Bioremediation of acid mine drainage using decomposable plant material in a constant flow bioreactor

M.A. Harris · S. Ragusa

Abstract Under stagnant conditions, the ability of 15 earth materials (non-lime) including various inorganic 2:1 and 1:1 layer silicates, an amorphous oxide, and two 'whole' soils were tested for their pH-buffering efficiency in an acid mine drainage (AMD) water. The purpose was to decrease AMD acidity to a level where sulfate-reducing bacteria (SRB) placed in it may be activated. Of all materials, a whole soil (a high cation-exchange capacity clayey mollisol containing 40% clay, and 4% soil organic matter) caused the greatest pH increases from 2.5 up to 5.5 units after 10 days in the AMD water. Influent AMD was then ameliorated at various speeds through an SRB driven bioreactor using a 50/50 weight over weight (w/w) combination of the mollisol and ryegrass (MR) as the pH buffer substrate. This substrate combination decreased the SRB acclimatisation period (from 50 days in a previous experiment utilising sludge + ryegrass) to <10 days in the present experiment. After causing pH increases from 2.8 to >6 units in 5 days, the buffer reduced the hydraulic retention time (HRT) of the constant-flow reactor from 12 days at flow speeds of 100 ml/day to 2 days at 25 ml/day, respectively. After 10 days, soluble Fe, Al and sulfate were all decreased >1,800-, >40- and 3-fold, respectively. This was a more efficient performance than the no-flow bioreactor of a previous experiment using sludge + ryegrass. This method of AMD rehabilitation is an alternative for localities that lack cheap sources of calcium compounds for chemical treatment, but have a similar soil type and copious quantities of fresh decomposable plant wastes.

Keywords Acid mine drainage · Bioreactor · pH buffering · Sulfate-reducing bacteria

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Introduction

Several methods have been applied to reduce the toxicity of drainage water from disused mine-sites. Using a gentle spray, James and Mrost (1965) have attempted to leach out soluble salts and oxidation products from pyritic material to relatively low water table, where they are fixed onto clay particles. However, such a measure does not always solve the problems of metal toxicity to plant life (Hore-Lacy 1978), especially where water tables rise in wet seasons. Most techniques involve the application of chemical precipitators such as calcium compounds. Precipitation of heavy metals by lime or limestone is one of the oldest and cheapest methods for removal of heavy metals from acid drainage solutions. Such measures have not been highly successful. Research has shown (Dean and others 1972) that Cu, Pb, Cd and Zn will begin to precipitate from dilute solutions at pH values exceeding 5.3, 6.0, 6.7 and 7.0, respectively. However, lime additions have not been found satisfactory for highly pyritic material as they are soon tend to form pans. The longevity of the oxidation process is calculated in decades rather than years (Elliott and others 1998) and sources of lime are not ubiquitous. Hore-Lacy (1978) observed that in one United States case where 2,500 tonne/ha of lime was added to the tailings at Bingham (the 'iron mountain'), the pH was back to a value of 3.5 after 3 months. Elliott and others (1998) concluded that the management of acid mine drainage should be realistically focused on prevention and control rather than the chemical treatment effluents. The present methods utilising lime are not cost-effective in the long term. Biological mitigation using sulfate-reducing bacteria (SRB) has been reported (Lyew and others 1994; Elliott and others 1998). Because substrates are necessary for the reduction process, costs may be prohibitive. For a microbial process to be economically feasible, the carbon and energy source should be cheap, widely available and highly

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effective (Hard and others 1997). Citing the high cost of (commercially available) organic acids as an SRB energy source, Elliott and others (1998) suggested further research into the use of an alternative carbon source in column bioreactors for potential large-scale use.

In South Australia, organic wastes comprise almost half of the annual output of household rubbish; a total of 97,546 tonnes (Turner 1993). A large fraction of this is cut grass that is wasted through the process of decomposition. Readily decomposable and finely ground ryegrass could be used as a cheap and effective energy source for SRB mitigation of acid mine drainage (Harris and Ragusa 2000). They found that under stagnant conditions, a lime-stabilised sewage sludge in combination with decomposable plant material and SRB substantially decreased the acidity of AMD that had a prior pH of <3.

However, the availability of carbon from plant matter depends on decomposition, and decomposition is extremely limited in acidic and anoxic conditions (Kalin and others 1993), which is typical of acid-sulphate minespoils. Further, Gyure and others (1990) showed that concentrations of organic acid (from decomposition of organic matter) greater than 5 mM completely inhibited SRB activity in sediment at pH 3.8. Harris and Ragusa (2000) found that finely ground, readily decomposable plant material placed in an AMD of pH 2.30 released some organic acids, but at such a high potency so as to have decreased the AMD pH to a value of 1.89. Subsequent inoculation of these samples with SRB did not result in establishment of viable colonies in this medium. This was probably one factor that delayed the activation of SRB for their sewage sludge/ryegrass experiment, where even for the most favourable treatment, SRB was not activated until a period of >50 days had elapsed after incubation had commenced, even for the most favourable sludge + ryegrass treatment. For the sewage sludge/ryegrass experiments (Harris and Ragusa 2000), readily decomposable plant material by itself did not significantly change the concentration of heavy metals in the AMD. Neither did the sludge-only treatments.

Van Breemen and others (1983) have concluded that alkalinity produced during sulfate reduction, but not removed from the soil as acid neutralising capacity (ANC; aq), causes ANC(s) to increase. The above-mentioned 'stagnant treatment' mitigation system (Harris and Ragusa 2000) is not always useful for field applications because flowing effluent can remove suspended acid neutralising components. Therefore, the high acidity (pH<3 for many acidic mine waters) must first be appreciably decreased before biological sulfate reduction can occur (Alexander 1977). SRB are known to have their optimum pH at around neutral to slightly alkaline (MacFarlane and Gibson 1961). Harris and Ragusa (2000) observed that SRB can operate in waters at significantly lower pH values, when such waters were previously provided with effective pH buffers. The buffer they utilised (a mixture of decomposable ryegrass and aerobically treated sewage sludge) took 50 days to increase the AMD pH values to 3.0 from its initial 2.3. With a continuous flow of new quantities of AMD influent into a column bioreactor, it would seem that the above-mentioned acclimatisation period would be longer. Substantially reducing this acclimatisation period would require a more efficient pH buffer for the AMD than that utilised by the above-mentioned authors. In a column experiment, Larsen and Schierup (1981) showed that the efficiency of straw to adsorb heavy metals from an AMD could be improved by increasing the pH with NaOH. They also point out that one disadvantage of using NaOH is that some organic constituents of the straw are more soluble under basic conditions.

When soil pH falls below a value of 4, clay minerals, and Al/Fe hydr(oxides) become the most effective proton sinks in the ground (Bruggenwert and others 1991). At 6 h after lowering the pH of a stream with sulfuric acid, Norton and others (1990) found that below pH 5, neutralisation was dominated by the release of Al. Upon progressive acidification of the soil, large amounts of Al ions are eventually liberated (Bolt 1976). This acidity can be used up as acid neutralising capacity (ANC) when the Al cations enter the adsorption sites of the clay minerals or of oxides (Bruggenwert and others 1991). For example, after testing the adsorptive power of Fe_2O_3 by adding to trace elements mobilised in fermented (anaerobic conditions) plant material, up to 50% of the Cu, Zn, Co and Ni was removed from solution at pH 7 (Toth 1968), although adsorption decreased with lower pH values. The same effect was observed for a sand coated with ferric oxide. The dissolution of Fe_2O_3, Al_2O_3 and MnO_2 is negligible at pH values of >5 (Van Breemen and others 1983). However, they are very strong buffers at pH<5 because oxides are the chief materials accepting protons (Bolt 1976; Barrow 1987). Therefore, they may be very important in buffering against hydrogen ions. The ability of these and other buffers to increase the pH of AMD needs to be investigated. It would seem from the foregoing that clay minerals or oxides added to AMD may increase the pH of such waters up to a level where the activation of SRB could be triggered.

