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Sediment Conditioning with Organic and/or Inorganic Carbon Sources as a First Step in Alkalinity Generation of Acid Mine Pit Lake Water (pH 2−3)

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Laboratory scale microcosm studies were conducted to determine the efficacy of controlled in situ saprobization of lake sediments as one opportunity for deacidification of artificial shallow lakes resulting from open cast lignite mining located in southeast Germany. Under lasting anoxic conditions iron and sulfate were removed from the lake water as a result of microbial iron- and sulfate reduction together with a subsequent precipitation of insoluble sulfide minerals to the lake sediment. The 2 L closed system microcosms were made up of a model of the sediment/water interface. They were filled with lake water and sediment and treated subsequently with different organic and/or inorganic carbon sources in combination with wheat straw. Just the water was characterized initially and finally 8 weeks later. The rise of pH together with acidity consumption was observed. In the microcosms treated with wheat straw and pyruvate, ethanol, Carbokalk, or Pfezi-granula the pH rose to 5.5 and 7.0, respectively, and iron- and sulfate reduction were observed. With wheat straw and ethanol or Pfezi-granula sulfate concentrations decreased from initial concentrations of 11.5−13.5 mmol L^{-1} to final concentrations of < 3 mmol L^{-1}. However, the iron concentration decreased significantly to a value of 0.01 mmol L^{-1} exclusively with Pfezi-granula and wheat straw. For the most reactive microcosms geochemical equilibrium calculations suggest precipitation of different sulfide minerals. The critical revision of the results obtained from this study indicate that ethanol or Carbokalk together with wheat straw are suitable for further upscaling in larger-scale microcosms.

Introduction

In the Lusatia Mining District situated in Brandenburg and East Saxony, East Germany, lignite was excavated intensively in opencast mines. The groundwater level has been drastically lowered over an area of approximately 2500 km^2 producing a volume deficit of 13 billion m^3. Open pits were left and tertiary substrates accompanied by pyrite and/or marcasite have been exposed to oxygen (*1*). As a result in the Lusatia Mining District the main problem when filling a pit with groundwater is the acidification. More than 100 pit lakes are formed in the last 50 years (*1*, *2*). At full capacity, these lakes will cover an area of 208 km^2 and hold approximately 4.5 billion m^3 of water. Approximately half of these lakes have pH-values in the range of 2.5−3 (*3*). The resulting lakes represent a special form of artificial shallow lakes defined as acid mine pit lakes or acidic mining lakes (AML). The environmental limiting conditions vary strongly in these lakes depending on morphometry and water quality. The reasons are very great differences in the removed lignite formation, the tilt technology for the dump material, the chemistry of the geological strata excavated, and the chemistry of the groundwater. The hydrochemical conditions of the AML are comparable with acid mine drainage (AMD). This is due to the weathering of pyrite in the lignite and in the accompanying geological formations. The precise reaction mechanism in the weathering of pyrite is not exactly clear. The weathering is influenced strongly by the humidity and buffer capacity of the dump as well as the temperature, the pH, and above all the activity of acidophilic bacteria (*4*, *5*). This process is represented in a greatly simplified manner (eq 1).

$$FeS_2 + 3.75O_2 + 3.5H_2O \rightarrow Fe(OH)_{3(amorphous)} + 2SO_4^{2-} + 4H^+ \quad (1)$$

A part of the produced acid is buffered by carbonates, silicates, and ion exchange processes as well as the solution of ferrous hydroxides and manganese oxides. These processes result in a high mineral acidity of seepage water and groundwater. Therefore these lakes have very low pH-values and high heavy metal concentrations (*6*−*8*). Under these conditions fish farming is excluded. Swimming and other sports are demanded by the European Union as long as the pH-value is below 6.

The tight restriction on use is a strong argument for the redevelopment of AML areas. For this reason the halting of the pyrite oxidation in the bordering dumps and the prevention of the acidity stream into AML is well presented in the literature, e.g. refs *9*−*11*. Furthermore the neutralization of lake water of AML is an important approach. The effort of anoxic on-side bioreactors is under discussion. An acidity consumption via desulfurication can occur (*12*−*14*). Together with subsequent metal sulfide precipitation (*15*, *16*) the metal ion concentration in the lake water can decrease simultaneously. In addition biological in-situ remediation strategies are alternatives. The addition of organic carbon controls in-lake alkalinity generating natural cycles. The microbial iron- and sulfate reduction serves as an example. Thereby the accumulation of reduced iron- and sulfur compounds in the sediment (*17*−*20*) leads to alkalinity generation (Figure 1). Not only this effect could be observed in micro- and mesocosm studies after addition of organic carbon (*21*−*25*) but also suitable organic carbon sources on microbial alkalinity generation were presented (*12*, *22*, *23*, *25*−*30*).

