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Microcosm Studies for Neutralization of Hypolimnic Acid Mine Pit Lake Water (pH 2.6)

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Ten microcosms of 0.088 m^3 water volume (0.3 m i.d. and 1.20 m height) were designed for neutralization studies representing hypolimnic ecosystem models for acid mine pit lakes. Sediment and water were collected from an acid lignite mine pit lake (Brandenburg, Germany) and filled into the microcosms. To determine the efficacy of controlled in situ organic carbon amendments as a possible neutralization method, sediment and water were treated with ethanol and Carbokalk with and without wheat straw. The water chemistry was monitored for 1 yr. At start-up and end of the experiments, the sediment was characterized. Iron and sulfate were removed with varying intensity from the water phase as a result of microbial iron and sulfate reduction together with a subsequent precipitation of unsoluble sulfide minerals to the sediment. The pH rose, and alkalinity generation and bacterial growth were observed. Neutralization rates were calculated using equivalents of accumulated total reduced inorganic sulfur together with the nonsulfidic reactive ferrous iron in the sediment. In the treated microcosms, the neutralization rates were between 6 and 15 equiv m^{-2} a^{-1}. Carbokalk was most effective in stimulating growth of sulfate-reducing bacteria and probably also served as inoculum. With Carbokalk together with wheat straw, the pH increased from 2.6 to around 6.5 within the whole microcosm. The critical revision of the results indicates that the application of Carbokalk (approximately 3.9 kg m^{-2}) together with the application of wheat straw (approximately 9.3 kg m^{-2}) is most suitable for further experiments in outdoor enclosures (mesocosms). For that case, the prediction of the water quality for a lake water column after multiple lake turnover events is presented based on batch reaction simulation using the geochemical model PHREEQC.

Introduction

In the Lusatian Mining District (Brandenburg, Germany) more than 100 pit lakes were formed in the last 50 yr (*1*). They cover an area of 208 km^2 and contain approximately 4.5 billion m^3 of water. Approximately half of these lakes have pH values in the range of 2.5−3 as a result of the weathering of pyrite in the lignite and in the accompanying geological formations (*1, 2*). This complex process is described elsewhere (e.g., refs *3* and *4*). These pit lakes generally have a high mineral acidity (Fe, Al, sulfate) and elevated heavy metal concentrations (*5*). This situation is comparable to other pit lakes (*6, 7*).

The tight restriction on use in a future recreational lake district is a good argument for the re-development of acidic pit lakes, and consequently, the neutralization is an important task. Worldwide efforts were made to develop in-situ neutralization techniques for acidic pit lake water (*8*−*11*) and water from acidic aquifers (*12*). Our approach is the manipulation of an ecosystem in such a way that the addition of organic carbon controls in-lake the alkalinity generation via the microbial iron and sulfate reduction (*13*). The general processes were presented earlier (*14*−*17*) and are outlined in Figure 1.

To evaluate this approach, different scales of model experiments were used as proposed in the scientific community (e.g., refs *18* and *19*). We started with smaller closed laboratory experiments in darkness (microcosms) to choose appropriate carbon sources for the anaerobic bacterial community near the water−sediment interface (*13*). In the present paper, the results of larger open microcosms exposed to higher acidity load per square meter and additionally to oxygen input from the atmosphere are presented. The interpretation of chemical changes in water and sediment together with the turnover of neutralization equivalents is combined with a critical view on cause-and-effect relationships between organic carbon load and response within the microbial community. These studies are essential for further mesocosm studies in outdoor enclosures in the acid mine pit lake AML 111 (Brandenburg, Germany).

Materials and Methods

Habitat. Typical yellow brown surface sediment (7.0 m water depth) and lake water from AML 111 of the Koyne/Plessa lignite field in the Lusatian Mining District (Germany) were used to construct the microcosms. Flooding of AML 111 was completed in 1969. The lake consists of three subbasins (*20*). Morphologically, it is a shallow lake with an average water depth of 4.5 m and a comparatively small hypolimnion. The maximum depth is 10.2 m. The photoautotrophic biomass is very limited in the epilimnion. Only in a few individual cases does the biovolume exceed 1 mm^3 L^{-1}. The phytoplankton population is dominated by chrysophyceae of the genus *Ochromonas*. Chlorophyceae of the genus *Chlamydomonas* are also common. The zooplankton consists mainly of heliozoa, ciliates, and rotifers (*21*). The pH of AML 111 lake water is approximately 2.6, while the electric conductivity averages 2.5 mS cm^{-1} ($\kappa_{25°C,AML\ 111}$, corrected to ref *22*). Both total inorganic (TIC) and total organic carbon (TOC) contents are less than 0.1 mmol L^{-1}. The chemical composition of the lake water and of the sediment varied little during the investigation period between 1998 and 1999 (*20, 23, 24*).

Carbon Sources. Ethanol (Merck, Germany) was used as pure substrate. In some treatments, it was substituted by an economical sugar industry byproduct named Carbokalk (Zuckerverband Magdeburg e.V., Germany). This is the solid precipitate of nonsugars after lime clarification of the extracted sugar beet juice. The lime content of Carbokalk varies around 50% (w/w) depending on dry weight. Cut wheat straw from ecological, pesticide-free cultivation served as a

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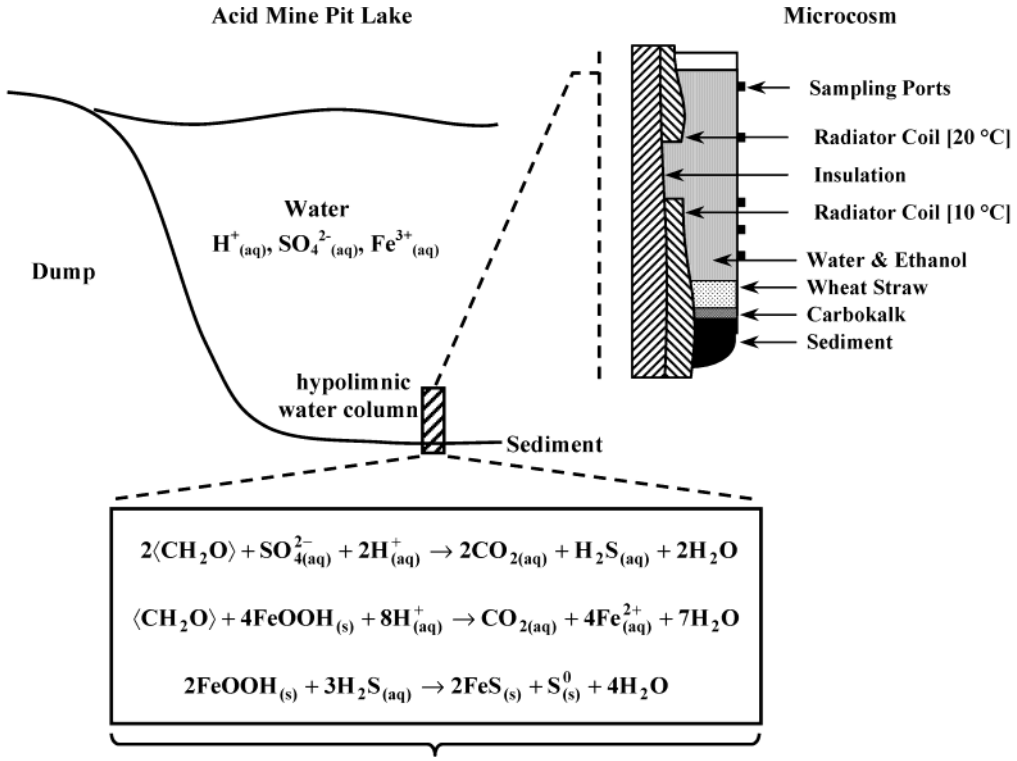

FIGURE 1. Schematic description of the microcosm design. Equations from refs *15*–*17*.

