

Detoxification of Acid Drainage Using Inorganic pH Buffers in a Constant Flow Bioreactor

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Abstract To substantially reduce the acclimatization period for SRB in a highly acidic medium, we studied some effective pH buffers for flowing AMD. Silicate buffers increased pH before SRB was introduced. A mixture of AMD and sewage-sludge-impregnated ryegrass from a previous incubation contained an enriched strain of *Desulfovibrio vulgaris*, one of two major SRBs. They were active at a pH above 5 at 30 °C for six days, producing a black coloration probably because of the precipitation of sulfide, thereby initially suggesting the concentrated presence of viable SRB. The in situ pH was 6.3. The odor of H₂S was detected from the inoculants. An up-flow bioreactor made from rigid PVC tubing with sampling ports placed 5 cm apart in the column was plugged with rubber seals. The treatment consisted of ground ryegrass mixed thoroughly with a mollisol soil. Freshly cut ryegrass ground to <1 mm diameter 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. An electrically powered peristaltic pump slowly pumped the AMD into the column from the bottom, until the top of the treatment material in the column was just covered with the influent. The pH was measured after 1 day. At the end of 20 days without any added lime, the oxide achieved the highest pH increase of all the buffers. The bioreactor supported sulphate reduction at continuous flow rates of 30, 60, 90, and 180 ml day⁻¹. The results of this study show that it is possible to establish an SRB population in a constant flow reactor, using readily decomposable material and a fast-acting soil-based buffer. It was concluded that cation exchange capacity (CEC), and not merely free lime, was the crucial buffer characteristic at the low pH level of <3.0 units. The CEC of the substrate used in this study was three times that of a sludge buffer used by Harris and Ragusa (2000). The only effective substrate difference (excluding the influent flow rate) between the present study and that of Harris and Ragusa (2000) is that of the buffers used. Hence, it was concluded that for pH increases, and decreases of soluble cations in AMD, CEC is

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a major determining factor among the buffering materials. Where sources of lime are 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.

Keywords Acid-drainage • Bioremediation • Sewage-sludge • Sulphate-reducing-bacteria

Highlights

- Inorganic buffers increase pH for rapid SRB detoxification of heavy metals in acid mine drainage
- SRB activated in flowing AMD.

1 Introduction

1.1 Effects of Sulfide Exposure

At metal mines, the target ore (like gold, silver, copper, etc.) is often rich in sulfide minerals. When the mining process exposes the sulfides such as Pyritic minerals (FeS_2) to water and air, oxidization to sulfuric acid occurs as reactions produce sulfuric acid. This acid can dissolve other harmful metals and metalloids (like arsenic) from the surrounding rock (Maiti 2003). According to Whitbread-Abrutat (2008), the most serious environmental concerns at orphaned and abandoned mines are acid rock drainage from underground workings, open pit mine faces, waste rock piles, and tailings storage areas that were left exposed to the elements or inadequately reclaimed, resulting in the contamination of water with dissolved metals and acidity. Sheoran et al. (2010) state that the pH of a given mine soil can change rapidly as the rock fragments weather. They observed that with pH levels as low as two, the sulfuric acid mobilizes aluminium, heavy metals and uranium from the rock, and oxidize, producing iron oxide as a by-product (hence the characteristic reddish-brown colour of AMD). Unweathered (or un-oxidized) mine soils which contain a significant amount of pyritic-S in excess of their neutralizers (carbonates) will rapidly drop the pH to a range of 2.2–3.5 after exposure to water and oxygen. When such toxic fluids flow into groundwater, streams and rivers, they destroy ecosystems and taint water in regions where freshwater already may be in short supply.

Vegetation achieves optimal growth in soil at a neutral pH, whilst carbonate (Ca/MgCO_3) bearing minerals and rocks tend to increase the pH as they weather and dissolve (Sheoran et al. 2010). When the soil pH drops to below 5.5, reduced legume and forage growth occur due to metal toxicities such as aluminium or manganese, phosphorus fixation, and reduced population of N-fixing bacteria (Sheoran et al. 2010).

1.2 Active or Passive Treatments? Justification

Treatment of acid mine drainage (AMD) and municipal wastewater (MWW) is central to maintaining global water resource quality. Untreated AMD causes water quality degradation in coal and metal mining regions worldwide (Bell and Donnelly 2006). Discharges of untreated MWW degrade water resources in many developing nations (Gadgil 1998; Kivaisi 2001). In developed nations, where MWW is generally addressed actively, treatment consumes considerable financial, material and energy resources (Muga and Mihelcic 2008). Compared to passive methods, conventional MWW and AMD treatment are energy-intensive with higher operational and maintenance costs (Younger et al. 2002; Muga and Mihelcic 2008).

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 problem of metal toxicity to plant life (Hore-Lacy 1978; Strosnider et al. 2009) especially where water tables rise in wet seasons. Most techniques involve the application of chemical precipitators such as calcium compounds. Precipitation by lime and limestone is one of the oldest and cheapest methods for removal of heavy metals from acid drainage solutions (Strosnider et al. 2009). Such measures have not been highly successful. Research has shown (Dean et al. 1972) that Cd, Cu, Pb and Zn will begin to precipitate from dilute solution at pH values exceeding 5.3, 6.0, 6.7, and 7, respectively. However, lime additions have not been highly successful for pyritic materials as they soon tend to form pans. The longevity of the oxidation process is calculated in decades rather than years (Elliott et al. 1998) and sources of lime are not ubiquitous. Hore-Lacy (1978) observed that in one United States case where 2500 t ha⁻¹ was added to the tailings at Bingham (the “Iron Mountain”) the pH was back to a value of 3.5 after 3 months. Elliott et al. (1998) concluded that the management of AMD should be focused on containment and prevention rather than the chemical treatment of effluents. The present methods utilizing lime are not cost-effective in the long term. Biological mitigation using sulphate reducing bacteria (SRB) has been reported (Lyew et al. 1994; Elliott et al. 1998). Because substrates are needed 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 effective (Hard et al. 1997; McCullough and Lund 2011).

Citing the high cost of commercial available organic acids as an energy source, Elliott et al. (1998) suggested further research into the use of alternate carbon source in bioreactors for potential large scale use. In South Australia organic waste comprises almost 50 % of all household rubbish, a total of 97, 546 tons (Handreck 1996). A large fraction of this is cut grass that is wasted through the process of decomposition. Readily decomposed and finely ground rye grass could be used as a cheap and effective energy source for the mitigation of AMD (Harris and Ragusa 2000).

