PRELIMINARY EVALUATION OF LOCALLY AVAILABLE ORGANIC SUBSTRATES FOR VERTICAL FLOW PASSIVE TREATMENT CELLS IN POTOSÍ, BOLIVIA

B. Santamaria\(^2\), M. R. Apoza Q., W.H. Strosnider and R.W. Nairn

Abstract: Vertical flow cells (VFCs) are key components of passive acid mine drainage (AMD) treatment systems and require organic substrates that create anaerobic conditions and encourage bacterial sulfate reduction. Sustainable and economical substrate options are often locally available organic waste materials. In the high elevation desert of Potosí, Bolivia, the low productivity landscape limits their availability. In this study, several locally available organic wastes were identified and evaluated as potential VFC substrates in a preliminary laboratory batch study to assist in passive treatment system design. Brewery waste, llama manure and cow manure were collected from three nearby locations. Two abandoned mine water discharges with different properties were collected near Cerro Rico de Potosí. Discharge water A had an initial pH of 2.96, a specific conductivity of 3.310 mS/cm and acidity of 1350 mg/L as CaCO\(_3\) equivalent. Discharge water B had an initial pH of 3.85, specific conductivity of 1.867 mS/cm and acidity of 1000 mg/L. Triplicate batch reactors were set up in 1-L cubitainers with each potential substrate exposed to each AMD, yielding a total of 18 bioreactors which were exposed for 9 days and sampled two times for analysis of anions and dissolved metals. Substrate samples were collected for microbial analysis including iron reducing bacteria (IRB), sulfate reducing bacteria (SRB), fermenters and nitrifiers. Cow manure reactors exhibited the greatest pH, alkalinity and specific conductance increases. Brewery waste reactors did not show any increase in pH. However, sulfate concentrations only decreased in brewery waste reactors, from 2595 mg/L in discharge water A and 1105 mg/L discharge water B to 1350±202 mg/L and 669±31 mg/L respectively. Discharge water A had a minimal impact on microbial groups tested. SRB populations were higher for brewery and cow manure reactors. Fermentative bacteria were higher in llama and cow manure reactors. Results from this short-term experiment suggest that llama and cow manure may be appropriate locally available substrates. Additional longer-term studies are needed to determine the best mix of substrates to be used in a VFC in Potosí, Bolivia.

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Introduction

Intensive mining activities near Cerro Rico de Potosí, Bolivia began in the 16th century with the arrival of the Spanish and has continued since then (Cunningham et al., 1996; Bartos, 2000; Archer et al., 2005). Ag, Sn, Pb and Zn ores have been mined through the years. Mining activities have contaminated nearby land, streams, and rivers (Strosnider et al., 2008). Ore processing tailings, waste rock and acid mine drainage (AMD) constitute sources of pollution and pose a great environmental threat to the area (Strosnider et al., 2007, 2008).

AMD is the water that emanates from mines and it is often characterized by low pH and elevated trace metals concentrations. One of the rivers most impacted by AMD is the Rio Pilcomayo, which flows from Bolivia to Argentina. Plants and animals, as well as water supplies and soils, have been detrimentally affected by mining activities (Archer et al., 2005). Several studies have been done in the upper Rio Pilcomayo watershed regarding the impacts of mining, including AMD discharge and stream water quality (Miller et al., 2002; Villarroel et al., 2006; Strosnider et al. 2007, 2008) and the impact on crops and drinking water (Miller et al., 2004; Archer et al., 2005). Some authors, like Villarroel et al. (2007) and Strosnider et al. (2008), indicate that levels of trace metals like Pb, As and Cu in water supplies exceed accepted minimum safe levels.

There is a consensus in the literature of the need for water treatment and pollution control in Potosí, Bolivia. One of the ways to treat AMD is to use passive treatment systems (e.g., Younger et al., 2002; Watzlaf et al., 2004). These, in contrast to active treatment systems, may entail greater initial capital investment but have less operational and maintenance costs (Johnson and Hallberg, 2005) and do not require ongoing fossil fuel or refined chemical inputs, which make them suitable for areas like Potosí. For net acidic mine waters, passive treatment systems often include vertical flow cells (VFC) or successive alkalinity producing systems (SAPS), commonly known as reducing and alkalinity producing systems (RAPS) (Watzlaf et al., 2004).