TABLE 1. Load of Organic Carbon Sources Used in Microcosms Expressed as Molar Units[a]

	organic carbon sources			
microcosms	wheat straw	substrate	$TOC_{substrate}$[b] (mmol L^{-1})	$TOC_{substrate}$ (mol m^{-2})
R	no			
S	yes			
E	no	ethanol	24	7.48
SE	yes	ethanol	24	7.48
Se	yes	ethanol	2.4	0.748
C1, C2, C3	no	Carbokalk	24	7.48
SC	yes	Carbokalk	24	7.48
Sc	yes	Carbokalk	2.4	0.748

[a] $TOC_{wheat\ straw}$ = 345 mol m^{-2}. [b] mmol L^{-1} for one-fourth of the water volume.

complex carbon and nutrient source. Information about the composition and function of ethanol, Carbokalk, and wheat straw in lower-scale microcosms is compiled in detail in ref *13*. The substrate dosage for the 0.088 m^3 microcosms was calculated using the balance of the equivalent concentrations. Complete oxidation of ethanol and the reduction of iron and sulfate were assumed. The organic carbon equivalents were calculated for ethanol and Carbokalk for a volume of 0.022 m^3 bottom water. For two of the microcosms, these dosages of organic carbon were lowered by a factor of 10. The addition of wheat straw occurred according to refs *8* and *13*. The quantity of wheat straw was recalculated for the treated water volume. The substrate combinations and dosages for the microcosms can be taken from Table 1.

Microcosm Design. The 0.088 m^3 scale microcosms consisted of open Duran glass columns (Schott, Germany), 1.50 m in height. Untreated (control) and treated microcosms each represented a lake section of an area of 0.071 m^2. The columns were filled with 14.8 ± 0.3 kg of homogenized fresh lake sediment (0.20 m sediment layer in each column) and 0.088 ± 0.0005 m^3 of lake water (1.25 m water layer). The columns were equipped with sampling ports set at 0.10, 0.30, 0.60, 0.75, and 0.90 m from the top. For final sediment sampling, the columns were divided 0.20 m above the column bottom via a detachable flanged system. Two separate thermostats were installed (Julabo Ltd., Germany) for the purpose of creating a synthetic temperature stratification similar to that of a lake. The temperature at the bottom 0.65 m of the column was set at 10.0 ± 0.2 °C. The temperature between 1.10 and 1.45 m was set at 20.0 ± 0.2 °C. After treatment with Carbokalk or ethanol, wheat straw was placed on the sediment surface of five microcosms (S, SE, Se, SC, Sc; see Table 1). Microscosms with Carbokalk alone were set up in triplicate to assess variability within a given treatment (C1, C2, and C3). The microcosms were incubated 1 yr in darkness to simulate lake bottom conditions (profundal). The experimental setup is shown in Figure 1.

Water Sampling and Analysis. Over a time period of 1 yr, water samples of the microcosms were collected with a syringe from different sampling ports on a monthly base. Oxidation–reduction potential (ORP), pH, acidity ($K_{B,8.2}$), dissolved sulfate, hydrogen sulfide, dissolved ferrous iron (Fe(II)), and total concentrations of dissolved iron (T-Fe) and dissolved aluminum (Al) and of TC/TOC and DC/DOC were determined according to ref *24*.

In situ *f* measurements were performed with an industrial glass electrode (Consort REFEX 2001, Belgium). The ORP was measured with a platinum electrode (Consort REFEX 2002, Belgium) and was calculated to the standard potential at pH 7 ($E_{H,pH7}$) assuming the theoretical slope of 59.1 mV/pH (eq 1; *25*):

$$E_{H,pH7}\,[mV] = (ORP + E_{ref}) - (7 - pH) \times 59.1 \quad (1)$$

$K_{B,8.2}$ was determined via volumetric titration according to procedure DIN 38409/H7 of the German Institute of Standardization using a 0.1 mol L^{-1} sodium hydroxide

solution. The $K_{B,8.2}$ is defined as the base capacity (or acidity) to the reference pH 8.2.

Sulfate was determined in filtrated water samples by ion chromatography (ICA-5000 System, GAT, Germany) against external calibration with diluted acidic sulfate standards (EN ISO 10304-1). The chromatographic system worked with a single-column ion exchange technique. Before analysis, the pH of the filtrate was adjusted between 2.4 and 2.6 with hydrochloric acid. After this, the hydrogen sulfide was removed by degassing the filtrate with argon.

H_2S was determined with an amperometric microelectrode (AMT, Germany). For the external calibration of the microelectrode, an on-line flow system combined with an electrochemical hydrogen sulfide generator (AMT, Germany) was used. Hydrogen sulfide and pH were used to calculate the sum of all sulfide species (ΣS^{2-}).

Fe(II) was determined photometrically with *o*-phenanthroline at a wavelength of 512 nm against an external calibration with diluted Fe(II) standards by a continuous-flow system (Skalar Analytical, San-plus, The Netherlands) that operated with a multichannel photometer and 5 cm flow-through cell. Before analysis, the dissolved Fe(II) was stabilized in the filtrate with sulfuric acid (25% w/w) between pH 2.4 and pH 2.6.

The total concentrations of iron (T-Fe) and aluminum (Al) in filtrated water samples were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 3000, Perkin-Elmer, Germany). The filtrates were stabilized in a diluted nitric acid solution (0.1% w/w). Analyses followed after external calibration with diluted multi-element standards.

The TC/TOC and DC/DOC fractions were determined in separate aliquots of the unfiltrated and filtrated water samples, respectively (C-Analyzer Dima-TOC-100; Dimatec, Germany). The total carbon and organic carbon were determined subsequently according to procedure DIN 38409 part 3 of the German Institute of Standardization. The inorganic carbon was degassed after addition of sulfuric acid (25% w/w).

To obtain total bacterial counts, water samples were fixed with 2% glutaraldehyde, filtered onto 0.2 μm polycarbonate filters, and stained with acridine orange according to standard procedures (*26*). Bacterial abundance and biovolumes were determined by epifluorescence microscopy using 1000× magnification and a Porton grid as outlined in ref *27*.

Most probable number (MPN) counts were performed to obtain viable counts for different functional groups of bacteria involved in iron and sulfur cycling. Media for iron-oxidizing bacteria (FeOB), sulfur-oxidizing bacteria (SOB), and sulfate-reducing bacteria (SRB) were as described in ref *28*. The medium for iron-reducing bacteria (FeRB) was adapted from a medium for *Geobacter metallireducens* (DSMZ no. 579, see also ref *23*). The medium was buffered with 10 mmol L^{-1} MES (2-morpholinoethanesulfonic acid) and poised at pH 6.

