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TECHNICAL ARTICLE

# Performance of a SAPS-Based Chemo-Bioreactor Treating Acid Mine Drainage Using Low-DOC Spent Mushroom Compost, and Limestone as Substrate

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**Abstract** Static and continuous experiments were carried out in a down flow chemo-bioreactor, modeled on successive alkalinity producing systems, for acid mine drainage treatment. Spent mushroom compost (SMC) was used as the immobilizing substrate and nutrient source for sulfate reducing bacteria (SRB). Operational success was assessed using effluent to influent ratios during continuous flow; these averaged: 0.0175, 0.12, and 0.071 for Fe, Cu, and Mn, respectively, and 0.11 for sulfate. In contrast, Mg was released by the SMC, increasing its concentration in the effluent. Acidity was consistently less than 1 mg/L. The pH of the effluent was higher than 7 for most of the experiment. SMC was shown to release sufficient organic carbon during the experiment to establish and maintain anaerobic conditions and sustain the SRB.

**Keywords** Acid mine drainage · Chemo-bioreactor · Spent mushroom compost · Performance ratio · Sulfate reduction

## Introduction

Passive bioremediation of acid mine drainage (AMD) using sulfate reducing bacteria (SRB) in bioreactors has received much attention over the last two decades (Johnson and Hallberg 2005; Neculita et al. 2007). Their treatment performances are, however, variable, and depend on a number of factors (Neculita et al. 2007). SRB mediated sulfate reduction mechanisms are summarized in Eqs. 1 and 2 (Dvorak et al. 1992; Neculita et al. 2007).

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \quad (1)$$

$$H_2S + M^{2+} \rightarrow MS(s) + 2H^+, \text{ where } M = \text{metal cations} \quad (2)$$

Most SRBs require near-neutral pH (Hao 2003), a suitable nutrient source, and a solid matrix on which they can grow and survive in extreme conditions. Simple organic carbon sources like ethanol and lactate have been used successfully in laboratory scale bioreactors as suitable electron donors for SRBs. However, these materials are too expensive for full scale treatment systems (Kaksonen et al. 2003; Neculita et al. 2007), and higher sulfate reduction rates can be obtained in a mixture of easily biodegradable organic carbon sources (Zagury et al. 2007).

A matrix medium with large pore spaces, low surface area, and a small void volume is preferred because it minimizes the plugging of the bioreactor, an important operational problem. Spent mushroom compost (SMC) has been used successfully as a growth matrix as well as a suitable organic carbon source for treating both metal and coal mine drainage (Dvorak et al. 1992; Stark et al. 1994). It shows good relative performance as an electron donor compared to other organic waste materials (Chang et al. 2000), and contains various organic matter, including lignin, cellulose, and hemicelluloses (Jordan et al. 2008), dissolved organic nitrogen (Guo et al. 2001), and nutrients (Bhattacharya et al. 2008). The polysaccharide content of SMC is generally degraded by hydrolytic fermentative anaerobes to alcohols and fatty acids that, in turn, support the growth of SRBs (Chang et al. 2000). Because of its

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slow degradation (Dvorak et al. 1992) and its bulk form physical property (Bhattacharya et al. 2008; Dvorak et al. 1992), it can be a suitable substrate in bioreactors for long term AMD bioremediation.

Although SRBs are generally inhibited by pH values below 5.5 and above 9 (Hao 2003; Widdel 1988), they have been used effectively to treat very acidic AMD, presumably because the alkalinity they generate creates near-neutral pH microenvironments (Das et al. 2009; Johnson and Hallberg 2005).

A field-based successive alkalinity producing system (SAPS), in which abandoned coal mine discharge sequentially flows downwards through an organic layer and limestone bed, was tested in the Hanchang coal mine of Kangwon province, Korea (Bhattacharya et al. 2008). Based on this work, a laboratory-scale SAPS-based chemo-bioreactor was constructed and tested. It is referred to as a chemo-bioreactor because, like the SAPS it models, it uses both chemical (acid neutralization by limestone) and biological processes. The objectives of this study were to examine the effectiveness of the chemo-bioreactor in removing metals and sulfate from AMD and to determine how long AMD could be effectively treated with only the labile carbon and nutrients available in the SMC.

## Materials and Methods

### Materials

A synthetic AMD was prepared to provide a consistent way to monitor the effects of treatment and to reduce the effects of possible interference by conflicting pollutants. It was prepared by adding salts to tap water (conductivity 260 ± 10 μS/cm; total dissolved solids (TDS) 132 mg/L) to generate a solution composed of: $FeSO_4{\cdot}7H_2O$, 2,330 mg/L; $CuSO_4{\cdot}5H_2O$, 1,850 mg/L; $MnSO_4{\cdot}H_2O$, 1,800 mg/L; $MgSO_4{\cdot}7H_2O$, 290 mg/L; $(NH_4)_2SO_4$, 950 mg/L, and $K_2HPO_4$, 750 mg/L. An analysis of this synthetic AMD is presented in Table 1.

