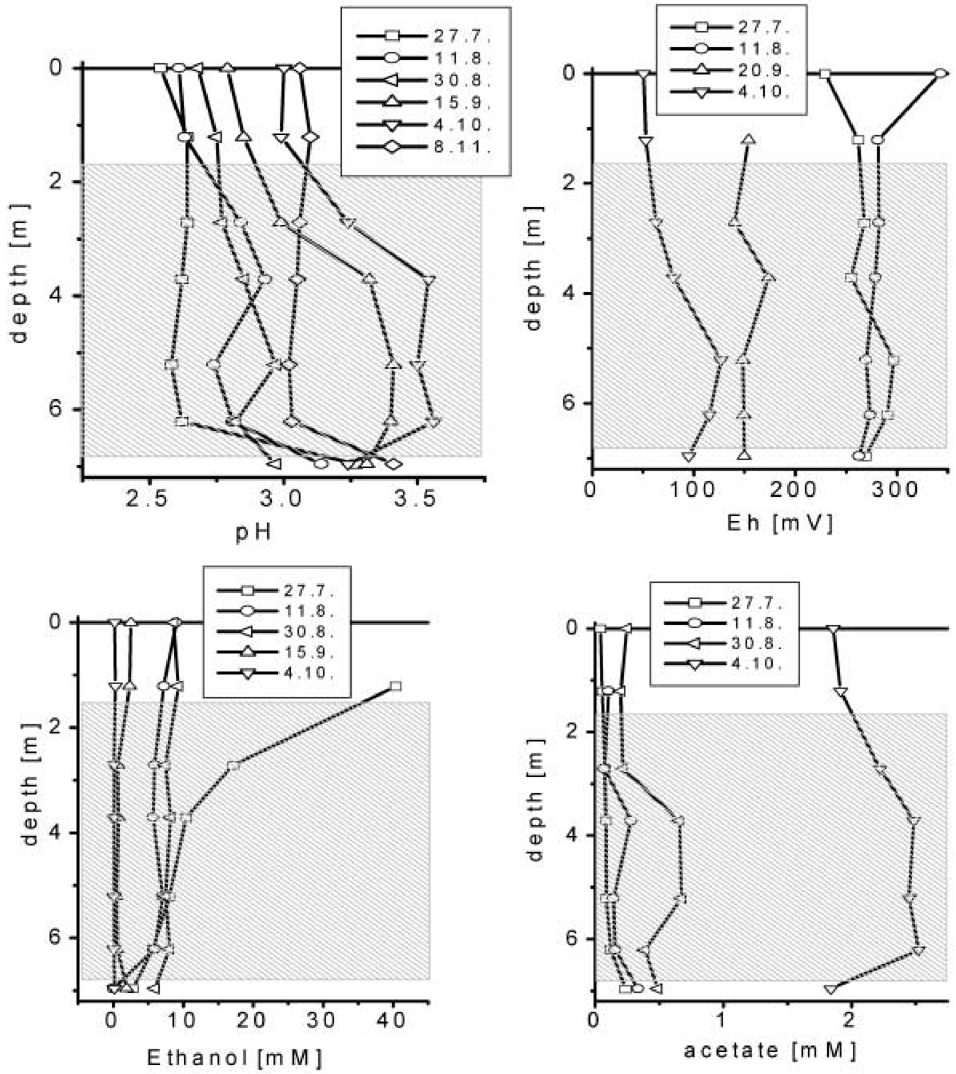


Figure 4. Vertical profiles of pH, Eh, ethanol, and acetate inside the reactor; the shaded areas indicate the straw layer

The only short-chain organic acids found in significant concentrations in the reactor were acetate and traces of butyrate (Figure 5). During ethanol addition, the ethanol concentration in the reactor was above 7 mM and acetate accumulated. The acetate increase continued when the ethanol addition was stopped and the concentration of ethanol decreased. The maximum acetate concentration was about one third of the maximum ethanol concentration.

To find out whether the observed pH changes in the reactor were caused by elevated concentrations of acetate, a laboratory experiment was conducted. Addition of 2.5 mM acetate to water from ML 111 only changed the pH from 2.55 to 2.6. Thus, the direct pH effect of the acetate was negligible. No signs were observed of clogging of the reactor and there were no visible precipitates in the straw layer.