Aim and hypothesis

Given adequate substrates and a long enough acclimatisation time (Harris and Ragusa 2000), dormant SRB colonies could become very active in extremely acidic, stagnant AD conditions (pH<3). The SRB placed in the harsher environment of a similar (in character) but flowing AMD would require a longer acclimatisation period (i.e. >50 days) than that in the above study. The aim of the present study was to decrease that time period. A more efficient pH buffer than that used previously is required. One objective of the present experiment was to identify materials that may more efficiently improve the quality of AMD. Some of these materials include oxides and other silicates. It is hypothesised that (1) clays or Fe/Al (hydr)oxides released in AMD of pH<2.5 may increase the pH of the AMD to a point where sulfate reduction by SRB is triggered, and (2) SRB will mitigate AMD of pH<3 in a constant flow column bioreactor energised by decomposable plant matter, but initially activated by an inorganic pH buffer.

Materials and methods

Properties of silicate buffers and whole soil buffers

A description of silicate buffers used in this study is shown in Table 1. The physical and chemical properties of the two whole soils are shown in Table 2. The first was an Alfisol (mainly illitic–kaolinitic) or red brown earth (RB) and the other a mollisol (MC; illitic–kaolinitic minerals). To increase its clay fraction, the RB was dug from the B horizon (10–20 cm depth) of a structurally degraded continuous wheat plot from the Waite Long-term trial, Waite Campus, University of Adelaide. The mollisol was a black earth (hence having a high clay content) from 0–4 cm of a natural fallow plot.

Processing of whole soils

After air-drying for 5 days at 30 °C, the clods were broken, gently crushed and thoroughly mixed. During mixing, any obvious organic matter and stones observed were removed from the samples. The soil was then passed through a 2-mm sieve, thereafter being stored in air tight plastic containers. The properties of the soils together with the test methods are listed in Table 2.

Inoculation

Inoculation is not necessary in order to initiate the sulfate-reduction process (Christensen and others 1996; Harris and Ragusa 2000), but was applied to shorten the initial lag phase (Christensen and others 1996). A mixture of AMD (Table 3) and sewage-sludge-impregnated ryegrass from a previous incubation containing an enriched strain of *Desulfovibrio vulgaris* was incubated for 20 days at 30 °C. This procedure was adapted from Nakamura (1989). After incubating *Desulfovibrio vulgaris* – one of two major SRBs active at a pH above 5.5 (Alexander 1976) at 30 °C for 6 days, Alexander found that the bacteria grew, and the medium turned black in colour. He had successfully used his enriched culture as an inoculum in a subsequent experiment. This effected a fourfold decrease in sulfates and the production of sulfides after only 3 days. The medium in the present study had turned black probably because of the precipitation of sulfide, thereby initially suggesting the concentrated presence of SRB. The in-situ pH was measured as 6.3. The presence of viable SRB was strongly suggested. In addition to the above-mentioned colour change, the smell of H_2S was detected from the inoculant. The presence of H_2S gas was confirmed by the lead acetate test: A filter paper dampened with lead (II) acetate solution was placed inside the tube. Lead (II) ions reacted with the H_2S gas to form brown lead (II) sulfide.

Aliquots of 20 g of the AMD-suffused sewage sludge and ground decomposable ryegrass containing this enriched culture were removed for use as an inoculant for the bioreactor. The inoculant was thoroughly mixed with the mixture already placed in the column. Continuous flow of the AMD influent (pH 2.3) through the column was started 2 days after inoculation.

The reactor

The reactor was made from rigid PVC tubing 500 mm high with an 80-mm internal diameter (Fig. 1). This upflow column bioreactor was designed by Elliott and others (1998), who achieved growth of SRB at pH 4, using sodium lactate as the energy source. Eight sampling ports spaced 5 cm apart in the column were plugged with rubber seals (Fig. 1). The treatment consisted of ground ryegrass mixed thoroughly with a mollisol soil. To avoid backflow of the mixture with possible clogging of the influent inlet, a 6-cm-thick gravel bed of 1 cm diameter quartz gravel was laid at the bottom of the column, which was overlain by a 4-cm-thick pure crushed quartz (250 μm) layer. Freshly cut ryegrass ground to <1 mm was hand-mixed with the mollisol in 1:5 ratio (w/w), and the mixture placed on the sand in the column to occupy the column volume, except for a 10-cm wide space at the top of the column. The AMD was slowly pumped into the column from the bottom, using an electrically powered peristaltic pump, until the top of the treatment material in the column was just

Table 1
Some properties of clay and soil buffers

Name	Abbreviation	pH	CEC (meq/100 g)	CaO (%)	SiO_2 (%)	FeO_3 (%)	Al_2O_3 (%)
Active bond	AB	10	75	1.0	56.0	4.5	16.2
Acid drainage	AD	2.3	–	–	–	–	–
Active gel	AG	10	75	0.9	56.0	4.6	16.0
Ball clay	BCL	4.7	n.d.[a]	0.2	58.2	1.0	26.4
Ceramic clay	CER	4.7	n.d.	0.2	58.2	1.0	26.4
Trufeed bentonite	TRP	7.0	80	0.02	61.0	2.9	14.8
Illite-kaolinite	ILK	6.5	n.d.	0.02	n.d.	n.d.	n.d.
Kaolinite	K10	7.2	6.5	0.03	65.4	1.1	21.8
Kaolinite	K15	7.4	n.d.	0.04	62.4	1.3	20.5
Kaolinite	K63	7.0	n.d.	0.03	62.4	0.9	22.1
China clay	CLH	8.4	n.d.	0.1	50.8	0.9	33.1
Oxide	OX	6.5	n.d.	0.03	35.0	28.0	14.5
Pure kaolinite	PURK	8.2	6.7	0.1	53.7	0.9	35.1
Red brown earth	RB	6.5	7.3	n.d.	n.d.	>2.0	n.d.
Mollisol	MC	7.2	59.1	n.d.	n.d.	n.d.	n.d.
Illite	IL	7.1	6.8	n.d.	n.d.	n.d.	n.d.
Kaolinite	Purk	7.2	7.1	n.d.	n.d.	n.d.	n.d.

[a]No data

Table 2
Some chemical and physical characteristics of the soils. *D* Dominant; *p* clay minerals present

Soil property	Alfisol	Mollisol	Test method
pH	6.5	7.2	Watson–Victor glass electrode (1:5 soil:water ratio)
Electrical conductivity (dS m^{-1})	0.12	0.23	Ionode glass electrode (1:5 soil:water ratio)
Organic C (%)	1.24	4.6	Leco C analyser
Total C (%)	1.4	4.61	Leco C analyser
Total N (%)	0.11	0.69	Leco N analyser
Inorganic C (%)	<0.1	<0.1	Difference
Exchangeable cations [cmol (+)/kg]:	7.3	59.1	1 N NH_4OAc (buffered at pH 7)
Ca	5.3	43.1	
Illite (total)	D	D	X-ray diffractometry
Kaolinite (total)	p	p	X-ray diffractometry
Randomly interstratified minerals (total)	p	p	X-ray diffractometry
Clay (%)	19	40.3	Pipette (Day 1965)
Silt: 2–20 μm (%)	30.8	20.0	Pipette
Fine sand: 20–200 μm (%)	44.8	17.2	Pipette
Coarse sand: 0.2–2.0 mm (%)	3.9	6.4	Pipette

covered with the influent. The pH was measured after 1 day.