The SMC was procured from the Indian Institute of Technology Rural Development Centre mushroom cultivation farm in Kharagpur, India, where they use it to produce commercial grade mushroom for the local market.

### Jar-Test Experiments

Jar tests were performed to establish the capability of SMC to produce alkalinity and liberate organic carbon, as well as any metal release. In two beakers, 33 g of SMC was placed followed by a layer of gravel and 1 L of distilled water. One set was acidified by adding 1(N) $H_2SO_4$ to lower its pH to 2.5. For the limestone jar test, 61 g of limestone were mixed with 1 L of distilled water. The SMC: water and limestone: water ratios were similar to those used in the chemo-bioreactor.

### The Chemo-Bioreactor and Its Operation

The column chemo-bioreactor used in this study is shown in Fig. 1. The down-flow chemo-bioreactor cylinders were made of transparent acrylic sheet. A is the reservoir; B and C are settlement tanks that prevented the entry of excessive total suspended solids (TSS) into the main reaction vessel D, where the substrate materials were present. This vessel was equipped with one sampling port just over the gravel layer (Fig. 1). Tanks *E* and *F* were for settling the precipitate coming from tank *D*. One peristaltic pump was placed after tank *D* to produce continuous flow. The operating temperature varied with the local atmosphere, from 16 to 30°C. Tank *D* (140 × 30 cm) was packed with 5.5 kg of limestone (5 cm) at the base followed by 2.95 kg of SMC (14 cm) and a gravel layer of 7 kg (5.5 cm) at the top, with a final reacting bed height of 19 ± 0.2 cm. The

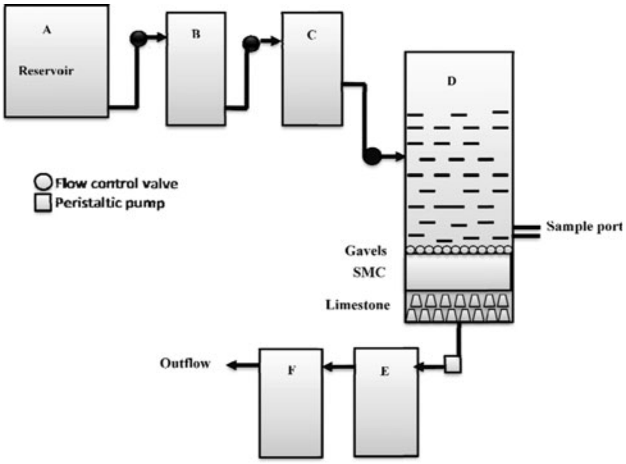


**Fig. 1** Schematic diagram of Chemo-bioreactor

**Table 1** Chemical parameters of the synthetic AMD

| pH | $SO_4^a$ | $Fe^a$ | $Cu^a$ | $Mg^a$ | $Mn^a$ | $COD^a$ | $DOC^a$ | $Acidity^b$ | $Cond.^c$ | $DO^a$ | $O.R.P.^d$ | $TS^a$ | $T.D.S^a$ | $Salinity^a$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2.21 | 2,251 | 467 | 463 | 29 | 567 | 240 | 0.01 | 1,394 | 3.90 | 3.80 | 538 | 1,243 | 2,040 | 2,450 |

*Units*: $^a$ mg/L; $^b$ mg/L(as $CaCO_3$); $^c$ mS/cm; $^d$ mV mass density of the SMC layer was 0.298 g $cm^{-3}$. The porosity was calculated to be 0.59 ± 0.01, using the final reacting bed volume and the calibrated liquid volume used to pack the bed.

The chemo-bioreactor was kept in a static condition for 21 days after having been filled with synthetic AMD, which was maintained at a height of 130 cm from the bottom of tank *D*. No inoculums or nutrient were added. Then the continuous flow experiment was initiated by activating a single channel peristaltic pump (PP-20-EX, Miclins, Chennai, India). A flow rate of 180 mL/h (0.0216 $h^{-1}$ dilution rate with respect to liquid volume) was maintained during the experiment (PIRAMID Consortium 2003), which is equal to 0.255 cm/h of superficial flow velocity and a hydraulic retention time of 74.5 h through the bed. The water column height was maintained at 130 ± 2 cm in tank *D* through the 12 week experiment because a long water column was expected to reduce the dissolved oxygen (DO) and thereby help establish an anaerobic environment.