The medium for fermentative bacteria was prepared in culture tubes with Durham tubes and butyl rubber septa. The composition was as described in ref *29* but without bicarbonate buffer and with N_2 atmosphere. Resazurin was used as redox indicator, and the pH was 7.0. Preparation, incubation, and evaluation of aerobic and anaerobic MPN cultures were performed according to ref *30*. MPN and their confidence limits were calculated with the program in ref *31*.

Sediment Sampling and Analysis. Undisturbed sediment cores with a diameter of 0.09 m were collected from the microcosms as compared to gravity corer techniques. The anoxic sediment cores were cut in 0.025 m layers to 0.10 m depth in an anaerobic chamber. pH and ORP were measured with an industrial glass and platinum electrode, respectively (Consort REFEX 2001/2002, Belgium). Prior to extraction and analysis of total reduced inorganic sulfur [TRIS; sum of acid-volatile sulfide (AVS), chromium-reducible sulfur (CRS), elemental sulfur (ES)], reactive iron (T-Fe_R), and reactive ferrous iron ($Fe(II)_R$, an aliquot of fresh wet sediments samples were stored in liquid nitrogen. For all other parameters, samples were prepared and analyzed immediately.

AVS, CRS, and ES were extracted sequentially in a modified three-step method according to refs *32*−*35*. The extracted hydrogen sulfide was trapped in a sulfur antioxidant buffer and was measured with differential pulse polarography (*36*).

For the extraction of T-Fe_R, which is classically defined as the iron fraction that reacts rapidly with hydrogen sulfide to form FeS_2 (*15*, *32*, *37*), different methods were described in the literature (*38*). We used the extraction scheme of Lovley and Phillips (*39*) yielding T-Fe_R and $Fe(II)_R$. We approximate the amount of non-sulfidic reactive ferrous iron (NS-$Fe(II)_R$) from the difference of $Fe(II)_R$ and the ferrous iron recalculated fom the AVS using a molar ratio of 1:1. NS-$Fe(II)_R$ produced as a result of microbial Fe(III) reduction may also be bound to the surfaces of iron(III) oxides or precipitate as carbonates and phosphates such as siderite ($FeCO_3$) or vivianite ($Fe_3(PO_4)_2$).

Total sulfur (T-S) was analyzed after high-temperature combustion in an oxygen gas stream. using the element analyzer vario-EL (Elementar, Germany).

Total iron (T-Fe) was determined by ICP-OES in aqueous solutions from aqua regia high-pressure microwave digestion (Mars5; CEM, Germany).

Determination of dry residues (T_R) as well as sediment densities (Δ) for individual sediment layers was performed by drying a defined balanced fresh wet sediment volume at 105 °C.

Total viable microbial biomass in sediment samples and Carbokalk was estimated using the phospholipid phosphate content determination. As described in ref *30*, the protocol of ref *40* was applied with the modifications introduced by ref *41*. Tests with AML 111 sediment yielded a relative standard deviation better than 10% for this method.

Viable bacterial counts in the sediments were determined by MPN assays as described above. All analytical results in the sediment except MPN values refer to dry weights. Statistical tests were performed using SigmaStat V2.03 (SPSS Science, Germany).

Calculation of Neutralization Rates. Neutralization rates (NR) were calculated via accumulation of TRIS and NS-$Fe(II)_R$ in the sediment (*36*). With this method, temporal variations of microbial activities were included. For each mole of TRIS, 2 mol of protons was consumed or 2 neutralization equivalents was produced (eq 2; *15*, *16*). According to eq 2, the same relation was assumed per mole of NS-$Fe(II)_R$ (eq 3; *16*, *17*). Here, the aqueous iron has to be fixed predominantly in iron phosphates or carbonates to generate alkalinity.

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

$$\langle CH_2O\rangle + 4FeOOH + 8H^+ \rightarrow CO_2 + 4Fe^{2+} + 7H_2O \quad (3)$$

By defining a reference area and a time relation for neutralization equivalents as compared to the control microcosm, neutralization rates per square meter and year can be determined (eq 4):

$$NR = \frac{V \times 20}{t \times A} \sum_{n}^{i=1} ([TRIS]_i + [NS\text{-}Fe(II)_R]_i)\Delta_i T_{R_i} \quad (4)$$

where NR is the neutralization rate (equiv m^{-2} a^{-1}), [TRIS]

TABLE 2. Initial Values of Aqueous and Sediment-Phase Key Parameters of the Control Microcosm (R)

water (mmol L^{-1})		sediment (μmol g^{-1})	
T-Fe	2.84	T-Fe	1620
		$T\text{-Fe}_R$	290
Fe(II)	0.018	$Fe(II)_R$	110
SO_4^{2-}	15.7	SO_4^{2-}	204
H_2S	<0.01	AVS	0.01
Al	1.39	CRS	8.10
		ES	5.29
		T-S	240
$K_{B8.2}$	16.5		
pH	2.6	pH	3.3
$E_{H,pH7}$ (mV)	543	$E_{H,pH7}$ (mV)	269

is the accumulation of total reduced inorganic sulfur (equiv g^{-1}), [NS-Fe(II)$_R$] is the accumulation of nonsulfidic reactive ferrous iron (equiv g^{-1}), Δ is the density of fresh sediment layer (kg L^{-1}), T_R is the dry residue of fresh sediment layer (%), V is the volume of fresh sediment layer (L), A is the area (m^2), t is the time of accumulation (a), i is the sediment layer, and n is the number of sediment layers.

Geochemical Modeling. The geochemical model PHREEQC (*42*) was used to simulate the neutralization of a simple lake water column induced by bacterial iron and sulfate reduction at standard temperature. In this conceptual simulation, two intervals per year representing summer and winter stratification are interupted by two mixing events that represent spring and fall turnover. The total volume of the lake water column is 1.0 dm^3. This volume is separated into the hypolimnic (0.165 dm^3) and epilimnic parts (0.845 dm^3), which represent the conditions in the pit lake AML 111. The hypolimnic water composition results from iron and sulfate reduction during stratification. This was simulated without a fixed O_2 and CO_2 fugacity. At the same time, the epilimnic part is under equilibrium conditions with O_2 ($10^{-0.7}$ atm) and CO_2 ($10^{-3.5}$ atm). After a mixing event during turnover, a new water composition resulted that was used as input to the next stratification event. The sequence of calculations is repeated until the water column is getting circum-neutral conditions.

As starting conditions, we used the typical water composition of AML 111 partly represented in the initial values of the control microcosm listed in Table 2. The simulation based on the neutralization rate of the microcosm SC (NR = 14.9 equiv m^{-2} a^{-1}). The irreversible reaction capabilities of PHREEQC were used to implement this neutralization rate in terms of chemical species involved in bacterial iron and sulfate reduction. To predict the water quality after multiple lake turnover events assuming equilibrium conditions, the PHREEQC codes "solution", "equilibrium phases", "reaction", and "mix" were combined. We used the PHREEQC database in which minor modifications have been made to make the data consistent with the tabulations in refs *43* and *44*. The database was modified according to additional solubility data for schwertmannite and jarosite (*45*, *46*) and Ni, Cu, and Co based upon values from the MINTEQ, WATEQ4F, and LLNL databases.

