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TREATMENT OF ACID MINE WATER BY SULFATE-REDUCING BACTERIA; RESULTS FROM A BENCH SCALE EXPERIMENT

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Abstract—Overflowing water from waterfilled mines and open pits in pyrite and coal mine areas is often acid and severely polluted by metals and sulfate. The effect of stimulating bacterial sulfate reduction in such systems in order to improve water quality was examined in a bench scale experiment. Polyacrylate cylinders with a sand bed and a layer of crushed stones at the bottom were filled with acid mine water, supplied with whey to support bacterial growth and incubated for 203 d. After an initial lag phase, pH started to increase, redox potentials dropped and dissolved concentrations of copper, zinc, iron and aluminium were reduced in cylinders supplied with whey. Simultaneously, a black sludge was formed. Acetate tended to accumulate as an undesired waste product. The observations indicate that sulfate-reducing bacteria (SRB) became active in the cylinders and contributed to metal removal. Inoculation with SRB shortened the initial lag phase, but was not necessary to initiate the sulfate-reduction process. The *in situ* treatment concept may be useful in relatively small and well defined mine water systems, where the process can be governed properly. Copyright © 1996 Elsevier Science Ltd

Key words—sulfate-reducing bacteria, acid mine drainage, pyrite mines, sulfide precipitation, whey, anaerobic water treatment

INTRODUCTION

Acid mine drainage (AMD) is one of the most serious environmental problems facing the coal- and metal-mining industry. AMD is formed when sulfide ores undergo chemical and biological oxidation processes, and is characterized by low pH-values and high levels of sulfate and metals. The oxidation of pyrite, which is the most abundant sulfide mineral, can be summarized by the following equations (Stumm and Morgan, 1981):

$$FeS_2 + 7/2O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (1)$$

$$Fe^{2+} + H^+ + 1/4O_2 \rightarrow Fe^{3+} + 1/2H_2O \quad (2)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (3)$$

Chemolithotrophic bacteria like *Thiobacillus ferrooxidans* oxidize ferrous iron (equation 2) at low pH-values, thereby increasing the overall reaction rate drastically (Stumm and Morgan, 1981). They may also attach to solid ore surfaces and attack sulfide minerals directly (Lundgren *et al.*, 1986).

The main sources of AMD in abandoned mine areas are usually old waste rock dumps and rock walls in tunnels and shafts. Open pits and underground workings will often be filled partly or completely with polluted water after the closure of a mine, and overflow from such systems may in some cases contribute significantly to the total transport of pollutants out of the area.

In Norway, copper is the most serious toxic element in mine drainage. Concentrations have periodically reached levels critical to the survival of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) in large recipient watercourses (Grande, 1991). AMD is a long term environmental problem since the oxidation processes may continue for decades or even centuries after the closure of a mine.

Tuttle and co-workers (1969) suggested to use sulfate-reducing bacteria (SRB) for mine water treatment by adding an organic waste product which stimulate their activity. Oxidation of an organic compound (CH_2O) coupled to sulfate-reduction and precipitation of metal cations (Me^{2+}) is summarized below.

$$2CH_2O + SO_4^{2-} + H^+ \rightarrow 2CO_2 + 2H_2O + HS^- \quad (4)$$

$$Me^{2+} + HS^- \rightarrow MeS + H^+ \quad (5)$$

Laboratory and pilot scale tests in bioreactors and artificial wetlands have proved that sulfate-reduction is effective in raising pH and removing metals and sulfate from mine waters (Hammack and Edenborn, 1992; Dvorak *et al.*, 1992; Hedin *et al.*,

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1989). A commercial scale SRB-reactor for the removal of zinc and sulfate from contaminated groundwater was constructed recently in the Netherlands after obtaining promising results with the process in laboratory and pilot scale experiments (Barnes *et al.*, 1991; Scheeren *et al.*, 1991).

A mine water treatment concept which has received little attention so far is *in situ* treatment in waterfilled underground workings and open pits by the use of SRB. The idea is to stimulate bacterial growth and metabolism directly in the contaminated water body and use the mine itself as a sedimentation basin for metal sulfide sludge formed by precipitation (Kuyucak *et al.*, 1991; Kuyucak and St-Germain, 1994). To further address this concept, we conducted a bench scale treatment experiment which is reported here. The main objectives were to find out whether an active SRB-population could be established in a mine water system supplied with whey and to what extent sulfate-reduction influenced water chemistry. The effects of substrate concentration and type of inoculum on the treatment efficiency was also tested.

The experiment was conducted at Løkken Works in central Norway. This former copper-zinc mine was closed in 1983, and a 6,000,000 m^3 underground mine (Wallenberg mine) was gradually filled with water over the next 9 yrs. The mine started to overflow in 1992. In order to reduce concentrations of metals and sulfate in the overflowing mine water, *in situ* treatment by sulfate-reduction has been considered as one possible treatment option.