They found that under stagnant conditions a lime-stabilized sewage sludge in combination with decomposable plant material and SRB substantially decreased the acidity of AMD that had a prior pH of <3.

1.3 Character of Substrate

Frömmichen et al. (2003) found that whilst pure and complex carbon sources can serve as suitable substrates for stimulating microbial reductive processes in (coal) pit lake sediment for alkalinity generation, complex substrates (i.e. straw, wood chips) were inefficient at remediation as acidic waters often lack the micro-flora that can degrade lignin. Sulphate-reducing bacteria are thus unable to use complex organic substrates such as starch, cellulose, proteins, and fats. Chang et al. (2000) tested different organic waste materials as electron donors for sulphate reducers and found that cellulose polysaccharides were the main components of the waste materials consumed in their reactors. SRB are dependent on other microbes that degrade these complex substrates and ferment them to products that can serve as substrates for SRB (Figuroa et al. 2004; Muyzer and Stams 2008). Thus Dvorak et al. (1992), Nagpal et al. (2000), and Tsukamoto et al. (2004) note that SRB use the easily degradable fraction of organic matter that contained short-chain low-molecular weight organic compounds (e.g. methanol, ethanol, lactate).

Lactate is the best substrate for SRB (Postgate 1984) and a superior electron donor compared to others (e.g. ethanol) in terms of moles of bicarbonate produced per mole of substrate consumed (3 vs. 2, respectively), since it is better at neutralizing the acidity in the treated effluent (Kaksonen et al. 2003). In Cuba, some detailed studies about the use of some types of biotechnological barriers such as bacteria or mushrooms that neutralize the acidic environmental contamination during the metal exploitation works in the area have been planned (Díaz de Villalvilla et al. 2003). The main drawback is that not all SRB species are capable of oxidizing lactate and ethanol to CO₂. Some SRB can only partially oxidize simple organic molecules to acetate, and very few can use acetate alone (Nagpal et al. 2000).

SRB activity rates are thus dependent on the nature of organic waste used, and, in particular, the bioavailability of organic carbon (Gibert et al. 2002). As naturally refractory organic substrates release carbon and other nutrients slowly, it would seem beneficial if combinations of labile and refractory substrates are used, as this decomposing mixture can continue to provide carbon after the initial labile carbon fractions are exhausted (Koschorreck et al. 2002; McCullough and Lund 2011). They observed that a good organic carbon source must both initiate and sustain SRB-based bioremediation.

The rationale for selecting 30 g L⁻¹ of sewage sludge as the lowest concentration evaluated was based on previous studies that indicated that this is the minimum concentration necessary to initiate bioremediation of AMD affected pit lakes (McCullough and Lund 2011; McCullough et al. 2006).

Thus the availability of carbon from plant matter depends on decomposition, and decomposition is extremely limited in acidic and anoxic conditions (Kalin et al. 1993), which is typical of acid sulphate mine-spoils. Gibert et al. (2002), in a review, found that the specific composition of the organic matter was a primary determinant of the efficacy of the passive SRB-based treatment system; in particular, the lability and biological availability of the material. However, the ability of the organic matter to be decomposed over a prolonged period is likely more important to bioremediation of AMD than initial lability and bioavailability. Harris and Ragusa (2001) also noted that the availability of carbon from plant matter was dependent on decomposition, which can be extremely limited in acidic and anoxic conditions.

Further, Gyure et al. (1990) showed that concentration of organic acids (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 readily decomposable finely ground plant material placed in an AMD of pH 2.30 released some organic acids, but at such a high potency 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, 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 experiment (Harris and Ragusa 2000), readily decomposable material by itself did not substantially change the concentration of heavy metals in AMD. Neither did the sludge-only treatments. Yet, Waybrant et al. (1998) observed that sewage sludge encouraged the highest levels of sulphate reduction over seven other organic carbon sources. Moreover, multiple studies have noted that mixtures of sewage sludge with multiple organic carbon sources generally promote higher sulphate reduction rates than single sources (Waybrant et al. 1998; Harris and Ragusa 2000; McCullough et al. 2006).

Van Breemen et al. (1983) concluded that alkalinity produced during sulphate reduction, but not removed from the soil as acid neutralizing capability (ANC; aq) causes ANC (s) to increase. Using a mixture of sewage sludge and green waste (leaf mulch, woodchips and sawdust). Kumar et al. (2013) reduced 4500 mg L^{-1} of sulphate to $<25 \text{ mg L}^{-1}$ in only 35 days. A laboratory experiment by Harris and Ragusa (2000) also found that (under stagnant conditions), a mixture of sewage sludge and plant material (fresh rye grass) was effective in initiating amelioration of acidity and metal concentrations of acid mine waters through sulphate reduction. They were able to increase pH levels from 2.3 to >3 within 30 days.

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 neutralizing components. Therefore the high acidity (pH < 3.0 for many acidic mine waters) must first be appreciably decreased before biological sulphate reduction can occur (Alexander 1977; McCullough and Lund 2011). SRB are known to have their optimum pH at around neutral to slightly alkaline (Church et al. 2007; Gyure et al. 1990). 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 utilized (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 bioreactor, it would seem that the above-mentioned acclimatization period would be longer. Substantially reducing this acclimatization period would require a more efficient pH buffer for the AMD than that utilized 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 et al. 1991). At 6 h after lowering the pH of a stream with sulfuric acid, Norton et al. (1990) found that below pH 5, neutralization was dominated by the release of Al. Upon progressive acidification of the soil, large amounts of Al ions are eventually liberated (Bolt and Bruggenwert 1976). This acidity can be used up as ANC when the Al cations enter the adsorption sites of the clay minerals or oxides (Bruggenwert et al. 1991). For example, after testing the adsorptive power of Fe_2O_3 by adding it to trace elements mobilized in fermented (anaerobic) plant material, up to 59 % of the Cu, Zn, Cu 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 of >5 (Van Breemen et al. 1983). However, they are strong buffers at $\text{pH} < 5$ because oxides are the chief materials accepting protons (Bolt and Bruggenwert 1976; Barrow and Bowden 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 which could trigger the activation of SRB.

