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TECHNICAL ARTICLE

Upper and Lower Concentration Thresholds for Bulk Organic Substrates in Bioremediation of Acid Mine Drainage

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Abstract Acidic pit lakes can form in open cut mine voids that extend below the groundwater table. The aim of this research was to determine what bulk organic material concentrations best stimulated sulphate-reducing bacteria (SRB) for acid mine drainage (AMD) treatment within a pit lake. An experiment was carried out to assess the effect of different substrate concentrations of sewage sludge on AMD bioremediation efficiency. Experimental microcosms were made of 300 mm long and 100 mm wide acrylic cores, with a total volume of 1.8 L. Four different concentrations of sewage sludge (ranging from 30 to 120 g/L) were tested. As the sewage sludge concentration increased, the bioremediation efficiency also increased, reflecting the higher organic carbon concentrations. Sewage sludge contributed alkaline materials that directly neutralised the AMD in proportion to the quantity added and therefore played a primary role in stimulating SRB bioremediation. The lowest concentration of sewage sludge (30 g/L) tested proved to be inadequate for effective SRB bioremediation. However, there were no measurable beneficial effects on SRB bioremediation efficiency when sewage sludge was added at concentrations >60 g/L. We compared our results with existing literature data to develop a conceptual model for remediation of AMD in pit lakes through organic material amendments. The model indicated that labile organic carbon availability was more important to the bioremediation rate than AMD strength, so long as iron and sulphate concentrations were not limiting. The conceptual model also indicates that bioremediation may still occur when only low concentrations of organic carbon are present in the pit lakes, albeit at a very slow rate. The model also demonstrates the presence of an organic material amendment threshold where excess organic carbon does not measurably influence the final outcome. The conceptual model defined is well supported by the results of the microcosm experiment.

Keywords SRB · Organic carbon · Sewage sludge · pH · Sulphate · Conceptual model

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Introduction

Given the high costs of backfilling open cut mining pits, many will be left upon cessation of mining, and will fill with surface and groundwater, forming pit lakes. Water quality in pit lakes is largely influenced by the nature of the ore type mined and the geology and hydrology of the surrounding catchment (Castro and Moore 2000).

Ever since the work of Tuttle et al. (1969) on the potential use of sulphate-reducing bacteria (SRB) to treat acid mine drainage (AMD), there has been a plethora of studies exploring the addition of organic materials for initiation of sulphate reduction to treat AMD problems. Poor allochthonous (carbon inputs into the pit lake from outside sources such as runoff from the vegetated catchment; McCullough et al. 2009) and autochthonous (carbon inputs from sources within the pit lake, such as microbial decomposition of particulate organic carbon, algal production; Peine and Peiffer 1998) contributions of organic carbon in pit lakes typically limit sulphate reduction. Organic substrates must therefore be amended to produce reducing conditions suitable for sulphate reduction and to serve as either direct or indirect sources of electron donors to the SRB (Castro et al. 1999; Wendt-Potthoff and Neu 1998).

Sulphate-reducing bacteria are 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 the reactors. SRB are dependent on other microbes that degrade these complex substrates and ferment them to products that can serve as substrates for SRB (Figueroa et al. 2004; Muyzer and Stams 2008). Frömmichen et al. (2003) found that whilst pure and complex carbon sources can serve as suitable substrates for stimulating microbial reductive processes in 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. SRB activity rates are dependent on the nature of organic waste used and, in particular, the bioavailability of organic carbon (Gibert et al. 2002). Naturally refractory organic substrates release carbon and other nutrients slowly, which is 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 et al. 2008). A good organic carbon source must both initiate and sustain SRB-based bioremediation.

Sewage sludge is commonly available in many remote mining regions (Kumar et al. 2011b) and has been internationally proposed for mine water remediation (Liang and Thomson 2009). Use of sewage sludge for bioremediation of acidic mine waters in pit lakes in remote locations seems to be an attractive and cost-effective option (McCullough 2008). However, many questions remain unanswered for field application of SRB-based bioremediation. For instance, is there a critical threshold, i.e. the amount of organic matter needed to initiate and sustain SRB activity? Further, is there a threshold above which further additions of organic material fail to enhance the rate of bioremediation? To our knowledge, there has been no determination of the optimum organic material dose required for acidic pit Lake Bioremediation by SRB. The present study was designed to gain a better understanding of the critical concentration of organic materials needed to both initiate and sustain SRB-based bioremediation. A conceptual model based on this and other published studies was then developed to illustrate the relationship between organic materials amendments dose and AMD treatment rate.