### Water Sampling and Analysis

Water samples were collected at 3 and 4 day intervals from the static and continuous flow experiments, respectively. All samples except for TSS analysis were filtered through a 0.45 μm nylon membrane filter to remove precipitates and other solid materials (ERMITE Consortium et al. 2004). Metal concentrations were quantified by atomic absorption spectrophotometry (GBC 932, Australia) after pretreatment with 1.5 μL of concentrated $HNO_3$ per 1 mL sample (Clesceri et al. 1998). Sulfate measurement was carried out using the turbidometric method (Csuros 1997) with a 420 nm spectrophotometer (Ocean Optics Inc. SD 2000). Acidity/alkalinity and TSS were determined by standard methods (Clesceri et al. 1998). Oxidation reduction potential (ORP), pH, DO, conductivity, total dissolved solids (TDS) and salinity were measured with a multi-parameter water analyzer (Orion 5–Star BENCHTOP MULTI, Thermo Scientific, USA) (PIRAMID Consortium 2003). Total organic carbon (TOC) was measured with a TOC analyzer (TOC-$V_{CPH}$, Shimadzu, Japan) using the filtered sample and was reported as dissolved organic carbon (DOC) (Khan et al. 1996). The water used for all of the analysis had a resistivity of 16–18 mega ohm-cm. Whenever possible, the pH, acidity/alkalinity, DO, TDS, TSS, conductivity, salinity and sulfate were determined on the same day that the samples were collected. Glassware and plastic-ware used in this study were cleaned by soaking in 10% nitric acid, rinsed several times with Milli-Q water, and finally oven dried.

## Results

### Data Analysis

Performance ratios were used to evaluate the performance of the chemo-bioreactor, e.g., metal $_{\text{effluent}}$: metal $_{\text{influent}}$ ($\text{metal}_{\text{ef}}$: $\text{metal}_{\text{in}}$) (Bhattacharya et al. 2008). Values equal to or more than one indicate non-performance or contaminant addition.

### Jar Tests

Results of the jar tests are summarized in Table 2. With circumneutral water, both limestone and SMC had high pH values. The pH and alkalinity of the SMC rose eventually, while it remained nearly constant for the limestone. DOC liberation from the SMC peaked within 72 h. Both Fe and Cu were seen to be released with acidified water, and little or no Mn was released from limestone. Release of Mg from SMC was seen to gradually increase, and within a week exceeded 150 mg/L.

### Static Tests

During the course of the 3 week static experiment, the SMC layer gradually turned dark. Gas bubbles were

**Table 2** Jar tests of spent mushroom compost (SMC) and limestone (LS)

| | SMC with circum-neutral water | | | SMC with acidified water | | | LS with circum-neutral water | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 h | 72 h | 192 h | 0 h | 72 h | 192 h | 0 h | 72 h | 192 h |
| pH | 6.77 | 7.59 | 7.79 | 2.5 | 3.18 | 4.24 | 9.47 | 9.48 | 9.51 |
| Alkalinity[a] | 114 | 180 | 208 | 98 | 67 | 86 | 327 | 321 | 332 |
| Fe[b] | 0.03 | 0.41 | 0.4 | 11.65 | 11.15 | 11.64 | 16.4 | 16.7 | 17.6 |
| Mg[b] | 1.45 | 1.92 | 3.34 | 81.8 | 107.2 | 164.2 | 1.31 | 1.35 | 1.45 |
| Mn[b] | 0.00 | 0.02 | 0.02 | 1.75 | 1.66 | 1.68 | 0.00 | 0.01 | 0.01 |
| Cu[b] | 0.00 | 0.01 | 0.00 | 3.05 | 3.18 | 3.77 | 0.91 | 0.95 | 1.55 |
| DOC[b] | 43.09 | 107.61 | 107.88 | 41.97 | 108.04 | 109.1 | – | – | – |

*Units*: [a] mg/L (as $CaCO_3$); [b] mg/L

**Table 3** Comparative study of static experiment (From *D* tank over spent mushroom compost bed)

| | Time (days) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 5 | 9 | 13 | 17 | 21 |
| pH | 2.3 | 4.4 | 4.4 | 6.46 | 6.81 | 7.16 |
| COD (mg/L) | 240 | 240 | 276 | 289 | 317 | 311 |
| DOC (mg/L) | 32.61 | 103.9 | 106.4 | 106.7 | 106.7 | 106.7 |
| Acidity (mg/L as $CaCO_3$) | 1,361 | 1,063 | 1,064 | 213 | 109 | 93 |
| Conductivity(mS/cm) | 3.90 | 3.93 | 3.11 | 3.11 | 3.25 | 3.3 |
| DO (mg/L) | 4.0 | 3.3 | 2.3 | 1.14 | 0.2 | 0.1 |
| O.R.P (mV) | 538 | 524 | 502 | 115 | 10 | −108 |
| TDS (mg/L) | 2,040 | 2,040 | 2,020 | 2,005 | 2,025 | 2,020 |
| TSS (mg/L) | 52 | 31 | 23 | 17 | 16.5 | 16 |
| Salinity (mg/L) | 2,520 | 2,500 | 2,550 | 2,550 | 2,480 | 2,480 |

