Pilot-scale passive bioreactors for the treatment of acid mine drainage: Efficiency of mushroom compost vs. mixed substrates for metal removal

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1. Introduction

The use of passive bioreactors continues to increase worldwide for the treatment of AMD (acid mine drainage)-impacted waters, which are typically characterized by low pH and high metal and sulfate concentrations. Passive bioreactors benefit the ease of installation and affordability, whereas the leading drawback is their relatively limited lifetime (~10 years) (Thomas et al., 2010).

For typical bioreactors, the overall metal removal efficiencies usually follow the order Al > Fe > Mn (Neculita et al., 2007), with little dependence on the type of substrate material. Precipitation of Al as gibbsite [Al(OH)3] at pH > 5 and of Fe as oxihydroxides, such as goethite (α-FeOOH), lepidocrocite (γ-FeOOH), hematite (Fe2O3), and magnetite (Fe3O4) at moderate pH (3.5) accounts for most of their removal. Mn is generally acknowledged as hard to treat due to various inhibitory factors, especially in the presence of high concentrations of Fe (Edwards et al., 2009). These inhibitory factors include the following: 1) the high solubility of Mn sulfide (alabandite, MnS) (Stumm and Morgan, 1996); 2) the inhibition of Mn precipitation under the condition of Fe/Mn >4 (Skousen et al., 2000); 3) the anaerobic reductive dissolution of Mn oxides by organics (Stone and Morgan, 1984), sulfides (Burdige and Nealson, 1986), and Fe(II) (Villinski et al., 2003); and 4) the relatively high pH (>8) required for chemical precipitation as oxides (Mariner et al., 2008).

The efficiency of AMD treatment in passive bioreactors relies on sulfate-reducing bacteria (SRB), which require a source of available organic carbon and appropriate environmental conditions (pH 5–8, ORP < -100 mV, and sulfate). Various waste materials (animal and poultry manure, compost, woodchips and sawdust, and chitinous materials) could be utilized as physical supports and substrates for sustaining the growth of anaerobic bacteria, including SRB. Bacterial activity improves mine water quality by increasing the pH and reducing sulfate and metal concentrations. Among various waste materials, mushroom compost, while yielding better efficiencies predicted by the physicochemical characterization of mushroom compost, steady state effluent quality was then noted for around 100 days before worsening in AMD source water occurred in response to seasonal variations in precipitation. Such changes in AMD quality resulted in performance deterioration in all reactors.
materials, spent mushroom compost, generally consisting of a mixture of rice straw, peat, animal manure (chicken, cow, or horse), gypsum, and limestone, is one of the most commonly used organic substrates for anaerobic bacteria in the first generation of passive bioreactors (Dvorak et al., 1992; Stark and Williams, 1994; Ji et al., 2008). However, supplementation of easily available organic carbon is often required to compensate its depletion after continuous operation and, therefore, to extend the bioreactor lifespan (Stark et al., 1994). Recently, very high Mn removal efficiencies (up to 100%) were found in treating synthetic mine drainage (pH 6.2; 90 mg L\(^{-1}\) Mn) (Edwards et al., 2009; Karathanasis et al., 2010) and in synthetic AMD (pH 2.2; 567 mg L\(^{-1}\) Mn) in the presence of Fe (Cheong et al., 2010). In addition, crab-shell chitin (high in carbonates and phosphates) was reported as a multifunctional (sorption media and substrate) amendment, offering significant benefits to a mushroom compost-limestone-based bioreactor, especially for Mn removal, when added in fractions of 50–100% (Robinson-Lora, 2009). However, highly variable Mn removal efficiencies have been reported in the literature, ranging from 23% (Reisman et al., 2009) to 86% (Venot et al., 2008) in pilot-scale testing. Apparently, these inconsistent results are related to the types of organic substrates used and to AMD quality and flow rate (Guo et al., 2001; Neculita et al., 2011).