Table 1 Physico-chemical characteristics of synthetic acid mine water

Parameter	Value
pH	2.8
EC (mS/cm)	5.9
ORP (mV)	350
Dissolved oxygen	7.4
Acidity $KB_{8.2}$ (mM)	28
Sulphate	1,800
Nitrate	65
Ammonia	50
Phosphate	0.1
Aluminium	100
Calcium	50
Copper	25
Iron	380
Potassium	170
Magnesium	100
Manganese	15
Nickel	2
Zinc	2

All values are in mg/L except otherwise indicated

Materials and Methods

Organic Material and Synthetic Mine Water

Secondary treated sewage sludge was collected from the Beenyup Wastewater Treatment Plant, Perth, Western Australia, for the microcosm experiment. Synthetic acid mine water (SAMW) was prepared by dissolving analytical grade chemicals in MilliQ water. Solute concentrations in the SAMW (Table 1) were selected to simulate routinely reported AMD affected pit lake water in the literature (Chang et al. 2000; Gray and O'Neill 1997; La et al. 2003; Prasad and Henry 2009).

Experimental Design

Fifteen microcosms were constructed from 100 mm diameter and 300 mm long clean and acid-rinsed acrylic tubes containing 1.8 L of SAMW. These microcosms were sealed with rubber bungs at the bottom and with removable PVC lids at the top to minimise atmospheric gas exchange, mimicking stratified lake conditions. Microcosms were placed in opaque black plastic tubs filled with water to evenly distribute temperatures. Microcosms were covered by opaque tarpaulins to exclude light and limit primary production, to simulate deep lake conditions. All experiments were carried out at a temperature of ≈25 °C and monitored for 60 days. Three replicate cores were allocated to each of the following treatments; control (untreated, 'C'), and four dosage levels of sewage sludge: 30 g/L, 'S30'; 60 g/L, 'S60'; 90 g/L, 'S90'; and 120 g/L, 'S120'. The rationale for selecting 30 g/L 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). All of the microcosms received SAMW inoculated with 5 % (v/v) bacterial inoculum from a successful bioremediated stock microcosm containing AMD, sediment, and organic matter (green waste and mulch). The inoculation was to overcome the absence of pit lake sediment and bacteria normally found in real AMD.

Sampling and Analysis

Sewage sludge was analysed for total Kjeldahl nitrogen (TKN), total carbon (TC), and total organic carbon (TOC) as per standard methods (APHA 1998). Physico-chemical measurements in treated and control microcosms were taken after sewage sludge addition, at day 1, then weekly to day 42, and finally at day 60. A Hydrolab Datasonde 4a multiparameter meter (Hydrolab, USA) was used for recording temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), and oxidation reduction potential (ORP; platinum reference).

A 180 mL water sample was taken for chemical analysis from each microcosm on days 1 and 60. An aliquot of this sample was filtered through 0.5 µm filter papers (Metrigard^{TM}, Pall Corporation). The filtrate was analysed for metals (Al, Ca, Mg, Fe, K, Mn, Na, Ni, Zn) and S following acidification with 1 % analytical grade nitric acid and analysed using an ICP-AES (Varian Vista-Pro, USA). Another aliquot of filtrate was analysed for sulphate and phosphate using ion chromatography (Metrohm 761 Compact, Switzerland). Ammonia (NH_3–N), NO_x–N (nitrate and nitrite) on filtered, and total nitrogen (TN) on unfiltered samples were analysed on a nutrient auto-analyser (Skalar, USA). DOC was measured as non-purgeable organic carbon (NPOC) on filtered samples using a TOC analyser (Schimadzu TOC-V CSH, Japan). Acidity ($K_{B8.2}$) was measured with an auto-titrator (Metrohm, Switzerland) using 0.1 M NaOH as the titrant.