MATERIALS AND METHODS

Experimental set-up

Transparent polyacrylate cylinders (inner diameter 20 cm, height 100 cm) with a 7 cm layer of quartz sand and a 25 cm layer of crushed gabbro stones at the bottom were used as mine water treatment vessels (Fig. 1). Quartz sand was taken from an AMD-affected stream in the mine area, and the crushed gabbro stones were from a local stone quarry. The mine water used in the cylinders was a mixture of 90% moderately polluted water from the Wallenberg mine and 10% heavily polluted drainage water from Gammelgruva ("The Old Mine"). Since the water from the Wallenberg mine had to be pumped up from a level 120 metres below ground level, it was not possible to avoid aeration. The reason for mixing two mine waters in the cylinders, was because similar mixing occurs naturally in the Wallenberg mine when drainage water from Gammelgruva and other parts of the old mining areas enter the system.

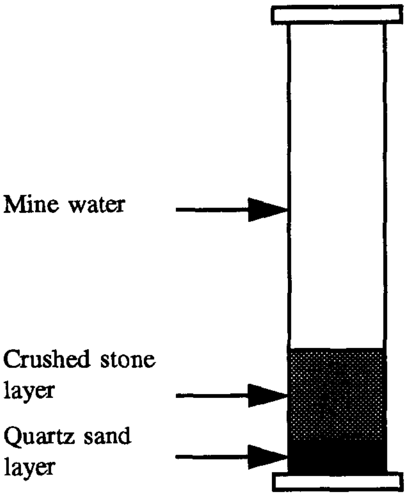

Fig. 1. Polyacrylate cylinder used in mine water treatment experiment.

Table 1. Experimental set-up of mine water treatment experiment

Cylinder designation	Amendments
W1	5.7% whey
W2	5.7% whey
W LD	0.2% whey
WCM 1	5.7% whey, 2.3% cow manure
WCM 2	5.7% whey, 2.3% cow manure
WSRB 1	5.7% whey, 4.5% SRB-culture
WSRB 2	5.7% whey, 4.5% SRB-culture
Control	No amendments

Inoculum and whey were added to the cylinders during set-up to initiate and support bacterial growth (Table 1). The abbreviations listed in Table 1 will hereafter be used when referring to the cylinders. Whey was obtained from a local dairy (Østlandsmeieriet, Dovre). Each cylinder was supplied with 1250 ml whey, except WLD which was supplied with 50 ml whey and the control which had no whey. Whey has been previously found to contain 50 g lactose, 6 g proteins, 6 g ash and 0.5 g fat per litre (Zadow, 1986).

Cow manure obtained from a local farm was used as inoculum in cylinders WCM 1 and 2. Cow manure has been previously found to contain SRB (Reynolds *et al.*, 1991). Each cylinder was supplied with 500 ml. The manure was partly mixed into the bottom sand bed and partly suspended in the water phase of the cylinders.

A mixed sulfate-reducing culture grown on lactose in a mineral salt medium (Widdel and Pfennig, 1981) was used as inoculum in cylinders WSRB 1 and 2. The mixed culture contained approximately 5×10^7 fermenting bacteria (strain Rb-k) per ml and 5×10^7 SRB (strain L-200) per ml, as determined by direct cell counting in a phase contrast microscope. Each cylinder was supplied with 1.0 litre mixed culture inoculum. Strain Rb-k was originally isolated from sediments in Raubekken stream, which receive AMD from the Løkken area, while strain L-200 was isolated from the waterfilled Wallenberg mine (Christensen *et al.*, 1994).

After addition of whey and inoculum, all the cylinders were sealed with gas-tight rubber O-rings and polyacrylate lids and incubated at 15°C in darkness for 203 d. Microscope slides coated with a thin layer of 2% Bacto-agar (Difco Lab., Detroit, MI, U.S.A.) were placed on top of the crushed stone layer at the start and retrieved at the end of the experiment for examination of biofilm formation. Holes plugged with butyl rubber stoppers in the cylinder walls allowed water samples to be taken from the free water phase and from interstitial water in the crushed stone layer and the sand bed during the experiment.