O_2, pH, Eh and H_2S in the Enclosure

Vertical profiles of pH and Eh (Figure 6) and oxygen saturation and H_2S concentration (Figure 7) were measured with multi-parameter probes in the enclosure. The first profile (17th July 1999) shows the conditions in the water column of the enclosure before the reactor was installed. The oxygen was saturated in the upper part and showed a slightly

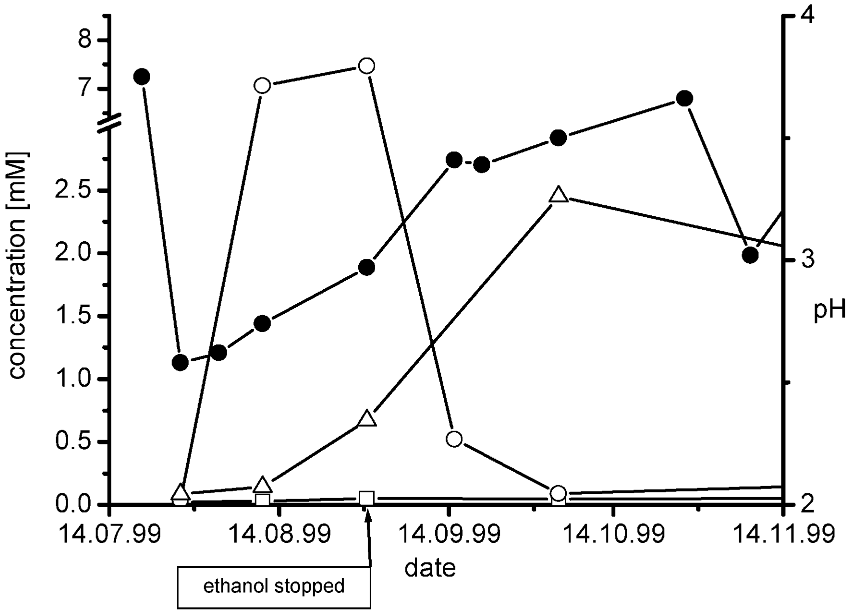


Figure 5. Time course of ethanol (O), acetate (Δ), butyrate (□) and pH (●) in the straw layer of the reactor at a depth of 5.2 m

oversaturated maximum at a depth of 4 m. This deep-oxygen maximum was not created by biological production, but is a relict of the spring circulation, when water temperature was lower, accompanied by higher concentrations of oxygen. The spring concentration was confined by thermal stratification, and diffusive and radiative warming of the water led to this oversaturation. The pH was about 2.6 in the surface water and increased to 2.8 near the bottom. The Eh increased slightly from the surface to the bottom, from 800 to 850 mV.

After 2 weeks of reactor operation (3 August 1999), the oxygen saturation and Eh had dropped and the pH profile had changed markedly. On August 8th, many Chironomid larvae were observed at the surface, probably trying to escape from the anoxic conditions at the bottom. H_2S accumulated in the water and on August 30, H_2S was detected by smell in all samples.

On September 1, oxygen was nearly depleted in the whole water column. The redox potential decreased from 550 mV at the surface to 350 mV near the bottom. The pH profile exhibited no significant changes. The general tendency of these changes continued until October 4th. The oxygen remained depleted, except near the surface where atmospheric oxygen was incorporated. The redox potential ranged between 350 and 400 mV. The pH increased slightly throughout the main water body and sharply increased between 5 and 6 m to a value near pH 3. On October 27, the water column was completely mixed. By the end of October, the whole water column below a depth of 1 m contained about 270 μM H_2S (Figure 7).

Chemical Changes in the Water Column

The water in the enclosure contained 8 μM nitrate at the start of the experiment. At a depth of 6 m, nitrate disappeared during the first week of the experiment. At the surface, no nitrate was detectable from September 1 (data not shown). These data indicate rapid denitrification of all nitrate present in the system.