Most of the presented measures appear energy intensive and uneconomic because of the extremely acidic habitats and the high water deficit in the Lusatia Mining District. Solely the biological in-situ remediation strategies seem to be favorable and environment-friendly. This behavior led to this contribution. We consider a carbon source mixture for use in alkalinity generation of a typical extremely acidic

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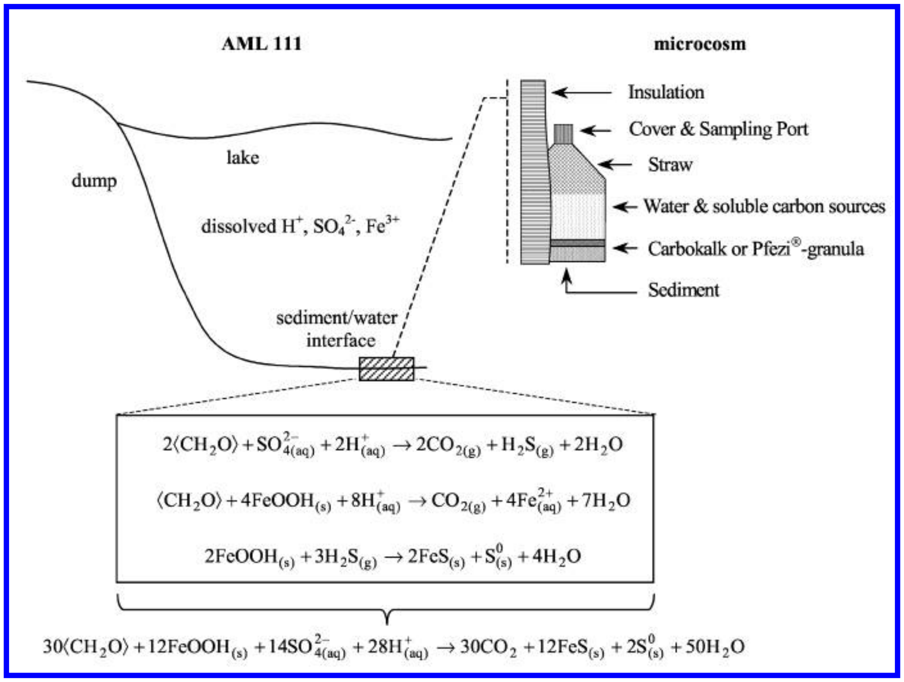

FIGURE 1. Schematic description of the microcosm design. Equations from the literature (*17*–*20*).

habitat (pH < 3) investigated by a microcosm approach. The interpretation of this approach is, besides we are restricted to the products of microbial processes, rigorous on the geochemical level. It allows us to give firm information about pH, alkalinity, and element concentrations and the efficacy of a certain carbon source mixture for further up-scaling studies.

Material and Methods

Habitat. Typical yellow brown surface sediment (7.0 m water depth) and epilimnic lake water from the AML 111 of the Koyne/Plessa lignite field in the Lusatia Mining District (Germany) were used to construct the microcosms. Flooding of the AML 111 was completed in 1969. The lake consists of three partial basins (*31*). Morphologically, the lake is a category 3 shallow water lake (*32*) 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 *Ochromonas* species. Chlorophyceae of the *Chlamydomonas* species are also common. The zooplankton consists mainly of heliozoa, ciliates, and rotifers. The pH of AML 111 lake water is approximately 2.6, while electric conductivity averages 2.5 mS cm^{-1} ($\kappa_{25°C,AML\ 111}$, corrected according to ref *33*). Both total inorganic and organic carbon 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 (*31*, *34*, *35*).

Carbon Sources. As pure substrates glucose, pyruvate, lactate, acetate, and ethanol were used (Merck, Germany). These expensive carbon sources were supplemented by economical agriculture byproducts. These byproducts were whey (Altmark-Käserei Uelzena Ltd., Germany), molasses (Diamant-Zucker-Fabriken Ltd. & Co., Könnern, Germany), Carbokalk (Zuckerverband Magdeburg e.V., Germany), and Pfezi-granula (Gesellschaft zur Verwaltung der Fermentation Neubrandenburg Ltd., Germany). Carbokalk is a sugar industry byproduct. It is the solid precipitate of nonsugars after lime clarification of the extracted sugar beet juice. The lime content of the Carbokalk is 50% (w/w). The Pfezi-granula consists of sugar beet chips, molasses, and cement. The cement content of the Pfezi-granula is 50% (w/w). All substrates were completely water soluble with the exception of the Pfezi-granula and the Carbokalk. Cut wheat straw from ecological, pesticide free cultivation served functionally as periphyton for the microorganisms of the sediment water interface. Additionally it serves as a complex carbon source. Information about composition of the agricultural byproducts and of the straw is outlined in Table 1 and further compiled in detail by ref *36*.