Sampling and analyses

Water samples (250 ml) were collected on days 0, 19, 54, 99 and 203. To obtain representative samples from the water phases on day 0, precipitates were allowed to settle for approximately 2 h before sampling. During the experiment, most samples were taken from the free water phase of the cylinders, since we wanted to monitor the effect of the sulfate-reduction process on the whole water body. Only a few spot samples were taken from the sand bed and the crushed stone layer. Redox potentials were measured immediately after sampling on days 19, 99 and 203, using a platinum electrode paired with a Ag/AgCl-reference electrode. pH was routinely measured for all the samples immediately after sampling. Unfiltered samples fixed with 1% (vol/vol) 7 N HNO_3 were analysed for metals and sulfur by inductively coupled plasma spectroscopy (ICP) at Landbrukets Analysesenter, 1432 Ås-NLH, Norway. Sulfide was measured spectrophotometrically as CuS in $CuSO_4$-HCl reagent at 480 nm (Cord-Ruwisch, 1985). Sulfate concentrations were calculated from the total sulfur content, assuming that the concentrations of other sulfide species in solution were negligible. Total organic carbon (TOC) was analysed in 100 μl samples on a Dohrmann DC-190 (Santa Clara, CA, U.S.A.) equipped with an IR-detector. Ammonium, nitrate and phosphate were analysed colorimetrically. Water samples for determination of total acidity and total alkalinity were brought to the laboratory in completely filled 50 ml serum bottles sealed with butyl rubber stoppers. Aliquots were titrated with 0.1 N NaOH to pH 8.3 to determine acidity, and with 0.1 N HCl to pH 4.5 to determine alkalinity. Total acidity and alkalinity were calculated according to Standard Methods (APHA, 1985). Volatile fatty acids (VFA) were analysed on a Hewlett-Packard 5890 Gas Chromatograph fitted with a fused silica capillary column and a Flame Ionisation Detector. Total bacterial counts were determined by EPI-fluorescence microscopy, using, 4,6-diamidino 2-phenylindole (DAPI) (Porter and Feig, 1980) for visualization of cells on 0.22 μm Nucleopore® filters.

The geochemical equilibrium model MinteqA2 (version 3.10), developed by the U.S. Environmental Protection Agency, Athens, GA, 1991, was used to aid in the interpretation of water chemical data.

RESULTS

The chemical composition of mine waters from Wallenberg mine and Gammelgruva, which was mixed 9:1 in the cylinders during set-up, is shown in Table 2. The water from Wallenberg mine was colourless, while the water from Gammelgruva had a reddish colour which indicated that soluble ferric iron species were present. Oxygen was present in all of the cylinders initially, as no attempts were made to avoid exposure to air during set-up. Yellow precipitates formed immediately after mixing of the two mine waters. In order to identify precipitated elements, we estimated theoretical start concentrations of metals and sulfate in the mixed mine water and compared these to measured dissolved concentrations (Table 2). It was found that Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+} and sulfate had been partly removed from the water phase by precipitation. The geochemical equilibrium model MinteqA2 indicated oversaturation of ferric iron minerals (goethite, lipidocrocit, jarosite, hematite) and gypsum, but not Mg^{2+}, Zn^{2+} or Mn^{2+}-compounds, in the mixed mine water.

Whey gave rise to a greenish colour in the water phase of the cylinders and cow manure to a dark brownish colour in WCM 1 and WCM 2. Both whey and the two types of inoculum increased pH in the water phase, as can be seen by comparing measured start values in the control cylinder with the amended cylinders (Table 2). Start concentrations of copper were lower in WSRB 1 and 2 than in other cylinders because of the content of sulfide in the SRB inoculum, which removed copper by precipitation during set-up.

During the experiment, black precipitates were formed in all of the cylinders supplied with whey. The precipitates appeared first at the interface between the sand layer and the crushed stone layer, and the black zone expanded upwards gradually. Above the crushed stone layer, the colour change was the result of the deposition of a black film on the inner surface of the polyacrylate walls, while the water phase itself was nearly colourless. The dividing line between the black zone and the unaffected water body above was always very distinct, indicating a stable stratification in the cylinders. Sporadic temperature measurements in the incubation room revealed a vertical temperature gradient, which probably contributed significantly to the stabilization of the water columns. The temperature gradient ranged from approximately 14°C at floor level to 19°C close to the ceiling.

Black precipitates were first seen in WSRB 1 and thereafter in WSRB 2, W1 and W2. All these cylinders became completely black over the course of the study. Towards the end of the experiment, black precipitates were also seen in WCM 1, WCM 2 and

Table 2. Water chemical parameters measured in mine water from Wallenberg mine and Gammelgruva, and start concentrations (theoretical and measured) in cylinders supplied with whey (W), whey low dosage (WLD), whey + cow manure (WCM) and whey + SRB (WSRB). Theoretical start concentrations were estimated from individual mine water concentrations and the mixing ratio, omitting a possible precipitation. Measured concentrations were determined two hours after set-up. All concentrations are in mg l^{-1}

	Concentrations in mine waters			Start concentrations in cylinders after mixing 9:1				
				Measured				
Parameter	Wallenberg mine	Gammelgruva	Theoretical	Control	WLD	W*	WCM*	WSRB*
pH	5.52	2.40	3.40	3.08	3.05	3.71	4.22	4.37
SO_4	2940	6600	3306	1002	936	888	857	896
Ca	500	382	488	194	—	211†	220†	202†
Mg	350	212	336	56.1	—	58.8†	59.8†	55.1†
Al	1.38	263	27.5	28.3	26.1	21.9	18.8	17.7
Fe	139	1160	241	1.89	—	29.0†	26.3†	28.0†
Cu	1.45	99.2	11.2	10.7	9.88	8.84	7.63	4.52
Zn	33.5	89.2	39.1	11.7	10.8	9.80	9.41	8.62
Mn	13.6	11.7	13.4	1.90	1.75	1.78	1.89	1.62

* = Average of two parallel cylinders.
† = Measured at day 19.