There are approximately 300 coal mines and more than 2000 metal mines presently closed in South Korea. Mine drainage from these sites has a wide range of pH values (2.1–7.4) and is generally dominated by Fe (0.3–508 mg L\(^{-1}\)), Al (0.4–129 mg L\(^{-1}\)), and Mn (0–59 mg L\(^{-1}\)) (Ji et al., 2008; MIRECO, 2011). From 1996 to 2002, 35 passive treatment systems at 29 mines were constructed to treat AMD, all of which utilized mushroom compost as a substrate material (Ji et al., 2008). However, a relatively recent investigation revealed highly variable removal efficiencies of acidity, sulfate, and metals, depending on the substrate age, metal loading, and mine drainage flow rates. Some systems also had various operational problems such as overflow, leakage, clogging, broken pipes, and inefficient sulfate and metal removal (Ji et al., 2008).

A comprehensive study was performed to identify alternatives to mushroom compost for AMD biological treatment in passive treatment systems (Neculita et al., 2011). Preliminary data obtained in laboratory batch bioreactors indicated that a mixture of substrates composed of cellulosic and organic wastes yielded higher removal efficiencies for Mn and other metals relative to mushroom compost (Neculita et al., 2011). This study is a follow-up investigation of the previous batch testing in an attempt to demonstrate the enhanced performance of substrate mixtures in treating a natural AMD source under scaled-up field conditions. The testing was performed with pilot field-scale column bioreactors at a closed mine site in South Korea over a 174-day period. The relative efficiency of a mixture of four substrates (cow manure compost, mushroom compost, woodchips, and sawdust) compared to mushroom compost for pH neutralization, sulfate reduction, and metal removal was examined, with special attention to Mn removal in both types of bioreactors.

2. Materials and methods

2.1. Organic substrates and their characterization

Cow manure compost, mushroom compost, sawdust, and rice straw were used as organic substrates. The composts (cow manure and mushroom) were obtained from a regional farmhouses located in Hongseong city, South Korea. Sawdust and rice straw were purchased from Hanyoung Corp. (Seoul, South Korea). Rice straw pieces were provided uncut. Organic substrates were used without pretreatment, while being kept refrigerated at 4 °C until use. The pH was measured by mixing the solids with deionized water at a 1:1 volume ratio (ASTM, 1995) and using a HACH pH-meter (HQ-40D). The water content of the organic substrates was measured by subtracting the weight of a sample dried at 105 °C for 24 h from that before drying. The total volatile solids (TVS), which are usually used as an estimate of the organic matter content of solid materials, were determined at 550 °C as per Karam (1993). Dissolved organic carbon (DOC) was extracted from solid samples using deionized water and a solid: liquid ratio of 1:1 and a 2 h mixing period, following the procedure outlined by Zagury et al. (2006), and determined using an Apollo 9000 TOC analyzer (Teledyne Tekmar, USA). Carbon—nitrogen—hydrogen content was measured with an elemental analyzer (Flash EA, Thermo Finnigan, USA). Before elemental analysis, solid samples were prepared by drying and grinding to a fine powder. The selected physicochemical properties of the organic substrates are presented in Table 1. Along with the substrates, creek sediment was used as a source of acclimated SRB, which was collected from the bottom of a creek approximately 1 km downstream from a coal mine located near Samchuk city (South Korea). The sediment was kept in a headspace-free bottle and refrigerated at 4 °C until use. Enumeration of SRB in organic materials and the creek sediment was performed using the Most Probable Number technique as per ASTM (2009).