At the beginning of the experiment, more than 90% of dissolved iron was ferric (Figure 8). Ferric iron in the bottom water was completely reduced to ferrous iron during the first week. The iron in the enclosure was completely reduced at the end of August, and remained in the ferrous state until the end of the experiment. After the first week, a slight mobilisation of sulfate was observed in the bottom water (Figure 9). During the course of the experiment, however, sulfate decreased at an approximately linear rate of 26 μmol L-1 d^{-1}. There was also a decrease in the sulfate concentration in the control enclosure, which demonstrates seasonal fluctuations of sulfate. Compared to the control, the reactor enclosure lost

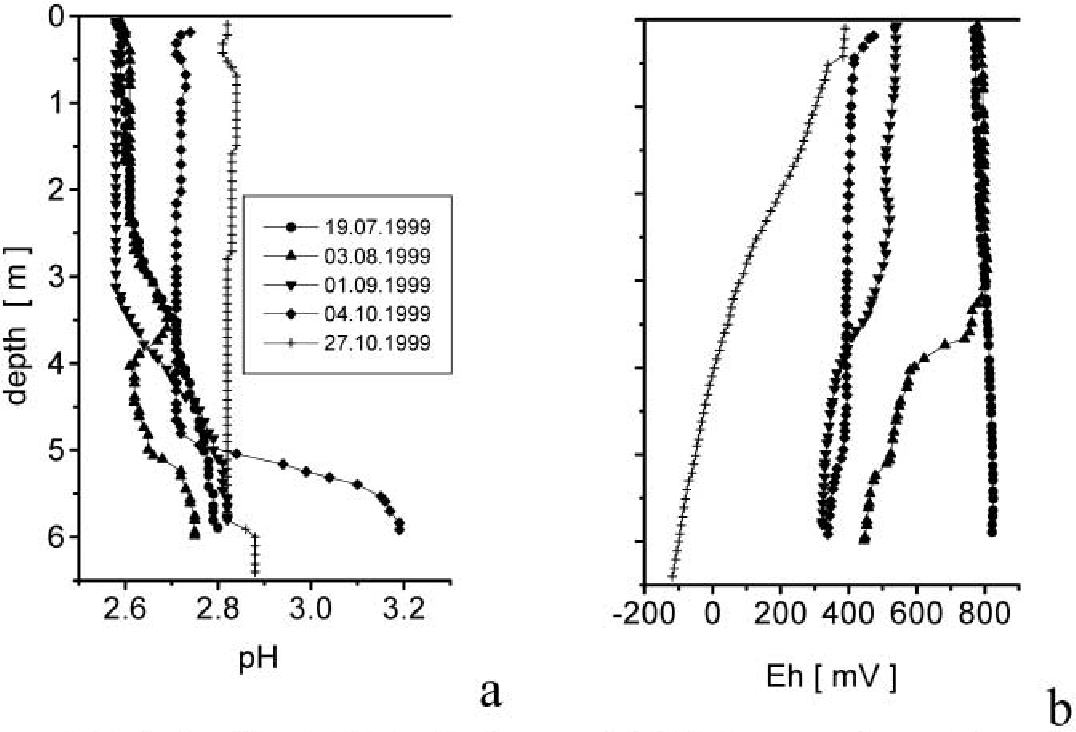


Figure 6. Vertical profiles of pH (a) and redox potential (b) in the water column of the enclosure measured by a multiparameter probe.

0.8 mM of sulfate during the experiment. On October 27, which was the first sampling date after complete mixing of the water column, the water in the whole enclosure was milky white (Figure 10), indicating the formation of elemental sulfur from oxidation of H_2S by oxygen introduced during mixing.

Test for preferential flow

Heterogeneous packing of the reactor might have created preferential flow channels, which can bias sampling from one single sampling port at each depth. To test if the quality of the water was homogeneous at a given depth, on one date we collected samples from all 3 sampling ports at each depth (Table 3). The values at the 1.2 m depth represents the analytical error, since this sampling port was located above the straw layer. The standard deviations for all depths were not different from the standard deviation at 1.2 m.

