Biological manganese removal from acid mine drainage in constructed wetlands and prototype bioreactors

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Abstract

Mine drainage waters vary considerably in the range and concentration of heavy metals they contain. Besides iron, manganese is frequently present at elevated concentrations in waters draining both coal and metal mines. Passive treatment systems (aerobic wetlands and compost bioreactors) are designed to remove iron by biologically induced oxidation/precipitation. Manganese, however, is problematic as it does not readily form sulfidic minerals and requires elevated pH (>8) for abiotic oxidation of Mn (II) to insoluble Mn (IV). As a result, manganese removal in passive remediation systems is often less effective than removal of iron. This was found to be the case at the pilot passive treatment plant (PPTP) constructed to treat water draining the former Wheal Jane tin mine in Cornwall, UK, where effective removal of manganese occurred only in one of the three rock filter components of the composite systems over a 1-year period of monitoring. Water in the two rock filter systems where manganese removal was relatively poor was generally pH 5, whereas it was significantly higher (~pH 7) in the third (effective) system. These differences in water chemistry and manganese removal were due to variable performances in the compost bioreactors that feed the rock filter units in the composite passive systems at Wheal Jane. An alternative approach for removing soluble manganese from mine waters, using fixed bed bioreactors, was developed. Ferromanganese nodules (about 2 cm diameter), collected from an abandoned mine adit in north Wales, were used to inoculate the bioreactors (working volume ca. 700 ml). Following colonization by manganese-oxidizing microbes, the aerated bioreactor catalysed the removal of soluble manganese, via oxidation of Mn (II) and precipitation of the resultant Mn (IV) in the bioreactor, in synthetic media and mine water from the Wheal Jane PPTP. Such an approach has potential application for removing soluble Mn from mine streams and other Mn-contaminated water courses.

1. Introduction

Soluble manganese (Mn (II)) is often found in considerably greater concentrations in mine drainage waters than in unpolluted streams and groundwater (Banks et al., 1997). Even though there are uncer-
tainties regarding the toxicity of manganese, recent research has shown that elevated concentrations of manganese are highly correlated to the toxicity of lake sediment pore water (Boucher and Watzin, 1999; Doyle et al., 2003). Besides its potential toxicity, the removal of this metal from surface- and groundwaters is desirable for several reasons. As with iron and aluminium, manganese hydrolysis also contributes to the total mineral acidity of mine waters (Eq. (1)).

\[
\text{Mn}^{2+} + 0.5\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{MnO}_2 + 2\text{H}^+ \tag{1}
\]

In addition, manganese in sources of water that are used for human consumption is undesirable because it imparts a metallic taste to water, stains laundry and water fixtures and, as Mn (IV) readily precipitates, manganese can block water distribution networks. For these reasons, the U.S. Environmental Protection Agency (EPA) has set a secondary maximum contaminant level for Mn of 0.05 mg/l. The EPA has also established guidelines limiting the concentration of Mn in acidic waters discharging from mines at maximum of 4 mg/l, as long as average discharges for a 30-day period do not exceed 2 mg/l. In the European Union, legislation under consideration may establish an Environmental Quality Standard for manganese of 0.03 mg/l.

With more stringent manganese discharge limits being discussed in Europe and in the US, effective means of removal of soluble Mn from mine waters are required. Even though manganese readily precipitates as Mn (IV), little oxidation of Mn (II) occurs in solutions below pH 8, as the kinetics for manganese oxidation are slow relative to that of ferrous iron. In addition, biological oxidation of Mn (II) does not proceed rapidly in the presence of Fe (II), and thus it is not removed significantly in aerobic wetlands where the concentration of ferrous iron exceeds 1 mg/l (Nairn and Prior, 2004). Manganese does not readily form an insoluble sulfide phase and thus it is not removed to a great extent in compost wetlands or by sulfate-reducing bioreactors, though it may precipitate in these systems as rhodocrosite (MnCO₃). As manganese is generally not efficiently removed by current passive technologies applied to remediate mine water, alternative approaches are needed.