1.4 Cuba: Camagüey Sulfide System

Because of its vast number of ore deposits, the Camagüey mining region (Fig. 1) is the most important in Cuba, constituting a district of epithermal deposits (Díaz de Villalvilla et al. 2003). They found that the nature of the rocks of the volcanic arc in Camagüey allows correlation with those of The Dominican Republic. There are several types of mineral deposits in Central Cuba including high sulfide (Golden Hill) and low sulfide systems (Beatriz, Florencia), Cu–Mo–Au porphyry (Palo Seco y La Purísima) and Au–Ag–Zn Skarns (La Purísima) deposits (2003; Simon et al. 1999).



Fig. 1 Physiography of Cuba. Note the high density of natural waterways in the heavily mined Camaguey sulfidic region. Acidic mine drainage threatens the integrity of surface water and ground water aquifers

The presence of several types of mineral deposits genetically distinctive in these formations are characterized by pyrite presence as main mineral, either disseminated or accompanied by quartz in veins (Díaz de Villalvilla et al. 2003). The high prices of gold and silver at the present time, and a variety of minerals such as gold, silver, lead, bismuth and nickel belonging to the telluride group suggests at least several decades of future mining operations. In Golden Hill, Florencia and Beatriz, the corresponding environmental studies have been defined in order to take the systematic measures to avoid or decrease damage as possible (López et al. 1998).

The deposits are small and located near each other, standing out as small elevations on the surface, with ores formed by pyrite as the main mineral (Díaz de Villalvilla et al. 2003). Typical oxidation zones reach 10–20 m depth with quartz, carbonates, and mineral-loamy products of the wall-rock alteration. The reserves are approximately 5–10 tons of gold. The veins usually have high gold contents and erratic distributions could be present. Small mining operations are needed to open pit facilities and benefit chord in the studied resources (Díaz de Villalvilla et al. 2003).

The above workers concluded that the oxidation of these sulfurs could generate acid drainage, which can potentially affect the superficial drainages of the area of influence in the mine and consequently enter in the food-chain. However, the presence of carbonates in the mineral bodies and associated with the wall rocks could directly limit the mobility of the metals and the acid drainage product of the water circulation and the pyrite presence during the mining activity (open pit) (Díaz de Villalvilla et al. 2003). Thus samples collected by Du Bray (2004), of stream sediment using the open-pit method and heap leaching show results of <5–12 silver ppm, 0.04–0.4 ppm of gold, <5 ppm cadmium, 18–81 ppm cooper, 1.4–4.2 % of iron 200–2.020 ppm manganese, 7–197 ppm lead, and 70–1.131 ppm zinc (Du Bray 2004).

1.5 Dominican Republic: Pueblo Viejo Sulfides

1.5.1 Physiography and Climate

The Dominican Republic occupies the eastern portion of the Caribbean island of Espanola (the nation of Haiti occupies the western portion). The Dominican Republic was estimated to be the world's 11th ranked producer of nickel in 2008 (Wacaster 2010). The Pueblo Viejo gold deposit ranks as one of the largest high-sulfidation acid epithermal deposits in the world (Mueller et al. 2008; Kuck 2009; Redwood 2009).

The central region of the Dominican Republic is dominated by the Cordillera Central Mountain Range, which runs from the Haitian border to the Caribbean Sea (Fig. 2). The highest point in the Cordillera Central is Pico Duarte at 3175 m. Pueblo Viejo is located in the eastern portion of the Cordillera Central (Fig. 2) where local topography ranges from 565 m at Loma Cuaba to approximately 65 m at the Hatillo Reservoir (Smith et al. 2008).

The mine was inadequately closed and reclaimed, resulting in the extensive formation of acid mine drainage (AMD), leading to a legacy of contaminated infrastructure, soil, and water at the site and surrounding area. Untreated and uncontrolled AMD resulted in the transport of high metal concentrations and acidity into local waterways (Kuck 2009). Other environmental, health, and safety hazards identified at the site included rusting machinery, oil and gasoline-contaminated soils, mercury-contaminated materials, asbestos, and local groundwater contamination (Kuck 2009). Two rivers run through the concession, the Margajita and the Maguaca. The Margajita drains into the Yuna River upstream from the Hatillo Reservoir while the Maguaca joins the Yuna below the Hatillo Reservoir. The flows of both rivers vary substantially during rainstorms (Smith and Stephenson 2011).

The Dominican Republic has a tropical climate with little fluctuation in seasonal temperatures, although August is generally the hottest month, and January and February are the coolest. Temperatures at the project site range from daytime highs of 32 °C to night-time lows of 18 °C. Annual rainfall is approximately 1800 cm, with May through October typically being the wettest months. The Dominican Republic is in a hurricane channel; the hurricane season is typically



Fig. 2 The eastern section of the Central Cordillera contains Pueblo Viejo, one of the world’s largest sulfidic gold mines, headquartered at Cotuí, within 150 km of Santo Domingo. It therefore can be seen that, as in Camaguey (Cuba), several river basins (at least 4) are at risk from toxic heavy metal pollution from exposed sulfidic wastes

August to November. As a result of previous mining and agriculture, there is little primary vegetation on the Pueblo Viejo site and surrounding concessions, but secondary vegetation is abundant outside of the excavated areas and can be quite dense (Smith and Stephenson 2011).

1.6 Aim and Hypothesis

Given adequate substrates and a long enough acclimatization time (Harris and Ragusa 2000), dormant SRB colonies could become extremely active in acid drainage (AD) conditions ($\text{pH} < 3$). The SRB placed in the harsher environment of a similar (in character) but flowing AMD would require a longer acclimatization 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 hypothesized that (1) clay or Fe/Al (hydr)oxides released in AMD of pH < 2.5 may increase the pH of the AMD to a point where sulphate reduction by SRB is begun, and (2) SRB will mitigate SRB of pH < 3 in a constant flow bioreactor energized by decomposable plant matter, but initially activated by an inorganic pH buffer.

2 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 (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 1–4 cm of a natural follow plot.

2.1 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 containers. The properties of the soils together with the test methods are listed in Table 2.