Conceptual Model

Using the results from the present study, earlier research on SRB-based bioremediation by bulk organic materials amendments in our laboratory, and published work (Frömmichen et al. 2004; Harris and Ragusa 2000, 2001; Kumar et al. 2011c; McCullough and Lund 2011; McCullough et al. 2006, 2008; Tuttle et al. 1969; Waybrant et al. 1998), we developed a conceptual model explaining the response of AMD to organic amendments.

Results and Discussion

The sewage sludge from the Beenyup wastewater treatment plant had a TKN of 5.1 g/kg, a TC of 45 g/kg, and a TOC of 15 g/kg on a dry weight basis. The total inorganic

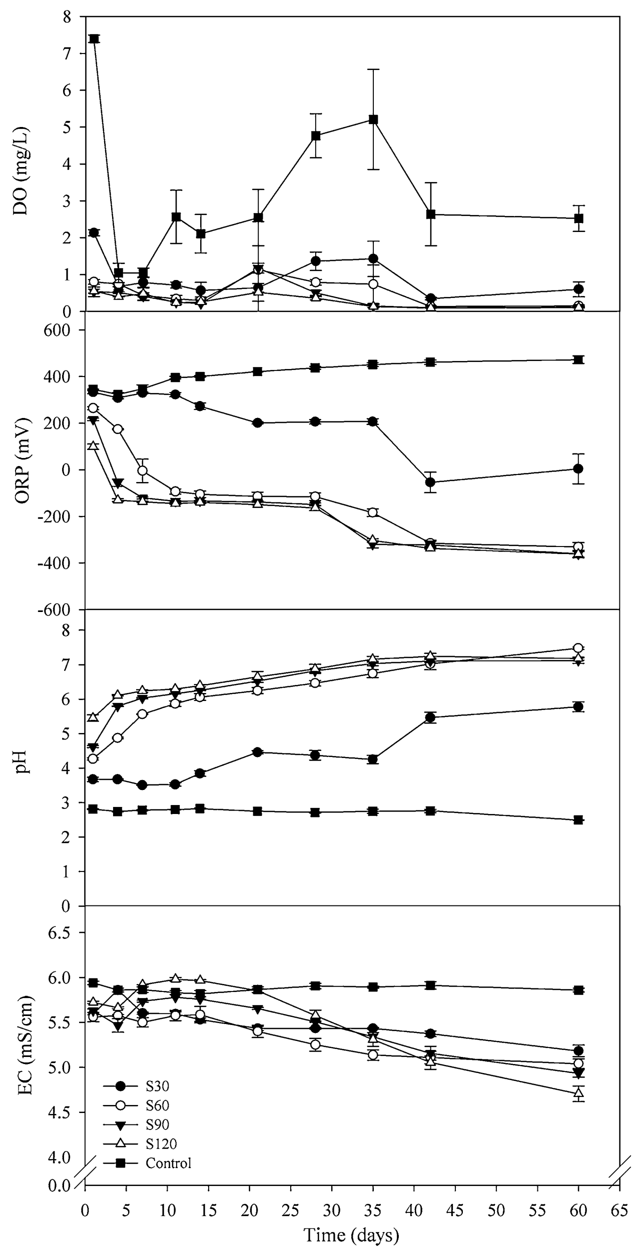

Fig. 1 Time trace data on mean (n = 3, ± SD) changes in synthetic mine water DO, ORP, pH, and EC following treatment with different sewage sludge concentrations

carbon (TIC) concentration was determined by the difference between TC and TOC and revealed that the sludge would contribute significant alkalinity. Thus, the combination of high TC and TOC indicated that the sludge would potentially be able to combat the acidic environment and initiate and sustain SRB activity until remediation was achieved (McCullough and Lund 2011).