Results and Discussion

Changes in Water Chemistry. With a temperature gradient of 20−10 °C along the microcosm depth, boundary conditions were adjusted, which were strongly simplified as compared to outdoor conditions. The constant temperature gradient almost excluded convection and advection within the microcosms. Mainly diffusive mass transport was expected between the different compartments. Before start-up, the initial chemical conditions were determined in the water column and the sediment (Table 2). The starting conditions in the water column were similar to the conditions observed in AML 111 after spring and autumn circulation. The water column was saturated with oxygen and showed negligible amounts of Fe(II) as compared to Fe(III). The pH was 2.6, and the TOC and/or TIC showed typical small values for acid mine pit lakes near 0.01 mmol L^{-1}.

After 1 yr, the pH was lowered from 2.6 to 2.4 in the control (R, Figure 2). The TIC (data not shown) and the contents of dissolved T-Fe and Fe(II) showed depth profiles with distinct gradients. Contrarily, for sulfate and TOC (data not shown), no gradients were recognized. In the bottom water, the content of T-Fe was around 0.2 mmol L^{-1} higher as compared to the initial conditions (2.84 mmol L^{-1}). In contrast to the initial conditions, Fe(II) was around 30% from T-Fe content. The TIC content increased approximately three times and reached 0.05 mmol L^{-1} in the bottom water. This indicates microbial Fe(III) reduction in the control microcosm, as it is also observed in untreated lake sediments of AML 111 (*24*). The sulfate content (14.0−15.1 mmol L^{-1}) had comparable values over the whole water column as compared to the initial values.

In the treated microcosms (Figure 2: S, E, C1−3; Figure 3: SE, Se, SC, Sc), changes in concentrations of the water column and the sediment were observed after amendment with the carbon sources ethanol or Carbokalk and wheat straw. For the observed pH values and redox conditions, microbially catalyzed solution and precipitation processes were responsible. As a result, we found raised values of pH (all microcosms), T-Fe, Fe(II), sulfide (S, E,C1−3, SE, Se, Sc), and sulfate (E, C1−3) as well as lowered values of $E_{H,pH7}$ (all microcosms), T-Fe, Fe(II), and sulfate (SC) especially in the bottom water column. How can these nonuniform results be interpreted?

After treatment of the microcosms, the microbially mediated reductive dissolution of iron hydroxides and/or (hydroxo)sulfates had started in the top sediment layer. Here an increase of the pH occurred with time. As a result, a strong proton flux was initiated temporary both from the bottom water and from the deeper sediment zones to the surface sediment layer. An increase of pH and decrease of $E_{H,pH7}$ in the water column close to the sediment resulted (*47*). Both the proton flux from deeper sediment and the external alkalinity load by the $CaCO_3$ of the Carbokalk (microcosms C1−3, SC, and Sc) led to a downward extension of the zone of elevated pH (data not shown). Now a pH-dependent transformation of (e.g., jarosite and schwertmannite to goethite) typical minerals in sediments of acid mine waters (*48*) could proceed. The result was the liberation of mineral-bound sulfate and the desorption of sulfate adsorbed at mineral surface (*49*−*51*). An evidence for this is the molar ratio of 1:2 for Fe:S in the interstitial water observed after 1 yr. This ratio is lower than the stoichiometric ratio in the minerals such as jarosite (molar Fe:S ratio = 3:2) and schwertmannite (molar Fe:S ratio = 8:1.25). All these processes led to a temporary increase of the iron and sulfate contents in the bottom water of the treated microcosms (*47*). Finally, we observed elevated sulfate concentration only in the microcosms E and C1−3. In the other microcosms, high sulfate-reducing activity reduced accumulation of sulfate in the water phase and sulfide was produced. This process became more intensive and led to pH values >5 in the top sediment layer. Solutions were supersaturated concerning Fe(II) and hydrogen sulfide and led to precipitation of FeS (Figure 4). The iron and sulfate concentrations both in the interstitial water and in the water column close to the sediment were reduced. This stage was very distinct at the end of the experiments in the microcosm SC (Figure 3). Here, the iron contents were lower than 5 μmol L^{-1}, close to the limit of determination (2 μmol L^{-1}). The depth profile of sulfate in the water column showed a deviating behavior as

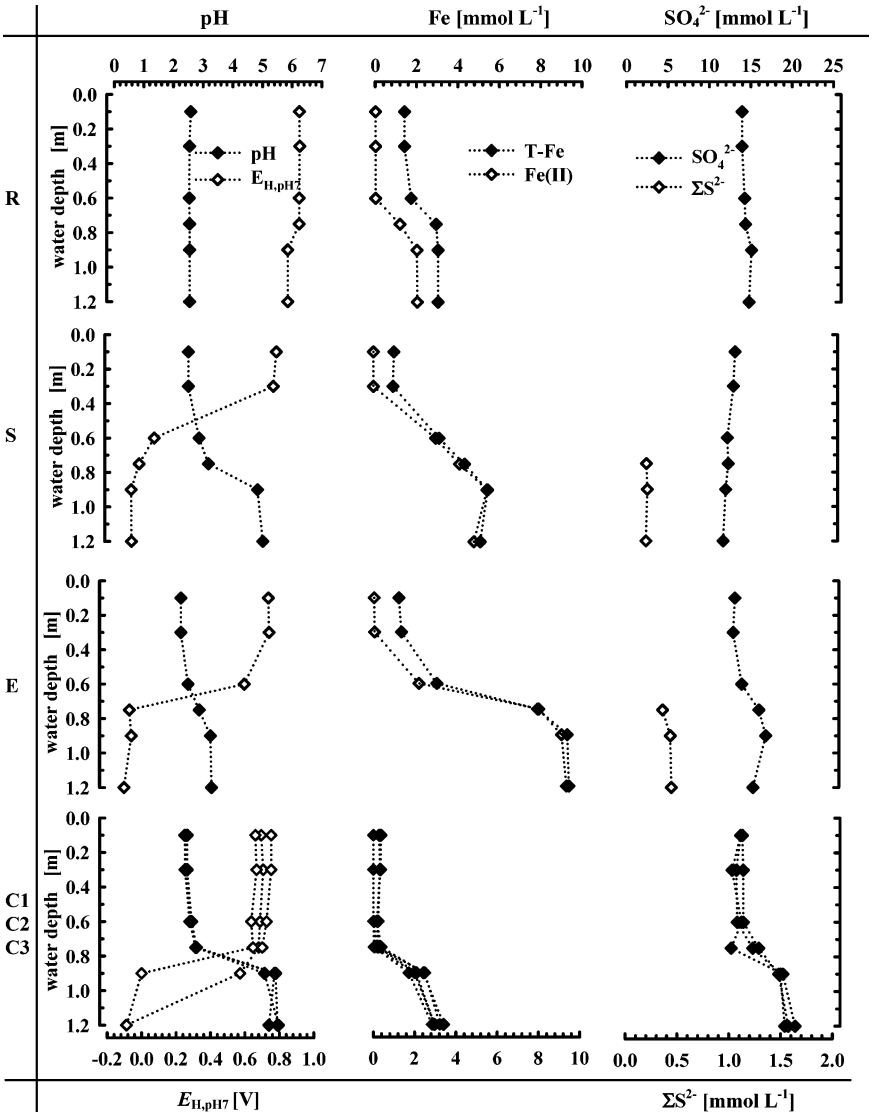

FIGURE 2. Water depth profiles of final pH and $E_{H,pH7}$ and concentrations of dissolved total iron (T-Fe), dissolved ferrous iron (Fe(II)), dissolved sulfate (SO_4^{2-}), and sum of dissolved sulfide (ΣS^{2-}) for the control (R) and the treated microcosms E, C1−3, and S (see Table 1 for abbreviations).

compared with all the other microcosms. In the other microcosms, the dissolution process in the sediment and the mass transport of Fe(II) and sulfate into the water column were more dominant until the end of experiments. The described processes were also observed in other microcosms with iron- and sulfate-rich sediments from acidic pit mine lakes (*11*, *13*) or sediments of eutrophic lakes (*52*). Strong concentration gradients for most of the considered parameters were observed in the middle part of the microcosms. Furthermore, in the upper part of the microcosms with the exception of the microcosm SC an acidic water body was observed. In those microcosms, the iron contents in the top water column were lower than initial values of the control microcosm (R). This is a result of iron hydroxide and (hydroxo)sulfate precipitates at the microcosm wall. These precipitations are typical for iron- and sulfate-rich acidic waters from pit mine lakes or acid mine drainage (*46*, *53*, *54*).