Table 3. Initial and final nutrient concentrations in the water phase of the cylinders

	TOC (mg l^{-1})		NH_4^+ (mg l^{-1})		PO_4^{3-} (mg l^{-1})	
Cylinder	Day 0	Day 203	Day 0	Day 203	Day 0	Day 203
W 1	1160	1000	6.7	6.4	10.6	4.1
W 2	1140	740	6.7	4.0	10.8	5.3
W LD	43	2.7	0.2	0.2	0.5	<0.1
WCM 1	1240	853	11.4	8.6	8.3	10.0
WCM 2	1160	782	9.6	5.6	8.8	4.8
WSRB 1	1060	217	8.7	2.0	9.6	1.3
WSRB 2	1040	749	8.2	8.0	9.4	1.5
Control	4.5	4.6	<0.1	<0.1	<0.1	<0.1

WLD, but the black zone did not expand above the top of the crushed stone layer in these cylinders. No black precipitates were observed in the control cylinder without whey.

Temporal changes in water chemistry in the cylinders during the experiment are shown in Fig. 2. pH increased, redox potentials dropped and metals were removed from the water phase of W1, W2, WSRB 1 and WSRB 2. Residual concentrations of both copper and zinc were below the ICP detection limit of 0.05 mg l^{-1}. Dissolved iron concentrations increased temporarily and decreased again towards the end of the experiment. Calcium and sulfate concentrations increased in W1, W2, WSRB 1 and WSRB 2 when reducing conditions evolved. Water chemical changes were less pronounced in the other cylinders, although copper was removed effectively from WLD.

We found significant differences in water chemistry between the two parallel cylinders WSRB 1 and WSRB 2 throughout the experiment. In WSRB 1, black precipitates showed up earlier, metals were removed more rapidly from the water phase and the residual TOC-concentration was much lower than in WSRB 2. The findings indicate that mineralization was delayed in WSRB 2 compared to WSRB 1, but the biological and chemical processes which took place in the two cylinders were similar. We therefore averaged the results and plotted them as one curve in Fig. 2

Spot tests showed that interstitial waters in the sand layer and the crushed stone layer were markedly different from the overlaying water with respect to pH and metal concentrations. At day 19, pH in the sand layer of WCM 1 was 5.89, as opposed to 3.74 in the free water phase. At the same time, pH in the crushed stone layer of all cylinders supplied with 5.7% whey was 0.8–2.2 units higher than in the free water phase. However, the pH-differences became gradually smaller towards the end of the experimental period. At day 19, water from the sand layer of W1 contained per litre (percentage of initial concentrations in parenthesis): Cu 1.20 mg (13.6%), Zn 1.28 mg (7.7%), Fe 73.1 mg (262%), Al 50.7 mg (232%), Ca 503 mg (243%) and Mn 27.0 mg (1525%). At day 99, water from the crushed stone layer of W2 contained per litre: Cu 1.70 mg (19.2%), Zn 3.54 mg (36.2%), Fe 31.2 mg (104%), Al 8.80 mg (40.4%), Ca 518 mg (241%) and Mn 21.6 mg (1207%).

Total bacterial counts in the water phase of cylinders supplied with 5.7% whey were initially between 2.0×10^7 and 3.4×10^7 cells per ml. Coccoid bacteria, originating from the whey, was the predominant morphotype. Cylinders inoculated with SRB (WSRB 1 and 2) contained 1.3×10^6 vibrio-shaped bacteria (similar to strain L-200) per ml in addition to the coccoids. Throughout the experiment (days 54, 99 and 203), total counts ranged from 1.0×10^6 to 2.5×10^7 bacteria per ml in all cylinders supplied with 5.7% whey. Biofilm formation was observed on the agar-coated microscope slides retrieved from the top of the crushed stone layer at the end of the experiment. Typical vibrio-shaped bacteria, similar to strain L-200, were seen on slides from WSRB 1 and 2. These cells appeared to be partly embedded in the agar layer and were coated with dark precipitates.