TABLE 1. Composition of Agricultural Byproducts Used in Microcosms Given as Percent Dry Weight (wt %)

	wheat straw	whey	molasses	Carbokalk	Pfezi-granula
dry weight (%)	98	61.6	82.4	67.4	97.7
limestones				≈50	
cement					50
molasses					25
sugar beet chips					25
TOC	44.4	28.2	38.2	3.2	19.2
TIC		<0.7	<0.1	9.0	0.59
TN	0.56	1.5	2.3	0.43	0.95
TP	0.16	4.3	0.2	6.7	1.1
o-phosphate		0.7	<0.1	1.0	0.3
sulfate		<0.1	0.8	0.7	2.3

The 2 L microcosms were treated with pure substrates (Merck) to get a final molar substrate concentration of 30 mmol L^{-1}. This concentration is nearly the highest value of substrate dosage found for iron- and sulfate reduction in synthetic media (*37*, *38*). There was an overdose of organic carbon with respect to the equivalent concentration of iron and sulfate in the water phase. The complex substrates were inserted into the microcosms with a defined mass concentration of 30 g L^{-1}. We considered that the orders of magnitude of the resulted molar concentration agreed with those of the pure substrates. The addition of straw occurred according to ref *25*. The quantity of wheat straw was recalculated for the treated water volume. The substrate combinations and dosages for the microcosms could be taken from Table 2.

Microcosm-Design. The 2 L scale microcosms were realized in closed Duran-glass bottles (Schott, Germany).

TABLE 2. Load of Organic Carbon Sources Used in Microcosms Expressed as Molar Units ($TOC_{wheat\ straw} = 44.5$ mol m^{-2})

	organic carbon sources			
			$TOC_{substrate}$	
microcosm	wheat straw	substrate	(mmol L^{-1})	(mol m^{-2})
R				
S	+			
SG	+	glucose-D-monohydrate	180	28.8
SP	+	sodium pyruvate	90	14.4
SL	+	sodium-DL-lactate	90	14.4
SE	+	ethanol	60	9.6
SA	+	sodium acatate	60	9.6
SW	+	whey	82	13.1
SM	+	molasses	96	15.5
SC	+	Carbokalk	54	8.64
SPfezi	+	Pfezi-granula	418	74.9

TABLE 3. Initial Concentrations of Dissolved Constituents and pH, E_{h7}, and Acidity of the Reference Microcosm (R)

parameter	replicates (mmol L^{-1})	
TIC	0.10	0.13
TOC		0.10
SO_4^{2-}	13.8	11.5
Fe(II)	nd[a]	0.056
Fe-G	2.60	2.06
Ca	6.21	5.79
Mg	1.34	1.27
Al	1.67	1.48
Zn	16.7	17.0
pH	2.5	2.5
E_{h7} (mV)	488	517
Aci (mequiv L^{-1})	20.9	16.0

[a] nd, not detectable.

Untreated (reference) and treated mesocosms represented a lake section of an area of 125 cm^2. The bottles were filled with 255 ± 25 g homogenized fresh lake sediment (2.0 cm sediment layer in each bottle) and 2.0 ± 0.01 L of lake water (16 cm water layer). Masses of 15.0 ± 0.01 g wheat straw (1.2 ± 0.01 kg m^{-2}) and different carbon sources (Table 1) were added to the treated microcosm. Each experimental mixture was set up twice as replicate. Mass transport between the microcosm and atmosphere could be excluded. The bottles were locked together with ETFE covers and Teflon-coated butyl rubber seals. The microcosms were incubated in darkness at room temperature (21 ± 2 °C) for the duration of 8 weeks. The detailed construction of the experimental setup is outlined in Figure 1.

Water Sampling and Analysis. After 8 weeks water samples of the 2 L microcosms were collected with a syringe under argon atmosphere in a glovebag.

pH, oxidation−reduction potential (ORP), acidity, and alkalinity were determined at the start and end of the experiments. In situ pH measurements in the lab were completed 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 E_{h7}. Alkalinity ($K_{S4.3}$) and acidity ($K_{B8.2}$) were determined via volumetric titration according to procedure DIN 38409/H7 of the German Institute of Standardization.

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 has 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.

Hydrogen sulfide was determined with an amperometric microelectrode (AMT, Germany). For the external calibration of the microelectrode we used an on-line flow system combined with an electrochemical hydrogen sulfide generator (AMT, Germany).

Iron(II) was determined photometrically with *o*-phenanthrolin at a wavelength of 512 nm against an external calibration with diluted iron(II) standards (*35*). We used a CFA system (Scalar Analytical, San-plus, Germany) which operated with a multichannel photometer and 5 cm flow through cell. Before analysis the dissolved iron(II) was stabilized in the filtrate with sulfuric acid (25% w/w) between pH 2.4 and 2.6.

The total concentrations of iron (T-Fe), calcium (Ca), magnesium (Mg), aluminum (Al), and zinc (Zn) in filtrated water samples were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). We used the Optima 3000 (Perkin-Elmer, Germany). The filtrates were stabilized in a diluted nitric acid (0.1% w/w). Analyses followed after external calibration with diluted multielement standards.

The TOC/TIC and DOC/DIC were determined in different aliquots of the unfiltered and filtrated water sample, respectively. In the first aliquot, the TC was transferred catalytically at 850 °C together with platinic wool to CO_2. The determination of CO_2 followed with infrared spectrometry after external calibration with mixed bicarbonate/phthalic acid standards. To a second aliquot was given sulfuric acid (25% w/w) to displace the TIC with an oxygen gas stream. The TOC was determined subsequently analogous to TC (DIN 38409 part 3). We worked with the C-Analyzer Dima-TOC-100 (Dimatec, Germany).