These include an abiotic Mn-removal system consisting of limestone-filled columns to significantly raise the pH for Mn oxidation and subsequent precipitation (Thornton, 1995) or a reactor filled with dolomite as a catalyst for Mn (II) oxidation (Johnson, 2003). Biological approaches, which rely on oxygenic photosynthesis to cause a rise in solution pH to above 8 (Eq. (2)), include columns of immobilized cyanobacteria (Bender et al., 1994) and small pools filled with rocks that have been colonized by algae (Phillips et al., 1995).

\[
6\text{HCO}_3^{-}(aq) + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 + 6\text{OH}^- \tag{2}
\]

The latter approach has been applied at the Wheal Jane pilot-scale passive treatment plant (“PPTP”). This study site was built in 1993 following a catastrophic spill of acid mine drainage from the Wheal Jane tin mine in Cornwall, England. Full details of the demonstration plant construction and operation can be found elsewhere (Hamilton et al., 1999; Whitehead and Prior, 2004). In brief, a small fraction (<5%) of the acid mine drainage (“AMD”, pH~3.5) water flows into the PPTP, where it is fed into three similar composite treatment systems. The main variations are the pretreatment that the AMD is subjected to prior to entering the “wetlands”. These are: (i) addition of lime (CaO) to adjust the AMD to a predetermined pH value (the “lime-dosed” or “LD” system); (ii) passage through an anaerobic (manure-based) cell to remove dissolved oxygen and then through an anoxic limestone drain (to passively add alkalinity; the “ALD” system); (iii) no pretreatment (the “lime-free” or “LF” system). In each system, AMD then flows (by gravity) through a series of five “aerobic cells” (to encourage oxidation and precipitation of Fe and concomitant removal of As), then through a single “compost bioreactor” (containing sawdust and cow manure) and finally through a rock filter (a series of 10 small algal ponds).

Following a shutdown period of about 2 years, the PPTP was recommissioned in spring 2000 for a multidisciplinary research project aimed at understanding the underlying principles of AMD remediation using this composite wetland approach. Here we report that two of the rock filter components of the PPTP were ineffective at removing soluble manganese, though the performance of the third system improved significantly during the 12-month period of monitoring. We also show that fixed bed column bioreactors, containing immobilized manganese-oxi-
dizing microorganisms, have potential as effective alternatives to “rock filter” technology for removing soluble manganese from mine waters.

2. Materials and methods

2.1. Rock filter description and sampling

A full description of the PPTP at the former Wheal Jane mine is given elsewhere in this issue (Whitehead and Prior, 2004). The rock filters are the final components of the PPTP systems and consist of a series of 10 algal ponds (referred to as RF1 to RF10). Each pond measures 4.5 by 9.75 m, and is lined with high-density polyethylene and filled with granite stones for colonization by freshwater algae. Water fills each pond to a depth of approximately 15 cm. The influent to the rock filters is supplied directly from the compost bioreactors that are positioned immediately ahead of these ponds in the composite system.

Mine water in the treatment system was analysed on site for pH, redox potential (as \(E_h\) values, i.e. corrected to the standard hydrogen electrode), temperature, conductivity and dissolved oxygen (DO\(_2\)). Grab samples (25 ml) of the water were taken directly into sterile plastic sample bottles, which were completely filled by immersion into the water. Samples were taken at approximately midway down the length of each pond and from the compost bioreactor influent and effluent (CB influent or CB effluent) from each of the three treatment systems. Samples for soluble metal analysis (acidified with HNO\(_3\)) and samples for dissolved organic carbon (DOC) were filtered through a 0.2 \(\mu\)m pore-sized nitrocellulose filters.

2.2. Manganese nodule collection

Ferromanganese nodules were collected from a trial mine adit located in the Gwydyr forest, north Wales. The irregular spherical-shaped, black-brown nodules, ca. 1–2 cm in diameter, were found in a pool within the adit (pH 6.9, soluble Mn of 30 mg/l, conductivity 149 \(\mu\)S/cm). These were transferred to the laboratory and stored at 4 °C until use. Similar nodules collected from a nearby adit (no longer accessible) are described elsewhere (Roberts, 1986).