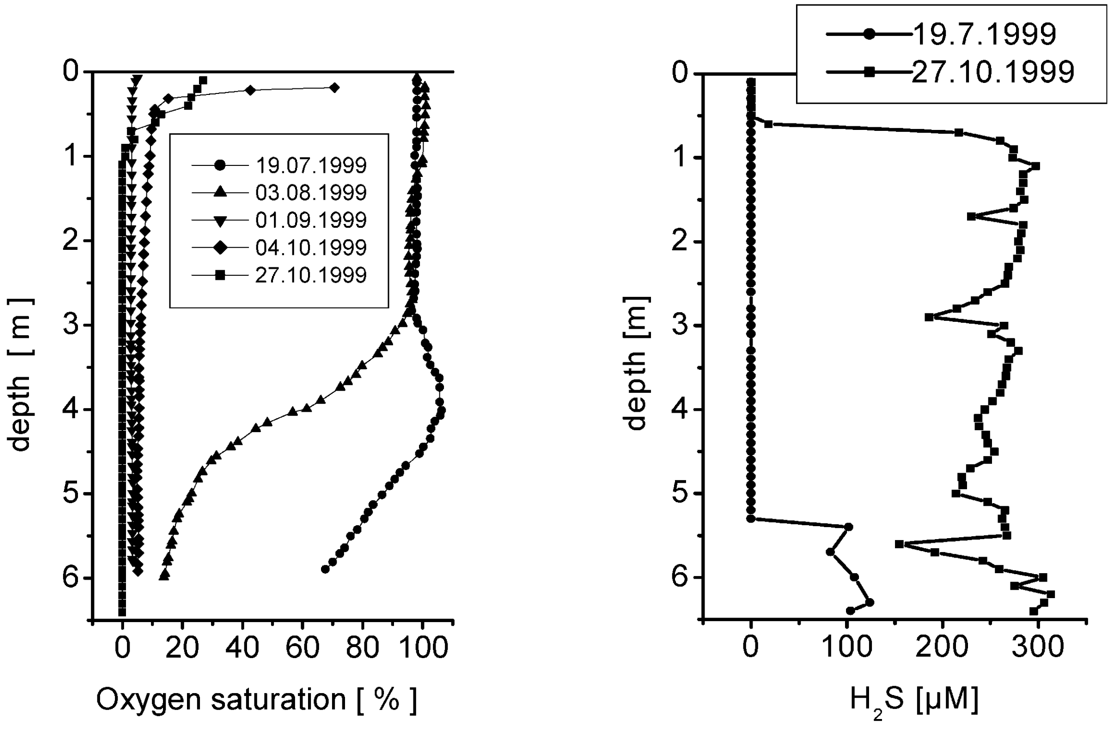
Discussion

Technical aspects of the reactor design and sampling

The test for preferential flow channels showed that samples from 3 different ports at a particular depth were not different. This indicates that the water near the wall of the reactor was uniformly distributed and that no preferential flow channels with different water quality existed. We cannot be sure that this was also true inside the straw package. This is an important issue since if we assume that the water in the inner part of the straw layer was not different from the water near the tube walls, we never had a zone with a pH higher than 3.8 in the system. The accumulation of H_2S shows that sulfate reduction took place. Microbial sulfate reduction is usually inhibited under acidic conditions. So far, no sulfate-reducing bacteria have been isolated that are capable of growth below pH 4 (Hard et al.1997). Even in mixed culture experiments with acid mine water and different carbon sources, sulfate reduction did not occur below a pH of 4.0 (Wakao et al. 1979). Attempts to isolate truly acidophilic sulfate-reducing bacteria have generally failed (Johnson 1998). However, sulfate

Table 3. Test for homogeneity inside of the reactor. Values are means ± standard deviations of samples from three different sampling ports at each depth.