### Table 1

<table>
<thead>
<tr>
<th>Organic material</th>
<th>Mushroom compost</th>
<th>Cow manure compost</th>
<th>Sawdust</th>
<th>Cut rice straw</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physicochemical parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.64–7.67</td>
<td>6.64–6.70</td>
<td>6.11–6.16</td>
<td>4.78–4.85</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>49.3</td>
<td>66.3</td>
<td>11.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Total volatile solids (%)</td>
<td>55.8</td>
<td>79.2</td>
<td>84.6</td>
<td>99.2</td>
</tr>
<tr>
<td>DOC (mg L(^{-1}))</td>
<td>3146</td>
<td>2011</td>
<td>2923</td>
<td>633</td>
</tr>
<tr>
<td><strong>Elemental analysis (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>2.5</td>
<td>2.2</td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>C</td>
<td>25.6</td>
<td>38.6</td>
<td>38.3</td>
<td>47.8</td>
</tr>
<tr>
<td>H</td>
<td>3.3</td>
<td>5.6</td>
<td>5.7</td>
<td>6.6</td>
</tr>
<tr>
<td>S</td>
<td>1.8</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>10</td>
<td>18</td>
<td>54</td>
<td>442</td>
</tr>
<tr>
<td><strong>Microbiological counts (cells mL(^{-1}))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfate-reducing bacteria (SRB)</td>
<td>1.7 × 10(^{10})</td>
<td>5.0 × 10(^{10})</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

- **Physicochemical parameters were calculated as average values from two replicates.**
composition of the mixture in reactors #1 and #2 was as follows: mushroom compost (20%), cow manure compost (10%), sawdust (20%), rice straw (10%), creek sediment (20%), and limestone (20%), whereas reactor #3 received 100% mushroom compost. Note that the mass of organic substrates was corrected for water content when mixed with other solids. To prevent clogging at the effluent port, the lower ends of the columns were filled with coarse quartz gravel (2–5 cm diameter), which consists almost entirely of quartz, to a height of around 30 cm. Also, an 80-mesh (0.177 mm) nylon disc was placed on the bottom of the gravel layer to prevent loss of organic particles to the effluent port. The columns were then saturated with the mine-site AMD, by allowing the water to flow vertically downward through the solid material, while slightly inclined or gently shaken to remove air pockets within the substrate layer. The AMD feed was then stopped for one week for bacterial acclimation. This acclimation period was necessary to grow a large population of SRB that would produce enough sulfide and alkalinity in order to withstand the shock of the AMD feed (Waybrant et al., 2002; Neculita et al., 2008). After acclimation, the operation began with downward vertical flow with a 4-day hydraulic retention time (HRT).

2.3. Sampling and analyses

The first sample was collected at the end of the first week, i.e. after the acclimation period. Samples were then collected weekly (for a total of 20 sampling-events) from the AMD reservoir and from the exit end of the effluent tubing, which drained to an effluent pond. Unfiltered samples were immediately measured for pH (Hach PHC101-01, USA), redox potential (Hach MTC101, USA), dissolved oxygen (DO) (LDOTM), electrical conductivity (CDC401), total dissolved solids (TDS) (CDC401), and salinity (CDC401) or were preserved for culturable SRB counts (at every two sampling-events). The recorded values of oxidation-reduction potential (ORP, mV) were converted into Eh by adding the reference electrode potential ($E_{\text{ref}}$) of 210 mV, corresponding to a 3 M KCl solution. A portion of the samples (about 10 mL) was filtered with 0.45 μm membrane mixed cellulose ester filters (Whatman, USA). The filtrate was then immediately analyzed for sulfide (methylene blue method), sulfate (sulfaVer 4 method), and ferrous iron (ortho-phenanthroline method) or was preserved for DOC, metal (Al, Fe, and Mn), and micronutrient (Na, K, Ca, and Mg) analysis in the laboratory. Detection limits of the methods were as follows: 5 μg L$^{-1}$, 1 mg L$^{-1}$, and 0.02 mg L$^{-1}$ for sulfides, sulfate, and ferrous iron, respectively. Metal concentrations were measured using inductively coupled plasma (HORIBA Jobin Yvon, France) on an acidified sample with concentrated HCl (1:9). Unless stated differently, all parameters were determined using standard methods (APHA, 2005). Culturable SRB enumeration was performed with an unfiltered sample using the Most Probable Number (MPN) technique as per ASTM (2009). A modified Postgate C was used as growth media, with a pH 7 and containing lactate as carbon source. More details on its composition are provided in Neculita et al. (2011). It should be noted that the MPN counts in the liquid samples might give only limited information about the bacteria in the reactor since the majority of the SRB probably lived in a biofilm.