Geochemical Modeling. The geochemical simulation program PHREEQC (*39*) was used to calculate the saturation indices (SI) of mineral phases. The initial composition of the water phase after addition of pyruvate, lactate, and acetate was calculated. The irreversible reaction capabilities were used in modeling the sulfate reduction process in a closed system. The PHREEQC database was used in which minor modifications have been made to make the data consistent with the tabulations in refs *40*−*44* and WATEQ4F (*45*). The database was modified according to additional solubility data for schwertmannite and jarosites (*46*, *47*) and Ni, Cu, and Co based upon values from the MINTEQ, WATEQ4F, and LLNL databases.

Results and Discussion

Changes in pH and E_{h7}. After filling the 2 L scale microcosms the initial conditions of the water of the reference system (R) were examined after acclimation of 2 days at room temperature (Table 3). In the two replicates only slight differences in the composition of the water phase were observed with regard to the iron and sulfate concentration and acidity, probably due to different adsorption behavior of the sediment and/or of the batch flasks toward dissolved iron and sulfate. With the decrease of these components the acid content was lowered in the water. Despite the slight differences between the two replicates an identical pH of 2.5 was observed. The microcosms treated with pure weak organic acids (SP, SL, SA) increased the pH near their log K-values (Table 4). The result was the change from the iron buffer to a buffer system dominated by the weak organic acids pyruvate, lactate, and acetate without any alkalinity generation. The measured initial pH (Table 4) could be recalculated with PHREEQC, and information about the speciation of the aqueous solution was available. The hydrochemistry was dominated by the weak organic acids. Pyruvate, lactate, or acetate effect most of the trace metals as a chelating agent. As a result of the raised pH the iron (SP) as well as the aluminum content (SL,

TABLE 4. Initial and Final (8 Weeks) pH and E_{h7} for the Reference Microcosm (R) and the Microcosms Treated with Wheat Straw (S), and with Wheat Straw and Synthetic Carbon Sources (SG, SP, SL, SA, SE)[a]

	initial				final			
experiment	pH replicates		E_{h7} (mV) replicates		pH replicates		E_{h7} (mV) replicates	
R	2.5	2.5	488	517	2.3	2.4	547	506
S	2.6	2.5	483	494	4.7	4.5	−138	−140
SG	2.6	2.6	488	494	3.9	4.3	−369	−274
SP	3.2	3.1	362	394	5.5	5.1	−159	−54
SL	4.0	4.0	326	341	3.8	3.8	311	272
SA	4.8	4.8	348	356	4.3	4.4	−369	−366
SE	2.7	2.5	490	490	5.6	5.8	−115	−98

[a] For abbreviations see Table 2.

TABLE 5. Initial and Final (8 Weeks) pH and E_{h7} for the Microcosms Loaded with Wheat Straw and Economical Agricultural Byproducts as Carbon Sources[a]

	initial				final			
experiment	pH replicates		E_{h7} (mV) replicates		pH replicates		E_{h7} (mV) replicates	
SW	2.6	2.7	467	482	4.5	5.0	−76	−112
SM	2.9	3.0	460	470	2.9	3.9	317	−177
SC	3.6	3.0	466	526	6.2	6.0	−248	−205
SPfezi	2.8	2.6	464	497	6.7	6.8	−102	−143

[a] Abbreviations see Table 2.

SA) was lowered down to 10% of the initial value of the reference microcosm (R). The addition of all the other amendments influenced initially the pH and therefore the hydrochemistry in a quiet simplified manner (Tables 4 and 5).

After 8 weeks the treated microcosms developed an environment characterized by lower E_{h7} (Tables 4 and 5). In all microcosms almost oxygen-free conditions were achieved. All these observations indicated strong respiratory microbial processes. The redox potential (E_{h7}) in nearly all treated microcosms was lower than 0 mV compared to the reference microcosm. The pH of the microcosm SL did not follow this trend. Here, the highest redox potential ($E_{h7} = 311$ mV) was registered after 8 weeks. The lowest E_{h7} were achieved in the microcosms SG and SA, respectively. But in both experiments a pH lower or equal to 4.3 on average was measured in the two replicates.

Contrary to the initial conditions in the untreated reference system (R) the pH of both replicates was lowered at an average of 0.15. This is possibly due to deposits of not further specified iron hydroxides and/or iron hydroxosulfates which were observed at the glass partition of the batch flasks (*36*). Most of the treated microcosms showed a pH more largely or equal to 4.3 (Tables 4 and 5). An exeption were the microcosms containing straw and glucose (SG), lactate (SL), acetate (SA), or molasses (SM). With glucose and straw in one replicate the pH-value increased only to 3.9. By using straw and lactate a pH-value of 3.8 was measured each. Compared to the initial values the pH of the microcosms SL and SA were lowered slightly as a result of the combination of substrate decomposition and chemical balances. High pH-values above 5 appeared by using straw in combination with pyruvate (SP) or ethanol (SE).