Variations in mine water DO over time with different sewage sludge treatments are shown in Fig. 1a. Levels of DO in all microcosms decreased during the first week and then began to increase in the control microcosms. Sewage sludge treatment microcosms maintained low DO until the end of the experiment, except for the lowest concentration (30 g/L), which showed slight DO increases towards the end of the experiment. This DO trend in sewage sludge microcosms was expected as the organic material's initial role is to reduce DO concentrations for SRB activity (Castro et al. 1999). Initially recorded DO decreases in the control microcosm could be due to the air tight lids, which would have prevented atmospheric gas exchange, and the fact that the inoculums contained some organic matter, but DO increased later after the organic material was depleted. The increases in DO could be due to opening of the microcosm lids for regular physico-chemical measurements and water sampling.

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 DO is removed (Castro and Moore 2000). SRB start reducing sulphate after most of the oxidising species have been consumed, at an anaerobic redox potential between −75 and −200 mV (Connell and Patrick 1968). Figure 1b shows ORP values during the experiment. Control ORP was stable at the initial value for the first few weeks, but thereafter increased slightly. This ORP increase could be attributed to ongoing oxidation of iron and chemicals used to prepare the SAMW under oxic conditions. ORP declined sharply in sewage sludge treatments (>60 g/L) by week 1, then continued to decrease slowly. Nevertheless, sewage treatment microcosms maintained ORP in the range conducive for sulphate reduction, as illustrated by the black precipitate, indicative of iron monosulphide formation (Church et al. 2007; Martins et al. 2008), that developed. Although the lowest sewage sludge (30 g/L) tested did not produce similar ORP declines, this treatment still demonstrated a slight ORP decrease from week 3 onwards. Consequently, the lowest concentration of sewage sludge used appeared ineffective at establishing conditions favourable for SRB to reduce sulphate in AMD within the timeframe of this experiment.

The pH in the control microcosms remained stable throughout the experiment, as the high ORP and the lack of available carbon appeared to limit SRB activity. In contrast, the sewage sludge treated microcosms showed pH increases from the inception of the experiment (Fig. 1c). The treated microcosms exhibited behaviour typical of SRB-based bioremediation, i.e. once the ORP declined, the pH increased. Even the S30 treatment showed slow increases in pH around week 3. The rapid increase in pH seen in all the treatments at Day 1 was too rapid for it to be derived from SRB activity; instead it is believed to be due to alkaline substances within the sewage that directly neutralised the water. The increases in pH are proportion to the amount of sewage added. Throughout the experiment, there were no significant differences between pH increases recorded for sewage sludge treatments at concentrations >60 g/L and there were no significant differences in final microcosm pH with sewage sludge concentrations >60 g/L. Bioremediation was rapid once the initial pH increased to >4.5, as SRB's are known to perform better in more neutral environments (Koschorreck 2008).

Figure 1d shows the changes in EC over time in the microcosms. EC in the control microcosms remained stable throughout the course of the experiment at ≈5.8 mS/cm. EC in the higher sewage sludge (S90 and S120) treatments initially increased before starting to decrease. The initial increases in EC are attributed to the substantial amounts of additional solutes contained in the high concentrations of sewage sludge. However, once sulphate reduction started, EC declined significantly. In the low sewage sludge treatments (S30 and S60), this pattern was less pronounced, reflecting the lower amounts of additional solutes added and the slower rates of bioremediation. EC dropped as the pH increased and ions precipitated from the water. In general, EC was stable in the absence of microbial reductive processes but decreased following pH increases caused by SRB activity, which agrees with Fyson et al. (2006).

Table 2 shows the acidity in the microcosms at day 60. Acidity remained unchanged in the control, as expected.

Table 2 Mean (n = 3, ± SD) final acidity and acidity removal efficiency (%) following treatment with different concentrations of sewage sludge

Treatment	Day 60 ($K_{B8.2}$)	Removal efficiency (%)
Control	27.4 ± 0.2	2 ± 0.7
S30	7.1 ± 0.7	75 ± 2
S60	0.5 ± 0	98 ± 0.1
S90	0.8 ± 0	97 ± 0.1
S120	0.7 ± 0	98 ± 0.1

*Initial acidity of SAMW = 28 mM

This also highlights the stability of the SAMW. All sewage sludge treatments besides S30 showed high levels of acidity removal, which corresponded well with observed pH increases and ORP decreases, indicating that sulphate reduction was the major alkalinity generating process. Further evidence for acidity reduction due to bacterial sulphate reduction was that all the microcosm cores had a strong sulphide odour and visible black precipitates, likely iron monosulphide (Church et al. 2007; Kumar et al. 2011c).