VFC rely on an organic substrate for alkalinity generation and removal of trace metals. These cells create an anaerobic environment that enhances the activity of sulfate reducing bacteria (SRB) for precipitation of trace metals as metal sulfides. Different organic substrates have been used in laboratory and field experiments but the effectiveness of each is site dependent (Waybrandt et al., 1998; Cocos et al., 2002; Waybrandt et al., 2002; Figueroa et al., 2004; Watzlaf et al., 2004; Pruden et al., 2007). Although there is no broadly applicable substrate
recipe, the organic matter used for these systems usually constitutes composted organic wastes that are available in the area of implementation. Potosí, being located at high elevation in a low productivity landscape, poses several challenges for the installation and operation of a passive treatment system. The limited availability of organic matter, low partial pressure of oxygen, increased sunlight intensity and extreme weather conditions are major challenges to overcome when designing a treatment system in an area like Potosí. Previous studies using organic wastes in Bolivia include the use of llama dung by Younger et al in La Paz (2002) and by the researchers in Centro de Investigacion Minero Ambiental-Agencia de Cooperación Internacional del Japon in Potosí (CIMA-JICA) (F. Llanos pers. comm. 2008). However, other wastes that are locally available, such as brewery waste and cow manure, have not been tested. These organic wastes were collected and tested in an initial, short-term laboratory study to examine their potential applicability and efficiency.

Methods

Collection of Substrate

Llama manure was collected at a farm located in Villa Collu on July 26th 2008. The llama manure was collected in two 5441-mL Whirl-Pack® sterile bags using a shovel and sterile gloves from the interior of a stockpile, where it was physically degraded. The area of collection was located approximately 2 km downstream from the Chimborazo mining sector of Cerro Rico de Potosí. Because Cerro Rico was historically host to thousands of small inefficient smelting operations (which likely dispersed heavy metals in its vicinity; Strosnider et al. 2007) and the llamas were feeding from surrounding areas, their diet and therefore feces may show some evidence of feeding in a naturally highly mineralized area affected by mining pollution.

Cow manure and brewery waste were collected from Hacienda Cayara on July 27th 2008. Each substrate was collected in four 5441-mL Whirl-Pack® sterile bags using a shovel and sterile gloves. Aged cow manure was collected from the center and bottom of the stockpile. The brewery waste was obtained by the Hacienda Cayara from the Cervecería Potosina, a local brewery in Potosí. It is used to feed cows during the dry season when the production of natural grasses is minimal and is the waste resulting from malt processing. After malt is cooked, it is separated by filtration. The resulting waste is then transferred to a collection pile, where it is held until sold to farmers.
Collection of Mine Water

Mine water used for construction of the batch reactors was collected from two different abandoned mine discharges on Cerro Rico: A (S 19. 589 093˚, W 65. 773 577˚) and B (S 19. 617 417˚, W 65. 736 409˚) on July 29th 2008. Discharge A is associated with natural and anthropogenic erosion of mineral-rich material (pallacos) from Cerro Rico and the Sn mining of that material that ceased decades ago (Bartos, 2000; Waltham, 2005). Discharge B emanates from an abandoned mine portal that is likely draining an active Zn mining operation (F. Llanos pers. comm., 2008). Discharges A and B correspond with sites “11” and “1” respectively in Strosnider et al. (2007). Discharge A drains to Rio Huayna Mayu and Discharge B drains to Rio Sucu / Kori Mayu, both tributaries of the heavily contaminated Ribera de la Vera Cruz at the headwaters of the Rio Pilcomayo (Strosnider et al., 2007, 2008).

AMD from Discharge A was collected in a 20-L cubitainer and Discharge B was collected in two 10-L cubitainers. Air was removed from the cubitainers to minimize oxidation of the mine water during transport. Dissolved oxygen (DO) was measured using a calibrated YSI Model 55 meter. Specific conductivity (SC), temperature and pH were measured using a calibrated Orion 1230 multimeter. Anion and metal samples were collected in HDPE bottles and analyzed upon return at the University of Oklahoma Center for Restoration of Ecosystems and Watersheds (CREW) laboratories. Dissolved metals were collected by filtering the AMD through a 0.45-um nylon syringe filter prior to acidification. Total and dissolved metal samples were preserved with analytical grade nitric acid. Metals were digested following method EPA 3015 and analyzed using a Varian Vista-Pro® inductively coupled plasma-optical emission spectrometer (ICP-OES) following EPA method 6010. Anion samples were analyzed using a MetrOhm® 761 compact ion chromatograph (IC), following method EPA 300. However, values for anions must be flagged since the recommended holding times for nitrite, nitrate and phosphate of 48 hr requiring preservation at 4˚C and for sulfate, chloride, bromide and fluoride of 28 days with no preservation (Pfaff, 1993) was exceeded.