Sampling methods – bioreactor

The interstitial water in the column was sampled with a syringe and needle, after removing the rubber seals in turn from the holes. The sampling regime was as follows: 5 ml were taken every day from the top surface of the water in the column (i.e. at 40 cm) and at 5 cm (the base of the column). Water samples were taken at 15, 25 and 35 cm (hereafter in this study referred to as ports 4, 7 and 10), on the 1st, 3rd and 5th day after each increase in AMD flow rate of the column incubation. The sampling plan was to continue every 3 days until a pH reading of at least 5.5 was simultaneously obtained from all ports above port 3 (i.e. at least 15 cm above the point of influent entry), at which the influent flow rate would be increased. Influent and effluent pH was measured with a Watson–Victor glass pH electrode model 5003.

Buffer study

To substantially reduce the acclimatisation period for the SRB in a highly acidic medium, an experiment was conducted to identify some effective pH buffers for flowing AMD. A cross section of earth materials (14 non-lime materials) comprising inorganic layer silicates, including ten bentonites, 1:1 clays, an amorphous oxide, and two 'whole' soils were to be tested for their buffering efficiency in AD. One of the whole soils – an alfisol (RB) containing 2% iron oxide (Desphande and others 1964) was selected because addition of iron oxides to a Ferralli–Haplic acrisol leads to an increase in buffering capacity against hydrogen ions (Yu 1985). The other whole soil was selected because of its high level of organic matter and clay content. The materials are listed in Table 1. Pulverised $Ca(OH)_2$ (<1 mm diameter), at rates of 0, 2.5, 5.0 and 7.0% (w/w), was mixed with each clay or soil. The samples were each submerged in stagnant AMD (pH<2.5) for 30 days. The pH of the AMD was recorded every other day for 30 days, or until no further changes occurred for consecutive readings.

Bioreactor experiment

The SRB bioreactor experiment was carried out under anaerobic conditions at mesophilic temperatures (30–35 °C) because the formation of sulfide by sulfate reduction in nature is enhanced by increasing water levels and rising temperatures (Alexander 1977). The pH was recorded daily throughout the incubation. Cation concentration of metals in the samples was monitored by ICPAES. The passage of AMD effluent in the proposed column was to occur through the material, which was to be shown in experiment 2, as the most effective buffer.

Results

Buffer study

The effects of the various buffers on the pH of the AD are shown in Fig. 2, where the relationship between pH and

Table 3
Concentration of cations in solution in an AMD at Brukunga, South Australia

Component	Concentration (mg/l)
Aluminium	46
Arsenic	0.2
Boron	<0.1
Calcium	500
Cadmium	<0.1
Cobalt	0.1
Chromium	<0.1
Copper	0.1
Iron	2,100
Potassium	<1
Magnesium	290
Manganese	110
Molybdenum	<0.1
Sodium	220
Nickel	0.3
Phosphorus	<1
Sulfur	2,700
Selenium	<0.1
Zinc	5.7
Acidity as $CaCO_3$ (kg)	2,006–2,602

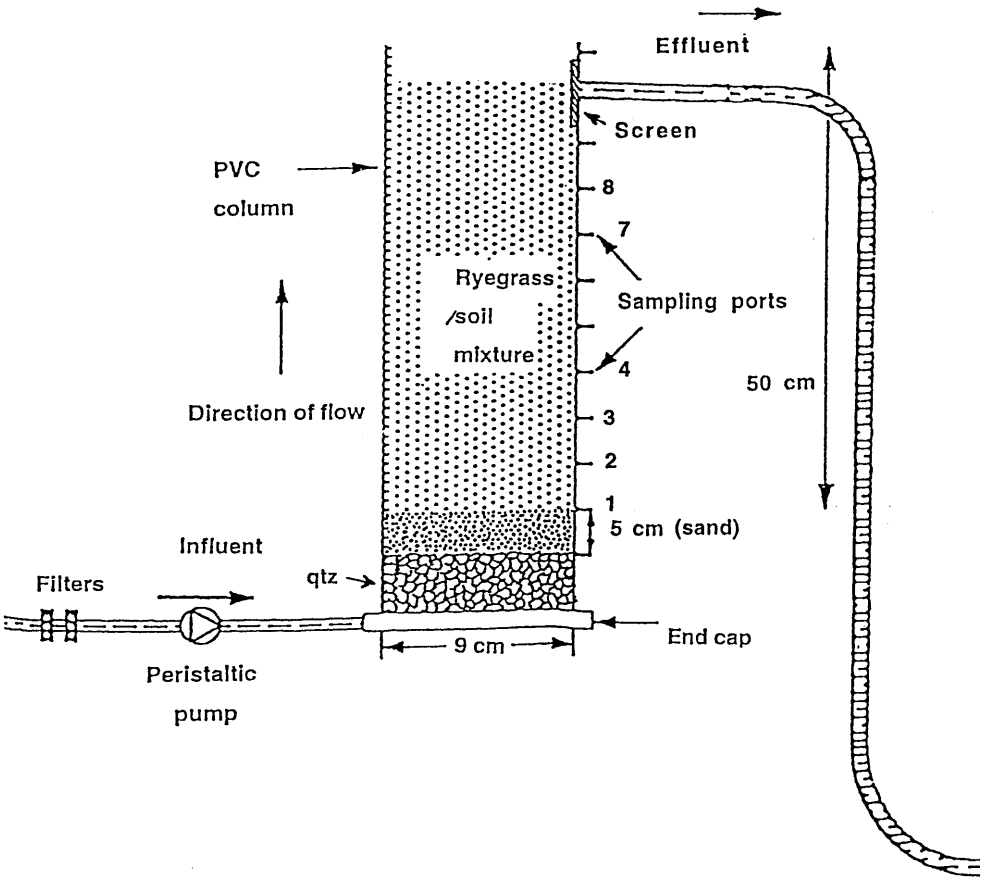

Fig. 1
Schematic diagram of SRB bioreactor using soil/ryegrass buffer nutrient for AMD mitigation. (Adapted from Elliot and others 1998)

different rates of lime application is observed. Incrementally higher lime rates did not necessarily cause an increase in the pH of the AMD. Although most buffers did not respond well to lime addition, even at the highest lime rate of 7.5%, it was in general, always the same ones that caused the pH increases at the various rates of added lime (Fig. 2). Of the 15 buffers studied, even at the highest lime application rate (7.5%) – only four – the mollisol whole soil (MC), the active bond clay (AB), the active gel clay (AG) and the oxide (OX), decreased the AMD acidity to pH>5. At the 5% added $Ca(OH)_2$ level, only three earth materials – the MC, the OX and the AG – accomplished AMD neutralisation at pH>5 (Fig. 2).