Beside pure carbon sources more economical agricultural byproducts were used. With the exception of straw and molasses (SM) in these microcosms a pH higher than 4.3 could be received (Table 5). The microcosms with straw and Pfezi-granular (SPfezi) and straw with Carbokalk (SC), respectively, produced the highest pH above 6 on average. Like the microcosm SE the E_{h7} indicated the typical zone for sulfate- and iron reducing processes.

Changes in Composition of Aqueous Phase. Corresponding to increased pH and decreased E_{h7} changes in concentrations of dissolved constituents were observed. Aqueous concentrations of T-Fe (up to 95% as ferrous species), sulfate, TOC, TIC, Al, and Zn as well as alkalinity and acidity are given in Figure 2. Generally the results showed that in the microcosms SP, SE, SC, and SPfezi the highest pH were achieved and alkalinity could be generated. In this view the microcosms SG, SL, SA, and SM were least active, and S and SW took an intermediate position.

Sulfate removal of noticeable success was observed only in the microcosms SE and SPfezi. The final sulfate concentrations were below 3 mmol L^{-1}. The black color of the whole batch flasks with the mixture SE suggests that sulfate removal was the result of microbial sulfate reduction. For the microcosm SPfezi the black color was only observed in the area of the sediment/water interface just as for the microcosm SP (*36*).

Compared to the other agricultural byproducts, the Pfezi-granula of the microcosm SPfezi reacted differently due to the content of cement (50 wt %). The highly dissolved calcium concentration (approximately 23 mmol L^{-1}) in the microcosm SPfezi indicated the dissolution of slaked lime ($Ca(OH)_2$). This proton consuming chemical dissolution dominated the microbial process. The concentrations of iron, aluminum, and the micronutrients were lowered near their detection limits and the acidity decreased below 1 mmol L^{-1}. However, the large rapid decrease of acidity as a result of the chemical behavior made use of this carbon source impractical to control microbial processes. Therefore microcosm SPfezi was eliminated for further upscaling.

Carbokalk and ethanol could be more suitable carbon sources. The acidity consumption and alkalinity generation in the microcosm SC using straw and Carbokalk was comparable to the microcosm SE using straw and ethanol. Concentrations of TIC, TOC, dissolved iron, aluminum, and micronutrients were in the same order of magnitude. However, there was a big difference in the dissolved concentration of sulfate. The sulfate content in the microcosm SC (19.3 mmol L^{-1}) was even above the initial values compared to most of the other microcosms. It seems that the microbial sulfate reduction within the microcosm SC was lower than within the microcosm SE. But this assumption will not explain the black color of the whole microcosm SC and the concentration of dissolved iron in SC. In addition to organic substances Carbokalk contains 7.49 mmol g^{-1} TIC (dry matter) mainly bound in calcium and magnesium salts. The dissolution of Carbokalk supported the increase of pH in the sediment more strongly as in the microcosm SE which might have resulted in the transformation of jarosite or schwertmannit into goethite (*48*) and the separation of mineral bounded sulfate. Jarosite, schwertmannite, and goethite are typical minerals of acid mine drainage (*46*, *48*) as well as of AML sediments in the Lusatia area (*49*−*51*). Furthermore the desorption of adsorbed sulfate from mineral surfaces represents an additional source for sulfate resulting in higher dissolved sulfate concentrations (*52*−*54*). The corresponding iron concentration between the microcosm SE and SC is restored to an increasing supply of bonding partners for the ferrous iron in the microcosm SC. Taking into account that strongly increased TIC iron carbonate (siderite) could form in addition to iron sulfides (amorphous FeS, mackinawite, or pyrite). In total 151 mmol L^{-1} TIC (60 g fresh Carbokalk) was inserted into the microcosms. We calculated that 5.18% were dissolved during the experiment based on the increased concentrations of dissolved calcium and magnesium.