Construction of Batch Reactors

Substrate dry weight was determined in triplicate prior to the construction of the batch reactors. Approximately 5 g of wet substrate was weighed and dried at 85˚C for 24 hours. Due to the high altitude of Potosí, CIMA-JICA has adapted standard methods for drying soils to consider the boiling point of water at this altitude (85˚C). The reactors were built with a single
substrate in triplicate for each AMD using 1-L cubitainers. Since more than one bag was collected for each substrate a composite was prepared by using the collected moist material, accounting for the dry weight for each bag. Cow and llama reactors were constructed by using moist substrate equivalent to 400 g of dry material; however, due to volume constraints, 200 g of dry material was used for the brewery waste reactors. After the addition of 600 mL of AMD, each reactor was incubated at approximately 12 °C, which is representative of possible VFC operational temperatures in this alpine climate. Care was taken to minimize head space in the cubitainers. The reactors were built on July 29th 2008 and the experiment started the same day.

Analytical Methods

Water Quality Analysis. Water quality analyses were taken at mid-point and end point of the experiment, August 3rd (Day 6) and 6th (Day 9) 2008, respectively. For substrate analyses, approximately 500 g of moist substrate was collected from each reactor. It was then centrifuged for 5 min at 3000 rpm on a Kokusan H-40F bench top centrifuge. Water samples were taken from the supernatant for analysis of dissolved metals for llama dung and brewery waste by filtering the AMD with a 0.45-um nylon syringe filter and preserved with analytical grade nitric acid. Due to filtering constraints, only total metals samples were collected for cow manure reactors. Anions samples were collected as time permitted.

Microbial Analysis. Microbial analyses were done at day 0, 6 and 9 of the experiment. Ten grams of moist substrate was collected from each reactor and 15 mL of 2% sodium pyrophosphate was used as a cell dislodger. The substrates were shaken on a horizontal shaker (Omron H3CA MH-4AIII) at 120 rpm for 45 min. They were then centrifuged for 2 min at 2000 rpm on a Kubota 5922 floor centrifuge. The supernatant was then diluted 1:10 with Butterfields solution and used for the serial dilutions. Microbial analysis included fermentative bacteria (FB), nitrate-reducing bacteria (NB), sulfate reducing bacteria (SRB) and iron reducing bacteria (IRB). IRB were enumerated by preparing the media as described by Cytoculture Environmental Biotechnology. SRB were analyzed by following the modified SM 9240D (Cytoculture Environmental Biotechnology). Media was prepared by transferring 9 mL into each test tube and by autoclaving at 120°C for 20 min on a Yamato sterilizer SM-300. The tubes were inoculated at 25°C for 14 days and a positive reaction was observed when the media color changed from yellow to green for IRB and as the formation of a black precipitate for SRB. NB
(BD-221830) broth and FB (BD-221687) broth were purchased from BD diagnostic systems. The NB and FB test tubes were inoculated and incubated for 24 hr at 37°C. Analyses of results followed those described by BD Diagnostics.

Results

Water Quality Parameters.

Water quality parameters for mine drainage discharges and reactor sampling points are summarized in Table 1. Discharge A had a higher pH and specific conductivity than discharge B. Specific conductivity increased in all reactors. Alkalinity generation was observed in all reactors except those with brewery waste. Cow and llama manure showed the greatest increase in specific conductivity, alkalinity and pH. Specific conductivity in brewery waste slightly decreased for discharge A and slightly increased for discharge B; pH did not change for both discharges. Values for dissolved oxygen varied within reactors but in general increased for those built with discharge water A and decreased for discharge water B.

Table 1. Values of specific conductivity, pH, dissolved oxygen and alkalinity for discharge water A, discharge water B and for batch reactors.