Phosphate and ammonium concentrations decreased in the water phase of all cylinders during the experiment, but were still relatively high at day 203 (Table 3). Nitrate concentrations were consistently below 0.1 mg l^{-1}. Volatile fatty acids were found in all cylinders supplied with whey at the end of the experiment, and acetate was the most abundant compound (Table 4). The acetate concentration in

Table 4. Volatile fatty acids, sulfide, total acidity and total alkalinity in the water phase of the cylinders at the end of the experiment (day 203)

Cylinder	Acetate (mM)	Propionate (mM)	Butyrate (mM)	Sulfide (mM)	Acidity (mequiv l^{-1})	Alkalinity (mequiv l^{-1})
W 1	15.5	0.6	0.4	2.1	17.4	8.8
W 2	6.9	0.5	0.4	1.5	13.0	5.0
W LD	<0.3	0	0	0	5.2	0
WCM 1	4.5	0	2.1	0	15.6	0
WCM 2	4.1	0.6	1.4	0	13.6	1.8
WSRB 1	3.9	0	0	1.8	2.6	14.2
WSRB 2	14.4	0	0.7	4.1	17.2	16.6
Control	<0.3	0	0	0	5.0	0

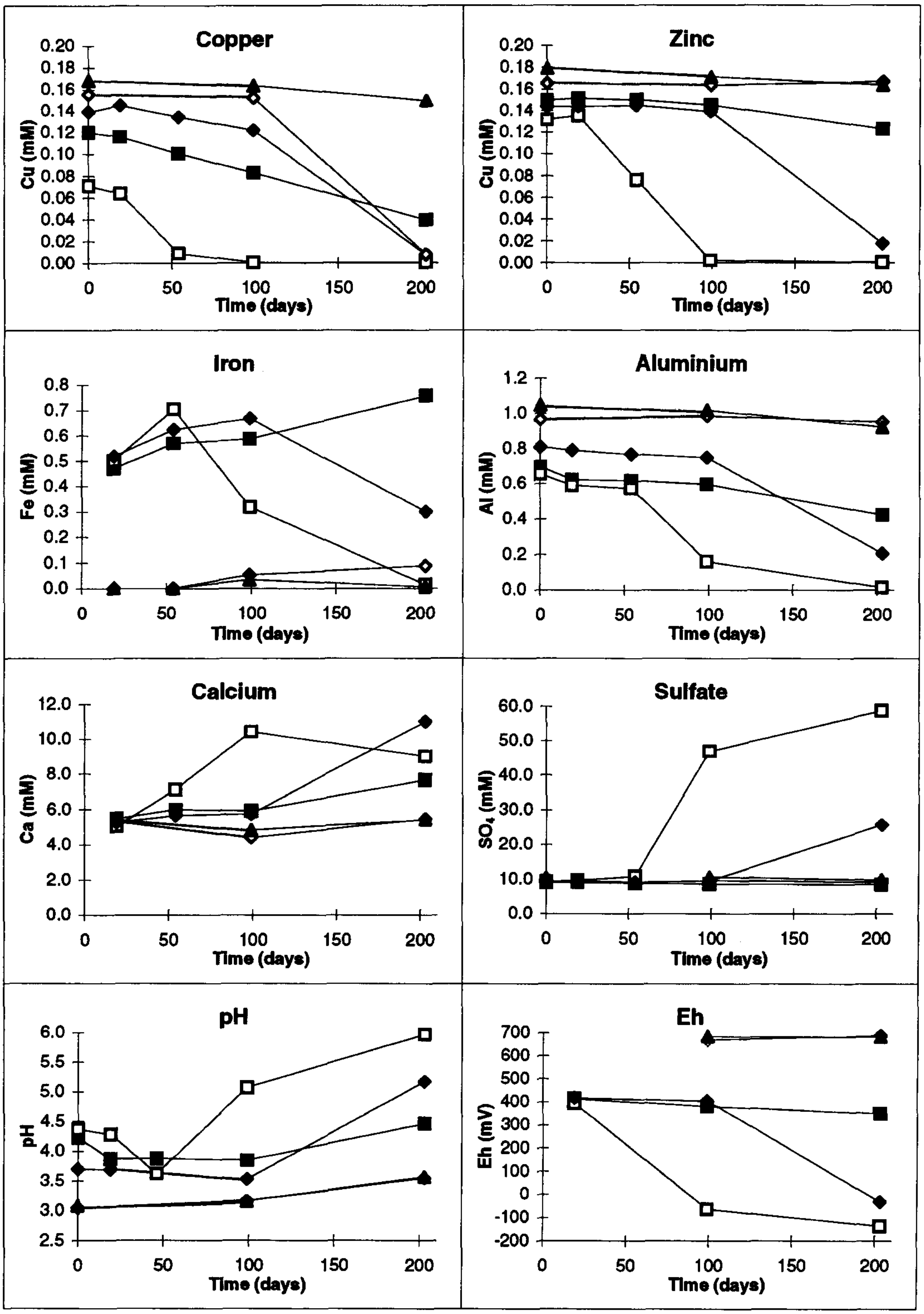

Fig. 2. Temporal variations of pH, E_h, metal and sulfate concentrations in the water phase of W1 and W2 (◆——◆), WLD (◇——◇), WCM 1 and WCM 2 (■——■), WSRB 1 and WSRB 2 (□——□) and the control cylinder (▲——▲) during the treatment experiment. Values from parallel cylinders are averaged. Average pH was calculated from average H^+-concentrations.