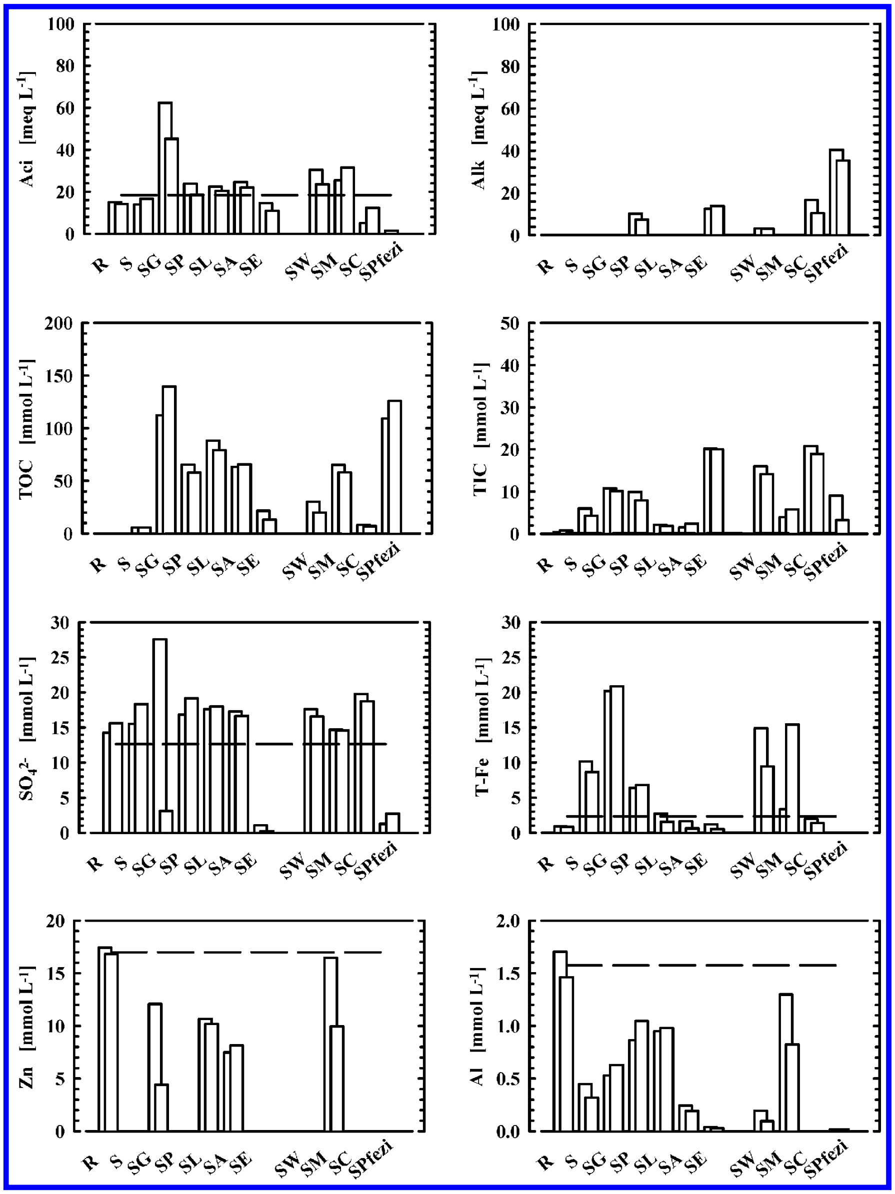

FIGURE 2. Final concentrations of acidity (Aci), alkalinity (Alk), total organic carbon (TOC), total inorganic carbon (TIC) and dissolved sulfate (SO_4^{2-}), dissolved total iron (T-Fe), dissolved aluminum (Al) and dissolved zinc (Zn) for the reference (R) and the treated microcosms (S, SG, SP, SL, SA, SE, SW, SM, SC, SPfezi; abbreviations see Table 2). Dashed lines − initial concentrations of the reference (R) given in Table 2. DOC and DIC concentrations are in the 90−95% range of TOC and TIC contents.

Importance of Wheat Straw. Dried plant material, like wheat straw, consists of about 90% lignins, cellulose, and lipids (*55*). Therefore, it is not an ideal electron donor. In the treatment of acidic mining wastewater different lignin- and cellulose containing plant materials such as straw and hay (*29*), mushroom compost (*12*), and sawdust (*28*) were tested as electron donors for iron and sulfate reduction. Except for the treatment with mushroom compost an increase of pH and a permanent activity in iron and sulfate reduction was observed only in combination with easy biodegradable substances, such as lactate or glucose. The reason for this is seen in the disturbed evolution of anaerobic microorganisms caused by an exclusive dosage of untreated biomass inhibitors of hydrophobic nature (*30*) and the unfavorable C/N ratio of nitrogen poor substrates such as wheat straw (*29*). Therefore, straw appeared as a low-reactive material that supports functionally the development of anaerobic biofilms.

In our experiments wheat straw alone (C/N ratio 93/1) was not an exceptional substrate. Together with the dissolved ammonia in the lake water a C/N ratio of 42/1 resulted by adding wheat straw into the microcosm. Within 8 weeks the C/N ratio stopped with 10/1 and 6/1, respectively (*36*). This C/N ratio together with the concentration of dissolved TOC (5.23 mmol L^{-1} on average) yielded ideal limiting conditions for the iron- and sulfate reduction (*29*, *37*, *38*, *56*, *57*). Nevertheless, with regard to C-dissimilation microcosm S belonged to the group of the less active microcosms SG, SL, SA, SM, and SW. Together with the carbon sources ethanol and Carbokalk a synergistic effect could be observed (Table 6). Therefore we suggest that straw supports the evolution of microbial communities mainly physical in the active microcosms SP, SE, SC, and SPfezi. The role of the physical support of wheat straw was also described for water denitrification in an up-flow reactor (*58*). Here, the water velocity played an important role in the denitrification performance of the system. The decrease in efficiency at higher velocities included wash-out of bacteria beside wash-out of extracellular enzymes and solubilized carbon sources.

Synergistic Effects of Carbon Source Mixtures. The synergistic effect combining carbon sources with wheat straw was seen clearly by replicated experiments of microcosms treated with ethanol or Carbokalk with and without straw.