### Table 2

<table>
<thead>
<tr>
<th>Component</th>
<th>Units</th>
<th>Values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>–</td>
<td>2.93–4.07</td>
</tr>
<tr>
<td>ORP</td>
<td>mV</td>
<td>461 ± 41</td>
</tr>
<tr>
<td>DO</td>
<td>mg L$^{-1}$</td>
<td>6.4 ± 2.0</td>
</tr>
<tr>
<td>Conductivity</td>
<td>mS cm$^{-1}$</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>TDS</td>
<td>mg L$^{-1}$</td>
<td>1118 ± 174</td>
</tr>
<tr>
<td>Salinity</td>
<td>–</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>mg L$^{-1}$</td>
<td>1142 ± 58.8</td>
</tr>
<tr>
<td>Al$^{3+}$</td>
<td>mg L$^{-1}$</td>
<td>97.2 ± 34.9</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>mg L$^{-1}$</td>
<td>29.0 ± 8.2</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>mg L$^{-1}$</td>
<td>310.7 ± 94.2</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>mg L$^{-1}$</td>
<td>170.0 ± 49.7</td>
</tr>
<tr>
<td>Na$^{+}$</td>
<td>mg L$^{-1}$</td>
<td>91.7 ± 146.5</td>
</tr>
<tr>
<td>K$^{+}$</td>
<td>mg L$^{-1}$</td>
<td>9.4 ± 4.4</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>mg L$^{-1}$</td>
<td>1277 ± 726</td>
</tr>
</tbody>
</table>

* Mean and standard deviations were calculated with $n = 20$.
MPN counting in the effluent gives information about detachment rates rather than active bacteria. All reagents were of ACS quality.

3. Results and discussion

3.1. Characterization of natural organic materials

Consistent with previously reported findings, the sawdust had a typical wood waste acidic pH (4.8) (Table 1), while other organic waste compost (mushroom and cow manure) had neutral pH values (6.6–7.7) (Neculita and Zagury, 2008). On the other hand, the pH of cut rice straw (pH 6.1) fell between the two groups of natural organic materials. Based on the TVS values, the sawdust consisted of more than 99% organic material and cut rice straw consisted of 85%, whereas the mushroom compost had only 56%, with the leftovers of this last substrate consisting of inorganic materials such as calcite and gypsum, in additions to ashes. Yet, the DOC concentrations (the higher DOC, the more organic carbon is available for microbial activity) released in water from mushroom compost (and eventually available for bacterial consumption) were the highest (3 g L⁻¹), which is within the typical range of 0.8–11 g L⁻¹ (Guo et al., 2001). This observation, together with a C/N ratio of 10 (the optimal value for the biodegradation of complex organic materials), indicated that the mushroom compost was the best substrate (Cheong et al., 2010), followed by cow manure compost. However, in a recent study, mushroom compost showed poor performance in sulfate removal and acid neutralization of mine drainage (Robinson-Lora, 2009). Furthermore, mushroom compost was proven to be a good substrate for AMD in the short term but a poor one over the long-term operation of passive bioreactors (Ji et al., 2008). Similarly, the poor performance of manure as a sole substrate has also been reported, despite its high DOC concentrations and the favorable C/N ratio (Zagury et al., 2006). In fact, in field passive systems, a higher initial DOC concentration could entail a faster depletion of the source of available organic carbon for anaerobic bacteria. Bacterial counts showed that culturable SRB were present only in compost (cow manure and mushroom; up to 2 × 10³ cells mL⁻¹), which is consistent with previously reported results (10² cells mL⁻¹ in composted poultry manure and leaf compost) (Neculita and Zagury, 2008).

