

Upper and lower concentration thresholds for bioremediation of acid mine drainage using bulk organic substrates

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Abstract

Acidic pit lakes may form in open cut mine voids that extend below the groundwater table and fill from surface and groundwater in-flows at the cessation of mining. Pit lake water quality may often be affected by acid mine drainage (AMD). Among the many remediation technologies available, sulphate reducing bacteria (SRB) based bioremediation using organic wastes appears to have significant potential towards ameliorating AMD effects of elevated acidity, metal and sulphate concentrations. A microcosm experiment was carried out under controlled conditions 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 30–120 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 plays 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 concentration >60 g/L.

Keywords: SRB, organic carbon, sewage, pH, acidity, sulphate.

Introduction

Considering the high costs involved in backfilling open cut mining pit voids, many will be left upon cessation of mining to fill with surface and ground waters to form pit lakes. Water quality in pit lakes is largely influenced by the nature of ore type mined, geology and hydrology of the surrounding catchment (Castro and Moore 2000). Among the different treatments available, stimulating naturally occurring alkalinity generating processes appear to be the most appropriate and feasible method (McCullough and Lund 2011). Typically, the low organic carbon concentrations in acidic pit lake waters hinder the natural development of alkalinity generating processes. Such organic matter limitation in pit lakes can be overcome by external addition of organic materials.

Ever since the work of Tuttle et al. (1969) on the potential of SRB to treat AMD, there have been a plethora of studies exploring addition of organic materials for

initiation of sulphate reduction to treat AMD problems. Acidic mine waters often contain high sulphate and iron concentrations. 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 limits the sulphate reduction rate. Organic substrates must therefore be amended not only to produce reducing conditions suitable for sulphate reduction, but also to serve as either direct or indirect sources of electron donors to SRB (Wendt-Potthoff and Neu 1998; Castro et al. 1999).

SRB are known for their inability to use complex organic substrates such as starch, cellulose, proteins, and fats. Hence, 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). Frommichen 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, the complex substrates i.e., straw, wood chips were inefficient for successful remediation as acidic waters often lack the microflora that are able to degrade lignin. SRB activity rates are dependent on the nature of organic waste used and in particular bioavailability of organic carbon (Gibert et al. 2002). Naturally refractory organic substrates release carbon and other nutrients slowly and this is particularly beneficial if combinations of labile and refractory substrates are used, as this decomposing mixture will then continue to provide carbon fractions 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. 2011a). 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? The aim of this study was therefore to understand the critical concentrations of sewage sludge needed to initiate and sustain SRB based bioremediation of AMD.

Materials and methods

Organic materials and synthetic mine water

Secondary treated sewage sludge collected from Beenyup Wastewater Treatment Plant, Perth, Western Australia was used for stimulating bioremediation. Synthetic acid mine water was prepared in laboratory by dissolving analytical grade chemicals in MilliQ water. Solute concentrations in the synthetic acidic mine water were selected to simulate routinely reported AMD affected pit lake water in the literature (pH 2.8, oxidation-reduction potential (ORP) 350 mV, electrical

conductivity (EC) 5.9 mS/cm, sulphate 1,800 mg/L, Al 100 mg/L, Cu 25 mg/L, Fe 380 mg/L, Mn 15 mg/L, Ni 2 mg/L and Zn 2 mg/L).

Experimental design

Fifteen microcosms were constructed from 100 mm diameter and 300 mm long acrylic tubes containing 1.8 L of synthetic acidic mine water. Microcosms were sealed with rubber bungs at the bottom and at the top were sealed with removable PVC lids to minimise atmospheric gas exchange. Microcosms were placed in opaque black plastic tubs which were filled with water to maintain an even temperature. The top of the microcosms were covered by opaque tarpaulins to exclude light and limit primary production. The experiment was carried out at a temperature of ~25 °C. Three replicate cores were allocated to each of the following treatments; control (untreated, 'C'), sewage (30 g/L, 'S30'), sewage (60 g/L, 'S30'), sewage (90 g/L, 'S90') and sewage (120 g/L, 'S120') and monitored for 60 days. All the microcosms received a 5% (v/v) bacterial inoculum from a successful bioremediated stock microcosm containing acidic mine water, sediment, and organic matter as green waste and mulch. The inoculation was to overcome the absence of pit lake sediment and bacteria normally found in real mine water.