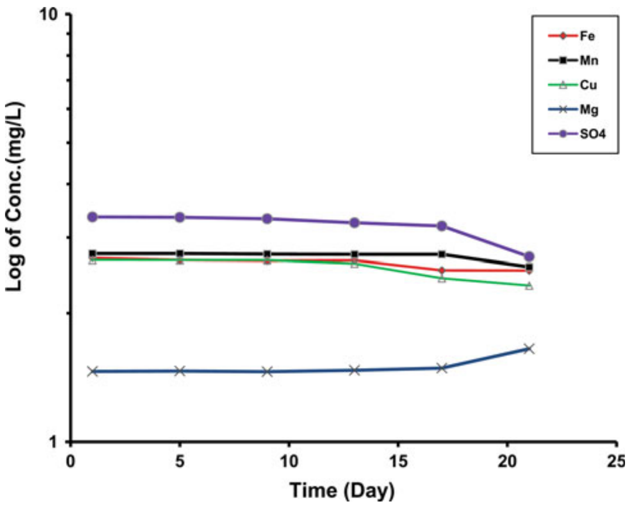


**Fig. 2** Dissolved metal and sulfate concentration during static experiment

observed moving up from the bottom of the SMC bed. Table 3 and Fig. 2 summarize the static experiment results. After starting to release organic carbon within 1 week, DOC rose to 106.7 mg/L and remained constant, indicating saturation of organic carbon release. DO and ORP decreased to 0.1 mg/L and—108 mV, respectively after 21 days. Fe and Cu also began to decrease after 13 days and showed a 29 and 54% decrease respectively, under static conditions. Mn concentration was reduced to 35% after 17 days. The concentration of dissolved Mg showed an increase during the same period and a similar tendency until the end (Fig. 2). Sulfate decreased only slightly until day 17, and then it decreased to 500 mg/L. The pH steadily increased, to 5.5, and the acidity decreased below 50 mg/L. The ORP of the water decreased after 13 days, while COD increased. However, conductivity, TDS, and salinity all decreased rather slowly. A period of 2 weeks was needed to establish and stabilize the chemo-bioreactor performance.

## Initial Stage of Continuous Flow

After 3 weeks of static experimental conditions, the continuous flow experiment was initiated. Table 4 presents the status of parameters at the beginning of continuous flow. Immediately following the end of the static experiment, the effluent into tank *E* was deep brown in color with an unpleasant odor. At this stage, the pH of the effluent was >7 at tanks *E* and *F* with lower acidity. Metal (ef): metal (in) remained within a range of 0.14–0.276 and 0.073–0.125 for tanks *E* and *F*, respectively, with the exception of Mg, which was between 3.57 and 4.35. No correlation was found between sulfate and conductivity; the ratios were between 0.337 and 0.26 for sulfate, and 1.23 and 1.27 for conductivity. COD decreased through the column, increased after passing through the limestone bed, and then decreased again. Conductivity, ORP, TDS, and salinity all slowly decreased.

## Water Chemistry During Continuous Flow

With time, the reactor bed in tank *D* turned black and gas bubbles were observed occasionally; however, the color of the effluent became faint and the odor of the effluent disappeared. During continuous flow experiments, 88–98% of all measured metals except Mg were removed and sulfate reduction averaged over 88% (Table 5). The removal of metals and sulfate during continuous flow is presented in

**Table 4** Reading at initial stage of continuous experiment

| | Samples from various tanks | | | |
|---|---|---|---|---|
| | *A* (Raw AMD) | D (over SMC bed) | *E* | *F* |
| pH | 2.26 | 3.05 | 7.14 | 7.19 |
| Sulfate (mg/L) | 2,251 | 1,899 | 757 | 584 |
| Fe (mg/L) | 466.5 | 462.3 | 129 | 34.11 |
| Cu (mg/L) | 463.1 | 310.3 | 110.1 | 58.03 |
| Mg (mg/L) | 28.9 | 30.08 | 102 | 125.96 |
| Mn (mg/L) | 567.2 | 551.7 | 79.2 | 68.3 |
| C.O.D (mg/L) | 240 | 199 | 311 | 288 |
| DOC (mg/L) | 0.01 | 106.78 | 106.69 | 106.7 |
| Acidity (mg/L as $CaCO_3$) | 1,374 | 1,009 | 89 | 89 |
| Conductivity(mS/cm) | 3.91 | 3.68 | 3.18 | 3.06 |
| D.O.(mg/L) | 4.00 | 0.5 | 0.00 | 0.3 |
| O.R.P (mV) | 553 | −110 | 83 | 107 |
| T.D.S (mg/L) | 2,030 | 2,080 | 1,910 | 1,910 |
| TSS (mg/L) | 1,230 | 42 | 194 | 55 |
| Salinity (mg/L) | 2,418 | 2,300 | 2,280 | 2,265 |