2.3. Removal of soluble manganese in shake flask cultures

A chemically defined liquid medium (MMS) designed for growth of Leptothrix discophora strain SS-1 (Nelson et al., 1999) was used for enrichment and growth of manganese-oxidizing bacteria. This medium contained 0.24 g sodium pyruvate as energy substrate and, just prior to use, Mn\(^{2+}\) was added to concentrations of 2 to 8 mg/l from a stock solution of 22.31 g MnSO\(_4\)/l deionised water. The final pH of the medium was 7.0.

Initial experiments to monitor manganese removal from MMS medium were performed in shake flasks. Two of the ferromanganese nodules were placed into replicate 250 ml conical flasks containing 100 ml of filter-sterilized MMS medium and the flasks were shaken (100 rpm) at 20 °C. When Mn removal from solution was nearly complete, 90 ml of the medium was removed and replaced with fresh MMS containing Mn at a concentration of 8 mg/l. These flasks were incubated as before. Samples were withdrawn from the shake flasks at regular intervals and concentrations of soluble Mn were determined (Section 2.5).

2.4. Commissioning and evaluation of manganese immobilization in a fixed bed bioreactor

A fixed bed bioreactor for immobilizing manganese from mine waters was set up by mixing the ferromanganese nodules (ca. 10%, v/v) with porous, acid-washed glass beads (8–16 mm) manufactured by Poraver, Germany (Kolmert and Johnson, 2001). The nodules/beads mixture was packed (to a depth of 14.5 cm) into a 25 by 10 cm perspex column, between 3.5 cm layers of acid-washed pea gravel (Fig. 5a). The base and cap of the column contained ports for aeration (applied at ca. 2 l/min), medium circulation and sampling. The column was filled to the top of the upper gravel layer surface with MMS medium, which was then recirculated through the column using a peristaltic pump until the soluble manganese had declined to <10% of the initial concentration, at which point the column was drained and fresh medium introduced. This sequence was repeated several times, and the column was observed to accumulate increasing amounts of brown-black, Mn (IV), precipitates (Fig. 5b). The external plumbing on the bioreactor was then reconfigured to allow the system to be tested...
in continuous plug-flow mode. In addition to studies with MMS medium, the bioreactor was fed with water collected from RF10 of the “ALD” system on 21 October 2001. Within 24 h of collection, this water (pH 5.3, Mn=6.1 mg/l, dissolved organic carbon=11.8 mg/l) was filter-sterilized (through cellulose acetate membranes, 0.2 μm pore size) and was stored in the dark at 4 °C until used.

2.5. Analytical techniques

Total soluble manganese (i.e. that which passes through a 0.2 μm pore size membrane) was detected colorimetrically with formaldoxime in a reducing medium (lower limit of detection is 0.1 mg Mn/l) and sulfate concentration was determined turbidometrically as the insoluble barium sulfate using Hydrocheck test kits (WPA, Cambridge, UK). Dissolved organic carbon (DOC) was measured using a Protoc Analyser (Pollution and Process Monitoring UK).

3. Results

3.1. Performance of the Wheal Jane PPTP rock filters

The composite, pilot-scale passive treatment plant (“PPTP”) at the former Wheal Jane tin mine in Cornwall, England, makes use of rock filters to promote oxygenic photosynthesis and the concomitant increase in mine water pH to promote the oxidation and precipitation of manganese and to polish the processed AMD (e.g. removal of organic carbon compounds draining the compost bioreactors).

The performance of the rock filters was monitored over a 1-year period. The rock filters from each of the three treatment systems were effective in removing DOC, which was introduced during passage of the mine water through the compost bioreactors. As the mine water passed through the LD rock filter, DOC decreased from a mean value of 24.2±7.3 mg/l (standard deviation) in the compost bioreactor effluent to 3.1±1.9 mg/l in the rock filter effluent. DOC decreased from 22.3±9.2 to 3.2±1.8 mg C/l in the “ALD” rock filters. In the one sample taken during the same time period from the LF rock filters (the LF system was shut down for a period of 1 year), the DOC decreased from 11.5 to 4.0 mg/l.