Depth	pH	Eh [mV]	SRP [µM]	Fe^{II} [mM]
1.21	2.64 ± 0.03	262 ± 35	40.7 ± 4.2	2.88 ± 0.03
2.71	2.62 ± 0.02	268 ± 19	44.6 ± 6.7	2.79 ± 0.08
3.71	2.62 ± 0.01	255 ± 10	37.5 ± 2	2.76 ± 0.16
5.21	2.58 ± 0.05	297 ± 55	40.7 ± 3.5	2.83 ± 0.15
6.21	2.62± 0.03	291 ± 25	57.9 ± 5.6	2.88 ± 0.08



a b

Figure 7. Vertical profiles of O_2 (a) and H_2S (b) in the water column of the enclosure

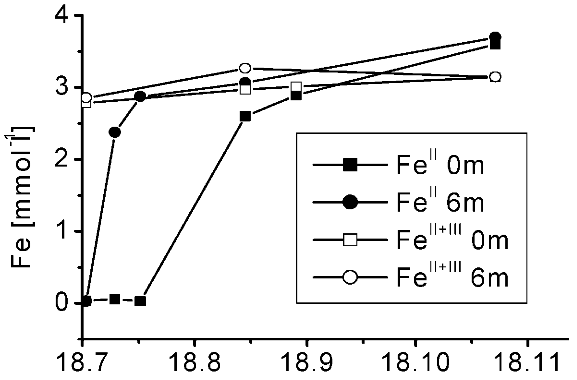


Figure 8. Concentration of dissolved ferrous iron (solid symbols) and total iron (open symbols) in the water of the enclosure at the surface (■, □) and in 6 m depth (●, O).

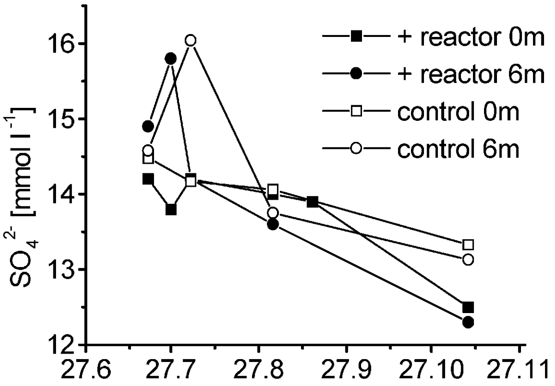


Figure 9. Concentration of sulfate in the water of the enclosure at the surface (■) and in 6 m depth (●). Open symbols are data from an untreated control enclosure.

reduction has been shown to proceed in extremely acidic environments. ^{35}S-tracer measurements in a volcanic lake in Japan indicated sulfate reduction in water with a pH of 1.8 (Satake 1977). In an acidic lake sediment, sulfate reduction was measured at pH 3.8 (Gyure et al. 1990). The authors obtained enrichment cultures at the in situ pH of 3.8 but failed to get pure cultures of acid-tolerant sulfate reducers.

Sulfate reduction was demonstrated in subsurface sands at a porewater pH around 4 (Ulrich et al. 1998). Using a most probable number method, sulfate-reducing bacteria (SRB) were counted in acid mine drainage with a pH between 3 and 4. Acetate and formate were used as substrates. However, the bacteria only grew in neutral media (Fortin et al. 1996). These results suggest that a low pH is

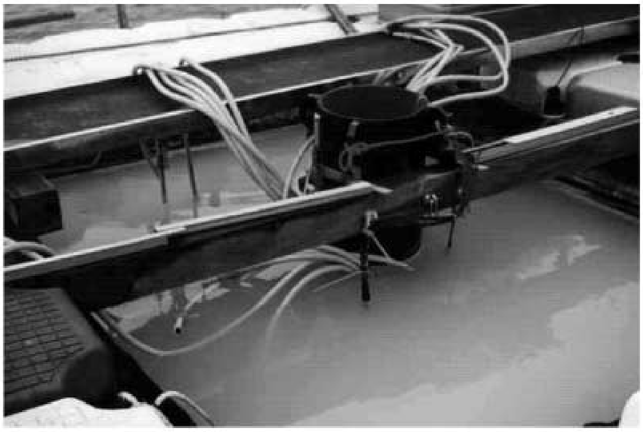


Figure 10. Sulfur "milk" in the enclosure on Oct. 27th

unfavourable to sulfate reducers but does not strictly prevent their activity. It is not unreasonable that sulfate reduction proceeded in our reactor under acidic conditions. Because of the microbial activities inside of the reactor, the redox potential in the reactor tended to be lower than in the free enclosure.