Table 1 Some properties of clay and soil buffers

Name	Abbreviation	pH	CEC (cmol (+)/kgcmol)	CaO	SiO ₂	Fe ₂ O ₃	Al ₂ O 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	4.5	16.2
Ball clay	BCL	4.7	n.d. ^a	0.2	58.2	1.0	26.3
Trufeed bentonite	TRP	7.0	80	0.02	61	2.9	14.8
China clay	CLH	8.4	n.d.	0.1	50.8	0.9	33.1
Oxide	OX	6.5	n.d.	0.3	5.0	28.0	14.5
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.
Pure kaolinite	PURK	8.2	6.7	0.1	53.7	0.9	35.1

^aNo data

Table 2 Some chemical and physical characteristics of two soils

Soil property	Alfisol	Mollisol	Test method
pH (1:5 sludge:water ratio)	6.5	7.2	Watson-Victor glass electrode
Electrical conductivity (dS m ⁻¹)	0.12	0.23	Ionode glass electrode
Organic C (%)	1.24	4.6	Leco C analyzer
Total C (%)	1.4	4.61	Leco C analyzer
Total N (%)	0.11	0.69	Leco N analyzer
Inorganic C (%)	<0.01	<0.01	Difference
CEC (cmolc (+)/kg)	7.3	59.1	1N NH ₄ OAC (buffered at pH 7)
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 (%)	31.7	36.1	Difference
Fine sand: 20–200 µm (%)	44.8	17.2	Pipette
Coarse sand: 0.2–2.0 mm (%)	3.9	6.4	Pipette

D Dominant; p clay minerals present

2.2 Inoculation

Inoculation was not necessary in order to initiate the sulphate reducing process (Christensen et al. 1996; Harris and Ragusa 2000; Koschorreck 2008; Koschorreck et al. 2011; Kumar et al. 2011), but was applied to shorten the initial lag phase (Christensen et al. 1996). A mixture of AMD (Table 3) and sewage-sludge impregnated ryegrass from a previous incubation containing an enriched strain of *Desulfovibrio vulgaris* one of two major SRBs active at a pH above 5 (Alexander 1976) at 30 °C for six days, Alexander (1976) 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 4-fold decrease in sulphates 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 at 6.3. The presence of viable SRB was strongly suggested. In addition to the above-mentioned colour change, the smell of H₂S was detected from the inoculants. The presence of H₂S 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 H₂S gas to form brown lead (II) sulfide.

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

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

Aluminium	46
Arsenic	0.2
Boron	<0.1
Cadmium	<0.1
Cobalt	0.1
Chromium	0.01
Copper	0.1
Iron	2100
Magnesium	290
Manganese	110
Nickel	0.03
Sulfur	2700
Selenium	<0.01
Zinc	5.7
Molybdenum	<0.1
Sodium	220
Phosphorus	<1
Acidity as CaCO ₃ (kg)	2006–2602

2.3 The Reactor

The reactor was made from rigid PVC tubing 500 mm high with an 80 mm internal diameter (Fig. 3). This up-flow bioreactor was designed by Elliott et al. (1998), who achieved growth of SRB at pH 4, using sodium lactate as the energy source. Eight sampling ports placed 5 cm apart in the column were plugged with rubber seals (Fig. 3). 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 quartz gravel was laid at the bottom of the column, which was overlain by a 4 cm thick pure washed quartz (250 μ) layer. Freshly cut ryegrass ground to <1 mm diameter 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 covered with the influent. The pH was measured after 1 day.

2.4 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 height) and at 5 cm (the base of the column). Water samples were taken at

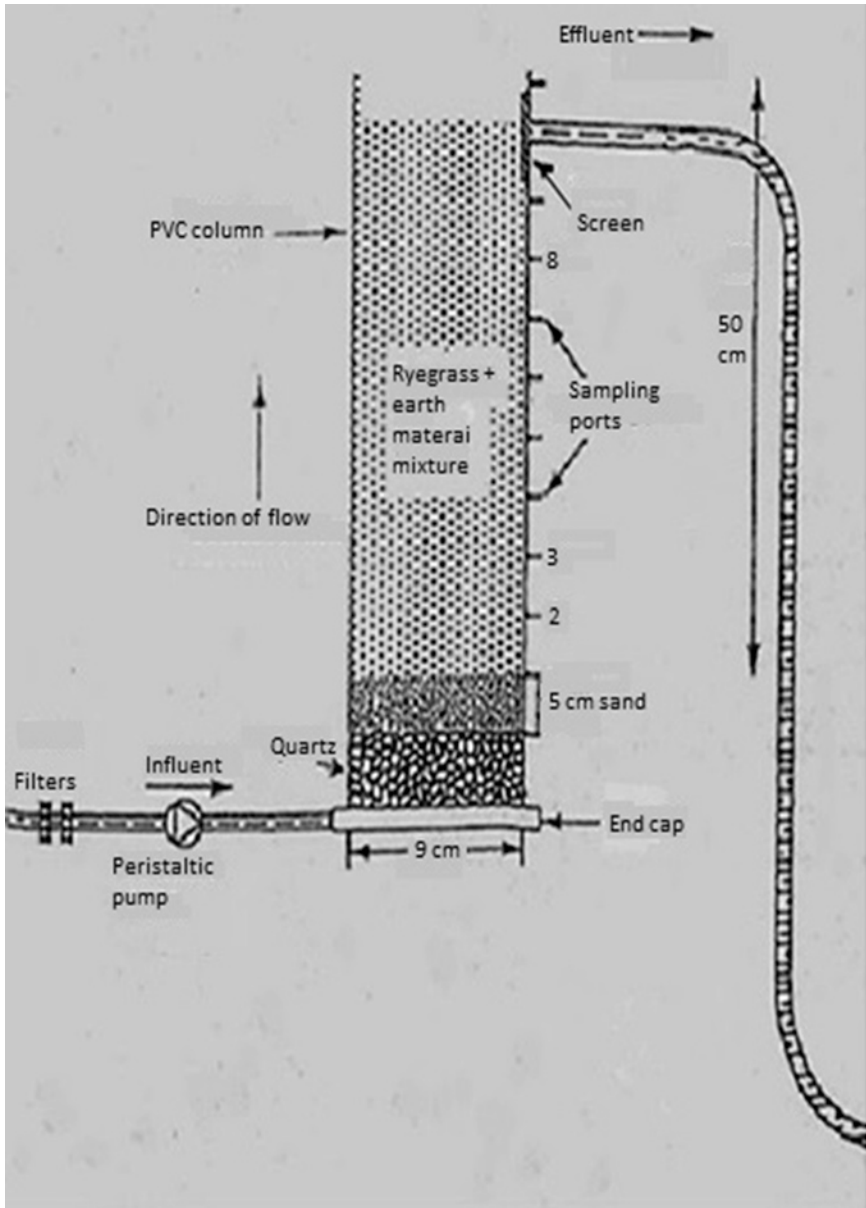


Fig. 3 Bioreactor containing nutrient source and a mollisol as a pH buffer to accelerate the proliferation of SRB (after Elliott et al. 1998)

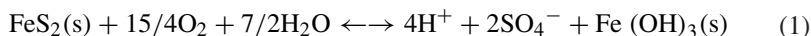
15-, 25-, 35 cm (hereafter in this study referred to as ports 4, 7, and 10) on the 1st, 3rd, and 5th day after each increase in AMD flow rate of the column incubation. The sampling plan was to continue every 3 days until a pH reading of at least 5.5 was simultaneously obtained from all ports above port 3 (i.e., at least 15 cm above the point of

influent entry), at which time the influent rate would be increased. Influent and effluent pH was measured with a Watson-Victor glass pH electrode model 5003.