3.2. Pilot-scale bioreactor tests

3.2.1. pH, Eh, DO, conductivity, TDS, and salinity

pH values sharply increased from <4 to a moderate range and remained in the moderate region (6.1–7.8) despite temporal variation throughout the entire testing period. The pH profile of the reactors generally followed the pH changes of the source AMD, with the exception of reactor #2, which registered an unusually low pH (4.6) on day 152 (Fig. 2). An increase in pH is typically observed in bioreactors (Waybrant et al., 2002; Neculita et al., 2008; Song et al., 2012). This increase is associated with the alkalinity increase that results from the formation of carbonate species either through dissolution of limestone from reactive mixtures or through the oxidation of readily available organic substrates by SRB, as well as with the dissolution of the surface-bound hydroxyl ion from substrate materials, under acidic conditions (Neculita et al., 2011). The deterioration of AMD quality, including a drop in pH values, is often observed at the end of the wet season in South Korea and is attributed to limited dilution of AMD, as the monsoon ends, in the summer (Kim et al., 2003).

The reactors quickly developed reducing conditions during the acclimation period, yielding Eh values of nearly +210 mV, at the first sampling. The Eh steadily decreased thereafter until day 80 and remained relatively constant reaching values as low as −147 mV (on day 117, #3) or −158 mV (on day 145, #1), before it began to rise and approach the initial values at the end of the
experiment. The increase in Eh toward the end would suggest the gradual inactivation of SRB as indicated by the increase of sulfate concentrations over the same period (Fig. 3). Eh values were also consistent with DO concentrations that were maintained at around 0 mg L\(^{-1}\) up to day 152 but increased to up to 2.4 mg L\(^{-1}\) on day 174.

The conductivity, TDS, and salinity, which are greatly influenced by concentrations of metals and sulfate leached from substrate materials, showed very similar breakthrough patterns throughout the experiment, consistent with previously reported behavior in a pilot-scale test (Guo et al., 2001). The peak values of the three parameters were recorded in a mushroom compost-based reactor (#3) on day 27 (9.1 mS cm\(^{-1}\), 4.9 g L\(^{-1}\), and 5.1\% of conductivity, TDS, and salinity, respectively). The bioreactors containing mixed substrates consistently yielded lower values than the mushroom compost reactor up to day 80. Hence, the data suggested that the mushroom compost released higher amounts of ionic components into the solution relative to the reactors #1 and #2 that also washed out metals, giving rise to a peak of their concentration in the effluent, but to a lower extent.

3.2.2. Sulfate, sulfide, DOC, and SRB counts

The mushroom compost released a significant amount of sulfate, yielding higher concentrations of sulfate in the early reaction times than the AMD source, especially for the reactor with 100% mushroom compost (Fig. 3). In a separate analysis, acid soluble sulfate, determined as per Sobek et al. (1978), showed a value of 27.5 g kg\(^{-1}\) in mushroom compost. Such a release of sulfate from mushroom compost was also reported in a previous study (31.1 g kg\(^{-1}\); Guo et al., 2001). The potential source of sulfate in the mushroom compost could be either cow manure or gypsum (CaSO\(_4\cdot\)2H\(_2\)O), or both, since the former is a base material of mushroom compost and the dissolution of the latter can be enhanced through an ion-pair mechanism and the common ion effect (Truesdale, 2011). In a previous study, as much as 7 g kg\(^{-1}\) of sulfate was found to originate from a composted poultry manure substrate (Zagury et al., 2006). However, after the initial leaching out of sulfate from the organic substrates, the system was able to consistently reduce sulfate concentrations in all three reactors below the values in the AMD within 50 days, after which it remained relatively stable until day 125, except for reactor #2. The average sulfate removal within this period was of 40, 48, and 20% for reactors #1, #2, and #3, respectively. The highest sulfate removal in the reactors with mixed substrates (#1 and #2) was observed on day 49, yielding a peak of 78 and 67% removals, respectively. Meanwhile, the sulfate level in the mushroom compost reactor (#3) was consistently higher than those in the mixed substrate reactor until day 125, with its greatest removal observed on the same day (47%). At the same time, the highest concentrations of produced sulfides were recorded on day 125, ranging from 26 (#3) to 51 mg L\(^{-1}\) (#2). Sulfides were measurable from day 14 onward, and their concentrations were generally higher in the reactors with mixed substrates than in the mushroom compost reactor, consistent with the greater sulfate reduction in the mixed substrate reactors.