<table>
<thead>
<tr>
<th></th>
<th>Date</th>
<th>SC (mS/cm)</th>
<th>pH</th>
<th>DO (mg/L)</th>
<th>DO (%)</th>
<th>Alkalinity (mg/L as CaCO3)</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>mean</td>
<td>sd.</td>
<td>mean</td>
<td>sd.</td>
<td>mean</td>
</tr>
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<td>Discharge A</td>
<td>7/29/2008</td>
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<td>N/A</td>
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<td>N/A</td>
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<td>6.8</td>
<td>0.02</td>
<td>1.53</td>
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<td>3.4</td>
<td>0.04</td>
<td>3.2</td>
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<td>8/5/2008</td>
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<td>4.7</td>
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<td>Cow A</td>
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<td>N/A</td>
<td>8.34</td>
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<td>0.28</td>
<td>7.4</td>
<td>0.1</td>
<td>4.1</td>
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<td>Llama B</td>
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<td>0.21</td>
<td>7.1</td>
<td>0.1</td>
<td>1.6</td>
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<tr>
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<td>0.10</td>
<td>3.8</td>
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<td>1.5</td>
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<td>Brewery Waste B</td>
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<td>0.04</td>
<td>3.5</td>
<td>0.04</td>
<td>4.3</td>
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<td>Cow B</td>
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<td>8.3</td>
<td>0.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Cow B</td>
<td>8/6/2008</td>
<td>29.4</td>
<td>0.7</td>
<td>8.2</td>
<td>0.1</td>
<td>0.3</td>
</tr>
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</table>
Anions.

Sulfate increased in llama and cow reactors and decreased up to 50% for brewery waste (Fig. 1). Chloride, bromide and phosphate increased in all reactors. Chloride increased from as low as 10 mg/L to 6300 mg/L in cow reactors (Fig. 2). Bromide increased to the greatest extent in the cow reactors, going from 12 mg/L in discharge B to 150 mg/L after 9 days of exposure to AMD. Nitrite also increased for discharge B reactors but had varying results for discharge A. Nitrate was below detection limits for llama dung and cow manure, and stayed constant for brewery waste reactors.

Figure 1. Sulfate concentration for reactors at beginning, middle and end-point of analysis; error bars represent standard deviation. Where error bars are absent n=1.

Metals.

Analyzed metals included Al, As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn (Tables 2 and 3). In the llama and brewery waste reactors, the removal was higher than 95% for dissolved Al. In the cow manure reactors, removal rates were lower for total Al. As, Mg, K and Na increased in all reactors. However, this increase was lower in the brewery waste reactors than in the llama and cow manure wastes for As and K. Ca increased in all reactors but to a lower extent in the cow manure reactors. The behavior of Cd and Co was similar for all reactors, with the greatest decrease in the llama and cow manure reactors. Cu decreased for all reactors, except for discharge B, where the concentration increased at 6 days, and then decreased again at
the end of the experiment. Iron decreased over 75% for all reactors. Ni, Mn and Zn decreased for all of the reactors but to a lesser extent with the brewery waste. Cr and Pb stayed mostly constant through the experiment.

![Chloride concentration for reactors at beginning, middle and end-point of analysis; error bars represent standard deviation. Where error bars are absent n=1.](image)

**Microbial Analysis.**

Discharge A had the lowest impact on the microbial community for all reactors (Fig. 3). In general, bacteria values were greater for llama manure reactors followed by cow and brewery waste except for SRB where all reactors had similar values. Specifically, cow manure reactor IRB concentrations decreased over the course of the experiment for discharge A but stayed constant for discharge B. Similar results were observed for llama and brewery reactors, where IRB decreased for both discharges. Fermentative bacteria only increased for cow manure reactors with discharge B and decreased for both discharges in llama and brewery waste reactors. Nitrate-reducing bacteria showed varying results. It increased for both discharges in cow manure reactors, for discharge A for brewery reactors and discharge B for llama manure reactors. SRB increased through the time of experiment in all reactors exposed to discharge A. Additionally, the characteristic smell for H₂S was observed in cow and llama manure reactors but not in brewery waste reactors.
Table 2. Initial mean dissolved and total metals for discharge A and batch reactors built with discharge A. All values are mg/L.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Date</th>
<th>Al</th>
<th>As</th>
<th>Ca</th>
<th>Cd</th>
<th>Co</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
<th>Na</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
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<tbody>
<tr>
<td>Dissolved</td>
<td></td>
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<tr>
<td>Initial</td>
<td>7/29/2008</td>
<td>16.0</td>
<td>0.01</td>
<td>120</td>
<td>0.74</td>
<td>0.11</td>
<td>0.001</td>
<td>0.2</td>
<td>53.4</td>
<td>12.3</td>
<td>19</td>
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<td>16</td>
<td>0.14</td>
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<td>51.7</td>
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<tr>
<td>Llama</td>
<td>8/3/2008</td>
<td>0.9</td>
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<td>296</td>
<td>0.01</td>
<td>0.02</td>
<td>0.002</td>
<td>0.09</td>
<td>1.3</td>
<td>1240</td>
<td>110</td>
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<td>Llama</td>
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<td>0.7</td>
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<td>0.02</td>
<td>0.003</td>
<td>0.07</td>
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<td>430</td>
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<td>0.007</td>
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<td>352</td>
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<td>2760</td>
<td>0.06</td>
<td>0.20</td>
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</table>