Table 3 shows the metals and sulphate concentrations in acidic mine water on day 1 and day 60 following sewage sludge treatment. Overall, metals and sulphate concentrations remained largely unchanged from the initial values in the Control, except for iron, which appeared to have precipitated from the water column. Iron may have precipitated as Fe(III) in the Control due to the oxic conditions present in these microcosms with no organic matter. The high metals and sulphate removal and pH increase that accompanied the sewage sludge treatments were most likely due to bacterial sulphate reduction. Solute concentrations increased following dosing with different concentrations of sewage sludge, as expected, as dissolved and decomposing components of sewage contributed to solute concentrations. However, day 60 metal concentrations in the sewage sludge treated systems showed that metals concentrations were reduced in the water column following bioremediation (McCullough and Lund 2011).

The Control microcosms had very low DOC concentrations of $\approx$16 mg/L to support SRB-based bioremediation processes (Table 4). Sewage treatments showed increasing concentration of TOC that paralleled increased sewage dosing. In the Controls, NH_4–N and total nitrogen concentrations increased slightly whereas nitrate concentrations decreased. A slight increase in NH_4–N and total nitrogen concentration in the Controls can be attributed to bacterial decomposition of organic material introduced with the inoculum. Phosphate concentrations remained largely unchanged in the Control. All sewage treatments also showed increases in NH_4–N and total nitrogen concentrations, but decreases in nitrate concentrations. Increases in NH_4–N and total nitrogen are attributed to bacterial decomposition and dissolution of organic nitrogen in the sewage.

A conceptual model based on this and earlier research is shown in Fig. 2. We propose that AMD water quality is not necessarily a limiting factor to treatment, unless iron and sulphate are in limited supply. Instead, rather than being a final variable determining success or failure of bioremediation in itself, AMD water quality appears to influence the rate of treatment and the quantities of organic materials that may be required. Amendment by organic materials (e.g. sewage) can provide initial neutralisation of the AMD by either reducing acidity (if acidity is high relative to the neutralisation potential) or (as in our study) increase pH following amendment (See Fig. 1c). In our experience, in most circumstances (except when using artificial AMD or potentially in newly flooded pit lakes), there is sufficient SRB present to quickly respond to the organic matter added (Kumar et al. 2011c; McCullough et al. 2006, 2008; McCullough and Lund 2011). Use of materials such as sewage provides an initial inoculum of SRB that can be useful when populations are likely to be low. As shown in Fig. 2, there is potentially an initial lag phase that could be caused by the slow build-up of SRB numbers or the use of organic materials that are low in labile organic carbon.

Tuttle et al. (1969) reported that fresh wood dust provided an initial input of labile organic carbon but overall, performed poorly compared to older partially decomposed wood dust, which appeared able to support SRB over a prolonged period. Partial decomposition appeared to favour establishment of anaerobic decomposers who were able to degrade the material at a steady rate, which in turn supplied SRB with a reliable source of labile organic carbon. This effect can also be seen in comparing the results of McCullough et al. (2006) and McCullough and Lund

Table 3 Mean (n = 3, ± SD) metals and sulphate concentrations following treatment with different concentrations of sewage sludge; sulphate in g/L; all other values are in mg/L