In contrast to effective DOC removal, the rock filters have, in general, performed poorly in terms of manganese removal from the mine water. Analysis of water samples taken from across the whole treatment system showed that little change in soluble manganese concentrations occurred within the aerobic cells (which are mostly highly acidic, pH<3, due to ferric iron hydrolysis). Comparison with changes in sulfate concentrations in the same sample indicates that the decrease in soluble Mn was due mainly to dilution of the AMD by rainwater (data not shown). However, concentrations of ~2–8 mg Mn (II)/l were still present in the water draining the compost bioreactors of each of the three treatment systems.

Four months after resumption of AMD flow through the LF system, samples of water taken from each algal pond of the three rock filter systems contained detectable concentrations of soluble Mn. In the 10 rock filter ponds of the LD and “ALD” systems, these were ~7 mg/l, while in the LF system, soluble manganese in RF1 (8 mg/l) was greater than in RF2–10 (2–5 mg/l).

In three further sampling events during the following year, again only the rock filter component of the LF system exhibited any manganese removal (Fig. 1). The mean soluble Mn concentration during this period fluctuated around 4 mg/l in both the LD and “ALD” systems, while in the LF rock filter it decreased from around 4 mg/l in RF1, to 2.5 to 0.5 mg/l in downstream ponds.

There was a large variation between the maximum Mn concentration and the minimum concentration in each of the three systems sampled on different dates (Fig. 1). However, maximum or minimum concentrations in each of the three rock filter systems were recorded on the same sampling occasion. Only in the LF system was there an improvement, in terms of removal of soluble Mn, of the performance of the rock filter component with time. Towards the end of the monitoring period, concentrations of Mn in some of the rock filter ponds were below the level of detection (0.1 mg/l) of the colorimetic method used (Fig. 2).

There was a strong relationship between the pH of the mine water as it flowed through the rock filters, and Mn removal. Increasing pH as the water traversed the ponds was only observed in the LF rock filter system, from a mean of about 6 in RF1 to above 7 in the remaining ponds (Fig. 1). In contrast, the mean pH
in the LD and “ALD” systems was between 4 and 5, and water pH in any rock filter unit in these two systems never exceeded 6. A good correlation \((R=0.7041)\) between pond water pH and relative (to the influent) manganese concentration in the rock filter pond waters was evident (Fig. 3).
3.2. Removal of soluble manganese by Mn-oxidizing microbes

As the rock filters were not consistently effective at removing Mn from mine water, an alternative treatment strategy based on bioreactors containing immobilized manganese-oxidizing bacteria was devised. In MMS medium inoculated with the ferromanganese nodules, soluble manganese declined from 3 mg/l steadily during incubation (Fig. 4), and this corresponded with the appearance of brown-black precipitates (and associated microbes) within cultures. The nodule-associated microorganisms also catalysed the removal of Mn when added to MMS in the range of the maximum concentration (8 mg/l) detected in the Wheal Jane treatment plant (Fig. 4). It thus appeared

Fig. 2. Improvement of the LF system rock filter component over a period of 1 year as indicated by decreasing concentrations of soluble Mn in the down-stream ponds, RF7 (shaded bars) and RF10 (striped bars), as compared to the first pond (RF1, black bars).

Fig. 3. Relative soluble Mn concentration in the rock filter pool samples (n=120) and the pH of the sample. A strong relationship between the two is indicated by a correlation coefficient (R) of 0.7041. The relative concentration was determined by dividing the soluble Mn in the rock filter sample by the respective compost bioreactor influent Mn concentration on that sampling date. Values below 1 indicate Mn removal while values above 1 indicate Mn mobilization.
that the microbes associated with the nodules were capable of oxidizing Mn (II) to Mn (IV), which readily precipitated from solution.