**Table 5** Mean chemical analysis of influent and effluent during continuous flow

| Parameters | Influent | Effluent (*F* tank) | Indian standards: 2,490 (maximum permissible limits) |
|---|---|---|---|
| pH | 2.31 | 6.27 | 5.5–9.0 |
| Sulfate (mg/L) | 2,213 | 245.28 | 1,000 |
| Fe (mg/L) | 459.27 | 8.04 | – |
| Cu (mg/L) | 462.18 | 55.41 | 3.0 |
| Mg (mg/L) | 28.46 | 202.57 | – |
| Mn (mg/L) | 566.9 | 40.29 | – |
| C.O.D (mg/L) | 238 | 243 | 250 |
| DOC (mg/L) | 0.01 | 54.07 | – |
| Acidity (mg/L as $CaCO_3$) | 26.97 | 0.28 | – |
| Conductivity(mS/cm) | 3.86 | 3.06 | – |
| D.O (mg/L) | 3.0 | 1.1 | – |
| O.R.P (mV) | 431 | 86 | – |
| T.D.S (mg/L) | 2,030 | 1,920 | 2,100 |
| TSS (mg/L) | 1,227 | 36 | 100 |
| Salinity (mg/L) | 2,495 | 2,285 | – |

Values are means of 24 influent and 28 effluent samples

Fig. 3. The $metal_{ef}$: $metal_{in}$ and $sulfate_{ef}$: $sulfate_{in}$ ratios indicate a high level of removal efficiency. The $Fe_{ef}$: $Fe_{in}$ ratio decreased from 0.076 to less than 0.0033 within 16 days of continuous flow, and remained at that level until the end of the experiment, with only minor fluctuations. The copper removal ratio was 0.125 at the start, and ranged from 0.143 to 0.105 during continuous flow; most of the time, the ratio ranged from 0.118 to 0.105. The ratio of manganese removal through the experiment was approximately 0.07, compared to 0.12 at the initial stage. Sulfate was removed at a high rate, with $sulfate_{ef}$: $sulfate_{in}$ averaging below 0.125, though periodic fluctuations occurred. This means that sulfate was being reduced to sulfide. In such a setting, sulfate removal is limited by the availability of dissolved carbon, a phenomenon called carbon drought (Bhattacharya et al. 2008). Carbon drought can represent a phase of inactivity of the mostly hydrolytic fermentative anaerobes that produce the simple carbon for the SRBs from the complex hydrocarbons of the SMC in the absence of reactive carbon molecules (Chang et al. 2000).

Initially, iron would have precipitated as iron hydroxide; as the system became reducing, these precipitates would have been transformed to iron sulfide and possibly carbonate. This could explain why the Fe reduction plot is not as steep as the sulfate reduction plot. The same could be true for copper removal.

The magnesium concentration in the effluent was higher than in the influent in the static experiment as well as during the initial stages of continuous flow. During continuous flow, the concentration of Mg was 4–9 times greater in the effluent than the influent. The jar test results showed that the magnesium was released from the SMC; fluctuations in the magnesium values during the continuous flow experiment are attributed to the release of magnesium from gypsum in the SMC and the inability of the chemo-bioreactor to treat such releases.

The effluent pH and acidity were maintained throughout the experiment at values >6 and <50 mg/L, respectively. ORP decreased. The COD of the effluent fluctuated, with an average value of 243 mg/L. This suggests that there was a balance between nutrient addition and ion concentration reduction during continuous flow. A slight decrease in conductivity, TDS, and salinity was observed over time.