After day 125, sulfate and ferrous iron concentrations in AMD significantly increased, and this deterioration in input water quality had an adverse impact on the biological activity, leading to increases in sulfate concentrations and decreases in sulfide concentrations and SRB counts (especially in reactor #2). Nevertheless, in the most efficient reactor (#1), the sulfate level remained well below that of the AMD throughout the entire experiment. DOC concentrations gradually decreased with time to reach final values of 5.2 (#1), 9.4 (#2), and 3.6 mg L\(^{-1}\) (#3), with the exception of an abnormally high value measured in reactor #2 on day 91 (609 mg L\(^{-1}\)). Apparently, DOC for all substrates was leached out in a similar manner. These results suggest that a substrate with a high initial DOC is likely to leach out a significant amount of DOC to the effluent, losing available substrate for microbial growth. Consequently, after some period of operation, the activity of anaerobic bacteria in the reactor could become limited by the lack of

Fig. 3. Sulfate reduction evolution during AMD treatment in pilot-scale bioreactors. Reactors #1 and #2: mushroom compost (20%), cow manure compost (10%), sawdust (20%), rice straw (10%), creek sediment (20%), and limestone (20%); Reactor #3: mushroom compost (100%).
SRB counts in reactor #3 are characterized by the highest values at early time, followed by a sharp drop from 6-log to 3-log, within three weeks (from day 125 to day 146). Wide variations in SRB counts were also observed in reactor #2, whereas reactor #1 was the steadiest.

3.2.3. Total Fe, Fe$^{2+}$, Al, and Mn

The major mechanisms involved in metal removal in passive bioreactors have been reported to be sorption and precipitation, with some minor contribution from filtration through precipitate formation (Neculita et al., 2007). The sorption saturation of mushroom compost during the treatment of synthetic AMD (pH 3.5, 48 mg L$^{-1}$ Fe, and 22 mg L$^{-1}$ Mn) under high flow rates could be reached in as few as 15 days (Stark et al., 1994), after which metal removal is dominated by precipitation. In the present study, metal removal efficiencies were consistent with the results from batch bioreactor testing (Neculita et al., 2011). They showed the following order: Al $>$ Fe $>$ Mn. Exceptions were recorded on two occasions, which were related to the mushroom compost-based reactor, in which Al concentrations in the effluents were always less than 5 mg L$^{-1}$, despite the concentration variation in AMD from 42.5 (day 125) to 172.5 mg L$^{-1}$ (day 49) (Fig. 4). One notable observation in the changes of total Fe concentration was a significant increase in reactor #2 toward the end of the reaction period (Fig. 4). This could be partially explained by the reactor failure as reflected by the release of significant amounts of Fe$^{2+}$ and sulfate, and the sharp decrease of effluent pH in the reactor. Furthermore, such changes in the geochemistry within the reactor possibly re-dissolve precipitated or adsorbed iron minerals and leach out a significant amount of iron, even exceeding the level in AMD source water. Nevertheless, from day 49 to the end of testing, in reactors #1 and #3 (or up to day 158 in reactor #2), satisfactory Fe removal was found, in the range of 68–92%, including day 49, when the total Fe concentration in AMD was the highest (235 mg L$^{-1}$). Meanwhile, Mn concentrations in input water varied from 13.8 (day 125) to 40.5 mg L$^{-1}$ (day 91), while in treated water it varied from 8.7 (#2 on day 125) to 47.4 mg L$^{-1}$ (#3 on day 158) (Fig. 4). Overall, the effluent quality of the mushroom compost-based reactor was even poorer than the AMD over the first two months because of leaching of sulfate and major cations. Mn release from this substrate material led to higher concentrations in the treated effluent than AMD during early reaction times. Similar results, i.e. marginal to very low Mn removal efficiencies, were obtained in laboratory column-bioreactors filled with Korean mushroom compost and operated at 20-d of HRT and a Fe/Mn ratio of 10 (Chang et al., 2000).