W1 was 15.5 mM, which corresponds to 37% of the remaining TOC. The water in WSRB 1 had a net alkalinity at the end of the experiment, while all others had a net acidity (Table 4).

Free sulfide was found in W1, W2, WSRB 1 and WSRB 2 at the end of the experiment (Table 4). Total sulfide production in the cylinders, and thereby sulfate consumption, can be estimated roughly by adding the molar concentrations of sulfide-precipitated metals and free sulfide. Sulfide-precipitated metals were estimated as the difference between theoretical start values and measured residual concentrations in the water phase at the end of the experiment. We found that the total sulfide production ranged from 6.4 to 9.3 mM in W1, W2, WSRB 1 and WSRB 2. This corresponds to the reduction of 614–893 mg l^{-1} sulfate, which is 19–27% of the theoretical start concentrations of sulfate.

Using the computer model MinteqA2, we found the water phases of W1 and W2 to be slightly oversaturated with respect to several minerals at the end of the experiment. Pyrite, sphalerite, covellite gypsum, diaspore and sulfur were expected to form as stable end products of precipitation under the given conditions.

DISCUSSION

A variety of chemical and biological processes are likely to take place and influence water chemistry when a mixture of mine waters is supplied with whey and inoculum. We found that rapid chemical precipitation processes occurred in all of the cylinders during set-up, and that these processes were responsible for greatly reducing the start concentrations of several metals and sulfate in the water phase. The computer model MinteqA2 predicted precipitation of ferric iron minerals and gypsum from the mixed mine water under the given conditions. It is not clear why also magnesium, zinc and manganese concentrations were reduced in the water phase simultaneously, but the most likely explanation is that oversaturation and precipitation occurred locally in the inhomogenous water phase during the mixing process.

Throughout the experiment, water chemical data and visual observations indicated that sulfate-reducing bacteria became active in the cylinders supplied with whey. The evidences include heavy metal removal from the water phase, decreasing redox potentials, formation of black precipitates and the presence of free sulfide in the water phase of cylinders W1, W2, WSRB 1 and WSRB 2 at the end of the experiment. Among the heavy metals, copper was always the first element to be removed, followed closely by zinc. CuS is highly insoluble over a broad pH-range and sulfide precipitation of copper ions is therefore a rapid and efficient process in acid mine waters. The temporal increase in iron concentrations which was observed in all of the cylinders supplied with 5.7% whey, was probably caused by the reduction of ferric precipitates formed during set-up (Kuyucak *et al.*, 1991). Whether ferric iron was reduced enzymatically by Fe(III) reducing bacteria (Lovley, 1991), sulfate-reducing bacteria (Coleman *et al.*, 1993) or chemically by sulfide produced by SRB cannot be determined from our data. Towards the end of the experiment, dissolved iron concentrations dropped drastically in cylinders supplied with whey and whey + SRB-inoculum. The relatively high sulfide concentrations found in these cylinders at day 203, strongly indicate that iron was removed by sulfide precipitation. A possible alternative iron precipitation process is the formation of siderite ($FeCO_3$) from ferrous iron and bicarbonate. However, since siderite has been reported to be unstable in the presence of sulfide (Coleman *et al.*, 1993), we find it unlikely that siderite was an important end product in our cylinders. Aluminium removal was probably caused by the formation of aluminium(oxy)hydroxides, as indicated by the geochemical equilibrium model MinteqA2. Both iron sulfide- and aluminium(oxy)hydroxide formation are strongly pH-dependent processes and did not become quantitatively important in the cylinders before the pH started to increase.

The increase in dissolved sulfate concentrations in W1, W2, WSRB 1 and WSRB 2 during the experimental period was unexpected and may also seem to be in contradiction with the findings that SRB were active in the systems and reduced sulfate to sulfide. It is not fully clear why the sulfate concentrations increased, but the most likely explanation is that an initial coprecipitation of ferric(oxy)hydroxides and sulfate occurred during set-up, and that these precipitates were dissolved when reducing conditions evolved. Coprecipitation of 1.5–4.7 moles of sulfate per mole iron in ferric(oxy)hydroxide precipitates was previously reported by Herlihy *et al.* (1989). Rough calculations indicate that less than 27% of the sulfate present at the start was reduced to sulfide over the course of the study, and it was therefore not surprising that a temporal increase in dissolved sulfate concentrations was seen.

We added a high dosage of whey (5.7%, vol/vol) to most cylinders to enable complete heavy metal removal. The presence of free sulfide and residual concentrations of VFA in the water phase of W1, W2, WSRB 1 and WSRB 2 at the end of the experiment showed that organic carbon was added in excess. The fact that acetate accumulated in the water phase over the course of the study indicates that fermentation and incomplete substrate oxidation proceeded much faster than complete oxidation under the prevailing conditions. The reason for this is not fully clear, but it is well known from enrichment studies that incompletely oxidizing SRB often grow faster than complete oxidizers (Widdel, 1988). The high concentrations of free sulfide found at the end of the experiment (40–150 mg l^{-1}) may also have inhibited bacterial acetate oxidation, since it was reported recently that 40 mg l^{-1} sulfide adversely affected acetate conversion rates by SRB in a laboratory reactor system built to treat sulfuric acid (Stucki *et al.*, 1993). The relatively high residual concentrations of TOC, sulfate and nutrients indicate that there was a potential for further sulfate-reduction in the cylinders.