### Carbon Availability

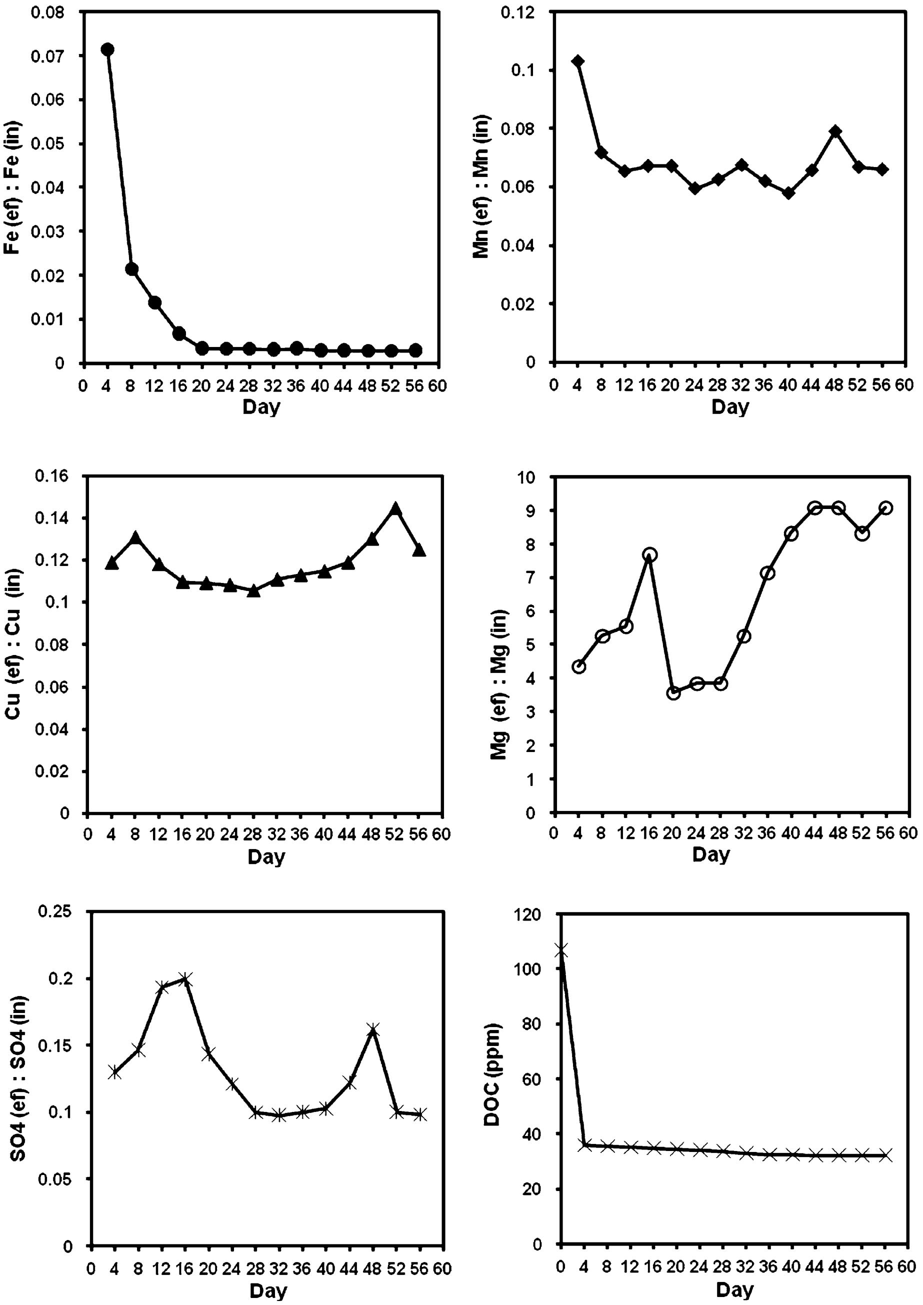
In the jar test, most of the DOC was released from the dried SMC within 3 days. During the static condition experiment, the DOC level peaked and then stabilized within 1 week. However, during continuous flow, the DOC level fell within the first week of the experiment (Fig. 3), and remained constant at 30 mg/L for nearly 8 weeks. With continuous flow, the DOC concentration in the chemo-bioreactor column decreased due to dilution. However, presumably the SMC continued to release DOC at a nearly constant rate, since sufficient DOC was available for the microorganisms present in the chemo-bioreactor and since metal and sulfate reduction continued. A total volume of 233 L of AMD were treated during continuous flow and 2.95 kg of SMC was introduced as a carbon and alkalinity source. That means 12.66 g of SMC was enough to supply the required organic carbon to treat 1 L of AMD.

## Discussion

The results of static and continuous flow demonstrate that the simple down flow reactor is capable of reducing metal and sulfate concentrations from AMD. Hydraulic retention time (HRT) strongly affects the performance of continuously running bioreactors where slow growing bacterial metabolism is involved (Dvorak et al. 1992). A HRT of 74.5 h was maintained throughout the continuous flow to allow time for microbial activity. Enhanced removal of sulfate and metal was observed during this time.

Throughout the static experiment, reducing conditions were established at the bottom of the reactor as heterotrophic bacteria consumed the oxygen, as confirmed by the low DO (just 0.1 mg/L) and negative ORP. Establishment of anaerobic condition and release of available organic carbon created the environment for SRB growth. The gradual darkening of the reactor bed was presumably caused by the formation of metal sulfides, such as iron

**Fig. 3** Metal $_{\text{influent}}$: Metal $_{\text{effluent}}$ and Sulfate $_{\text{influent}}$: Sulfate $_{\text{effluent}}$ status during continuous flow



monosulfides (Smith and Melville 2004). Sulfate concentrations decreased as anaerobic microorganisms, including sulfate reducing bacteria, began to act and $H_2S$ was produced, using sulfate as a terminal electron acceptor coupled with metal sulfide formation. The bubbles formed were likely $H_2S$ and $CO_2$ from limestone dissolution. A slow decrease in conductivity indicates that dissolved ion concentrations decreased faster than nutrient release and suggests that the chemical and biological processes might have complemented each other.

During continuous flow, Fe (ef): Fe (in) ranged from 0.071 to 0.0029, with an average of 0.0039. This is better than the 0.32 reported by Dvorak et al. (1992) in his system (with a HRT of 5 days) and the 0.01–0.02 reported by Bhattacharya et al. (2007). Average sulfate (ef): sulfate (in) ranged from 0.2 to 0.982 with an average of 0.122. In contrast, Bhattacharya et al. (2007) reported an average of 1, with a minimum value of 0.4, and Dvorak et al. (1992) reported 0.826. Average values of 0.1175 and 0.0726 for Cu and Mn were measured, respectively. However, Mg concentrations increased, with Mg (ef): Mg (in) averaging 5.88 during continuous flow. Effluent water quality met Indian standards for effluent discharge in inland surface water and for irrigation (IS: 2490), except for copper (Table 5).