With respect to differences in the pH evolution, the treated microcosms can be divided into two main groups. The eight microcosms E, C1−3, S, SE, Se, and Sc belonged to the first subgroup. Here the pH increased to values between 4.3 and 6 in the bottom water. With a final pH < 4.3, the entire water column of the microcosm E is separated within the first group. The second subgroup comprised only the microcosm SC where a pH ≫ 6 was observed throughout the whole water column. In conclusion, both Carbokalk and ethanol alone were not able to stimulate biological deacidification processes to the extent necessary for a neutralization of the bottom water. With respect to ethanol, this was evident already from the small closed microcosm experiments as discussed in ref *13*. In particular, wheat straw had an important role in the promotion of the neutralizing processes. Microcosms with wheat straw alone or in combination with ethanol or Carbokalk achieved pH values higher than in microcosms without wheat straw. The reason for this behavior is hard to clarify. Besides the physical support of wheat straw as substratum for microbial biofilms as it was stated for upflow reactors for denitrification (*55*), we assume a chemical buffer system at the straw surface generated by fermentative bacteria. But currently we have no analytical evidence for this assumption. In addition, higher phosphorus concentrations in straw-treated microcosms (*47*) may have had a stimulatory effect.

Only in the microcosm SC, the pH rose from 2.8 to near 6.5 in the entire water column. Here, TIC of the bottom water (100 mmol L^{-1}) was 3-fold higher than in the interstitial water (30 mmol L^{-1}). In the whole water column, the sulfate

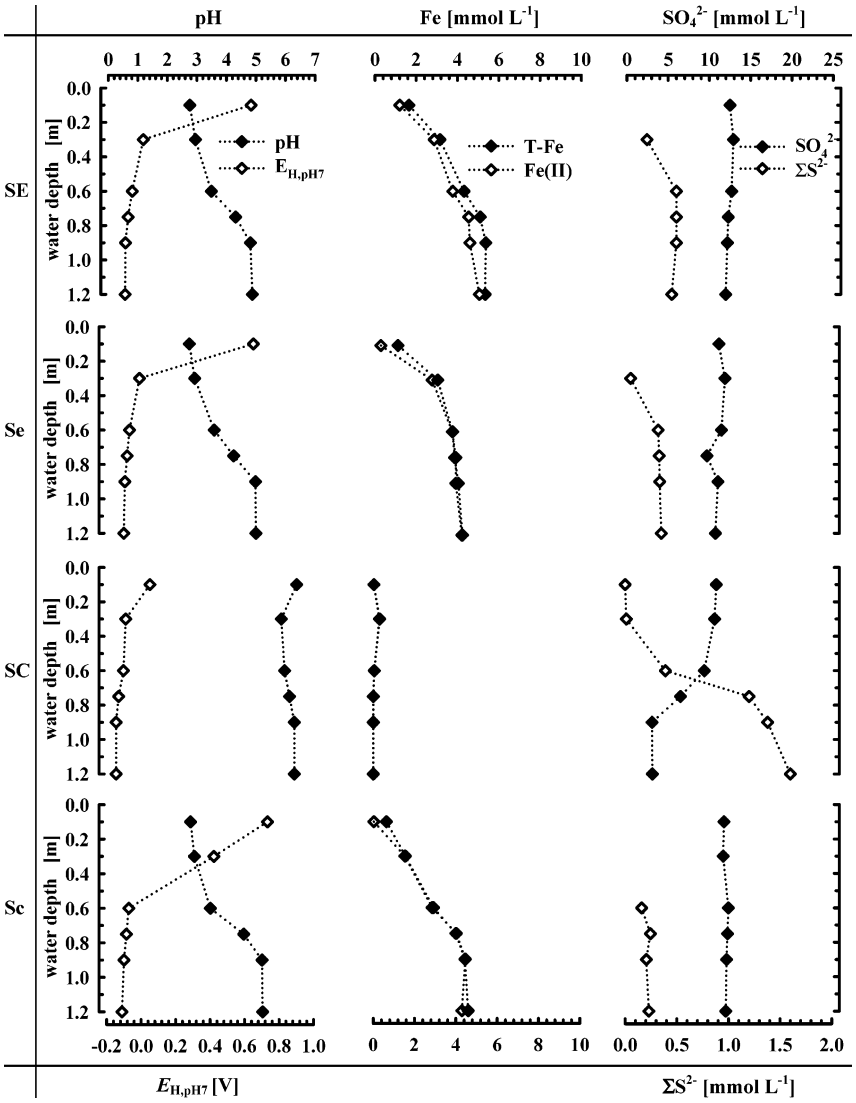

FIGURE 3. Water depth profiles of final pH and $E_{H,pH7}$ and concentrations of dissolved total iron (T-Fe), dissolved ferrous iron (Fe(II)), dissolved sulfate (SO_4^{2-}), and sum of dissolved sulfide (ΣS^{2-}) for the control (R) and the treated microcosms SE, Se, SC, and Sc (see Table 1 for abbreviations).

content was lowered from 1190 to 685 mmol, and the iron content was lowered from 304 to 6.33 mmol. This corresponded to high amounts of TRIS and NS-$Fe(II)_R$ in the sediment (Figure 3).