TABLE 6. Final Aqueous Composition for the Microcosms Treated with Ethanol or Carbokalk with and without Wheat Straw

	(mmol L^{-1})			
	ethanol		Carbokalk	
	+straw	−straw	+straw	−straw
Fe(II)	0.68	4.17	nd[a]	0.68
SO_4^{2-}	2.22	21.5	16.7	21.0
H_2S	0.33	nd[a]	3.68	nd[a]
TIC	23.5	2.04	35.4	18.7
TOC	14.0	49.8	8.3	0.63
pH	5.9	3.0	6.5	6.3
E_{h7} (mV)	−43	369	−152	190
Aci (mequiv L^{-1})	17.1	19.4	6.94	9.51
Alk (mequiv L^{-1})	11.3		20.2	9.23

[a] nd, not detectable.

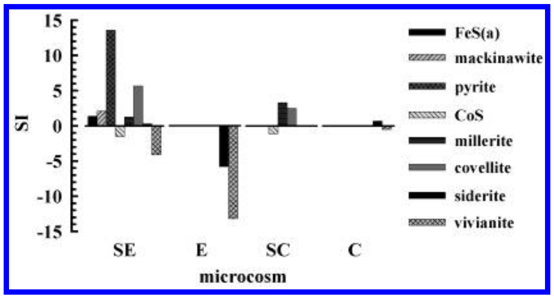

FIGURE 3. Saturation indices (SI) of the key minerals for the microcosms treated with ethanol and Carbokalk with and without wheat straw.

The results are given in Table 6.

A pH-value of 3.0 was achieved with exceptional addition of ethanol to the microcosm. But no black metal sulfide precipitants could be observed in the water phase macroscopically. Merely the sediment changed to gray color. Black spots and a slight deep black sediment/water interface could be observed. This explains iron and sulfate reducing processes that remained limited to the sediment. Not bound iron and sulfate were transported into the water. The dissolved sulfate and iron contents were 21.5 mmol L^{-1} and 4.17 mmol L^{-1}, respectively, clear above the initial values and above those of the microcosm SE. Compared to the microcosm SE the TOC concentration of 49.8 mmol L^{-1} was three times higher, and the TIC concentration of 2.04 mmol L^{-1} met only approximately 10% of the values of the microcosm SE. No alkalinity generation could be observed in the water phase.

With an exceptional addition of Carbokalk the microcosm reached a pH of 6. No metal sulfide deposits were optically observed. Between the Carbokalk and the lake sediment a slight deep black layer had developed. Compared to the ethanol experiment the concentration of dissolved sulfate increased very strongly (21.3 mmol L^{-1}), whereas the TOC content remained very low (0.63 mmol L^{-1}). The concentration of TIC achieved 50% of the value of the microcosm SC. The same effect was observed in alkalinity generation. We suggest that the corresponding small dissolved ferrous iron content in the experiment without straw was the result of siderite. The geochemical model showed that the solution was slightly supersaturated with respect to siderite (Figure 3, $SI_{siderite} = 0.7$).

It can be summarized that an additional acid buffer capacity of about 10 mequiv L^{-1} represents the synergistic effect. The acid buffer capacity was raised to about 50% in the microcosm SC. In the microcosm SE acid buffer capacity was only produced with a mixture of ethanol and wheat straw.

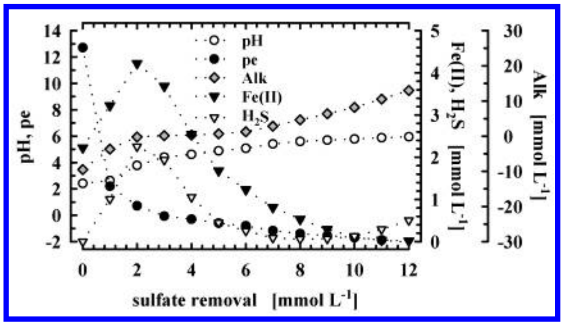

FIGURE 4. Irreversible batch reaction modeling: Concentrations of dissolved hydrogen sulfide (H_2S) and total ferrous iron (Fe(II)), pH, pe, and alkalinity (alk) as a function of sulfate removal; pe = ($nF/2.303RT$) E_h.

The microcosms SE and SC showed the most efficient results also under economical aspects. Therefore, these microcosms were selected for upscaling experiments in microcosm studies of 88 L column of which the results will be presented in a following paper. Compared to ethanol the Carbokalk has the advantage of an inherent buffer capacity resulted from the carbonates.

Geochemical Modeling. First, in the most successful microcosms SE and SC and their equivalents without wheat straw respectively, the saturation indices (SI) of the water phase were calculated (Figure 3). Second, the water composition after sulfate reduction was calculated exemplary using irreversible batch reaction capabilities of PHREEQC (Figures 4 and 5). In total both calculations processes, which controlled the aqueous ion concentrations, could be recognized without expensive geochemical analysis of mineral phases.