The addition of SRB-inoculum to the cylinders had a positive effect on the mine water treatment process. The lag phase was shorter and heavy metals were removed earlier and more efficiently than in the other cylinders. In contrast, cow manure appeared to have a negative effect. The reason for this is not clear. Sulfate reduction was also found to take place in cylinders supplied with whey only. It seems most likely that SRB in these cylinders originated from the sand layer, although viable SRB previously have been found in mine water from the Wallenberg mine, which was used in the cylinders (Christensen *et al.*, 1994).

To improve growth conditions for SRB, we put a layer of sand and a layer of crushed stones at the bottom of the cylinders. A sand layer in the culturing vessel was previously found to be of vital importance for the sulfate-reduction process in laboratory scale AMD treatment experiments (Kuyucak *et al.*, 1991). pH increased and copper concentrations decreased in interstitial waters from the sand and crushed stone layers during an early phase of the experiment. Simultaneously, dissolved concentrations of iron and manganese increased drastically, indicating that reductive dissolution of oxides/hydroxides occurred. The finding that black precipitates started to occur at the interface between the sand layer and the crushed stone layer indicate that growth conditions were indeed more favourable than in the free water phase. The extensive biofilm formation on microscope slides kept at the top of the crushed stone layer during the experiment suggest that a large fraction of the bacteria was attached to solid surfaces. Attachment to surfaces has been shown to increase bacterial tolerance to cupric copper (Hicks and Rowbury, 1988) and oxygen tolerance among SRB (Fukui and Takii, 1990).

The results indicate that it might be feasible to stimulate bacterial sulfate-reduction *in situ* in waterfilled mines and open pits. Sulfide precipitation of heavy metals has some important advantages over hydroxide precipitation, including lower solubility of metal sulfides over a broad pH-range and higher sludge density. In conventional lime treatment plants, sludge handling costs may often exceed lime costs. In contrast, no sludge handling is necessary when using the *in situ* treatment concept, since the underground mines and open pits are both "bioreactors" and sedimentation basins. The amount of organic compounds added to a mine water system in order to stimulate the process should be balanced with the metal content and acidity to ensure proper metal removal and avoid a harmful load of excessive organic material and nutrients in the effluent. Such calculations are often difficult, however, because different water qualities may prevail in different parts of the system. Further, the organic carbon demand in a particular mine water system cannot be calculated from water-chemical parameters alone, since oxidized precipitates may act as a sink for sulfide ions and thereby increase the organic carbon demand. This was indeed the case in our experiment, where precipitates rich in ferric iron were present initially. It should also be kept in mind that remobilization of metals like iron and manganese may occur under anoxic and reducing conditions.

The *in situ* treatment concept may be useful in relatively small systems or in defined parts of larger systems where it is possible to monitor water chemistry and thereby govern the process properly. Contaminated seepage streams can be diverted into such system for metal precipitation and neutralization. As observed during our experiment, sediments and solid surfaces will tend to be colonized first by the sulfate-reducers and a high surface area to water volume ratio will probably be beneficial. Waste rock is occasionally back-filled in old underground mines. This increases the surface area to water volume ratio and might be favourable for bacterial growth.

CONCLUSIONS

Dairy whey stimulated bacterial sulfate-reduction when added to acid mine water in a bench scale treatment experiment. pH increased and heavy metals were removed from the water phase after on-set of the sulfate-reduction process. The results indicate that it might be feasible to use SRB for treatment of acid mine water *in situ* in waterfilled mines and open pits.

Inoculation of the experimental cylinders with SRB was not necessary to initiate sulfate-reduction, but shortened the lag phase significantly. Most of the bacterial activity took place in the crushed stone layer at the bottom of the cylinders, where growth conditions were found to be more favourable than in the free water phase.

In situ treatment by SRB is a possible low cost, low maintenance concept for acid mine water treatment. Sludge handling costs can be minimized or eliminated, since the mine is used as a sedimentation basin. The amount of organic compounds added to a mine water system should be balanced with the metal content and acidity to ensure proper metal removal and avoid a harmful load of excessive organic material and nutrients in the effluent. The *in situ* treatment concept may be particularly useful in small mine water systems where the sulfate-reduction process can be properly governed.

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REFERENCES

APHA (1985) *Standard Methods for the Examination of Water and Wastewater*, 16th edition. American Public Health Association, Washington D.C.

Barnes L. J., Janssen F. J., Scheeren P. J. H., Versteegh J. H. and Koch R. O. (1991) *Simultaneous microbial removal of sulfate and heavy metals from waste water*. Presented at the first European Metals Conference, Bruxelles, Belgium, 15–20 Sept. 1991.