Metal/Sulfate	Control		S30		S60		S90		S120	
	Day 1	Day 60	Day 1	Day 60	Day 1	Day 60	Day 1	Day 60	Day 1	Day 60
Al	102 ± 2	98 ± 1.4	58 ± 2	0.2 ± 0	39 ± 3	BDL	20 ± 0.3	BDL	1.6 ± 1.1	BDL
Cu	25 ± 0.5	22 ± 0.5	12 ± 0.4	BDL	8 ± 0.5	BDL	5 ± 0.6	BDL	2.1 ± 0.3	BDL
Fe	495 ± 7	270 ± 30	370 ± 12	170 ± 20	300 ± 5	BDL	240 ± 5	0.1 ± 0	185 ± 3	0.2 ± 0
Mn	25 ± 0.4	24 ± 0.3	21 ± 0.6	18 ± 0.5	18 ± 0.4	1.9 ± 0.2	15 ± 0.2	1.3 ± 0.1	13 ± 0.2	1.0 ± 0.1
Ni	3.2 ± 0.2	2.7 ± 0	2.1 ± 0.1	0.1 ± 0	1.8 ± 0	0.1 ± 0	1.5 ± 0	0.1 ± 0	1.1 ± 0	0.1 ± 0
Zn	20 ± 2.5	5 ± 0.4	4.7 ± 0.2	0.2 ± 0	4.2 ± 0.2	0.1 ± 0	3.5 ± 0.2	0.1 ± 0	2.5 ± 0.1	0.1 ± 0
SO_4	3 ± 0.5	2.9 ± 0.8	2.9 ± 0.2	2.3 ± 0.5	2.8 ± 0.5	0.9 ± 0.5	2.8 ± 0.3	0.6 ± 0.1	2.8 ± 0.3	0.7 ± 0.2

BDL below detection limit; Limits of detection: Al 0.1 mg/L, Cu 0.05 mg/L, Fe 0.05 mg/L, Mn 0.01 mg/L, Ni 0.02 mg/L, Zn 0.05 mg/L

Table 4 Mean (n = 3, ± SD) nutrient concentrations following treatment with sewage sludge; all values in mg/L

Nutrient	Control		S30		S60		S90		S120	
	Day 1	Day 60	Day 1	Day 60	Day 1	Day 60	Day 1	Day 60	Day 1	Day 60
NH_4–N	18 ± 0.3	38 ± 3	39 ± 1	112 ± 2	57 ± 1	165 ± 7	77 ± 3	153 ± 6	97 ± 1	147 ± 7
NO_x–N	25 ± 0.3	4 ± 0.4	23 ± 0.1	0.1 ± 0	21 ± 1	0.2 ± 0.1	18 ± 1	0.7 ± 0.1	16 ± 0.2	0.2 ± 0
TN	45 ± 3	176 ± 30	107 ± 2	235 ± 74	150 ± 1	400 ± 2	191 ± 14	314 ± 51	187 ± 34	300 ± 60
PO_4-P	0.3 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.04 ± 0	4 ± 0.4	0.03 ± 0	10 ± 1	0.03 ± 0	16 ± 1
DOC	16 ± 1	10 ± 1	59 ± 14	33 ± 3	70 ± 4	47 ± 1	95 ± 5	61 ± 5	113 ± 5	64 ± 6

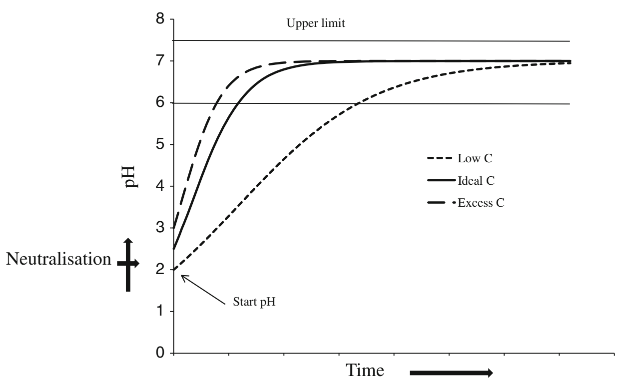

Fig. 2 Conceptual model showing response of synthetic mine water to different concentrations of organic carbon amendments

(2011), who conducted similar experiments on the same pit lake water using green waste but had very different outcomes. Using microcosms, McCullough et al. (2006) found that green waste was very successful in remediating AMD affected pit lake waters while McCullough and Lund (2011) found the converse. In the former study, green waste was sourced from an outside storage facility where it was allowed to partially degrade in an open environment while in the latter, the green waste was developed by drying fresh plant material. The effectiveness of sewage was also different in these aforementioned studies, with McCullough and Lund (2011) using relatively fresh sewage mainly from the large town of Bowen (Queensland) treatment plant, while the other study used sewage from drying beds in the small town of Collinsville (Queensland). The Collinsville sewage had been allowed to dry in the sun for an extended period and was likely to be overly degraded and incapable of providing sustained release of labile organic carbon and was therefore ineffective at treating the pit lake water. A similar effect was recorded by Kumar et al. (2011c) when sewage was stored under refrigeration for too long before use.