At the lowest lime application rate (2.5%), only the mollisol caused the AMD pH to increase to >5 (Fig. 2). Although the ANC (acid-neutralising capacity) of the AG is comparable to that of the mollisol, and the oxide, the mollisol is therefore seen to be the most significantly responsive to low level lime spiking (Fig. 2). Thus at just 2.5% added lime, the pH of the mollisol-treated AMD rose dramatically from 2.2 to 5.9 units, whereas the oxide increased AMD pH from 2.2 to a value of under 5.0. This represents (after 2.5% added lime) proportional pH increases of 38, 33 and 28% for MC, OX and AG, respectively. The control (AD) pH did not change significantly even at the highest lime rate because its pH, even after a 7.5% addition of lime, still remained unchanged at <2.5 units after 5 days and up to 25 days (Figs. 2 and 3). This shows that without an adequate buffer (in combination with lime) in AMD, the quantities of lime applied to neutralise the acidity becomes increasingly large.

With increasing rates of added lime, the pH increases became less substantial for all the above-mentioned three highly responsive buffers (i.e. MC, OX and AG). Whereas there are significant pH increases up to the 5% lime addition for all three responsive buffers, after that, at the 7.5% lime treatment, pH increases for them are small or negligible. The AB clay had previously not increased the pH of the AMD, even when 2.5% $Ca(OH)_{2\ was}$ added, but increased markedly when 5% $Ca(OH)_2$ was added (Fig. 2). In general, the more responsive the buffer, the earlier it peaks (i.e. stabilises; Fig. 2). While the MC-treated AMD peaked at just 5% added lime, the less responsive AB increased the AMD pH with 5% added lime and did not peak, even at 7.5% added lime. This increase (for AB) occurred from pH 4.5 to >6 units when 7.5% lime was added, with a pH improvement of 33%.

At the end of 20 days without any added lime, the oxide achieved the highest pH increases of all buffers (Fig. 3). The oxide was at least equally as responsive as the MC without lime during the 20-day period. In the first 5 days, the pH of the MC-treated AMD exceeded that of the oxide-treated AMD (Figs. 2 and 3). Because of its quicker action, the mollisol and not the oxide was chosen as a potential buffer for use in the planned bioreactor. The oxide was, in addition to the above-mentioned reasons, found to have inhibited the activation of SRB. No evidence of SRB was detected after a 30-day period following a subsequent trial

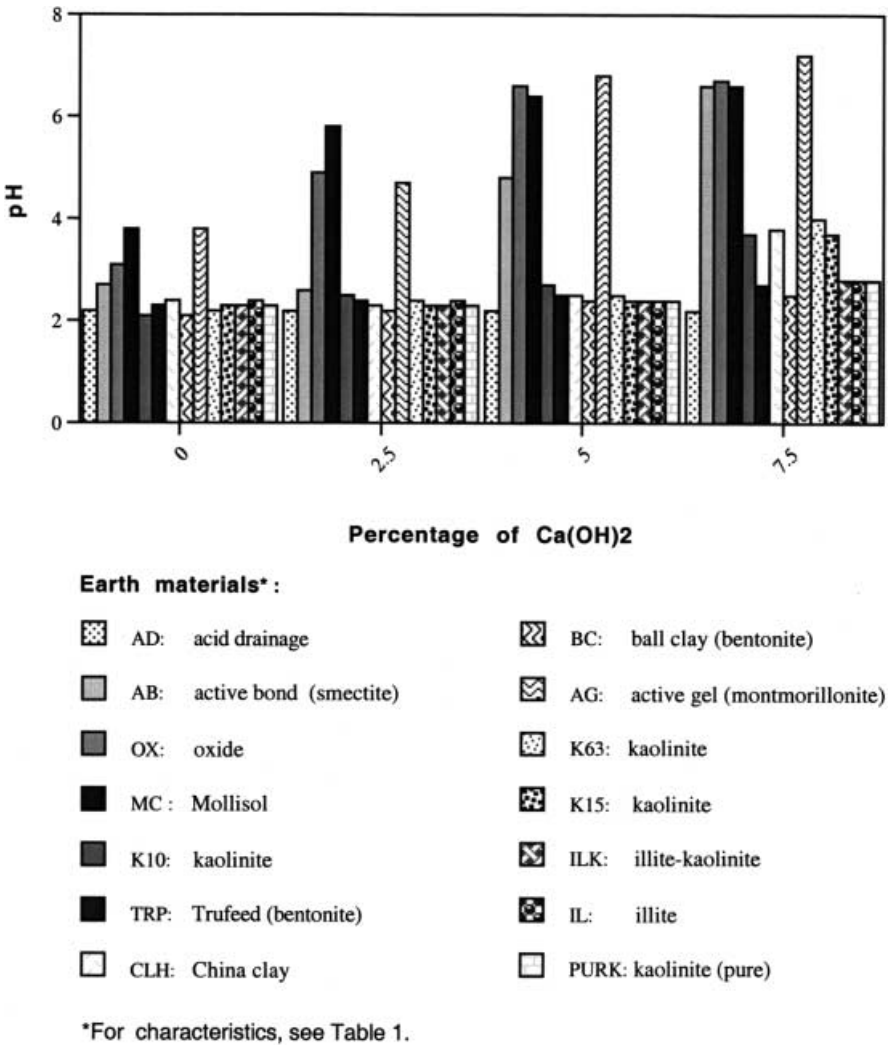

Fig. 2
Changes to pH of acid drainage (AMD) after mixing with various earth materials and various quantities of $Ca(OH)_2$

inoculation of the oxide-treated AMD in stagnant conditions (not illustrated here). However, for the mollisol, the waiting period for SRB activation was only 5 days.
Even without added lime, the MC, OX and AG increased the AMD pH substantially from 2.3 to >3.0 units at 5 days. These materials thereby exhibited a high ANC (Fig. 2). However, the AG, although a reasonably good buffer at 0 or 2.5% added lime, changed the physical characteristics of the AMD from watery to a highly viscous consistency because of its swelling. At the end of 21 days, the AG–AMD container was observed to be fully occupied with a swelling, sticky mass of material, which caused difficulty in visually demarcating the boundary between active gel clay and liquid AMD. If used in the bioreactor at that stage, this viscous AMD would have clogged the tubing in the pumping system. A pre-filtering would be required, which may lead to potential waste disposal problems. Because the objective of this experiment was to improve the water quality and generally expedite the remediation process for AMD, the above-mentioned uncertainties associated with the AG resulted in its rejection as a buffer for the bioreactor experiment.
Although the active bond clay (AB) did not swell appreciably, a pH peak of 5.0 units was not even approached until 5.0% of Ca(OH)2 had been added to the AMD (Fig. 2). The buffering response of the AB without lime was, therefore, very slow or non-existent because it did not, after 20 days, increase the AMD pH to a value as high as even 3.0 units (Fig. 3). Such a small decrease in acidity was insufficient, and was even less than that of the buffer used by Harris and Ragusa (2000) for SRB activation in the less drastic environment of a no-flow reactor system (compared with the upcoming through-flow system). On the basis of all the above-mentioned results, the MC was the chosen buffer for the following investigations.