3.3. Commissioning and performance of a fixed bed Mn-oxidizing bioreactor

Subsequent to the shake flask experiments, which showed that soluble Mn could be effectively removed by the microbially colonized freshwater nodules, a fixed bed bioreactor was established with the ferromanganese nodules (ca. 10%, v/v) as inoculum (Fig. 5a). The synthetic medium MMS was recirculated through the column until increasing amounts of brown-black (Mn (IV)) precipitates accumulated (Fig. 5b), indicating the Mn-oxidizing microbes had accumulated in the bioreactor.

Representative data showing removal of soluble manganese from MMS medium with the fixed bed bioreactor run in plug-flow and recirculating modes are shown in Fig. 6. In both cases, over 90% of soluble Mn was removed within 4 h. When run in
plug-flow mode, 50% of soluble manganese was removed with a flow rate of 400 ml/h, and 68% when the flow rate was lowered to 195 ml/h. No manganese removal by the bioreactor occurred when pyruvate was omitted from the MMS, nor when the pH of the MMS was lowered to 5 (data not shown).

When the filtered water from algal pond 10 of the “ALD” rock filter was spiked with Mn (II) and passed through the bioreactor, about 50% of the soluble Mn was removed within 200 min (data not shown). If, however, pyruvate was added or if the pH of the rock filter water was raised to 7, no significant removal occurred within 400 min.

4. Discussion

Manganese is the second most abundant heavy metal in the lithosphere, occurring at between 0.002% and 10% (w/w) in terrestrial ecosystems, ca. 0.2 μg/l in marine waters, and an average of 8 μg/l in freshwaters (Ehrlich, 1996). As with many other metals, greatly enhanced concentrations of soluble manganese can occur in waters draining coal and metal mines and this includes the Wheal Jane system (Neal et al., 2004; Whitehead et al., 2004). This study has focussed on Mn removal from the abandoned Wheal Jane tin mine AMD using wetland-based treatment, and preliminary results from a fixed bed bioreactor containing Mn-oxidizing microorganisms.

Manganese removal at the Wheal Jane pilot passive treatment plant was designed to occur in the final component (the rock filter) of the composite remediation scheme. Through oxygenic photosynthesis, the pH of the water should be raised sufficiently high enough for Mn (II) oxidation to occur and Mn to be removed from water as insoluble Mn (IV), and possibly also as rhodocrosite (MnCO3). This, however, did not occur in two of the three systems (the LD and “ALD” systems) at the PPTP.

A major reason for the contrasting trends observed in Mn geochemistry found within the algal ponds is that the necessary elevation in pH resulting from oxygenic photosynthesis only occurred in the LF system, as illustrated in Fig. 1. The discrepancy in Mn removal is due, in the main, to the variation in the performance of the compost bioreactors in each of the three systems. A similar observation was made during an earlier assessment period of the PPTP (Hamilton et al., 1999). Water draining these bioreactors in the “ALD” and LD systems contained greater concentrations of both ferrous iron and sulfide (Johnson and Hallberg, 2004). Microbiological (and abiotic) oxidation of these species in the algal ponds caused the pH of the water to decline, which is counterproductive to the objective of increasing water alkalinity. As the treatment plant is built in a temperate climate that receives considerable rainfall, alkalinity resulting from the oxygenic photosynthetic activity of the algal-colonized rocks appeared to be insufficient to
overcome the additional acidity produced in the waters that entered the rock filters in the LD and “ALD” systems. In addition to the decrease in pH caused by the oxidation of ferrous iron and hydrolysis of the Fe (III) produced, soluble Fe (II) in the rock filters is undesirable as it is known to reductively dissolve manganese oxides (Villinski et al., 2001).