![Fig. 4. Metal removal evolution during AMD treatment in pilot-scale bioreactors. Reactors #1 and #2: mushroom compost (20%), cow manure compost (10%), sawdust (20%), rice straw (10%), creek sediment (20%), and limestone (20%); Reactor #3: mushroom compost (100%).](image-url)
3.2.4. Na, K, Ca, and Mg

The significance of measuring these ions was, apart from ensuring the micronutrients are present to sustain SRB activity, to assess the influence of treatment system on water chemistry. Previously reported results showed that passive biological treatment systems (e.g., wetlands) could act as sink of Na, K, Ca and Mg from inputs of acid mine drainage (Wieder and Lang, 1984).

The bioreactors released large amounts of Na during early reaction times, as indicated by significantly higher Na concentrations in the effluent as compared to AMD. Such a release of Na was more apparent in the mushroom compost reactor, indicating the major source of Na was mushroom compost (Fig. 5). Na concentrations dropped quickly after the initial leaching phase before they increased again on day 91, which could be attributable to the temporal Na shock in AMD source water that occurred on day 34 and 49. A similar leaching effect was also observed for K, but its concentration in all reactors dropped to below 30 mg L\(^{-1}\) after day 49 since the K concentration in AMD was constantly maintained at a very low level.

Ca concentrations in the AMD varied between 176 and 420 mg L\(^{-1}\), with less fluctuation than Mg, which ranged between 95 and 274 mg L\(^{-1}\). The concentrations of Ca and Mg in the reactors varied considerably during the reaction period, but in general, the Ca concentrations remained higher than those in the AMD, while no such observation was obvious for Mg except early on in the reaction time. This indicates that the substrate materials contributed to the overall amount of Ca to some extent throughout the reaction period, but the release of Mg was rather short-lived. Increase in Ca concentrations is likely (and mainly) due to limestone dissolution. A notable observation in the Ca and Mg profiles was the sharp increase of concentrations between days 124 and 158, which may be related to a 0.6 unit decrease of the pH in AMD occurring over the same period (Fig. 3). In other words, the deterioration in AMD quality strongly affected the condition of all reactors and led to the release of high concentrations of Ca and Mg, especially in reactor #2.

3.2.5. Fe/Mn ratio effect on Mn removal efficiency

In addition to the possible reasons provided above for the very poor Mn removal in passive bioreactors, the Fe/Mn ratio in fed water may also play an important role in Mn removal within bioreactors (Skousen et al., 2000). Mn is ubiquitous in AMD-impacted waters, but it is one of the most problematic metals since it does not readily form sulfidic minerals and requires high pH (>8) for chemical oxidation to the less soluble Mn(IV) form (Mariner et al., 2008). At pH <8, the kinetics of Mn oxidation are very slow, requiring a secondary polishing treatment of bioreactor-treated waters (Mariner et al., 2008). Some studies that reported successful Mn removal to date were performed with a treated effluent with low Fe concentrations (Mariner et al., 2008), or with water with high Mn concentrations (up to 567 mg L\(^{-1}\); Cheong et al., 2010), i.e. a Fe/Mn ratio <2. The Fe/Mn ratio in previous studies varied from 0, i.e. AMD did not contain Fe (Edwards et al., 2009; Karathanasis et al., 2010) to 0.8 (Cheong et al., 2010) and up to 10 (Chang et al., 2000).

In the present study, the Fe/Mn ratio in AMD water varied from 1.7 to 3.5 within the first 104 days, except on day 76 (4.8) and day 49 (6.2) (Fig. 6). Better removal of Mn was observed during this period, with the highest removal efficiencies of 49% (#1) to 61% (#2). After day 104, the Fe/Mn ratio increased progressively up to 8, and Mn concentrations in treated water increased up to twice the original concentrations in AMD, regardless of the type of substrate material. In some cases where the bioreactor effluent had a higher level of Mn than that of source AMD (e.g., day 24), the excess Mn appears to originate from the reductive dissolution of solid Mn-bearing minerals or of Mn complexes associated with substrate.