The aqueous sulfide concentrations and the black color of the microcosms SE and SC indicated metal sulfide precipitation. In fact the water of the microcosm SE is supersaturated with respect to amorphous ironsulfide $FeS_{(a)}$, mackinawite (FeS), millerite (NiS), and covellite (CuS). We suggest that these widespread sulfide phases controlled the concentrations of Fe, Ni, and Cu. For sphalerite SI values could not be calculated since the Zn concentration was below the detection limit. In the microcosm SC the Zn and Fe concentrations were also below the detection limit. A supersaturation to metal sulfides was calculated for the mineral phases millerite and covellite controlling the concentration of Ni and Cu. The high supersaturation for sulfide minerals indicated a tendency for further metal sulfide precipitation. In the microcosms E and C H_2S was not detected. The SI values for these two microcosms were calculated with respect to carbonate and phosphate mineral phases. Siderite ($FeCO_3$) and vivianite ($Fe_3(PO_4)_2$) are common in anoxic sediments (*59*, *60*). In the microcosm C the SI value of siderite was 0.7. The solution is in equilibrium to siderite with a tendency to a slight supersaturation. This observation and the lowered iron content in the water compared to the initial value suggest that the Fe concentration was controlled by siderite precipitation. The carbonate concentration is an important factor for the siderite precipitation. In the microcosm C dissolution of carbonates from the Carbokalk has occurred. The solution was slightly undersaturated with regard to solid carbonates ($SI_{calcite} = -0.2$).

To simulate microbial reduction processes in pit lake water we subtracted sulfate and added sulfide and carbon dioxide irreversibly. Iron reduction in the water phase was calculated by subtracting ferric iron and adding ferrous iron. The ferrous iron flux controlled by microbial reduction processes in the sediment was considered with an additional transfer of ferrous iron to the aqueous solution. We used a positive/negative transfer of sulfate, sulfide, carbon dioxide, ferric,

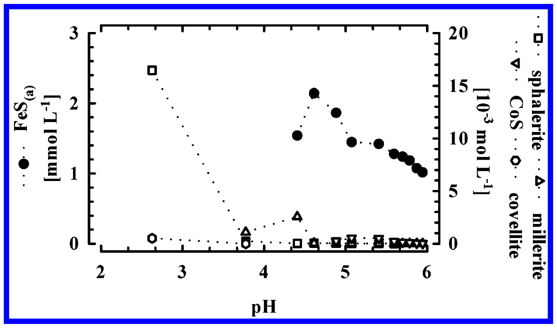

FIGURE 5. Irreversible batch reaction modeling: Amount of the precipitated FeS$_{(a)}$, sphalerite, covellite, millerite, and CoS as a function of pH given in Figure 4 as a result of subsequent sulfate removal.

and ferrous iron to pit lake water from the AML 111 in 12 varying amounts (12 mmol L^{-1} in 12 steps). The relative proportion or molar ratio between the reactants followed the global reaction outlined in Figure 1. The relative ratio of sulfate, sulfide, carbon dioxide, and ferrous iron were −1, 1, 4, and 1. The widespread metal sulfides such as amorphous iron sulfide (FeS$_{(a)}$), sphalerite (ZnS), millerite (NiS), CoS, covellite (CuS), and calcite ($CaCO_3$) were allowed to precipitate to equilibrium (SI = 0). The carbon dioxide partial pressure was raised with respect to closed system modeling.

The calculation results for the final relative ratio given above are outlined as a function of sulfate removal (Figure 4) and the pH (Figure 5). With the start of sulfate removal (*x*-axis, Figure 4) hydrogen sulfide concentration was raised continuously. Identical is the trend of the iron concentration as a result of the iron reduction from the solid phase. The pH and the alkalinity increased continuously and the pe (calculated from E_h) fell drastically to zero. With further formation of hydrogen sulfide supersaturation of most of the metal sulfides mentioned above occurred (data not shown). The precipitation of covellite (CuS), sphalerite (ZnS), and millerite (NiS) controlled the concentrations of Cu, Zn, and Ni. As a result the sulfide and metal concentrations were lowered in the aqueous phase. With further sulfate removal the mineral phases CoS and FeS$_{(a)}$ precipitate quantitatively from the aqueous phase. The precipitation of molar amounts of Cu, Zn, Ni, Co, and Fe from the aqueous is outlined as a function of pH (Figure 5). After sulfate removal of 12 mmol L^{-1}, the solution had a pH of 5.94. The water chemistry is comparable to the microcosm treated with ethanol and wheat straw (Table 6). However the molar ratios between sulfate, sulfide, carbon dioxide, and iron used in that model differed from the global equation in Figure 1. This equation summarizes though the microbial sulfate reduction in the aqueous solution (*17*−*19*) as well as the microbial (*19*, *20*) and chemical (*17*−*19*) iron reduction processes but does not represent the carbon and mineral components of our microcosm system.

It will be an interesting task in the future to complete this global equation with regard to the mineral composition of the AML sediment and the most efficient carbon source mixtures wheat straw/ethanol and wheat straw/Carbokalk. Furthermore the verification of applicability of the presented remediation strategy in an open system microcosm which represents a 1.5 m water column will be the next step on the way for a case study in the AML 111.

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