Substrate Overdosage

The accumulation of organic intermediates shows that substrate consumption was slower than substrate addition. The bottleneck in the consumption of ethanol was the oxidation of acetate by sulfate-reducing bacteria. These bacteria were not limited by their organic substrate but by another factor, probably the low pH. During the experiment, two additional stress factors developed: H_2S and acetate. It is known that H_2S inhibits sulfate reduction in pure cultures (Okabe et al. 1992) and bioreactors (Mizuno et al. 1998). The same is true for acetate, which under acidic conditions can cross the cell membrane and act as an uncoupler (Goodwin and Zeikus 1987). Thus, the substrate overdosage in our reactor could have produced a negative feedback mechanism, reducing microbial activity. Consumption of ethanol was inhibited. Only a few species of sulfate reducers are capable of significant growth on acetate (Colleran et al. 1994). In a laboratory study with acid mine water, acetate enhanced methanogenesis, but not sulfate reduction (Ueki et al. 1991). Therefore, acetate has to be regarded as an undesired side product in acid mine water treatment. Laboratory studies are necessary to evaluate the dependence of sulfate reduction on H_2S and acetate concentration. With this knowledge, the reactor can be run with an optimum substrate concentration, combining high reaction velocity with low toxicity.

What happened in the enclosure?

In the beginning of the test, much ethanol passed the reactor and reached the enclosure, which led to oxygen consumption in the enclosure. The surplus ethanol was additionally mixed into the enclosure through the bypass in our water circulating system, which drew water from 0.5 m above ground and released surplus water 2.5 m above ground. It is not clear, however, how this oxygen depletion and reduction could take effect even at the surface, since the whole water column was thermally stratified. The stratification was maintained by heat conduction from the surrounding lake through the walls of the enclosure, which also prevented turbulent diffusion.

The complete anoxia of the enclosure allowed the accumulation of H_2S. Ferrous iron was also present in the water column. The first steps of the whole remediation reaction, microbial reduction of iron and sulfur, took place.

The disappearance of ferric iron (Figure 8) suggests that soluble ferric iron was the main substrate of iron reduction according to equation (2):

$$[CH_2O] + 4\,Fe^{3+} + H_2O \Rightarrow CO_2 + 4\,Fe^{2+} + 4\,H^+ \qquad (2)$$

If crystalline iron is used, equation (3) is valid:

$$[CH_2O] + 4\,FeOOH + 8\,H^+ \Rightarrow CO_2 + 4\,Fe^{2+} + 7\,H_2O \quad (3)$$

The source of iron is important since it determines whether protons are produced (2) or consumed (3). A net alkalinity gain is only obtained if solid phase iron is used. From our data, we can not say to what extent the added crystalline iron was used. Proton production from the reduction of soluble ferric iron offers an explanation for the low pH in the reactor, although sulfate reduction took place. A net alkalinity gain in the reactor is only possible after all soluble ferric iron is reduced. This has to be taken into account during the start up phase of a reactor.

Microbial sulfate reduction consumes two protons per sulfate:

$$2\,[CH_2O] + SO_4^{2-} + 2H^+ \Rightarrow 2\,CO_2 + H_2S + 7\,H_2O \qquad (4)$$

Fe^{2+} and H_2S are only stable under anoxic conditions. To prevent diffusion and reoxidation in the oxic zone and to preserve an alkalinity gain, monosulfide or pyrite has to be formed:

$$Fe^{2+} + H_2S \Rightarrow FeS + 2\,H^+ \qquad (5)$$

$$FeS + S^0 \Rightarrow FeS_2 \qquad (6)$$

S^0 is formed during oxidation of H_2S:

$$2\ FeOOH + 3\ H_2S \Rightarrow 2\ FeS + S^0 + 4\ H_2O \qquad (7)$$

This gives equation (1) as the overall reaction of pyrite formation. However, the desired precipitation of iron sulfide could not take place since the pH was too low. Precipitation of FeS in solutions with the chemical composition like the reactor water needs a pH of at least 5 to take place. There you can find a slight oversaturation of FeS with low positive saturation indices (SI; $0<SI<1$). In the anoxic waters of our reactor system, we calculated an SI lower than –2 for FeS, a precursor for the FeS_2.