Bioreactor experiment

Appearance

The bioreactor supported sulfate reduction at continuous flow rates of 30, 60, 90 and 180 ml/day. Initially, a test flow rate of 60 ml/day was applied. The pH values rose from 2.3 at port 1 (near the basal entry point of AMD influent), to a value of 5 at the surface of the water (45 cm from the base) in the column (port 10) after just 4 days. The usual signs of SRB were not detected for a further 20 days. The water became cloudier. The red coloration was gradually

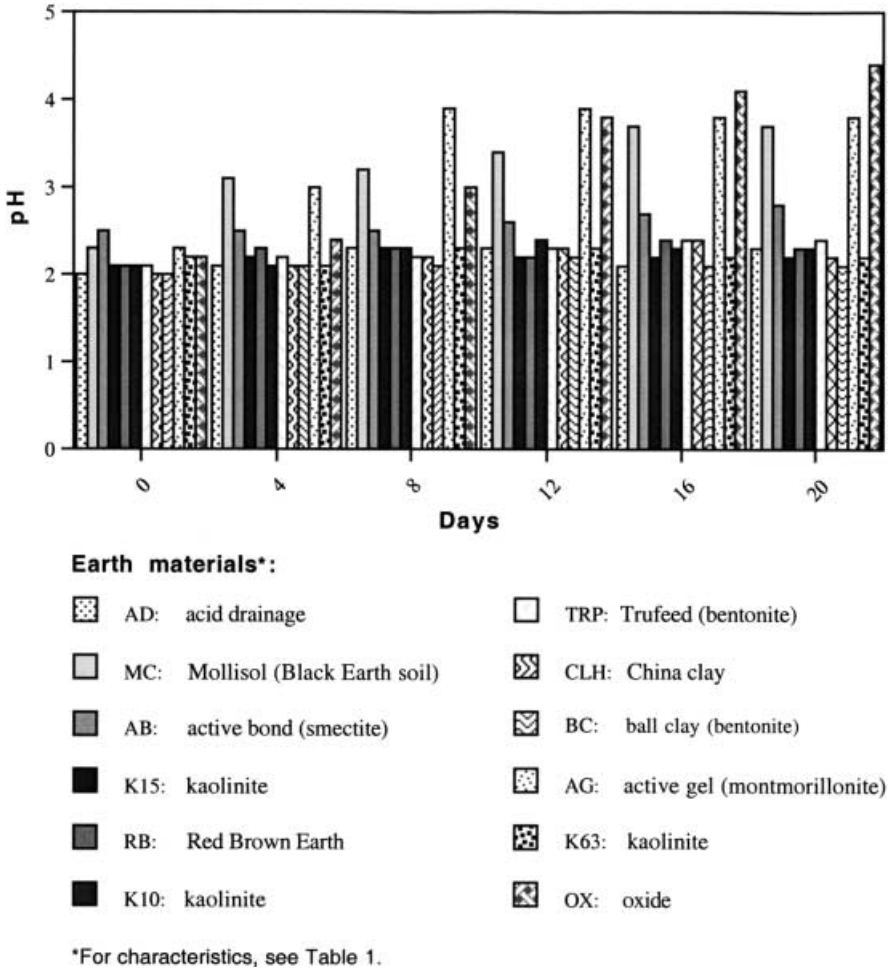

Fig. 3
Changes to pH of acid drainage (AMD) without $Ca(OH)_2$, but containing various earth materials

lost. The suspended matter of a water sample settled out after 20–40 days. Dissection of the column on the 20th day did not detect an odour of H_2S, nor the characteristic blackening coloration by iron sulfides, although the influent had a very high measured concentration of Fe ions. This suggested that while the buffer (MC) worked effectively to reduce AMD acidity, high flow conditions inhibited SRB respiration. The flow rate was consequently reduced, and the experiment was conducted at half the influent test rate (i.e. 30 ml/day). At day-5, the column began to darken in the middle with a strong odour of H_2S when any of the ports 3 through 8 were slightly opened. Hydrogen sulfide occurs as a respiration product of sulfate reduction. However, adaptation periods for the reactor to adjust increased with each higher influent flow rate, which was suggested by an immediate drop or a steadying drop in pH values from all ports on the day after the flow rate increased.

Changes in pH and adaptation of SRB to acidic conditions

The response of the reactor to low pH conditions was detected by sampling from column ports on the second day and every third day after the influent flow rate was increased. Figure 4 and Table 4 show the changes in pH at three different levels in the bioreactor. pH changes were less marked in the upper zone of the reactor. The zone of highest pH readings was not, as had been expected, furthest away from the influent inlet at the water surface at port 10 (45 cm), but was located at about three-quarters the distance up (port 7, 30 cm) from the base (Fig. 4, Table 4). As the influent flow period lengthened after the first day of each flow rate increase, the front of pH increase advanced downwards towards the inlet valve at the base of the column. The zone of the maximum pH values never at any time reached the surface of the AMD (Fig. 4).
After 3 days of operation at 30 ml/day, the pH at 15 cm above the influent inlet (port 4) was 5.4 (Fig. 4). The pH had risen to 5.7 1 day after the influent flow rate was increased to 60 ml/day (day 5). Thereafter, it took 4 additional days to recover to the pH of 5.9 on day 10 (Table 4). When the influent flow rate was again increased to 90 ml/day, the pH did not drop after 1 day (as it did when the rate had been increased from 30 to 60 m/day). Instead, the pH levelled off, and after 3 days began to rise to its highest values of the study prior to day 18, at 15 cm (Fig. 4). In general, as long as the influent flow rate through the reactor increases over time by small rates of 30 ml/day, pH at all ports also increases incrementally at least until day 15. This suggests that the SRB colony was becoming acclimatised to the slowly increasing acidic conditions in the column. However, between days 15 and 18, during the

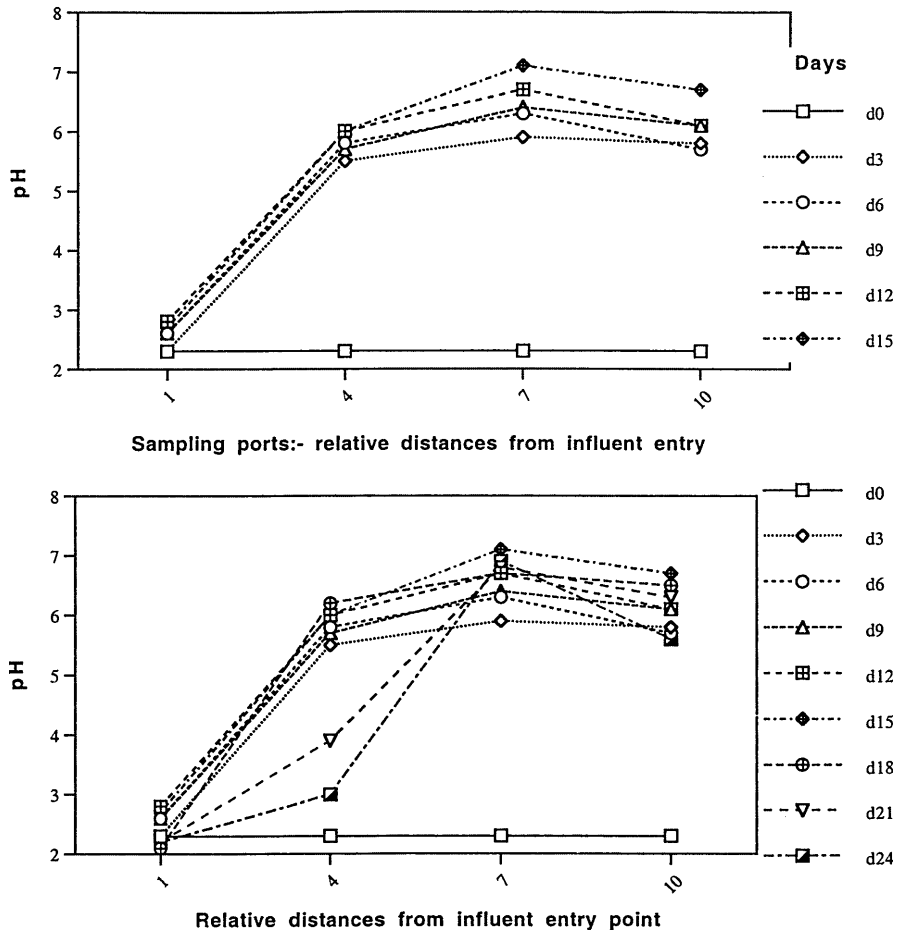

Fig. 4
pH changes with time and distance from AMD influent entry point in an SRB-driven bioreactor