*Samples taken prior to reactor construction

Table 3. Initial mean dissolved and total metals for discharge B and batch reactors built with discharge B. All values are mg/L.

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<th>Sample</th>
<th>Date</th>
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<th>Cd</th>
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<th>Cr</th>
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<td>Initial</td>
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<td>0.01</td>
<td>30</td>
<td>63.7</td>
<td>4.4</td>
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<td>0.02</td>
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<td>0.31</td>
<td>0.24</td>
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*Samples taken prior to reactor construction
Figure 3. MPN results (cells / g dry substrate) for substrate bacteria analysis, (A) IRB; (B) FB; (C) SRB; and, (D) NB.

Discussion

Several wastes were collected and analyzed in Potosí, Bolivia in an initial evaluation of performance as possible substrates for a passive treatment VFCs. The duration of study was limited so results must be viewed with caution. Previous studies have demonstrated the need for longer exposure times to determine optimal substrate for AMD remediation (Waybrandt et al., 1998; Wildeman et al., 1994a; Wildeman et al., 1994b; Waybrandt et al., 2002; Zagury et al., 2006). Even though these authors do not agree on a minimum time for assessing substrate performance, Zagury et al. (2006) found that a minimum time of 70 days was needed to compare single substrates performance against different substrates mixes. Other authors have shown that a time of 28 to 52 days was enough to analyze metal removal and sulfate reduction rates (Wildeman et al., 1994a; Wildeman et al. 1994b; Gilbert et al., 1999). In addition, it has been observed that at lower temperatures the oxidation-reduction potential increased (Wildeman et al., 1994a), which may decrease the rate of sulfate reduction and metal removal (Neculita et al.,
However, the data reported here represent only an initial effort and they do help to deliver insights and illuminate avenues for future experiments regarding the implementation of passive treatment systems in Potosí.

In batch reactors where limestone is not added, sulfate reduction and organic ligand exchange contribute to the generation of alkalinity (Wieder, 1993; Waybrandt et al., 1998, 2002). Although the values of alkalinity were high for llama dung and cow manure, these values would likely drop in a long term experiment, as was observed to occur in systems built with spent mushroom compost in Pennsylvania (Watzlaf et al., 2004). In general, substrates with lower abiotic acid neutralization capacity (ANC) have lesser changes in pH and alkalinity (Wieder, 1993). It can be inferred from the data that brewery waste may have a lower ANC than llama dung and cow manure as observed with changes in pH and alkalinity during time of experiment. As with other substrate properties, the ANC of the organic components will be depleted over time, potentially causing the high pH and alkalinity values for llama dung and cow manure to drop.

Sulfate values increased for llama and cow reactors and decreased for brewery waste. Previous studies have shown that manures contain high concentrations of sulfate which at the beginning of experiments may lead to an underestimation of \( \text{SO}_4^{2-} \) reduction due to \( \text{SO}_4^{2-} \) leaching (Waybrandt et al., 1998; Zagury et al., 2006). Gilbert et al. (1999) suggest the use of controls built with only water in order to be able to compare the changes in metals and anions concentrations due to natural components from the organic wastes. Brewery waste did not show an increase in pH and the presence of \( \text{SO}_4^{2-} \) reduction is generally accompanied by an increase in pH (Waybrandt et al., 1998, 2002). However, microbial analysis showed that SRB were indeed active during the experiment, indicating that \( \text{SO}_4^{2-} \) reduction is likely occurring in the brewery waste reactors as well as in llama and cow reactors. Other researchers have observed a drop in sulfate concentrations in reactors after 20 days of analysis (Waybrandt et al., 1998), suggesting that a longer retention time may be necessary in order to observe sulfate reduction. Chloride, phosphorus and nitrogen compounds are naturally present in organic wastes and the high values observed in the reactors came from composted material. A similar behavior was observed by Stewart et al. (1998) where the leaching of inorganic nitrogen increased during the first period of incubation of spent mushroom compost (SMC), and then decreased later.
VFC performance depends on anaerobic conditions which promote microbial activity and chemical reactions that enhance alkalinity generation. The organic layer of VFCs creates anoxic conditions because of the biotic and abiotic oxygen demand of organic substrates (Watzlaf et al., 2004). In some reactors, DO was present in higher quantities than was measured in the original AMD. It appears that there was insufficient aerobic heterotrophic bacterial activity to strip DO in the brewery waste. DO stripping is a key function of VFC substrate and therefore brewery waste alone is likely not a suitable substrate.