2.5 Buffer Study

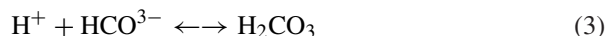
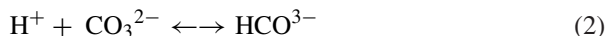
Need for buffers

A buffer was selected to remove H^+ ions from the AMD because the introduction of hydrogen ions by acid mine drainage changes the acidity of a stream as follows:



However, pH is not always a good indicator of acid mine drainage; pH measurements may not detect heavy acid mine drainage in a stream because of high alkalinity due to dissolved carbonates. Assessing the excess of hydrogen ions over basic ions, “total acidity,” is a better measurement of acid mine drainage.

To substantially reduce the acclimatization period for the SRB in a highly acidic medium, an experiment was conducted to identify some effective pH buffers for flowing AMD because acid mine drainage depletes the buffering ability of water by neutralizing carbonate and bicarbonate ions to form carbonic acid (H_2CO_3) as follows:



As exposure to acid mine drainage depletes the acid neutralizing capacity (ANC) of the carbonate buffering system, thereby completely destroying it below a pH of 4.2 (Eq. 4),



a cross-section of earth materials (14 non-lime materials) comprising inorganic layer silicates including 10 bentonites, 1: clays, an amorphous oxide, and two “whole” soils were to be tested for their buffering efficiency in AMD. Such buffers were chosen because of widespread availability of clays, and because Cravotta (2003), Watzlaf et al. (2003) observed that alkalinity produced by dissolution in limestone zones representative of real-world ALD performance provided sufficient alkalinity to produce net-alkaline effluent. One of the “whole” soils—an alfisol (RB) containing 2 % iron oxide (Deshpande et al. 1964) was selected because addition of iron oxide 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. Pulverized $Ca(OH)_2$ (<1 mm diameter) at rates of 0-, 2.5-, 5-, 7 % (w/w), was mixed with each clay or soil. The samples were each submerged in stagnant AMD (pH < 5) for 30 days. The pH of the AMD was recorded every day for thirty days, or until no further changes occurred for consecutive readings.

2.6 Bioreactor Experiment

The SRB bioreactor experiment was carried out under anaerobic conditions at mesophilic temperatures (30–35 °C) because the formation of sulfide by sulphate reduction is enhanced by increasing water levels and rising temperatures (Alexander 1977; Tsukamoto et al. 2004). 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.

3 Results

3.1 Buffer Study

The effects of the various buffers on the pH of the AMD are shown in Fig. 4, where the relationship between pH and different rates of lime application can be 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 liming rate of 7.5 %, it was, in general, always the same ones that caused the pH increases at various rates of added lime (Fig. 4). Of the fifteen buffers studied, even at the highest 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 $\text{Ca}(\text{OH})_2$ level, only three earth materials—the MC, OX, and AG—accomplished AMD neutralization at pH > 5 (Fig. 4).

At the lowest lime application rate (2.5 %), only the mollisol caused the AMD pH to increase to >5 (Fig. 4). Although the ANC of the AMD is comparable to that of the mollisol and the oxide, the mollisol is seen as the most significantly

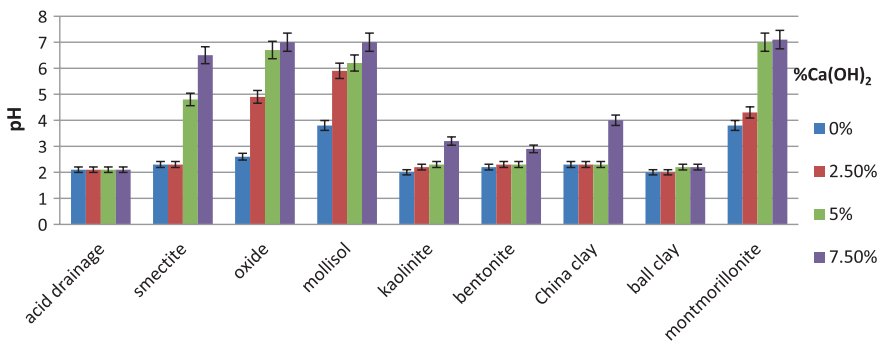


Fig. 4 Changes to AMD pH after addition of earth materials plus addition of calcium hydroxide

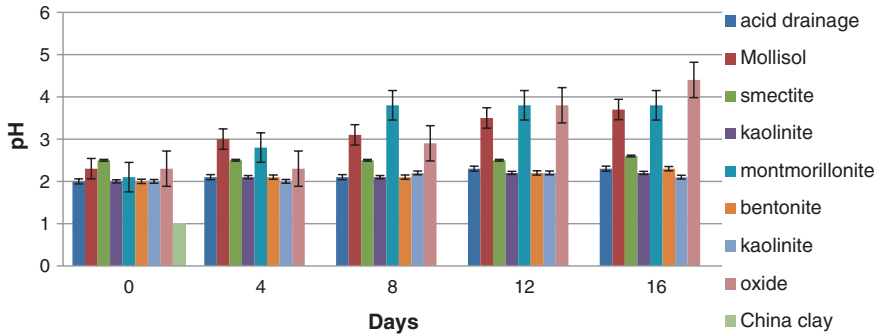


Fig. 5 Changes to pH of acid drainage after addition of non-calcic earth materials

responsive to low-level lime spiking (Fig. 4). Thus at just 2.55 of 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 pH (AMD) 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. 4 and 5). This shows that without an adequate buffer (in combination with lime) in AMD, the quantities of lime applied 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 AMD, even when 2.5 % of $\text{Ca}(\text{OH})_2$ was added, but increased markedly when 5 % $\text{Ca}(\text{OH})_2$ was added (Fig. 4). In general, the more responsive the buffer, the earlier it peaks (i.e., stabilizes; Fig. 4). While the MC-treated AMD peaked at just 5 % added lime, the less responsive AB increased the pH of the AMD 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 increase of all the buffers (Fig. 5), being 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. 4 and 5). 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 reasons, found to have inhibited the activation of the SRB. No evidence of SRB was detected after a 30-day period following a subsequent trial inoculation of the oxide-treated AMD in stagnant conditions (not illustrated here). However, for the mollisol, the waiting period for SRB was only 5 days. Even without added lime, the MC, OX, and AG substantially increased the AMD pH from 2.3 to >3.0 units at 5 days. These