Sampling and analysis

Sewage sludge was analysed for total Kjeldahl nitrogen (TKN), total carbon (TC) and total organic carbon as per standard methods (APHA 1998). Physico-chemical measurements in treated and control microcosms were taken after sewage sludge addition, at day 1 and then weekly to day 42 and finally at day 60. A Hydrolab Datasonde 4a multiparameter meter (Hydrolab, USA) was used for recording temperature, pH, EC and 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™, 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 on ICP-AES (Varian Vista-Pro, USA). Another aliquot of filtrate was analysed for sulphate using ion chromatography (Metrohm 761 Compact, Switzerland). Acidity (KB8.2) was measured using an auto-titrator (Metrohm, Switzerland) using 0.1 M NaOH as titrant.

Results and discussion

Sewage sludge from the Beenyp wastewater treatment plant contained TKN 5.1 g/kg, TC 45 g/kg and TOC 15 g/kg. ORP in the range of -100 mV is critical for establishment and activity of SRB for acidic mine water bioremediation (Castro and Moore 2000). Figure 1a presents the changes in acid mine water ORP values during the course of the experiment. Control ORP was relatively stable throughout the experiment at >300 mV. ORP declined sharply in treatments (>60 g/L) by week 1 and after this period continued to decrease slowly. More importantly these microcosms maintained ORP in the range conducive for sulphate reduction which was depicted from the black colour of the microcosms that are indicative of iron monosulphide formation (Church et al. 2007; Martins et al. 2008). The S30

treatment did not produce a similar ORP decrease, but showed slight decrease in ORP from week 3 onwards. The S30 treatment was ineffective in establishing the ORP conditions (-75 to -200 mV) favourable for SRB bioremediation within the time frame of this experiment (Connell and Patrick 1968).

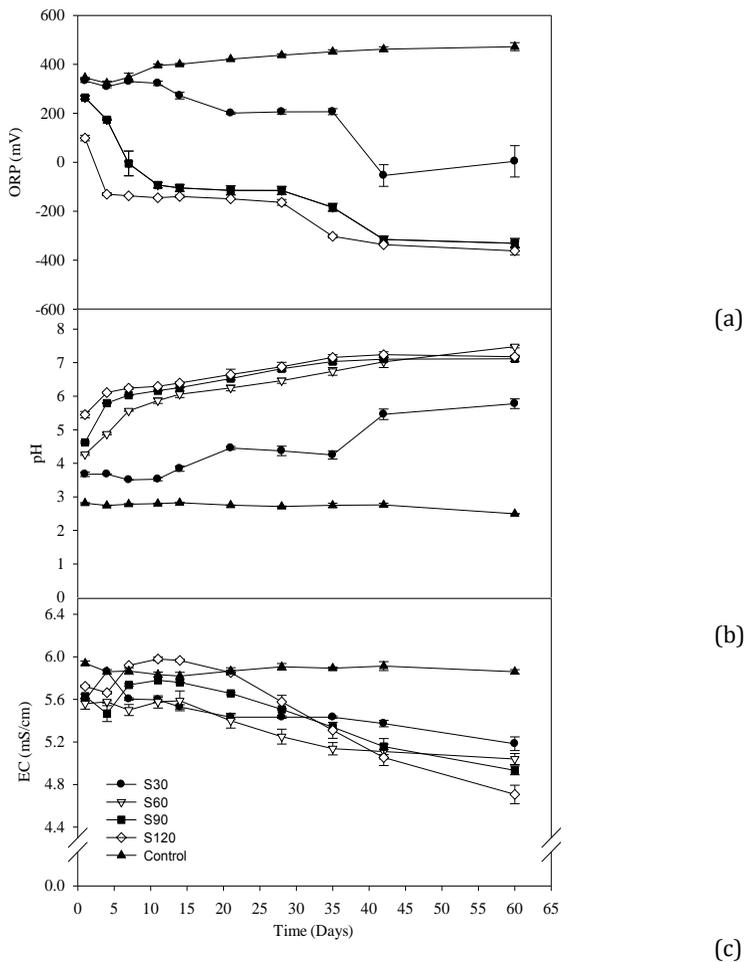


Figure 1 Time trace data on mean ($n=3$, \pm S.D.) changes in acidic mine water (a) ORP, (b) pH and (c) EC following treatment with different sewage concentrations.