Neutralization Equivalents and Alkalinity Generation. In the treated microcosms, the amount of TRIS and $Fe(II)_R$ as well as the ratio between the different species varied with the organic carbon source. They were strongly elevated with respect to the values of the control system at the beginning and end of the experiments. The main part of the reduced minerals were found in the top 5 cm of the sediment. In microcosms treated with wheat straw, AVS predominated with values between 5.37 and 583 μmol g^{-1} in the top layer of the sediment. Here, the CRS ranged from 16 to 191 μmol g^{-1}, and ES ranged from 3.51 to 71.8 μmol g^{-1}. $Fe(II)_R$ and $T\text{-}Fe_R$ showed values between 111 and 534 μmol g^{-1} and 170−585 μmol g^{-1}, respectively. $T\text{-}Fe_R$ was dominated by $Fe(II)_R$. With respect to the TRIS contents, microcosms can be divided in two groups similar to the arrangement by pH evolution of the water column. The first group comprises the microcosms S, E, C1−3, SE, Se, and Sc with values smaller than 500 μmol g^{-1} TRIS. The second group is represented again only by the microcosm SC with TRIS values larger than 700 μmol g^{-1}. The first group can still be divided in two subgroups with TRIS contents either below 100 μmol g^{-1} (E, C1−3) or above 200 μmol g^{-1} (S, SE, Se, Sc). Higher TRIS accumulation in the wheat straw-amended microcosms indicate the importance of wheat straw but also a correct substrate ratio to the second carbon source. This is supported by the evolution of microorganism communities (Figures 5 and 6). The most successful microcosm was the microcosm SC. Compared with the microcosms S or C1−3 in which straw or Carbokalk was amended alone, the sediment of SC showed the highest TRIS value (Figure 4). It was dominated by the AVS fraction (iron monosulfide sulfur). CRS (pyrite sulfur) was observed in the microcosms S, C1−3, and SC. ES and $T\text{-}Fe_R$ were of minor importance as compared to other species. The difference between $\delta^{34}S$ of ES, AVS, and CRS in the top two sediment layers is lower than 2‰ (*56*). This indicates that microbes played a predominant role in the formation of ES. A pure chemical oxidation of sulfide yields lighter ES as compared to AVS. The depletion can be up to 8‰ in the $\delta^{34}S$ of ES (*57*, *58*).

The alkalinity of TRIS neutralization equivalents in the sediment of the microcosms was used for calculating the neutralization rate. For this, we used a TRIS:H^+ ratio of 1:2. This equivalent ratio is valid for a global carbon source [CH_2O] where no consumption of protons is observed via sulfate reduction in the aqueous phase (Figure 1) if precipitation with dissolved Fe(II) to FeS follows. The microcosms were

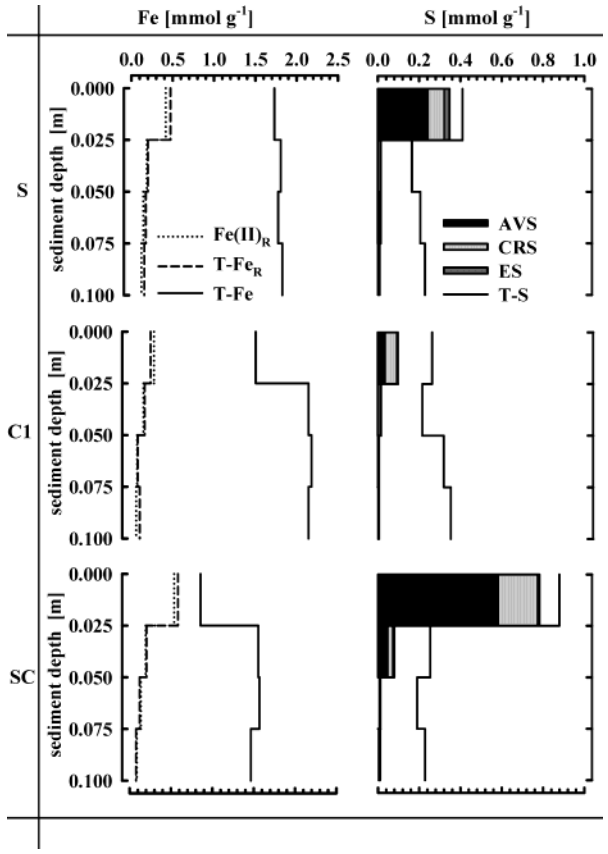

FIGURE 4. Sediment depth profiles of final amount of reactive ferrous iron ($Fe(II)_R$), reactive iron ($T\text{-}Fe_R$), total iron (T-Fe), acid volatile sulfide (AVS), chromium-reducible sulfur (CRS), elemental sulfur (ES), and total sulfur (T-S) for the treated microcosms S, C1, and SC (see Table 1 for abbreviations).

amended with complex carbon sources. Therefore, this assumption is a good estimate that was also used in refs *59* and *60*. With respect to iron reduction, which led to reduced $NS\text{-}Fe(II)_R$, we used the same equivalent ratio. The neutralization rates for microcosms S, C1−3, and SC are between 6 and 15 equiv m^{-2} a^{-1} (Table 4). They were sufficient to elevate the pH to around pH 7 exclusively in the microcosm SC. Here, the different acidity-forming processes of the aqueous phase listed in Table 3 could be overcome completely. These acidity-forming processes are differently weighted and summarized within a titration of $K_{B8.2}$. At start-up in all microcosms, $K_{B8.2}$ recalculated per area was close to 20.4 equiv m^{-2}. This indicates that $K_{B8.2}$ represented the highest possible acidity amount including acidity from proton concentration, the balancing between sulfate species, and the precipitation of $Fe(OH)_3$ and $Al(OH)_3$ (Table 3). The fact that the neutralization equivalents of the microcosm SC (14.9 equiv m^{-2}) were sufficient to neutralize the aqueous phase means that $K_{B8.2}$ as a static acidity indicator was not representative for the dynamic behavior of a microcosm with an anoxic water zone. An inverse calculation of the acidity according to Table 3 replacing $Fe(OH)_3$ precipitation by Fe(III) reduction yields an acidity of 13.3 equiv m^{-2}, which agreed with the observed processes in microcosm SC.

The neutralization rates in the microcosm SC are 1−2 orders of magnitude higher as compared to those published for untreated mine pit lakes of different age, atmospherically acidified lakes, and other limnic systems of different trophic levels (Table 4). For treated acidic mine pit lakes, no information on neutralization rates were found. The rates listed in Table 4 were recalculated from in-situ accumulation rates of reduced iron and sulfur minerals or from sulfate reduction rates determined with ^{35}S (*61*). For the neutralization rates of three untreated mine pit lakes of the Plessa/Grünewalde mining district (*60*), differences of 1 order of magnitude between these two methods were observed, which may indicate in-situ sulfide re-oxidation. The neutralization rates of our microcosms are within the range determined in a freshwater creek receiving acid mine drainage (*62*).

Microbial Biomass and Populations. Total bacterial numbers in the water column did not show a distinct trend with time in any of the treatments. In microcosms without wheat straw (R, E, C1−3), bacterial numbers (10^5−10^6 mL^{-1}) were within the range of mesotrophic or dystrophic lakes (*63*), and the Carbokalk-treated microcosms initially showed higher bacterial numbers than R and E. Differences in bacterial counts between 60 and 120 cm water depth were not significant (*t* test, $\alpha = 0.05$). This implies that the temperature difference of 10 °C had little influence on general water microbiology. Microcosms with wheat straw had significantly higher bacterial numbers than those without (Mann−Whitney rank sum test, $P = <0.001$). They were approximately 10-fold higher and fell within the range of eutrophic lakes according to ref *63*. In addition, values at 120 cm depth were significantly higher (Mann−Whitney rank sum test, $P = <0.001$) than those at 60 cm depth in these microcosms, indicating that wheat straw markedly increased bacterial numbers in the water, especially in its close vicinity. After 52 weeks, bacterial biomass in the water column was also determined. Again wheat straw-amended microcosms had significantly higher bacterial biomass, the factors even exceeding those of the bacterial numbers. This indicates that, in addition to increased abundance, individual cells were also larger in wheat straw-treated microcosms. We did not test if wheat straw served as inoculum for special types of bacteria; however, from geochemical and microbiological points of view, it turned out to be an important source of carbon and nutrients in the microcosms.