materials therefore exhibited a high ANC (Fig. 4). However, the AG, although a reasonable 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 which made it difficult to visually demarcate the boundary between the active gel 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 have been required, which might have led 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 % $\text{Ca}(\text{OH})_2$ had been added to the AMD (Fig. 4). The buffering response of the AB without lime was therefore very slow or non-existent because it did not, after 21 days, increase the AMD to a value of even 3.0 units (Fig. 5). Such a small increase 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 upward-through-flow system). On the basis of all the above-mentioned results, the MC was chosen to buffer fluids in the following investigations.

3.2 *Bioreactor Experiment*

3.2.1 Appearance

The bioreactor supported sulphate 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 of AMD influent), to a value of 5.0 in the surface of the water (45 cm upwards from the base) in the column (port 10) in 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 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 or 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⁻¹). 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 sulfide 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.

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

3.2.2 Changes in pH and Adaptation of SRB to Acidic Conditions

The response of the reactor to low pH conditions was detected by sampling at 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. The 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 about three quarters of the distance up 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 one 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 cm 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 flow rate had been increased from 30- to 60 ml/day). Instead, the pH leveled 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 increments of 30 ml day⁻¹, pH at all ports also increases incrementally at least until day 15. This suggests that the SRB colony was becoming acclimatized to the slowly increasing acidic conditions in the column. However, between days 15 and 18 during the initial period after the influent rate was doubled from 90- to 180 ml day⁻¹, 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 at a larger increment at port 7 than at port 10 at 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 at day 18 (Figs. 4 and 5). The subsequent pH recovery for 180 ml day⁻¹ did not begin in the zone furthest (45 cm) above the influent inlet, 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).

A laboratory experiment by Harris and Ragusa (2000) also found that a mixture of sewage sludge and plant material (fresh rye grass) was effective in initiating amelioration of acidity and metal concentrations of acid mine waters through sulphate reduction. They were able to increase pH levels from 2.3 to 3 within 30 days. With the organic materials we have tested, 60 days is usually sufficient to achieve a pH of 7, as also shown by Kumar et al. (2011), McCullough and Lund (2011), McCullough et al. (2006). This reflects the sigmoidal response of pH (Fig. 2) where early treatment phases can be slow, most likely due to limited initial supplies of labile organic carbon, which then rapidly accelerate up to pH 6 before reaching an asymptote near pH 7. This does not appear to be an artefact of completing experiments once pH neutrality is acquired, because microcosm pH does not seem to increase above 7–7.5 regardless of how long the experiment had been allowed to continue (Harris and Ragusa 2001; McCullough and Lund 2011). McCullough and Lund (2011) further note that this threshold pH does not appear to be due to limited sulphate or labile organic carbon and further research is required to understand the cause of this phenomenon.

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 level above, which contained viable SRB. At day 24, pH in the quartz-gravel-sand layer at the bottom was still unchanged at 2.3, as opposed to pH of 4.0 at 15 cm above the surface of the sand layer, i.e., at the base of the lowest one-third of the column substrate interstices (Fig. 4). Throughout incubation, the quartz-gravel-sand layer 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.

3.2.3 Cation Concentration

The concentration of the cations Al, Cu, Fe, Mg, Mn and sulphate was monitored by ICPAES. During this study, even the most efficient zone in the column experienced marked decreases in cations and sulphate concentration. After just 6 days of operation the following decreases had occurred at just 15 cm above the influent inlet (Fig. 7):

1. Fe from 1800- to 70 mg L⁻¹
2. Sulphate down from 2700- to 1330 mg L⁻¹
3. Although Al concentration had doubled from 32- to 62- by day 6, it had decreased to 1.8 mg L⁻¹ by day 9.

In contrast the metal concentrations were comparable in the quartz-gravel-sand interstices (port 1) to that for the raw AMD (Tables 3 and 4). Changes in the AMD pH with cation concentration after the first 10 days at the lowest influent flow rates

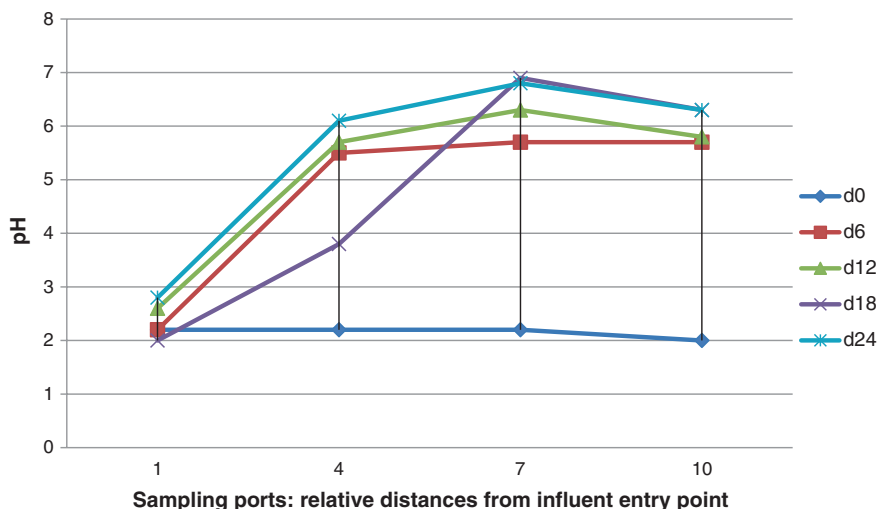
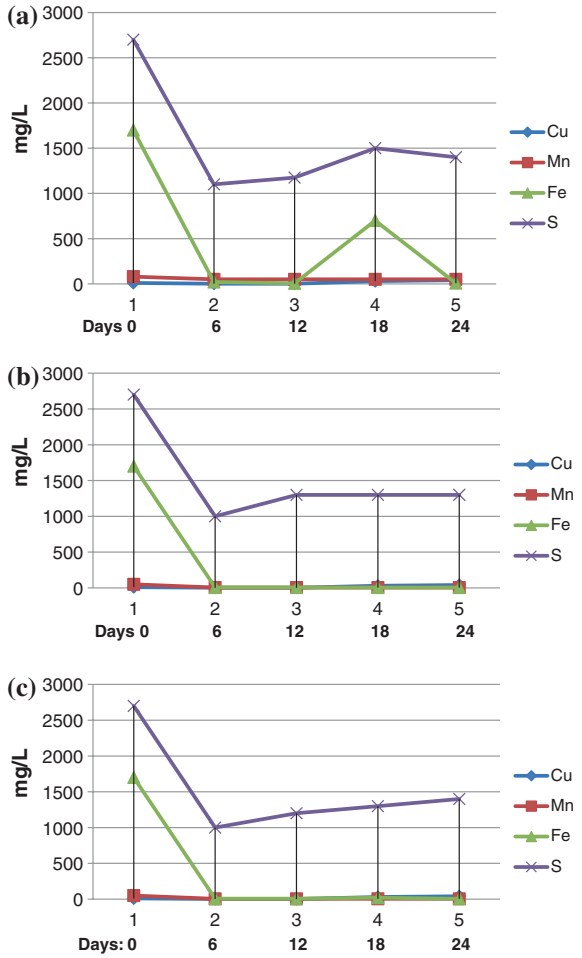