At the end of the stratified period, oxygen was introduced into the water column, and some of the H_2S was probably immediately oxidised to elemental sulfur.

$$2\ H_2S + O_2 \Rightarrow 2\ S^0 + 2\ H_2O \qquad (8)$$

Since at that time, H_2S was still present and oxygen was absent below a depth of 1 m, it appears that the reoxidation was limited by oxygen. During the following winter, H_2S and ferrous iron in the enclosure were subsequently reoxidised (data not shown).

Assuming sulfate reduction took place in pH-neutral microniches inside of the straw, we would expect immediate precipitation of sulfides at these microsites. The fact that H_2S escaped from the point of its formation demonstrates that either formation was faster than precipitation or that the conditions were not suitable for precipitation. This would indicate that sulfate reduction took place under highly acidic conditions. Future studies have to look for neutral microsites and the formation of sulfides inside the reactor.

Another possible source of H_2S was the sediment. We think, however, that the sediment was not a significant source of H_2S for several reasons. The accumulation of about 7 mol of H_2S in the water column would require a sulfate reduction rate of 17.5 mmol H_2S m^{-2} d^{-1}. This is not only much higher than the measured sulfate reduction rate of 0.5 mmol H_2S m^{-2} d^{-1} but also a high rate than in natural aquatic habitats (Sass 1997). We never observed significant H_2S accumulation in enclosures without a reactor, even under anoxic and reduced conditions in the water column (Frömmichen 2001). The conditions in the sediment, neutral pH, and high amounts of reactive ferric iron favoured the precipitation of iron sulfides rather than the accumulation of free H_2S. In a follow up experiment in 2000, we measured H_2S concentrations as high as 0.7 mM inside of the reactor while no H_2S was detected outside the reactor (unpublished results).

The occurrence of sulfate reduction was not only shown by the formation of H_2S but also by consumption of sulfate. Compared to the control, about 22 mol of sulfate disappeared from the water during the experiment, but only 7 mol of H_2S were formed. The missing 15 mol of sulfur were either reoxidised to elemental sulfur or precipitated as iron-sulfides either in the straw or at the sediment surface. Since the reactor is still operating and cannot be opened, the fate of the missing sulfur has not yet been determined. Regardless of its terminal fate, and assuming no other sink for sulfate than sulfate reduction was present and no oxidation occurred, the reduction of 22 mol of sulfate would remove 44 mol of acidity from the system (equation 3). This is a considerable percentage of the total acidity of the 395 mol proton equivalents (Frömmichen 2001) initially present in the water of the enclosure. If all of the reduced sulfur could be precipitated as pyrite, the reactor would be able to neutralise the enclosure in a reasonable time.

Sulfate-reducing bacteria can consume ethanol according to the equation:

$$2\ C_2H_6O + 3\ SO_4^{2-} + 6\ H^+ \Rightarrow 4\ CO_2 + 3\ H_2S + 6\ H_2O \quad (9)$$

During the experiment, a total of 154 mol ethanol was added to the reactor. Assuming exclusive use by sulfate reducers, this ethanol can reduce 231 mol of sulfate. However, most of the ethanol added was obviously not used by sulfate-reducing bacteria but by other processes. These processes probably included complete oxidation with oxygen by aerobic heterotrophs, nitrate reduction, fermentation, and microbial iron reduction, the last of which also contributes to alkalinity generation. Aerobic respiration was not important since the reactor was anoxic.

Conclusions

The reactor worked well from a technical point of view. The design of the water circulating system has to be improved to reduce mixing of the water in the enclosure.

The microbial processes of sulfate reduction and iron reduction took place even under acidic conditions in the bulk water phase.

Accumulation of toxic intermediates due to substrate overdosage could have inhibited microbial processes. The substrate concentrations have to be lowered and monitored during the reactor run in order to obtain optimum turnover rates.

During the start up phase of the reactor, no net alkalinity gain was obtained

The pH never reached a sufficiently high level to precipitate iron sulfides in the water column. The alkalinity gain could thus not be conserved after mixing of the lake in autumn. Future reactor designs have to create zones or periods with a pH above pH 6 and a sufficiently high acid capacity to enable the precipitation of sulfides.

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