pH in the control microcosms remained stable throughout the experiment, as the high ORP and lack of available carbon appeared to limit SRB activity. Whereas, the sewage sludge treated microcosms showed increases in pH from inception (Figure 1). The treated microcosms exhibited behaviour typical of SRB based bioremediation i.e., once the ORP declined then 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. 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 1c shows the changes in EC over time in the microcosms. EC in the control microcosms remained stable throughout the course of the experimental at ~5.8 mS/cm. EC in treated microcosms were more variable. EC in higher sewage sludge (S90 and S120) treatments initially increased before starting to decrease. The initial increase in EC could be attributed to the high concentration of sewage sludge also introduced substantial additional solutes to the microcosms. 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 additional solutes added and the slower rates of bioremediation.. EC dropped along with pH increase, which is explicable as pH increases lead to removal of ions from the water mainly through precipitation reactions. The overall trend noticed for acid mine water EC with different treatments i.e., stable values in the absence of microbial reductive processes and decrease in EC following pH increases due to SRB activity is in agreement with that reported elsewhere (Fyson et al. 2006).

Figure 1 shows the acidity remaining in the microcosms at day 60 and the removal. Control acidity remained unchanged as expected in the absence of bioremediation. This also highlights the synthetic mine water's stability. All sewage sludge treatments besides S30 showed high levels of acidity removal which corresponded well with pH increase and ORP decrease indicating 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 strong sulphide odour and visible black precipitates (likely iron monosulphide), which are good indicators of SRB activity (Church et al. 2007; Kumar et al. 2011b).

Table 1 Mean ($n=3$, \pm S.D.) 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 \pm 0.2	2 \pm 0.7
S30	7.1 \pm 0.7	75 \pm 2
S60	0.5 \pm 0	98 \pm 0.1
S90	0.8 \pm 0	97 \pm 0.1
S120	0.7 \pm 0	98 \pm 0.1

Table 2 shows the removal efficiency of metals and sulphate removal efficiency from acidic mine water following sewage sludge treatment

Table 2 Mean ($n=3$, \pm S.D.) metals and sulphate removal efficiency (%) following treatment with different concentrations of sewage sludge on day 60.

Metals/ sulphate	Control	S30	S60	S90	S120
Al	4 \pm 1	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0
Cu	13 \pm 2	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0
Fe	46 \pm 6	65 \pm 5	100 \pm 0	100 \pm 0	100 \pm 0
Mn	6 \pm 1	28 \pm 2	92 \pm 1	95 \pm 0.3	96 \pm 0.3
Ni	0	98 \pm 0.2	98 \pm 1	97 \pm 0.3	96 \pm 0.3
Zn	0	96 \pm 1	100 \pm 0	98 \pm 0.4	100 \pm 0
SO ₄	3 \pm 3	25 \pm 8	72 \pm 2	79 \pm 3	76 \pm 1

Overall, metals and sulphate concentrations remained largely unchanged in the control except for iron, which appeared to have precipitated and was removed from water column. Iron precipitation as Fe(III) occurred due to the oxidising conditions present in control and absence of abundant SRB and IRB bacterial communities. High metals and sulphate removal efficiencies recorded with sewage sludge treatment were most likely due to bacterial sulphate reduction and corresponding pH increase.

Conclusions

The microcosm experiment results indicated that increases in sewage sludge concentration increased direct alkalinity contributions which neutralised initial acidity which supported subsequent SRB based bioremediation leading to pH increase and metal removal. The lowest sewage sludge treatment (30 g/L) struggled to initiate and sustain the bioremediation process. This indicates there may be a minimum threshold of sewage sludge required before bioremediation is likely to be effective. 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. These higher doses would be much more difficult to achieve in a field trial. However, it remains unknown whether the additional organic material added would provide any longer term benefits.

