

# Treatment of synthetic acid mine drainage using rice wine waste as a carbon source

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**Abstract** Spent mushroom compost (SMC) is commonly used as a carbon source for passive treatment systems in South Korea; however, it has some drawbacks, such as sulfate release from itself. Consequently, investigations to identify effective substitutes for SMC are necessary. In this study, batch experiments were conducted for 27 days to evaluate the efficiency of rice wine waste (RWW) for reducing sulfate and removing dissolved metals within synthetic acid mine drainage (AMD). The results showed that RWW could be more suitable than SMC, which even released sulfate in the early stage of the experiment, for sulfate reduction by sulfate-reducing bacteria. Both materials produced similar results with respect to the removal of dissolved metals, such as Fe and Al. Furthermore, a mixture of SMC and RWW showed the greatest efficiency in sulfate removal. Overall, both RWW and the mixed carbon source showed comparable performance to SMC, which indicated that RWW had a great potential for use as a carbon source for AMD treatment.

**Keywords** Acid mine drainage · Spent mushroom compost · Rice wine waste · Sulfate reduction rates

## Introduction

Prolonged exposure of reactive sulfide minerals such as pyrite to oxygen and water in the absence of sufficient

neutralizing minerals generates acid mine drainage (AMD). AMD is characterized by low pH and high concentrations of sulfates and dissolved metals (Neculita and Zagury 2008) and, consequently, poses substantial risks to the environment. The CIPB (Coal Industry Promotion Board) of Korea reported that approximately 153 km of local streams were contaminated by 48,000 tons/day of AMD spilled from 152 nearby coal mines (CIPB 2000). In Korea, operations at 300 coal mines and more than 900 metal mines have been interrupted or stopped since 1989 (Cheong et al. 1998; Ji et al. 2008). Taking advantage of the low installation cost and the relative ease of maintenance at abandoned mine sites in remote locations, the passive treatment system to control AMD is widely adapted; a total of 35 passive treatment systems were installed in 29 closed or abandoned mines from 1996 to 2002 (Ji et al. 2008).

One of the main mechanisms behind the passive treatment system is application of sulfate-reducing bacteria (SRB) that can reduce sulfate to hydrogen sulfide while oxidizing organic compounds or the carbon source. Much of the hydrogen sulfide generated during this process reacts with dissolved metals in AMD to produce metal sulfides. SRB use sulfate as an electron acceptor and the carbon source as an electron donor (Pfenning et al. 1981). AMD is generally deficient in carbon; external addition is required to induce SRB activity (Costa et al. 2009). Accordingly, selection of a suitable carbon source is important and has a direct effect on the efficiency of passive treatment systems, especially successive alkalinity producing systems (SAPS).

Agricultural wastes are advantageous in terms of cost and recycling and have primarily been considered as carbon sources. To date, diverse agriculture wastes such as animal manure, vegetative compost, wood chips, sawdust, sugar and spent mushroom compost (SMC) have been

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applied as potential carbon sources (Amos and Younger 2003; Gibert et al. 2004; Costa and Duarte 2005; Zagury et al. 2006; Neculita and Zagury 2008). In addition, wastes from the food industry including molasses (Teclu et al. 2009) and whey (Christensen et al. 1996) were investigated as electron donors to sustain sulfate reduction during the treatment of AMD. In recent studies, mixtures of carbon sources have been found to yield higher efficiency for AMD treatment than single carbon sources (Gibert et al. 2004; Zagury et al. 2006; Neculita and Zagury 2008; Neculita et al. 2011).

In Korea, passive treatment systems in operation are usually filled with SMC, largely used as a unique carbon source in the first-generation bioreactors (URS 2003). However, recent laboratory tests have shown that SMC exerts poor performance for sulfate removal (An 2010; Robinson-Lora and Brennan 2010). Moreover, some studies showed significant increases in sulfate concentration in the initial stages of the process with SMC (An 2010; Neculita et al. 2011). Accordingly, investigations of alternative carbon sources to replace SMC are needed.