Table 4
Changes in pH with distance from an influent AMD after 10 days in an SRB bioreactor

Location	Distance (cm)	Average pH	pH change (units)
Port 2	5	2.3	+0.1
Port 3	10	2.7	+0.4
Port 4	15	5.8	+3.4
Port 5	20	6.8	+4.5
Port 6	25	6.5	+4.1

initial period after the influent rate was doubled from 90 to 180 ml/day, the pH began to fall at most ports (Fig. 4). This suggested an inability of the SRB colony to cope with such a large, sudden increase in acidity. Even though pH dropped by a larger increment at port 7 than at port 10 on day 18, the absolute value was still the highest at port 7. In addition, port 7 (30 cm) was the only position at which pH did not continue to fall after day 18 (Figs. 4 and 5). The subsequent pH recovery for 180 m/day did not begin in the zone furthest above the influent inlet (i.e. at 45 cm), but was observed at 30 cm (Fig. 4). This not only occurred at day 21, just 5 days after the largest influent rate increase, but while pH values were still dropping at the other ports above and below (Figs. 4 and 5).

With respect to pH, the interstitial waters in the quartz sand–gravel zone at the bottom were markedly different from the interstitial water in the substrate layer above, which contained viable SRB. At day 24, pH in the quartz/gravel/sand layer was still unchanged at 2.3, as opposed to pH of 4 at 15 cm above the surface of the sand layer, i.e. at the top of the lowest one-third of the column substrate interstices (Fig. 4). Throughout incubation, the quartz/sand/gravel zone water never exceeded a pH of 2.4 (Fig. 4). Acidity at a distance of 10 cm up from the surface of the sand bed was also monitored. Even at this height up inside the substrate, pH remained at <3 at day 10 (Table 4), while at just 5 cm further up, the value was 5.8 units. This suggested that SRB were not as active at 10 cm and below, as they were further up the column. Continuous fresh additions of AMD below 10 cm would have been a more demanding environment for SRB.

Cation concentration

The concentration of the cations Al, Cu, Fe, Mg, Mn and sulfate was monitored by ICPAES. During this study, even the least efficient zone in the column experienced marked decreases in cations and sulfate concentration. After just 6 days of operation, the following decreases had occurred at just 15 cm above the influent inlet (Fig. 6).

1. Fe from 1,800 to 70 mg/l.
2. Sufate down from 2,700 to 1,330 mg/l.
3. Although Al concentration had doubled from 32 to 62 by day 6, it had decreased to 1.8 mg/l by day 9.