Metal removal has been analyzed extensively in passive treatment field systems and laboratory experiments (Wieder, 1993; Waybrandt et al., 1998, 2002; Watzlaf et al., 2004). Metals concentrations in experiments carried out by Waybrandt et al. (1998) dropped quickly and remained low. In this experiment, Al, Cd, Co, Ni, Mn, Fe and Zn all decreased in the reactors. Although SRB activity was observed, other removal mechanisms may also be responsible for the metal removal. Metals removal mechanisms include adsorption to organic matter, organic ligands, anions and iron hydroxides as well as precipitation as metal sulfides (Fristoe and Nelson, 1983; Fletcher and Beckett, 1987; Carroll et al., 1998; Omoike and Vanloon, 1999; Olivie-Lanquet et al., 2001; Younger et al., 2002, Norton et al., 2004; Gibert et al., 2005; Luo et al. 2008).

Adsorption of metals to organic materials in constructed wetlands varies with pH (Machemer and Wildeman, 1992). Metals can bind in limited amounts at pH 4 to 6 to humic and fulvic acids present in organic substrates (Wieder, 1993; Gazea et al., 1996). Some studies suggest competition for adsorption sites between metals of interest. Wieder (1993) and Gazea et al. (1996) observed that Mn, Zn, Cd, Ni and Co do not easily bind to fulvic and humic acids. These authors also found that Fe, Zn, Cd, Ni and Co adsorb prior to Mn. Similar results were observed by Machemer and Wildeman (1992) who found that Fe and Cu adsorb better than Zn and Mn to organic substrates. In addition, Al adsorbs abiotically to particulate organic matter and dissolved organic carbon (DOC) present in organic wastes (Wieder, 1993; Omoike and Vanloon, 1999). Cd was most likely removed via organic ligands and adsorption to bacterial cells (e.g. Fristoe and Nelson, 1983). Adsorption of these metals to the substrate seems to be likely for these metals since it has been observed that it is an important process (Fristoe and Nelson, 1983; Fletcher and Beckett, 1987; Machemer and Wildeman, 1992; Omoike and Vanloon, 1999).
However, this process is limited by the finite number of sorption sites available and long-term performance is dependent upon other removal mechanisms.

Although As is not a natural component of the wastes it may have been adsorbed to its organic component. Studies done in China have shown accumulation of As in pig manure from feed additives used in swine production (Li and Chen, 2005) which could be the source for cow manure since it came from a dairy farm. Although no information is available of food additives used in cow manure, llamas may have been exposed to a different source. As mentioned before, llamas were feeding in areas impacted by mining activities and the higher concentration of As in its manure may come from bioaccumulation. As well, manure with high concentration of As has been used as land fertilizer and thus accumulation in crops is a concern (Li et al., 2009). Although no information is available regarding hops used in the brewery, it is known that cow and llama manure are used extensively as land fertilizers (Hacienda Cayara, per. Comm. 2009). Additionally, As-oxygen ions have low binding affinity and can be easily dissolved at neutral pH (Younger et al., 2002). This may explain the increased in As levels for all reactors, where the exposure of manure and brewery waste to ambient conditions may have increased the levels of As by re-dissolution from the organic waste exposed to AMD.