Waybrant et al. (1998) found that all of the eight organic matter types they tested reduced sulphate, with sewage sludge the fastest to achieve high levels of sulphate reduction. The mixture of sewage sludge and green waste (leaf mulch, woodchips and sawdust) in this study reduced 4,500 mg/L of sulphate to <25 mg/L in only 35 days. 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 (see Kumar et al. 2011c; 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 finishing experiments once pH neutrality is achieved as microcosm pH does not seem to increase above 7–7.5 regardless of how long the experiment is allowed to run (see McCullough and Lund 2011). 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. A review by Gibert et al. (2002) 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. For example, Harris and Ragusa (2001) noted that the availability of carbon from plant matter was dependent on decomposition, which can be extremely limited in acidic and anoxic conditions.

Tuttle et al. (1969) and Kumar et al. (2011c) also observed that excessive additions of labile carbon can be counterproductive, encouraging decomposers to only utilise labile substrates and not decompose refractory forms of carbon usable by SRB. In these studies and also those of McCullough and Lund (2011) and McCullough et al. (2006), where different concentrations of organic matter were tested, low organic material concentrations produced no to a very slow remediation rates. High concentrations were often no faster in remediating AMD than lower organic material concentrations. Typically, there appears to be an ideal organic amendment dose that produces effective remediation rates without overtreatment. In addition to supplying appropriate amounts of the proper organic materials for bioremediation, it is also likely necessary to maintain anoxic or lower redox lake sediments to prevent oxidation of sulphate reduction products and acid generation (Koschorreck et al. 2007). Although rarer in Europe, such seasonal (e.g. Bermúdez et al. 2007; Kumar et al. 2009) or even meromictic conditions (Castendyk 2011; Moreira et al. 2009) are common in many mid-latitude tropical and arid area pit lakes. Alternatively, they can be deliberately engineered as part of mine closure planning (Kumar et al. 2011a) by, for example, engineering the pit lakes design to establish temperature or chemical stratification (Fisher and Lawrence 2006; McCullough et al. 2012). Completely mixed lake systems will see a continuous influx of atmospheric oxygen to the sediments, which may need to be compensated for by increased organic materials dosing rates (Koschorreck et al. 2011).

Conclusions

The microcosm experiment results indicated that increases in sewage sludge concentration increased direct alkalinity contributions, which neutralised initial acidity and supported subsequent SRB-based bioremediation, leading to a further pH increase and metal removal. The lowest sewage sludge treatment (30 g/L) struggled to initiate and sustain the bioremediation process. This indicates that there may be a minimum threshold of sewage sludge required before bioremediation is likely to be effective; further studies are needed to test and optimise this combination of organic and inorganic carbon. Conversely, the highest treatments (90 and 120 g/L) of sewage sludge, whilst successful in remediating the acidic mine water, appeared to offer little advantage over the 60 g/L treatment in terms of efficacy. Such high doses would be more difficult to achieve in a field trial. However, it remains unknown whether the additional organic material might provide any long term benefits.

The conceptual model indicated that the AMD water quality is not a significant limiting factor to bioremediation success or even rate as long as iron and sulphate are present at sufficient concentration and there is an adequate supply of labile organic carbon and some inorganic carbon ($CaCO_3$) for initial neutralisation. Although, AMD concentration may marginally affect the rate of remediation, this limiting factor can often be overcome by maintaining an adequate supply of labile organic carbon for the microbial processes. Consequently, the major factor that appears to determine SRB success is meeting this organic materials dose threshold. Our model indicated that too little organic material significantly affected the rate of remediation whereas too much labile organic carbon does not seem to have any profound beneficial effect. Further studies are required to test these conclusions; we believe that field-scale SRB remediation research focused on the effects of different AMD concentrations and organic materials amendment doses are needed if pit lake bioremediation is to become a viable long term sustainable solution.

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