When bacterial biomasses between Carbokalk- and ethanol-treated microcosms at equivalent carbon concentration were compared, the Carbokalk treatments showed higher bacterial biomass. Thus Carbokalk was more efficient in stimulating bacterial growth in the water than ethanol.

Microbial biomass in the sediments was only determined at the end of the experiments. Since the sediment at the sampling site contained only 23 nmol g^{-1} phospholipid phosphate (grab sample, February 1998) and the added Carbokalk had only 14 nmol g^{-1} phospholipid phosphate, we can infer biomass production in the sediments of all microcosms. These values correspond to approximately 9.2×10^9 and 5.6×10^9 cells g^{-1}, respectively, using the conversion factors of ref *64* determined for aquifer sediments. Except in the control (R), maxima developed at the surface, which is also typical for natural sediments. When all biomass values with and without wheat straw were compared, microcosms with wheat straw showed significantly lower biomass values (median 46.9 P g^{-1}) than those without wheat straw (50.1 nmol P g^{-1}; Mann−Whitney rank sum test, $P = 0.01$). Probably in the wheat straw-treated microcosms nutrients and carbon sources were efficiently consumed by the proliferating microbial community in the water column and on the wheat straw itself (which we were unable to determine), so that less was available for the sediment. However, when expressed as cell numbers using the above-mentioned conversion factors, the median values without and with wheat straw, although statistically different, correspond to approximately 1.88 and 2.0×10^{10} cells g^{-1}, a fairly small difference for bacterial numbers.

Generally, the microbial biomass in the microcosms was within the range known from pristine rivers or marine

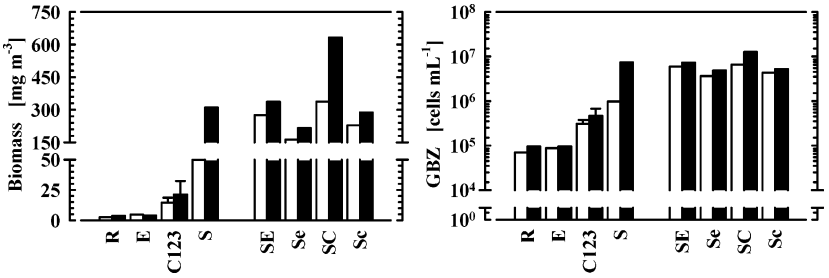

FIGURE 5. Final total bacterial biomass (biomass) and total bacterial counts (GBZ) in the aqueous compartment at 0.6 m (white bars) and 1.2 m (black bars) water depth for the control (R) and the treated microcosms (see Table 1 for abbrevations).

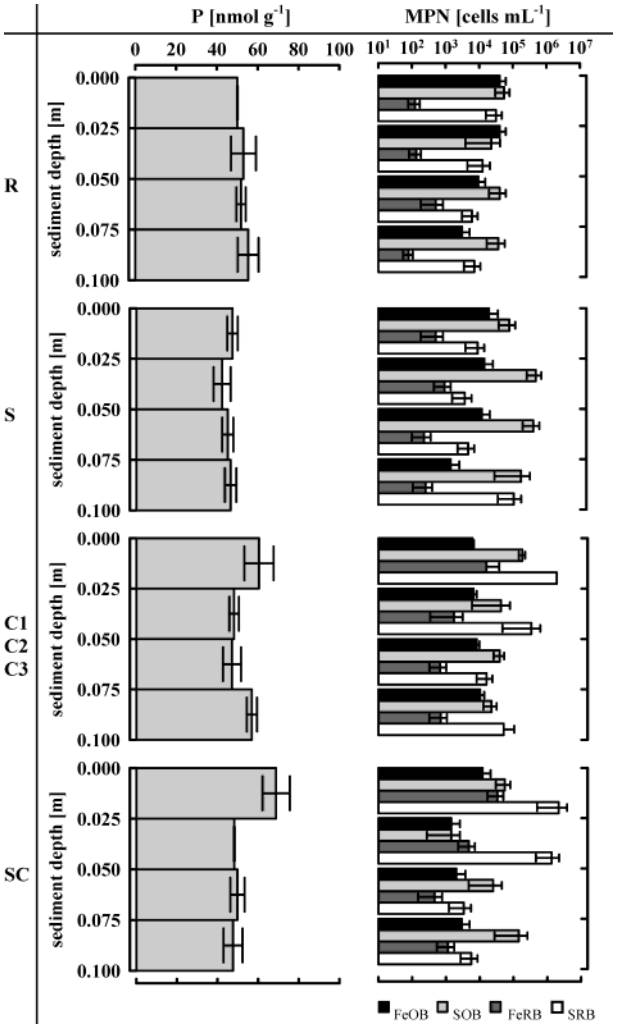

FIGURE 6. Sediment depth profiles of final most probable number (MPN) and final biomass (phospholipid-P concentrations) for the control (R) and the treated microcosms S, C, and SC. FeOB, iron-oxidizing bacteria; SOB, sulfur-oxidizing bacteria; FeRB, iron-reducing bacteria; SRB, sulfate-reducing bacteria. SRB at 0−0.025 m in C and SC represent minimum values. Correct cell numbers could not be determined due to insufficient dilution (see Table 1 for abbreviations).

sediments (*65*) despite the organic enrichment. Since the sediments were anoxic, we assume that the biomass predominantly consisted of bacteria.

Viable counts of bacteria involved in iron and sulfur cycling in water samples were highly variable, probably because of the presence of flocs. Lithotrophic iron- and sulfur-oxidizing bacteria were generally more abundant than anaerobes. Numbers of sulfate reducers were around 10 cells mL^{-1} except the June sample from SC with 6.4×10^4 cells mL^{-1} (data not shown). This confirms that the predominant site of reaction was the sediment. The role of biofilm growth on the wheat straw bed could not be resolved. Since biological material was not really attached to the wheat straw, reproducible sampling was not possible.

TABLE 3. Acidity-Producing Processes in Water of a Microcosm and the Remaining Acidity[a]

acidity producing process	acidity (equiv m^{-2})
chemical balances	
protolysis	3.16
$HSO_4^- \rightarrow SO_4^{2-}$	1.61
iron reduction	3.34
iron mineral deposits	
$Fe(OH)_{3(amorph)}$, ferrihydrite, goethite	10.1
schwertmannite[b]	8.58−9.25
$Fe_8O_8(OH)_x(SO_4)_y$; $\{1.0 \le y \le 1.75; x = 8 - 2y\}$	
jarosite[c]	≈6.69
$H_xNa_yK_zFe_3(OH)_6(SO_4)_2$; $\{x + y + z = 1\}$	
aluminum mineral deposits	
$Al(OH)_3$	5.24
alunite	≈3.49

[a] Acidity was calculated starting with the water chemistry of Table 2 and stopping with aqueous or dissolution equilibrium at pH 7. [b] From ref *46*. [c] From ref *45*.