Fig. 6 Changes to pH with distance from AMD entry point in an SRB-driven bioreactor

(30–60 ml day⁻¹) are also shown (Table 4). 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 small amounts. Secondly, as the distance away from the influent entry point increased, metal cation and sulphate 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. 7). This indicates that effects of an increasing influent flow rate on the metal and sulphate concentration became less marked with distance from the influent entry point. The pH changes inversely with toxic metal concentration over time (Fig. 6).

These observations, the fourth one in particular, suggest the presence of a growing SRB colony despite the increasingly acidic environment (caused by increasing flow rates), even though the nutrient substrate was being used up. An example is shown in Fig. 7a, at the 15 cm port (the 3rd port nearest the influent inlet) where the largest influent increase (on day 16, i.e., from 90- to 180 ml day⁻¹) caused the sulphate concentration to rise most sharply at 18 days from 1300- to 2200 mg L⁻¹ (a 59 % increase). It was brought back down to 1750 mg L⁻¹ 3 days later by day 21. The general trend describes a gradual lowering of sulphate concentration over time. At the lower column levels (and to some extent at the highest column levels) the largest sudden change to the sulphate and Fe regime coincided with the largest increase in influent flow rates (Fig. 7). At three quarters of the way up the column, sudden changes in influent flow rates caused the smallest perturbations in sulphate and other toxic ion concentrations (Fig. 7).

Fig. 7 Cation concentration at distances from AMD influent entry. **a** 15 cm above influent inlet, **b** 30 cm above influent inlet, and **c** 45 cm above influent inlet



4 Discussion

4.1 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 pH units. Only when the added lime was increased to 7.5 % did the pH of the RB-treated AMD exceed 3.0 units. A previous study indicates that a kaolinite sludge increased the pH of an AMD from 2.3 to 2.6 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 acclimatization period for SRB activation, it was not necessary to prolong the experimental 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 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, and Singh et al. (2003) found that buffering capacity increased in ascending order of clay content. The two pure clays that were among the three most effective buffers were the active bond and active gel, both being smectites (montmorillonites) with high CEC's. 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 more greatly than any other factor in the soil (Tisdall and Oades 1982).

4.2 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 soluble cations decreases, and vice versa (Fig. 6). 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 stage 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 Ponnampereuma (1972), who found that green manures added to soils caused a remarkable rise in pH two weeks after submergence. Singh et al. (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. Their explanation 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 SRB. This is corroborated by the findings of Katyal (1977). Using *Sesbania aculeata* as green manure under flooded conditions, he recorded a decrease in (metal) toxicity and favourable changes to pH.

Strong evidence for SRB activity was observed, including production of H₂S, the blackening of the substrate buffer, and the lead acetate test. The sulphate reduction was not as great at the water surface 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 sulphate 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 in 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 sulphate 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, sulphate, although less concentrated at port 7, was concentrated at 1300 mg L^{-1} at the water surface in the reactor. The two cations most affected by distance from the inlet port are S and Fe (Fig. 7). As distance increases further up the column from 30- to 45 cm, the differences in sulphate reduction become smaller (Fig. 7a–c). SRB activity did not increase as much between 20- and 45 cm (the water surface) as it did between 15- and 30 cm above the inlet port. A change in sulphate concentration from a decreasing one in the column to an increasing trend at the water surface can be seen (Table 4). Alexander (1977) concluded that even though SRB are active at $\text{pH} > 5.5$, they are most active at a neutral pH. The area around port 7 showed consistently greatest sulphate reduction. Nevertheless, such pH changes in this case are a symptom, not the cause of decreasing SRB activity at the water surface.

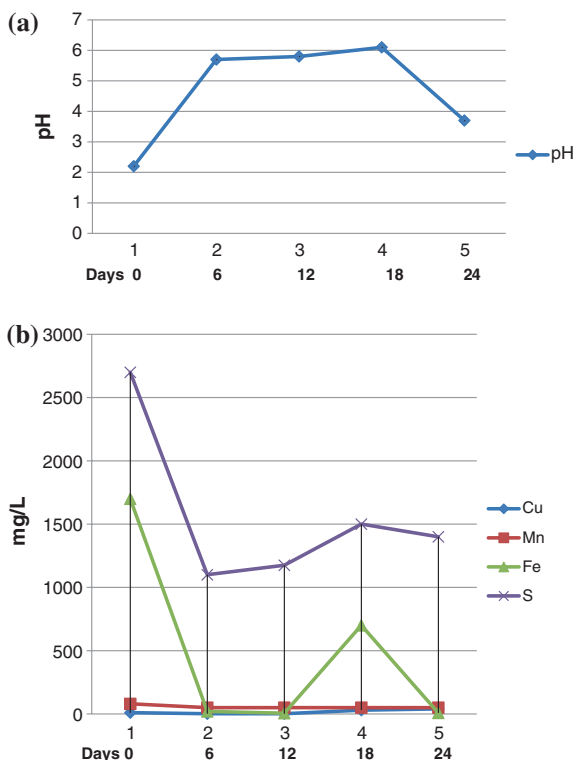
The answer may lie in the increasing consumption of oxygen in the upper layers of the AMD. Anoxic to anaerobic ORP is critical for initiation and continued SRB activity for AMD bioremediation (Strosnider and Nairn 2010) and can be, in fact, indicative of SRB activity. Anaerobic bacteria begin to use other electron acceptors in decreasing order of their electrochemical reduction potential once dissolved oxygen (DO) is removed (Castro and Moore 2000). SRB start reducing sulphate after most of the oxidizing species have been consumed, at an anaerobic redox potential between -75 and -200 mV (Connell and Patrick 1968).