SRB need a pH range of 5–9 for optimal growth (Postgate 1984) and microbial sulfate reduction cannot be relied onto neutralize all of the acidity. That is why limestone is added to supplement alkalinity generation (Dvorak et al. 1992). In the jar test, it was observed that both limestone and SMC act to increase the pH and neutralize acidity. Both of these acted to maintain the pH and alkalinity during the static and the initial stages of the continuous experiment and facilitate sulfidogenesis. However, limestone produces alkalinity at a much higher rate and seemed to be exhausted (or armored) earlier than the SMC. It was apparent that at the later equilibrium of the continuous experiment, the acidity of the water was being neutralized by the combined action of the SMC, limestone, and the alkalinity produced by microbial metabolism.

Although SMC released much greater amounts of DOC initially, it generated DOC throughout the continuous flow experiment. Exhaustion of SMC occurred slowly, and the SMC was able to produce both alkalinity and organic carbon for an extended period. Long HRT would allow other heterotrophic bacteria to break down complex and fixated organic carbon such as cellulose released from the SMC. These simple products could be used by SRBs over a long period of time. However, most of the organic carbon released from the chemo-bioreactor was not used.

Jar test results showed that the maximum DOC available from the SMC was 107.88 mg/L, which is low, per Guo et al. (2001), and that 99% of the DOC came out with the effluent during the start of continuous flow. However, although just over 30 mg/L of DOC was released from the chemo-bioreactor during the continuous experiment, this was sufficient to support the sulfate reduction rate. It seems that the SMC slowly released biodegradable DOC, and microorganisms present in the chemo-bioreactor could use these low concentrations of biodegradable organic carbon. However, SMC characteristics differ and its compositional variability should be taken into account when assessing the applicability of any substrate (Jordan et al. 2008).

The efficiency of SMC utilization was relatively high in this chemo-bioreactor; the ratio of water treated to the weight of SMC used was 78.98. Dvorak et al. (1992) reported utilization values of 19.4 and 33.91 in their Palmerton system (16 weeks) and 238.23 for their Pittsburgh system (50 weeks). The DOC curve (Fig. 3) shows that our continuous flow experiment could have been successfully extended for an even longer period, demonstrating the value of the long HRT. Alternatively, continuous addition of degradable organic carbon could be used as a long term solution to boost the treatment efficiency.

Decomposition and dissolution of some of the SMC components may explain the release of magnesium into the effluent water throughout the experiment. SMC contains 18 g $kg^{-1}$ of Mg, on average (based on 63 SMC samples, Jordan et al. 2008). It can be concluded that the amount of Mg ion being leached from the SMC greatly exceeded what was being removed within the system.

Electric conductivity represents the total ionic strength of the water. Conductivity decreased slightly during both the static and continuous experiments, indicating that more ions were precipitating or being offgassed than were being released by the SMC and limestone.

Metal removal is inversely proportional to redox potential except in the case of Al (Cheong et al. 1998). During both continuous and static experiments, redox potential was seen to decrease in tank *D*. Increased pH and decreased redox potential point toward microbial sulfate reduction (Jong and Parry 2006). As the pH of the reactor was maintained at a value $> 5.5$ and a decrease in redox potential was observed from the last stage of the static experiment to the end of the continuous experiment, it is clear that after establishment of anaerobic conditions, SRB colonized the reactor and reduced sulfate to sulfide.

A preliminary cost estimate was carried out for the total water treated and the results are equivalent to a capital cost of Rs. 50,000 (USD 1065) and an annual operating cost of Rs. 314 (USD 6.69), based on a market price of limestone of Rs. 1,245 (USD 26.5) per MT and a price of Rs. 15,000 (USD 319) per MT for spent mushroom compost, without taking into account personnel costs. This cost is less than reported in similar studies by Foucher et al. (2001) and Tsukamoto et al. (2004).

## Conclusions

From the experiment, we can conclude that:

- For all metals except magnesium, metal removal rates were greater during continuous flow. Effluent metal and sulfate concentrations were well below permissible effluent limits.
- The overall activity was the combined effect of chemical and biological processes.
- Organic carbon release from the SMC greatly decreased after 8 days, yet remained sufficiently high to maintain the desired level of bacterial activity. Longer HRT and experiment durations can be tried to optimize SMC utilization. Since process slow-down was observed, it can be presumed that an external supply of DOC would improve the process rate.
- SMC use introduces Mg into the water, but Mg is not considered to be a water contaminant in India.

This study shows that a gravity-fed chemo-bioreactor containing limestone and SMC can be a simple and cost effective technology to treat AMD, targeting metals and sulfate at the same time. The long water column helps to maintain anaerobic conditions and provides a higher surface area to water volume ratio, favorable to bacterial growth. Released unutilized DOC from the chemo-bioreactor could lead to another environmental problem, but allowing the effluent to flow through a microbial bed capable of degrading organic carbon may remove DOC and any metals remaining in the effluent.