Although the mean soluble Mn concentrations in algal ponds 3 to 10 of LF rock filter were quite low, the minimum and maximum values varied quite substantially. This is due to the apparent increasing improvement of the rock filter as indicated by the decreasing concentration of soluble Mn in each of the algal ponds during the year studied. Mine water flow through the LF system was restarted in June 2001, and some removal of Mn was noted in October of that year. Even smaller concentrations of Mn were noted in the algal ponds in April 2002, and in July and September 2002 soluble Mn was below 0.1 mg/l. The poor removal of soluble Mn observed in late autumn and early spring, as opposed to during the summer months, may have been due to different rates of oxygenic photosynthesis at these times. Further monitoring of the PTPP is required to make firm conclusions of the impact of seasonality on rock filter performance.

Thermodynamically, Mn (II) should oxidize spontaneously to Mn (IV) in aerated, neutral pH waters, but the activation energy required is relatively high, and this greatly slows down the process, causing Mn (II) to be far more stable in nonacidic waters than is ferrous iron. In contrast, the adsorption of Mn (II) onto MnO₂ is much more rapid. The “activation energy barrier” for Mn oxidation can be overcome biologically. Many microorganisms are known to be able to catalyse the oxidation of Mn (II), and at least some of these are known to utilize the energy available from the reaction (Ehrlich, 1996).

Here, Mn-oxidizing microbes have been used to catalyse the removal of Mn, as an alternative to the rock filters. The existence of marine “ferromanganese nodules” has been known for some time, and these are mined as an ore of the metal (Ehrlich, 1996). Iron/manganese-rich nodules may also occur in freshwaters, though these tend to be much smaller than their marine equivalents (Ehrlich, 1996). These nodules are formed by microbial oxidation of Mn (II) and the subsequent precipitation of the resulting Mn (IV) (and co-precipitated iron and aluminium). The nodules collected from an abandoned mine in north Wales also appeared to be comprised of Mn-microbes as they catalysed the removal of Mn (in a concentration range of that found at the Wheal Jane treatment plant) from medium that had been designed to encourage the growth of Mn-oxidizers.

Immobilization of these microbes onto glass beads packed into a column created a bioreactor that was also capable of removing Mn from solution. In addition to the decrease of soluble manganese, the accumulation of a black-brown precipitate implied that insoluble Mn (IV) was accumulating in the column, though this was not measured directly. That the microbes in the column were actively oxidizing the Mn (II) and causing it to precipitate, rather than Mn (II) merely accumulating on the manganese oxides in the bioreactor, was apparent when pyruvate (the carbon and energy source for the microbes) was eliminated from the medium and little or no Mn (II) removal was observed. Similarly, when the pH of the medium was lowered to 5, the microbes were apparently inactivated and again no removal occurred.

When water from the “ALD” rock filter was passed through the column, Mn (II) was oxidized and removed from the solution. This appears to be in contrast to the data obtained when MMS at pH 5 was used. This discrepancy, however, may be explained by the organic energy sources available. At low pH, pyruvate is less readily utilized by microorganisms, since it occurs as undissociated pyruvic acid rather than pyruvate. The biological toxicity of small molecular weight organic acids at low pH has been described elsewhere (Alexander et al., 1987). At this time, it is unclear what components in the “ALD” rock filter water were acting as energy sources for the Mn-oxidizers in the bioreactor.

The increasingly efficient performance of the bioreactor in terms of removing soluble Mn (II) may be due both to the increasing numbers of immobilized Mn (II)-oxidizers in the bioreactor and to the abiotic uptake of Mn (II) onto MnO₂ precipitates, and subsequent oxidation of the metal by sessile bacteria. What is clear is that a simple fixed bed bioreactor containing Mn (II)-oxidizing/depositing microorganisms can be highly effective in catalysing the removal of the metal from a synthetic, metal-rich wastewater. So far, the limited tests of the technology for...
bioremediating actual low pH, Mn-rich mine waters have shown considerable promise.

Aside from catalysing Mn removal at a pH lower than the rock filters, a major advantage offered by metal-removing bioreactors is that they occupy a far smaller “footprint” than, for example, the rock filters at the Wheal Jane passive treatment plant. Land availability frequently restricts the application of passive mine water treatment systems, and low-maintenance biological systems are attractive alternatives in such circumstances.

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