Christensen B. A., Johannessen M. and Nygaard K. (1994) Biological fixation of metals in mine drainage and ore waste. Norwegian Institute for Water Research, technical report. ISBN 82-91374-24-4.

Coleman M. L., Hedrick D. B., Lovley D. R., White D. C. and Pye K. (1993) Reduction of Fe(III) in sediments by sulfate-reducing bacteria. *Nature* **361**, 436–438.

Cord-Ruwisch R. (1985) A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. *J. Microbiol. Methods* **4**, 33–36.

Dvorak D. H., Hedin R. S., Edenborn H. M. and McIntyre P. E. (1992) Treatment of metal-contaminated water using bacterial sulfate reduction: results from pilot scale reactors. *Biotechnol. Bioeng.* **40**, 609–616.

Fukui M. and Takii S. (1990) Survival of sulfate-reducing bacteria in oxic surface sediment of a seawater lake. *FEMS Microbiol. Ecol.* **73**, 317–322.

Grande M. (1991) Biological effects of metal pollution from the mining industry. Norwegian Institute for Water Research, technical report. ISBN 82-577-1878-5.

Hammack R. W. and Edenborn H. M. (1992) The removal of nickel from mine waters using bacterial sulfate reduction. *Appl. Microbiol. Biotechnol.* **37**, 674–678.

Hedin R. S., Hammack R. W. and Hyman D. M. (1989) Potential importance of sulfate reduction processes in wetlands constructed to treat mine drainage. pp. 508–514. In: D. A. Hammer (Ed.), *Constructed wetlands for wastewater treatment*. Lewis Publishers, Chelsea, MI.

Herlihy A. T. and Mills A. L. (1989) Factors controlling the removal of sulfate and acidity from the waters of an acidified lake. *Water Air Soil Pollut.* **45**, 135–155.

Hicks S. J. and Rowbury R. J. (1988) Col V plasmids and the resistance of *Escherichia coli* to divalent copper ions. *Letters in Appl. Microbiol.* **6**, 15–18.

Kuyucak N., St-Germain P. and Wheeland K. G. (1991) *In situ bacterial treatment of AMD in open pits*. Proc. Second Int. Conf. on the abatement of acidic drainage, Montreal, Canada, Sept., 16–18, 1991. Vol. 1, 335–354.

Kuyucak N. and St-Germain P. (1994) *In situ treatment of acid mine drainage by sulfate reducing bacteria in open pits: scale-up experiences*. Proc. Third Int. Conf. on the abatement of acidic drainage, Pittsburgh, PA, April 24–29, 1994. Vol. 2, 303–310

Lovley D. R. (1991) Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiol. Rev.* **55** (2), 259–287.

Lundgren D. G., Valkova-Valchanova M. and Reed R. (1986) *Biotechnology and Bioengineering Symp.* No 16, 1986. J. Wiley & Sons, Inc.

Porter K. G. and Feig Y. S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* **25**, 943–948.

Reynolds J. S., Bolis J. L., Machemer S. M. and Wildeman T. R. (1991) "Preprint extended abstract". Presented before the Division of Environmental Chemistry. Am. Chemical Society, Atlanta, GA, April 14–19, 1991.

Scheeren P. J. H., Koch R. O., Buisman C. J. N., Barnes L. J. and Versteegh J. H. (1991) New biological treatment plant for heavy metal-contaminated groundwater. Presented at the First European Metals Conf., Bruxelles, Belgium, 15–20 Sept. 1991.

Stucki G., Hanselmann K. W. and Hürzeler R. A. (1993) Biological sulfuric acid transformations: Reactor design and process optimization. *Biotechnol. Bioeng.* **41**, 303–315.

Stumm W. and Morgan J. J. (1981) Aquatic Chemistry, An Introduction Emphasizing Chemical Equilibria in Natural Waters. Second Edition. J. Wiley & Sons, Inc.

Tuttle L. H., Dugan P. R. and Randles C. I. (1969) Microbial sulfate reduction and its potential utility as an acid mine water pollution abatement procedure. *Appl. Microbiol.* **17**, 297–302.

Widdel F. (1988) Microbiology and ecology of sulfate- and sulfur-reducing bacteria. In: A. J. B. Zehnder (Ed.), *Biology of anaerobic microorganisms*, pp. 469–585. J. Wiley & Sons, Inc.

Widdel F. and Pfennig N. (1981) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. I. Isolation of new sulfate-reducing bacteria enriched with acetate from saline environments. Description of *Desulfobacter postgateii* gen. nov., sp. nov. *Arch. Microbiol.* **129**, 395–400.

Zadow J. G. (1986) Utilization of milk products: whey. In: R. K. Robinson (Ed.), *Modern Dairy Technology, Advances in Milk Processing*. Elsevier Science, New York.