In this experiment the AMD did not completely fill the column. In colder months, the top few centimeters of sediments in a lake bed may be oxidized (Herhily and Mills 1985), and SRB is inhibited above the oxidation depth. This is because the process of sulphate reduction requires low oxidation-reduction potentials (Eh) characteristic of anaerobic habitats. Herhily et al. (1987) 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 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 oxidized (Herhily et al. 1987). In the present study, oxygen was always present in the column. During its setting up, no attempt was made to avoid exposure to air. There was an air-filled space above the water surface. The dissolved oxygen in the top layer of the water would have inhibited SRB and microbial sulphate reduction. The difference between the sulphate 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 utilized buffers containing similar proportions of clay (40 %), but the sludge in the previous study contained 2.5 % CaCO_3 (MA Harris, unpublished data), as opposed to <0.01 CaCO_3 for the mollisol used in this study (Table 2). Even without the high proportion of CaCO_3 of the sludge, the mollisol is a significantly more efficient pH buffer. The CECs of the two buffers differ markedly at 18.4 and 59.1 cmol (+) kg^{-1} respectively. 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, and may have resulted from the organic C fractions of the two buffers. CEC can be reduced by as much as 20–50 % by removal of the organic phase, which normally constitutes just 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 the inherent influence of organic matter on the CEC of the mollisol was three times that of its effect on the sludge (Harris and Ragusa 2000). This would be the case because the clay fraction of both soils were dominantly kaolinite-illites. By further increasing the organic C fraction, the addition of decomposable plant material would have increased the CEC 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, with the greater native organic fraction, would have exhibited greater increases in CEC.

As the capacity of the bioreactor exceeded 2 L, the 20 g L^{-1} inoculation rate of sewage sludges was seemingly less than half the minimum rate of 30 g L^{-1} minimum required according to Kumar et al. (2013). Thus Kumar et al. (2013) selected 30 g L^{-1} of sewage sludge as the lowest concentration evaluated based on previous studies that indicated that this is the minimum concentration necessary to initiate bioremediation of AMD affected pit lakes (McCullough and Lund 2011; McCullough et al. 2006). Therefore, the explanation of the success of this low rate of sewage sludge used in this study may be the fact that it had been already been SRB-activated, having been aliquots from a prior SRB-producing experiment by

Fig. 8 Variations of pH with AMD concentration of soluble cations in an SRB-driven bioreactor



Harris and Ragusa (2000). Such priming of the substrate therefore promises a possible lowering of the 30 mg L^{-1} minimum concentration found for sewage sludge which had not been previously exposed to SRB (Fig. 8).

5 Conclusions

The results of this study show that it is possible to establish an SRB population in a constant flow reactor, using rapidly decomposable material and a fast-acting soil-based buffer. Under such conditions, the SRB can become acclimatized 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 buffer/substrate materials of the present study proved to be far more effective than those of Harris and Ragusa (2000). It seems CEC, and not merely free lime, was the crucial buffer characteristic at the low pH level of <3.0 units. The CEC of the substrate used in this study was three times that of a sludge buffer used by Harris and Ragusa

(2000). The only effective substrate difference (excluding the influent flow rate) between the present study and that of Harris and Ragusa (2000) is that of the buffers used. Hence, it can be concluded that for pH increases 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.

6 Geotechnological Applications

6.1 A Procedure for the Hydrogeological Confinement of Acid Mine Drainage

By sealing off the source of contaminants, hydrogeological barriers can reduce or prevent the spread of AMD. The objective is to “buy time” required for detoxifying before re-introducing the liquid into the environment. To this end Navarro et al. (2000) dug a perimetrical ditch to catch and drain surface runoff. To determine the dimensions and positioning of the ditch, they identified the existence and propagation of leakages by noting the surge of contaminated water through contiguous aquifers and by analytical data from piezometers.

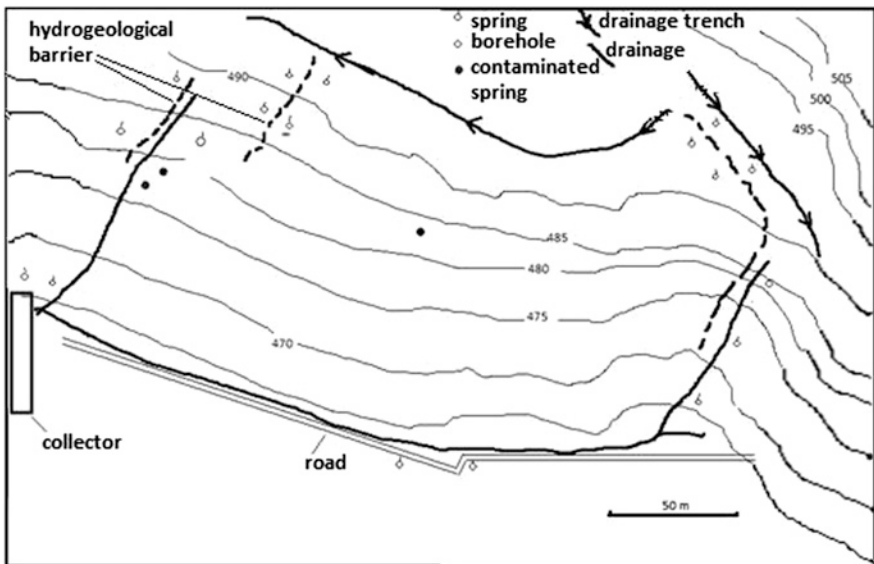


Fig. 9 Hydrogeological containment ground for aqueous contaminants emanating from mine waste tailings heap (after Navarro et al. 2000)

Their method includes control of contamination by preventing water from entering the waste-disposal site, and catching the inevitable outputs of contaminated water for subsequent treatment. The advantage of impermeable screens is the avoidance of high energy costs as pumping then becomes unnecessary. Initial costs of the impermeable barriers however can be extremely high, depending on the areal extent of the waste. The above workers however by taking advantage of natural hydrogeological barriers, reduced construction costs (Fig. 9).

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