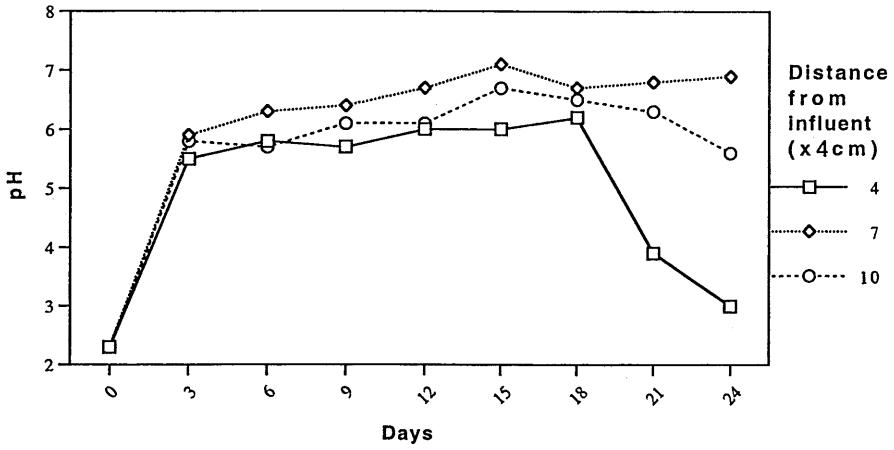

Fig. 5
pH changes in AMD at three levels in an SRB-driven bioreactor

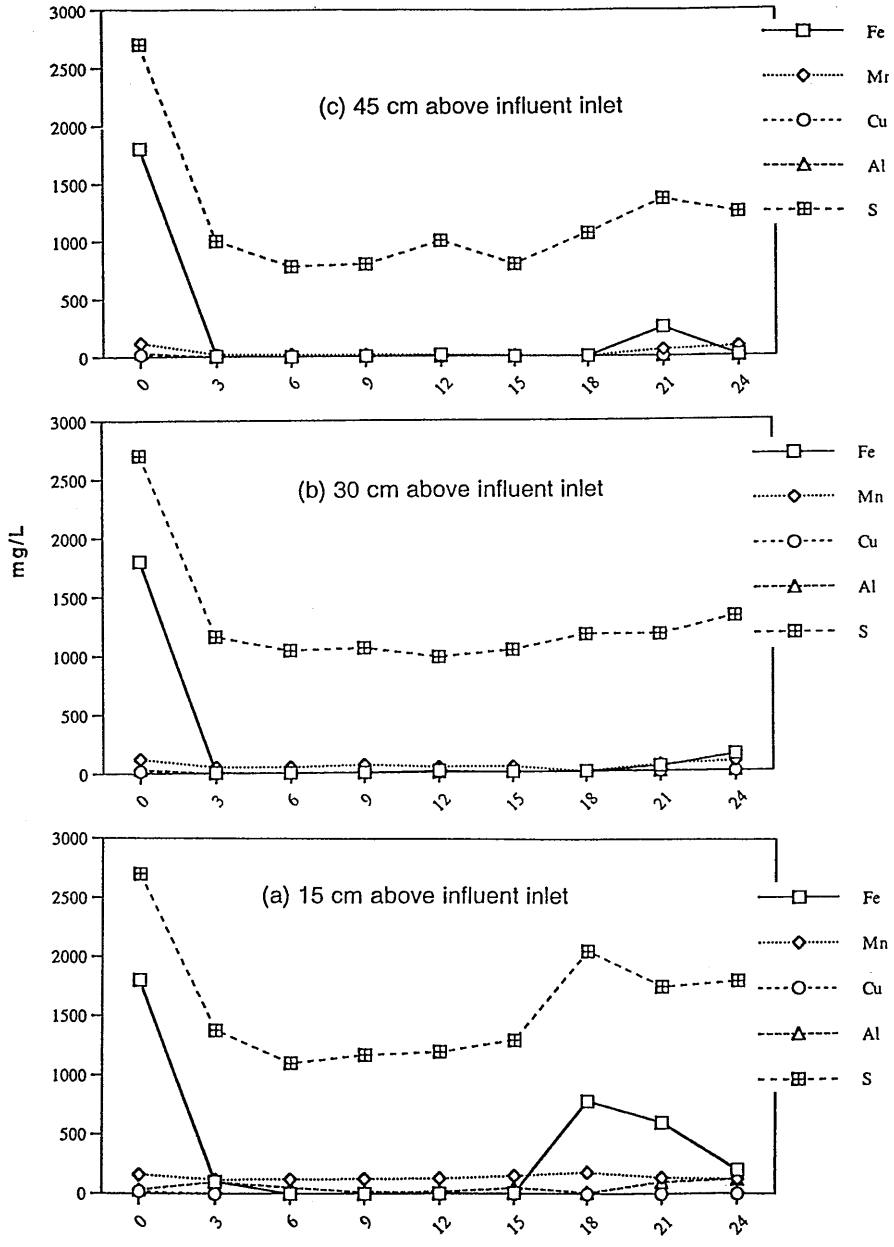

Fig. 6
Cation concentration at distances from AMD influent entry

In contrast, the metal concentrations were comparable in the quartz gravel–sand interstices (port 1) to that for the raw AMD (Tables 3 and 5). Changes in the AMD pH with cation concentration after the first 10 days at the lowest influent flow rates (30–60 ml/day) are also shown (Table 5). These and other data (such as Fig. 5) highlight four observations.

Firstly, as the incubation progressed, the concentration of heavy metal cations in the AMD appeared to decrease, but by increasingly smaller amounts. Secondly, as the distance away from the influent entry point increased, metal cation and sulfate concentration in the AMD decreased. Thirdly, with increasing distance up the column from the influent entry point, the cation concentration perturbations following increased flow rates in the AMD decreased in magnitude (Fig. 6). This indicates that effects of an increasing influent flow rate on the metal and sulfate concentration became less marked with distance from the influent entry point. The pH changes inversely with toxic metal concentration over time (Fig. 7). These observations, the fourth one in particular, suggest the presence of a growing SRB colony, despite the increasingly acidic environment (from increasing flow rates), even though the nutrient substrate was being used up. An example is shown in Fig. 6a, at the 15-cm port (the third port nearest the influent inlet), where the largest influent rate increase (on day 16, i.e. from 90 to 180 ml/day), caused the sulfate concentration to rise most sharply at 18 days from 1,300 to 2,200 mg/l (a 59% increase). It was brought back down to 1,750 mg/l 3 days later, by day 21. The general trend describes a gradual lowering of sulfate concentration over time. At the lower column levels (and to some extent at the highest column levels), the largest sudden change to the sulfate and Fe regime coincided with the largest increase in influent flow rates (Fig. 6). At three-quarters of the way up the column, sudden changes in influent flow rates caused the smallest perturbations in sulfate and other toxic cation concentrations (Fig. 6).

Discussion

Buffer study

The MC soil of this study contained 40% clay, whereas the other whole soil (RB) used in the buffer study contained only 19% clay (Table 2). Throughout the buffer study, the pH of the RB-treated AMD always remained at <4.0 units. Only when the added lime was increased to 7.5% did the pH of the RB-treated AMD exceeded 3.0 pH units. A previous study indicates that a kaolinitic sludge increased the pH of an AMD from 2.3 to 2.6 units over a 40-day period (Harris and Ragusa 2000). This is a small increase, and is not dissimilar to that of the RB of the present study. The RB clay fraction was dominated by illite, which is generally unreactive. Because one of the aims of this experiment was to shorten the acclimatisation period for SRB activation, it was not necessary to prolong the experimental time period merely to identify the peak characteristics of the less efficient buffers. The buffering study was, effectively, stopped at 20 days, when some of the materials had already begun to markedly increase the AMD pH to a far greater degree than kaolinites and illites did. Yu (1985), who observed the buffering capacity of three soils consisting of 30, 40 and 65% clay, found that buffering capacity increased in ascending order of clay content. The two pure clays that were among the three most effective buffers of this study were the active bond and the active gel, both are smectites with high CECs. The third material, a whole soil, i.e. the mollisol, contained high fractions of illite and kaolinite. The native organic matter was 4.6% (w/w), and on a w/w basis, organic matter influences CEC far greater than any other factor in the soil (Tisdall and Oades 1982).

Table 5
The effect of distance from influent on pH of SRB-treated AMD and soluble cations in a constant-flow bioreactor after 10 days

Sampling port distance (cm)	pH	Fe	S	Al	Mg
5	2.3	1,840	2,700	33	320
10	2.7	1,390	2,400	59	320
15	5.8	70	1,330	62	320
20	6.8	0.72	580	0.6	300
25	6.5	0.4	1,040	0.6	330

Bioreactor experiment

The pattern of AMD cation concentration and AMD pH during this incubation is seen to be, in general, an inverse relationship. As pH increases, the concentration of the soluble cations decreases, and vice versa (Fig. 7). The rise in AMD pH began before the detection of any evidence of SRB activities in the column. Although evidence from the prior buffer experiment indicates that the MC by itself would have caused a rise in the AMD pH, the pH was at all times below a value of 4.0 units. The second phase of this study must have been the combination of MC submerged with rapidly decomposable plant material that initially caused the rise to >4.0 units in the pH of the AMD. Qualitatively, this is in agreement with the findings of Ponnamperuma (1972), who found that green manures added to soil caused a remarkable rise in pH within 2 weeks of submergence. Singh and others (1992) found that pH increases after submergence of an acid soil (pH 5.6) with and without green manure went up to 6.0 and 7.1 in pH values, respectively. The explanation by Singh and others (1992) was that organic reducing substances formed during the decomposition of green manures may reduce Fe and Mn oxides. Protons are consumed in the course of oxide reduction, which causes soil pH to rise. The process of reduction could have accounted for the decrease in concentration of the soluble form of those metals 3 days after incubation began, but prior to the activation of the SRB. This is corroborated by the findings of Katyal (1977). Using *Sesbania aculeata* as green manure under flooded conditions, Katyal (1977) recorded a decrease in (metal) toxicity and favourable changes to pH.