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References

- APHA (1998) Standard methods for the examination of water and wastewater. 20th edn, American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC, USA. 1,220 p
- Castro JM, Moore JN (2000) Pit lakes: their characteristics and the potential for their remediation. *Environmental Geology* 39: 1254–1260

- Castro JM, Wielinga BW, Gannon JE and Moore JN (1999) Stimulation of sulfate-reducing bacteria in lake water from a former open-pit mine through addition of organic waste. *Water Environment Research* 71: 218–223
- Church CD, Wilkin RT, Alpers CN, Rye RO and McCleskey RB (2007) Microbial sulfate reduction and metal attenuation in pH 4 acid mine water. *Geochemical Transactions* 8: 10
- Connell WE, Patrick WHJ (1968) Sulfate reduction in soil: effects of redox potential and pH. *Science* 159: 86–87
- Figuroa L, Seyler J, Wildeman T (2004) Characterization of organic substrates used for anaerobic bioremediation of mining impacted waters. In: Jarvis A (Ed.) *Proceedings of International Mine Water Association Conference*. Newcastle, England p 43–52
- Frömmichen R, Kellner S, Friese K (2003) Sediment conditioning with organic and/or inorganic carbon sources as a first step in alkalinity generation of acid mine pit lake water (pH 2–3). *Environmental Science and Technology* 37: 1414–1421
- Fyson A, Nixdorf B, Kalin M (2006) The acidic lignite pit lakes of Germany - microcosm experiments on acidity removal through controlled eutrophication. *Ecological Engineering* 28: 288–295
- Gibert O, de Pablo J, Cortina JL and Ayora C (2002) Treatment of acid mine drainage by sulphate-reducing bacteria using permeable reactive barriers: a review from laboratory to full-scale experiments. *Reviews in Environmental Science and Biotechnology* 1: 327–333
- Koschorreck M (2008) Microbial sulphate reduction at a low pH. *FEMS Microbiology Ecology* 64: 329–342
- Koschorreck M, Frömmichen R, Herzsprung P, Tittel H and Wendt-Potthoff K (2002) The function of straw for in situ remediation of acidic mining lakes: results from an enclosure experiment. *Water, Air, and Soil Pollution* 2: 97–109
- Kumar RN, McCullough CD, Lund MA and Newport M (2011a) Sourcing organic materials for pit lake bioremediation in remote mining regions. *Mine Water and the Environment* 30: 296–301
- Kumar RN, McCullough CD, Lund MA (2011b) How does storage affect the quality and quantity of organic carbon in sewage for use in the bioremediation of acidic mine waters? *Ecological Engineering*, 37:1205–1213
- Martins MSF, Santos ES, Barros RJB and Costa MCSS (2008) Treatment of acid mine drainage with sulphate-reducing bacteria using a two stage bioremediation process. In: *Proceedings of the 10th International Mine Water Association (IMWA) Congress*. Czech Republic, IMWA.
- McCullough CD (2008) Approaches to remediation of acid mine drainage water in pit lakes. *International Journal of Mining, Reclamation and Environment* 22: 105–119
- McCullough CD, Lund MA (2011) Bioremediation of Acidic and Metalliferous Drainage (AMD) through organic carbon amendment by municipal sewage and green waste. *Journal of Environmental Management* 92: 2419–2426
- McCullough CD, Lund MA, May JM (2008) Field scale trials treating acidity in coal pit lakes using sewage and green waste. In: Rapantova N & Hrkal Z (Eds.), *Proceedings of the 10th International Mine Water Association (IMWA) Congress*. Karlovy Vary, Czech Republic, p 599–602
- McCullough CD, Steenbergen J, te Beest C and Lund MA (2009) More than water quality: environmental limitations to a fishery in acid pit lakes of Collie, south-west Australia. In: *Proceedings of the International Mine Water Conference*. Pretoria, South Africa, International Mine Water Association, p 507–511
- Muyzer G, Stams AJM (2008) The ecology and biotechnology of sulphate-reducing bacteria. *Nature Reviews in Microbiology* 6: 441–454
- Peine A, Peiffer S (1998) In-lake neutralization of acid mine lakes. In: *Acidic mining lakes*, Geller W; Klapper H & Salomons W (eds) Springer-Verlag, Berlin, Germany, 47–63 pp

- Tuttle JH, Dugan PR, Rendles CI (1969) Microbial sulfate reduction and its potential utility as an acid mine water pollution abatement procedure. *Applied Microbiology* 17: 297–302
- Wendt-Potthoff K, Neu TR (1998) Microbial processes for potential in situ remediation of acidic lakes. In: *Acidic Mining Lakes*, Geller W; Klapper H & Salomons W (eds) Springer, Berlin, 269–284 pp