Viable counts in the sediment were generally much higher than in the water column. However, the numbers of fermentative bacteria were still low (10^2−10^3 cells mL^{-1}) except the treatments SC and Sc with 6.3×10^4 cells mL^{-1}. Probably the combined addition of abundant carbon and nutrient sources (wheat straw) and acid buffering capacity against acids formed during fermentation (Carbokalk) favored their growth. Fermenters are considered beneficial because they generate low molecular weight organic compounds from complex carbon substrates, which are not directly attacked by most sulfate-reducing bacteria (*66*). Numbers of iron and sulfur bacteria in treatment R resembled those of the untreated lake sediment, with no pronounced depth gradient. All treatments with Carbokalk showed highly increased numbers of sulfate-reducing bacteria with maxima in the upper 2.5 cm (>10^6 cells mL^{-1}). This might be due to the pH-elevating effect of the Carbokalk, since most sulfate reducers known to date are neutrophilic, and from acid mining lake samples, generally higher numbers of these bacteria are obtained on pH-neutral media (*28*). In addition, Carbokalk may serve as a source of inoculum. We found that air-dried Carbokalk stored under aerobic conditions contained 10^7 g^{-1} sulfate-reducing bacteria and 2×10^6 g^{-1} iron reducers growing at pH 6. Which of the bacteria are predominantly active during biological neutralization remains an open question, and we are well aware that cultivation-based methods may greatly underestimate in-situ bacterial populations. The combination of ethanol and wheat straw seemed to decrease the numbers of iron-oxidizing bacteria to ≤10^3 cells mL^{-1}. Compared to all other

TABLE 4. Neutralization Rates in Sediments of Limnic Lake Systems[a]

limnological systems	neutralization rate (equiv m^{-2} a^{-1}) TRIS & NS-Fe(II)$_R$[b]	neutralization rate (equiv m^{-2} a^{-1}) SRR	ref
pyrite acidic			
AML 111 microcosms S/C/SC	6.1/12.6/14.9		this work
AML 111 sampling site (7 m water depth)		<0.001	*28*
AML 111 middle basin (10.2 m water depth)	0.121	0.12−3.19	*28*
mining district, Rauberweiher	0.004−0.107		*60*
mining district, Plessa/Grünewalde	0.020−0.137	0.8−3.87	
freshwater creek receiving acid mine drainage		1.24−165	*62*
atmospherically acidic	0.124		*68*
	0.074−0.104		*69*
	0.010−0.159		*59*
oligotrophic		0.045−3.79	*70*
mesotrophic		0.24−13.9	
eutrophic		1.61−15.3	
hypertrophic		4.23−11.2	

[a] TRIS and NS-Fe(II)$_R$: calculation via accumulation of reduced iron and sulfur minerals. SRR: calculation via sulfate reduction rate of the ^{35}S tracer incubation method based on ref *61*. [b] Neutralization rates via NS-Fe(II)$_R$ only for AML 111 microcosms available.

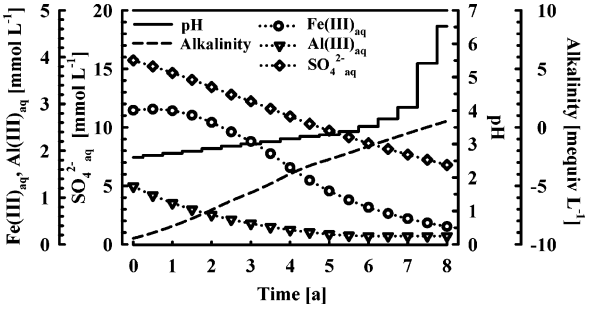

FIGURE 7. Irreversible batch reaction simulation: pH, alkalinity, and dissolved ferric iron ($Fe(III)_{aq}$), aluminum ($Al(III)_{aq}$), and sulfate ($SO_4^{2-}{}_{aq}$) as a function of time in the whole water column after multiple lake turnover events. Neutralization rate with respect to the microcosm SC (14.9 equiv m^{-2} a^{-1}).

columns, the difference was statistically significant (Mann−Whitney rank sum test, $P = <0.001$). Although the underlying mechanism is unknown, this effect can be regarded as beneficial since reoxidation of Fe(II) is generally a problem in biological neutralization of acidic mining lakes (*30*, *67*).

In conclusion, the addition of Carbokalk, ethanol, and wheat straw alone or in combinations stimulated microbial growth and activity. Carbokalk was of special importance for the establishment of a sulfate-reducing population. To which extent this effect was caused by its special organic carbon composition, its alkalizing properties, or its richness in sulfate-reducing bacteria deserves further study.

Geochemical Modeling. To predict water quality with respect to sulfate and iron reduction in lower-scale microcosms, we used earlier the irreversible reaction capabilities of PHREEQC (*13*). Here the basis was the ratio between sulfate, iron, and carbon dioxide from the global equation outlined in eq 2. From this study, detailed information about reduced sulfur and iron species in the sediments were gained. So the previously assumed ratios between sulfate, iron, and carbon dioxide were carefully revised with respect to the microcosm SC. Further assumptions with respect to the conditions in the AML 111 using the described methods were made to get a more realistic simulation for predicting the neutralization time of a lake water column. For that case, the evolution of water chemistry is plotted against time for a whole lake water column after turnover events (Figure 7). Results show that microbial iron and sulfate reduction simulated using the described method raised the pH to 6.5 after 8 yr. This is due to the final pH of the microcosm SC. Dissolved sulfate concentration reached a minimum with 6.79 mmol L^{-1}. This is nearly to the final average concentration (7.2 mmol L^{-1}) of the microcosm SC. Final dissolved aluminum concentrations are around 0.8 mmol L^{-1}, and iron concentration are at a nearly constant value of 0.38 mmol L^{-1}. The dissolved concentrations of aluminum and iron are higher as compared to the microcosm SC. A reason for that are neglected adsorption processes at the microcosm wall. During the evolution of lake water chemistry over a simulated period of 8 yr in the hypolimnic part, hydrogen sulfide was produced and the aqueous phase was supersaturated to FeS and other metal sulfide of minor interest. FeS precipitated and decreased the iron content (data not shown). In the epilimnic part, the iron concentration was controlled by equilibrium with amorphous ferric hydroxides. The decrease of aluminum to its low value was contolled by precepitation of different aluminum minerals in the whole water column (e.g., gibbsite, alunite). Sulfate was lowered both by sulfate reduction in the hypolimnic part and precipitation together with aluminum and iron in the hypolimnic and epilimnic part. Generally the simulation results compare favorable with pH and sulfate and with some minor restrictions with iron and aluminum. However, this simulation is not applicable for prognoses in pit lake ecosystems treated with Carbokalk and wheat straw because of its simplicity. Nevertheless it gives a first idea about the order of remediation time, and it has some relevance for the next experimental phase in field-scale mesocosms.

Implication for AML 111 Mesocosms. The addition of Carbokalk, ethanol, and wheat straw alone or in combinations stimulated chemical and microbial neutralization processes. As reported from previous small-scale microcosms (*13*), wheat straw and Carbokalk acted synergistically in terms of alkalinity generation. A special effect of Carbokalk was the beneficial influence on sulfate-reducing bacteria. The comprehensive understanding of the underlying mechanisms should be the subject of further studies. The fact that complete neutralization occurred in open systems amended with a combination of wheat straw and Carbokalk at moderate temperatures suggests that field treatments with this substrate combination might be successful for outdoor mesocosm experiments. For that case, a neutralization time of 8 yr has been simulated assuming constant neutralization rates. Continuing work will prove the manipulation of a lake water column to induce a temporary stable anoxic hypolimnion under natural conditions. Additionally advective−dispersive one-dimensional transport and kinetics of biogeochemical reactions will be implemented in a more refined biogeochemical model for treated acid pit lakes.

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