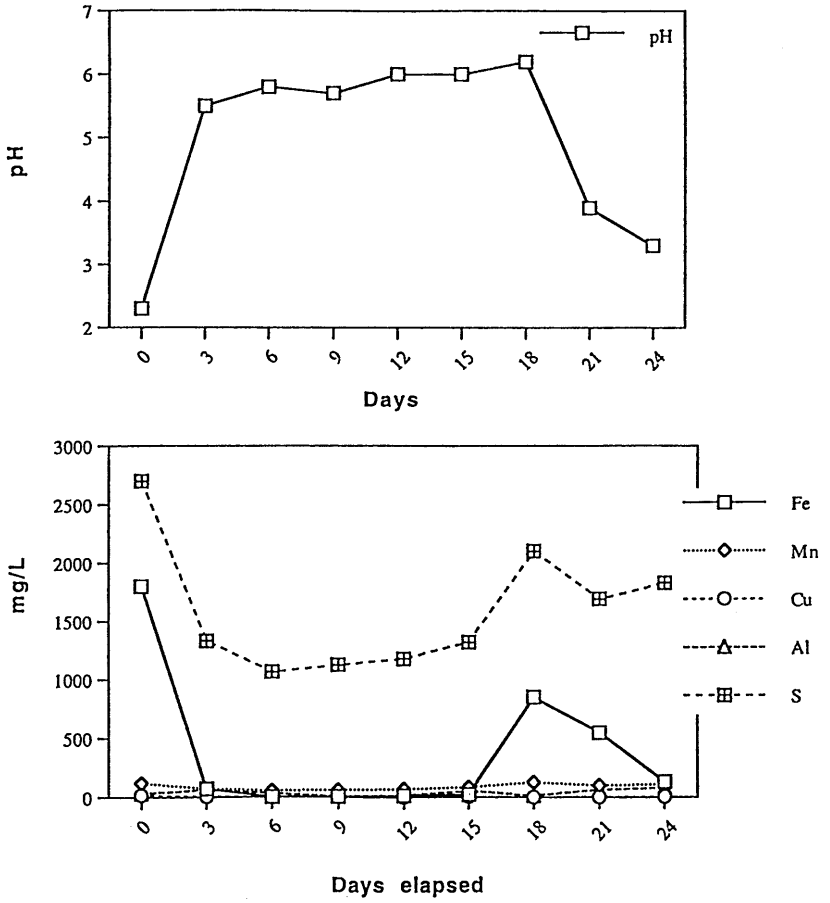

Fig. 7
Variations of pH with AMD concentration of soluble cations in an SRB-driven bioreactor

Strong evidence for SRB activity was observed. The typical SRB signs were observed, such as production of H_2S, the blackening of the substrate-buffer and the lead acetate test. The sulfate reduction was not as great at the surface of the AMD water as it was at port 7. Large temporal and spatial differences in the pH and soluble cations in a uniformly blended material were detected.

1. The front of pH increase advanced downwards in the column towards the influent as the incubation proceeded.
2. The sulfate reduction in the influent increased with distance away from the zone of fastest flow (i.e. from where it entered the column).
3. A general increase in pH values towards the top of the column always occurred during this study. The highest pH values were never at any time detected at the water surface, or near the surface. The highest pH values always existed at some distance below the surface zone (but always within the top half of the column).
4. The major inflection point for sulfate and other cations appear in the above-mentioned upper region, between ports 3 and 5, where the pH values begin to rise markedly, i.e. about a third of the distance down the column from the water surface.

However, the pH values, although lower at the surface than at 5–8 cm below the surface, never actually fall below the threshold for SRB growth (pH 5.5) in either of these zones. In this uppermost zone, sulfide reduction would not have been greatly hindered after day 3 as a result of pH changes. Even at day 10, sulfate, although less concentrated at port 7, was concentrated at 1,300 mg/l at the water surface in the reactor, only half the concentration between days 1 and 3.

The two cations most affected by distance from the inlet port are S and Fe (Fig. 6). As distance increases further up the column from 30 to 45 cm, the differences in sulfate reduction (Fig. 6a–c) become smaller. The activity of SRB did not increase as much between 30 and 45 cm (the water surface), as it did between 15 and 30 cm above the inlet port. A change in sulfate concentration from a decreasing one in the column to an increasing trend at the water surface can be seen (Table 5). Alexander (1977) concluded that even though SRB are active at pH>5.5, they are most active at a neutral pH. The area around port 7 showed consistently greatest sulfate reduction. Nevertheless, such pH changes in this case are a symptom, not the cause of decreasing SRB activity at the water surface.

The answer may lie in the consumption of oxygen in the upper layers of the AMD. In this experiment, the AMD did not completely fill the column. In colder months, the top few centimetres of sediments in a lake-bed may be oxidised (Herlihy and Mills 1985), and SRB is inhibited above the oxidation depth. This is because the process of sulfate reduction requires low oxidation–reduction potentials

(Eh) characteristic of anaerobic habitats. Herlihy and others (1987) further found that maximal rates of SR occurred at the surface of shallow lake sediments in summer, but at 3–7 cm in other seasons. They explained that this was because the biological oxygen consumption (by aerobic organisms) increased in summer, leading to anoxic conditions at the surface. In this study, the top of the reactor was left open to the air to simulate field conditions. Because the AMD was highly acidic, few aerobic organisms would have survived at the surface. At the higher column levels near the water surface, more dissolved oxygen would be present, causing an oxygen surplus in the upper zones of the water. The alkalinity may be consumed as the reduced sulfur species diffuse into oxic regions and are oxidised (Herlihy and others 1987). In the present study, oxygen was always present in the column. During its setting up, no attempts were made to avoid exposure to air. There was an air-filled space above the water surface. The dissolved oxygen in the top layer of water would have inhibited SRB and microbial sulfate reduction. The difference between the sulfate reduction rate in the AMD at 30 and 45 cm could thereby be resolved.
Compared with that of a previous study using aerobically treated sewage sludge as a pre-SRB-stage buffer (Harris and Ragusa 2000), the lag phase (i.e. pre-SRB activation time) of this study was shorter. The toxic cations were removed earlier and more efficiently than in that study. Both studies utilised buffers containing similar proportions of clay (40%), but the sludge in the previous study contained 2.5% $CaCO_3$ (M.A. Harris, unpublished data), as opposed to <0.1% $CaCO_3$ for the mollisol used in this study (Table 2). The mollisol is a significantly more efficient pH buffer, even without the high proportion of $CaCO_3$ of the sludge. The CECs of the two buffers differ markedly at 18.4 cmol (+) kg^{-1} and 59.1 cmol (+) kg^{-1}, respectively, for the sludge and mollisol. The CEC of the mollisol Ca is four times as high as that of the sludge Ca. These significant CEC differences must have caused the differing adsorptive capacities. These CEC differences may result from the organic C fraction of the two buffers. As stated previously (Tisdall and Oades 1982), soil organic matter exerts an influence on CEC far in excess of its proportion by weight in the soil. CEC can be reduced by as much as 20 to 50% by the removal of the organic phase, which normally constitutes only 3–5% of the soil mass. The organic C fractions for the mollisol and the sludge were 4.6 and 1.4%, respectively. They both contained the same proportion of clay. It is reasonable to conclude that inherent organic matter influence on CEC for the mollisol was three times that of its influence on the sludge (Harris and Ragusa 2000). This would be the case because the clay fraction of both soils were dominantly kaolinitic illites. By further increasing the organic C fraction, the addition of decomposable plant material would have increased the CEC of the mollisol in this experiment, and also of the sludge in a previous study (Harris and Ragusa 2000). However, during the incubation with green manure, the mollisol, already having a larger native organic fraction, would have exhibited greater increases in CEC.

Conclusions

The results of this study show that it is possible to establish an SRB population in a constant flow reactor, using rapidly decomposable plant material and a fast-acting soil-based pH buffer. Under such conditions, the SRB can become acclimatised to the high AMD acidity, even when the hydraulic retention time (HRT) is shortened.
Had the above-mentioned buffer of a previous study been used in this experiment, it would have been expected to have been significantly less effective because of the more drastic conditions of a moving acidic effluent. Yet, the materials of the buffer/substrate of the present study proved to be far more effective than those used by Harris and Ragusa (2000). It seems CEC, and not merely free lime, was the crucial buffer characteristic at the low pH level of <3 units. The CEC of the substrate used in this study was three times that of a sludge buffer used Harris and Ragusa (2000). Because the only effective substrate difference between the present study and that of Harris and Ragusa (2000), except the speed of the influent, is that of the buffers used: it can be concluded that for pH increases and decreases of the soluble cations in the AMD, CEC is a major determining factor among the buffering materials. Where sources of lime are unavailable or insufficient, the use of medium to high organic C soil-based buffers with small quantities of lime can be an effective alternative for activating SRB in a reactor.

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