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## References

Bhattacharya J, Yim GJ, Ji SW, Lee HS, Cheong YW (2007) SAPS influent and effluent geochemical parameters related to AMD pollution control: a case study. In: Proceedings of the IMWA annual meeting, Cagliari, pp 403–407

Bhattacharya J, Ji SW, Lee HS, Cheong YW, Yim GJ, Min JS, Choi YS (2008) Treatment of acidic coal mine drainage: design and operational challenges of successive alkalinity producing systems. Mine Water Environ 27:12–19

Chang IS, Shin PK, Kim BH (2000) Biological treatment of acid mine drainage under sulphate-reducing conditions with solid waste materials as substrate. Water Res 34:1269–1277

Cheong YW, Min JS, Kwon KS (1998) Metal removal efficiencies of substrates for treating acid mine drainage of the Dalsung mine, South Korea. J Geochem Explor 64:147–152

Clesceri LS, Greenberg AE, Eaton AD (1998) Standard methods for the examination of water and wastewater, 20th edn., method 3111. American Public Health Assoc, Washington, DC

Csuros M (1997) Environmental sampling and analysis: lab manual. CRC Press, Boca Raton, 307 pp

Das BK, Roy A, Koschorreck M, Mandal SM, Wendt-Potthoff K, Bhattacharya J (2009) Occurrence and role of algae and fungi in acid mine drainage environment with special reference to metals and sulfate immobilization. Water Res 43:883–894

Dvorak DH, Hedin RS, Edenborn HM, Mclntire PE (1992) Treatment of metal-contaminated water using bacterial sulfate reduction: result from pilot scale reactors. Biotechnol Bioeng 40:609–616

ERMITE Consortium (Younger P, Wolkersdorfer C, eds) (2004) Mining impacts on the fresh water environment: technical and managerial guidelines for catchment scale management. Mine Water Environ 23(Suppl 1):S2–S80

Foucher S, Battaglia-Brunet F, Ignatiadis I, Morin D (2001) Treatment by sulfate-reducing bacteria of Chessy acid-mine drainage and metals recovery. Chem Eng Sci 56:1639–1645

Guo M, Chorover J, Rosario R, Fox RH (2001) Leachate chemistry of field—weathered spent mushroom substrate. J Environ Qual 30: 1699–1709

Hao OJ (2003) Sulfate reducing bacteria. In: Mara DD, Horan NJ (eds) Handbook of water and wastewater microbiology, chap. 28. Academic Press, London, pp 459–469

Johnson DB, Hallberg KB (2005) Acid mine drainage remediation options: a review. Science Total Environ 338:3–14

Jong T, Parry D (2006) Microbial sulfate reduction under sequentially acidic condition in an upflow anaerobic packed bed bioreactor. Water Res 40:2561–2571

Jordan SN, Mullen GJ, Murphy MC (2008) Composition variability of spent mushroom compost in Ireland. Bioresource Technol 99:411–418

Kaksonen AH, Franzmann PD, Puhakka JA (2003) Performance and ethanol oxidation kinetics of a sulfate-reducing fluidized-bed reactor treating acidic metal-containing wastewater. Biodegradation 14:207–217

Khan E, Babcock Jr RW, Stenstrom MK, Suffet IH (1996) Biodegradable organic carbon for characterizing reclaimed and treated wastewaters. In: Proceedings of the 69th annual water environment federation conference and Expo, Dallas, TX, vol 4, pp 617–628

Neculita CM, Zagury GJ, Bussiere B (2007) Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: critical review and research needs. J Environ Qual 36:1–16

PIRAMID (passive in situ remediation of acidic mine/industrial drainage) Consortium (2003) Engineering guidelines for the passive remediation of acidic and/or metalliferous mine drainage and similar wastewaters. European Commission 5th framework RTD project # EVK1-CT-1999-000021, Univ of Newcastle Upon Tyne, UK, 151 pp

Postgate JR (1984) The sulphate-reducing bacteria, 2nd edn. Cambridge Univ Press, Cambridge, 159 pp

Smith J, Melville MD (2004) Iron monosulfide formation and oxidation in drain–bottom sediments of an acid sulfate soil environment. Appl Geochem 19:1837–1853

Stark LR, Wenerick WR, Williams FM, Stevens SE Jr, Wuest PJ (1994) Restoring the capacity of spent mushroom compost to treat coal mine drainage by reducing the inflow rate: a microcosm experiment. Water, Air, Soil Poll 75:405–420

Tsukamoto TK, Killion HA, Miller GC (2004) Column experiments for microbiological treatment of acid mine drainage: low-temperature, low-pH and matrix investigations. Water Res 38: 1405–1418

Widdel F (1988) Microbiology and ecology of sulfate and sulfur-reducing bacteria. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. Wiley, New York City, pp 469–586

Zagury GJ, Neculita CM, Bussiere B (2007) Passive treatment of acid mine drainage in bioreactors: short review, applications, and research needs. In: Proceedings of the 60th Canadian geotechnical conference and 8th joint CGS/IAH-CNC specialty groundwater conference, Ottawa, Canada, pp 1439–1446