![Fig. 5. Micronutrient evolution during acid mine drainage (AMD) treatment in pilot-scale bioreactors. Reactors #1 and #2: mushroom compost (20%), cow manure compost (10%), sawdust (20%), rice straw (10%), creek sediment (20%), and limestone (20%); Reactor #3: mushroom compost (100%).](image-url)
materials (Fig. 6). The sharp increases of Mn in the reactor effluents observed toward the end of the reaction time presumably resulted from the dissolution of Mn-sulfides in response to a pH decrease of source AMD. Very optimistic results generated in small scale experiments (Cheong et al., 2010) have not yet been confirmed in larger scales of practical significance, especially at a higher Fe/Mn ratio and under continuous flow. In fact, laboratory column-reactors tested for short periods of time (less than 6 months) do not provide pertinent data applicable to field-scale treatment systems. As a leading example, the chitinous materials, which apparently seemed to be the most promising substrate for Mn retention in laboratory passive systems (Robinson-Lora, 2009), failed to provide consistent results at a larger pilot-scale (Reisman et al., 2008).

4. Conclusions

The efficiency of a reactive mixture (in duplicate) comprised of four organic components (cow manure compost, mushroom compost, sawdust, and rice straw) was evaluated relative to mushroom compost alone (control), in three pilot-scale passive bioreactors fed for 174 days with AMD from a closed South Korean mine site. Results indicated that mixed-substrates could yield comparable performance to mushroom compost for reducing sulfate and removing metals from AMD, while providing better effluent quality in an initial phase of reaction (1–2 months). Deterioration of AMD quality, which occurred after 100 days of operation, presumably due to the reduced dilution of AMD with the onset of the dry season, led to performance deterioration of all reactors, but the reactors slowly recovered the treatment efficiencies toward the end of testing. The reactors were effective for pH neutralization and metal removal, showing a relative removal efficiency of Al > Fe > Mn. However, the mushroom compost reactor released significant amounts of sulfate and major cations that impaired the effluent quality at early reaction times and reduced overall treatment efficiency. Nevertheless, after the initial leaching out of sulfate from the organic substrates, the system was able to reduce sulfate concentrations in all three reactors below the values in the AMD within 50 days, after which it remained relatively stable until day 125. Furthermore, DOC loss from mushroom compost was more significant than the mixed substrates, suggesting that performance limitation due to a shortage of available organic carbon could occur sooner in mushroom compost reactors. Satisfactory Mn removal was achieved when the Fe/Mn ratio in the solution was small (<3.5), regardless of the substrates used. The overall results of this study suggested that the use of mixed substrates could be a viable option to overcome the limitations of mushroom compost during AMD treatment.

Acknowledgments

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Karathanasis, A.D., Edwards, J.D., Barton, C.D., 2010. Manganese and sulfate removal in an initial phase of reaction (1–2 months). Deterioration of AMD quality, which occurred after 100 days of operation, presumably due to the reduced dilution of AMD with the onset of the dry season, led to performance deterioration of all reactors, but the reactors slowly recovered the treatment efficiencies toward the end of testing. The reactors were effective for pH neutralization and metal removal, showing a relative removal efficiency of Al > Fe > Mn. However, the mushroom compost reactor released significant amounts of sulfate and major cations that impaired the effluent quality at early reaction times and reduced overall treatment efficiency. Nevertheless, after the initial leaching out of sulfate from the organic substrates, the system was able to reduce sulfate concentrations in all three reactors below the values in the AMD within 50 days, after which it remained relatively stable until day 125. Furthermore, DOC loss from mushroom compost was more significant than the mixed substrates, suggesting that performance limitation due to a shortage of available organic carbon could occur sooner in mushroom compost reactors. Satisfactory Mn removal was achieved when the Fe/Mn ratio in the solution was small (<3.5), regardless of the substrates used. The overall results of this study suggested that the use of mixed substrates could be a viable option to overcome the limitations of mushroom compost during AMD treatment.

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