The presence of DO in some of the reactors could explain the decrease in dissolved Fe. Fe(II) in the presence of oxygen oxidizes and precipitates as Fe(III)-hydroxide when pH is raised to > 3.5 (Hedin et al., 1994; Watzlaf et al., 2004). However, the orange precipitate associated with ferric hydroxide precipitation was not observed in the organic substrates, suggesting that Fe was being removed by other mechanisms. Other Fe removal mechanisms include combination with biotic and abiotic organic ligands (Fletcher and Beckett, 1987) and the formation of FeS (Kolmert and Johnson, 2001). In addition, metals like As (Mohan and Pittman, 2007), Cd (Olivie-Lanquet et al., 2001; Carroll et al., 1998), Pb (Carroll et al., 1998), and Zn (Fletcher and Beckett, 1987; Norton et al., 2004; Gibert et al., 2005) can adsorb and co-precipitate with Fe(III)-hydroxides. Although no visual sign of Fe(OH)₃ precipitation was observed some metals may have co-precipitated in small amounts with Fe(OH)₃. In a different note, dissolved Al and Fe can react with phosphate to form a stable solid (Omoike and Vanloon, 1999) later coming out of solution. This may have been a plausible removal mechanism due to the high concentration of phosphates in manures.
Another removal mechanism for metals in VFCs is precipitation as metal sulfides. Taking into consideration their solubility product, metals precipitate in the following order CuS, PbS, ZnS, CdS, FeS and MnS (Hedin et al., 1994; Watzlaf et al., 2004). Therefore, the changes observed in concentrations of Cu, Pb, Cd, Zn and Fe could also be due to precipitation as metal sulfides (Fletcher and Beckett, 1987; Younger et al., 2002; Norton et al., 2004; Gibert et al., 2005). Mg, Ca, K and Na are natural base cations present in the diets of llamas and cows and their presence in the batch reactors may be due to quantities in the composted materials. Figueroa et al. (2004) also observed an increase of sulfate, Zn and Mn in effluent water during the first 40 days of their experiments, suggesting that it takes time for the systems to equilibrate.

As mentioned above this short study was not designed to determine which organic waste will be most appropriate in terms of metal removal and microbial activity performance, instead this study served to examine the potential use of locally available substrates. It has previously been shown that the long-term performance of a passive treatment system relies in the competition and dependence between microbiological communities (Hallberg and Johnson, 2003; Logan et al., 2005). FB, NB, SRB and IRB depend on heterotrophic bacteria for the hydrolysis of complex carbohydrates present in the organic substrates. However, certain organic wastes used for the construction of these systems contain a high amount of simple sugars which may be depleted over time (Waybrandt et al., 1998, 2002), inducing the early activity of SRB and IRB for the reduction of sulfate and ferric iron, respectively. Current results suggest that conditions were favorable for sulfate reduction as shown by an increase in SRB population but unfavorable for iron reduction. Neculita et al. (2007) showed that SRB prefer an environment with pH 5 to 8 and with a maximum zinc concentration of 40 mg/L. Higher Zn concentrations like the ones found in the AMD of Potosí can be toxic for SRB (Kaksonen and Puhakka, 2007; Neculita et al., 2007). Moreover it has been suggested that metals like zinc, cadmium and nickel can have a synergistic effect having a higher toxicity at lower concentrations (Neculita et al., 2007; Koschorreck, 2008). In contrast, Koschorreck et al. (2003) and Koschorreck (2008) observed that sulfate reduction and SRB can be measured at pH as low as 3, suggesting that these bacteria can be active at acidic pH.

IRB use simple sugars to reduce ferric iron (Fe(III)) to ferrous iron (Fe(II)) (Lovley, 1991). Since previous studies have shown that manures contain a high amount of simple sugars, the decrease in IRB numbers could have been due to the initial lack of ferric iron. Increase in the
numbers of NB may have been due to the favorable anaerobic conditions and the availability of nitrogen in the system. It has been reported that manures contain a high amount of nitrogen (Stewart et al., 1998; Waybrandt et al., 1998). However, a longer experiment time should be done to analyze further behavior of microbial communities.

Initial results suggest that brewery waste is not appropriate for treatment of AMD when used alone. Llama dung and cow manure seem to be a better choice of substrate to use for high strength AMD in extreme environmental conditions like the high Andes in Bolivia. A mixture of the three substrates may be the best choice to use in a passive treatment design. However, a longer exposure time is recommended to analyze the stability of alkalinity generation, metal removal and microbial activity in these organic wastes. Further recommendations include the testing of different mixes of these organic materials depending on availability in Potosí.

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