Rice wine waste (RWW) which mainly consists of rice or wheat is a fermented organic material generated as a by-product during rice wine production. It contains sugar, protein and starch (Kim 2011). Kim (2011) reported that RWW had a potential to be used as a carbon source to feed probiotic bacteria, indicating that it could be used as a carbon source for SRB during treatment of AMD. In addition, since production of rice wine is widespread in South Korea RWW is available at free of cost. According to Costa et al. (2009), the ethanol as a component of wine wastes can be a carbon source for SRB. Although the main ingredient of RWW used in this study is different from that of wine waste used by Costa et al. (2009), their findings suggest that RWW could find its application in biological treatment of acid mine drainage.

Therefore, this study was conducted to evaluate the potential of RWW to reduce sulfate and to remove dissolved metals from synthetic AMD, comparing to that of SMC.

## Materials and methods

### Materials

SMC and RWW were used as the carbon source for SRB. These materials were obtained from a local farm located in Hongseong and a Brewery in Chuncheon, South Korea, respectively. Both SMC and RWW were kept refrigerated at 4° C until use. After mixing with deionized water, at the solid to liquid ratio of 1:1 (ASTM 1995), pH of the carbon sources was measured using a pH meter (HACH, HQ-

**Table 1** Composition of synthetic AMD fed into batch-scale reactors

Component	Values
pH	3.0
ORP (mV)	202.6
Fe (mg L <sup>-1</sup> )	192.2
Al (mg L <sup>-1</sup> )	104.1
Sulfate (mg L <sup>-1</sup> )	1,140

40D). The contents of carbon and nitrogen were measured using an elemental analyzer (Flash EA, CE Instruments). The pH of SMC was neutral (7.62), while that of RWW was acidic (4.83). The carbon and nitrogen content of SMC were 22.92 and 2.43 % (C/N = 9.4), while those of RWW were 47.27 and 7.73 % (C/N = 6.1), respectively.

The limestone, obtained from an open-pit mine in Gangwon-do, was crushed to pass through the 100-mesh sieve to be used as neutralizing and buffer material. XRF (Epsilon 5) showed that the main composition of this limestone was CaO (50 %) and MgO (1 %).

The SRB was obtained from a passive treatment system in South Korea that was suspected to contain SRB based on its black color and H<sub>2</sub>S odor. Until use as the inoculum, the bacterial community was grown and maintained in Postgate B medium (Postgate 1984).

Synthetic AMD (Table 1) was prepared by mixing deionized water with reagent grade chemicals (FeS·O<sub>4</sub>·7H<sub>2</sub>O for Fe and Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>·14-18H<sub>2</sub>O for Al). Fe and Al are the metals commonly observed in AMD from abandoned coal mines in Korea. Ji et al. (2008) investigated the operating status of passive treatment systems in South Korea. They analyzed Fe and Al concentrations in water samples collected from 27 coal mines and 2 metal mines. To more realistically simulate aerobic conditions in the field, synthetic AMD was not bubbled with N<sub>2</sub>. Also, 1 N sulfuric acid was used to adjust the pH and add sulfate to synthetic AMD.

### Setup of batch experiments

The capacity of SMC and RWW to promote sulfate reduction and metal removal was assessed as follows. For each carbon source, a set of 18 glass bottles of 120 mL was prepared. First, reactive mixtures were poured into the bottles at the amounts shown in Table 2. Each reactor was then filled with about 110 mL of AMD and tightly sealed. The sealed bottles were placed in a water bath at 23° C for 27 days. Duplicate reactors were prepared for each reactive mixture. The reactors R1 and R2 were prepared to compare the efficiency of SMC with that of RWW for reducing sulfate and dissolved metals, whereas the reactors R4 and R5 without SRB medium were used as controls. The

**Table 2** Composition of reactive mixtures evaluated in batch experiments

	Reactive mixture (g)				
	R1	R2	R3	R4	R5
Spent mushroom compost	3	–	1.5	3	–
Rice wine wastes	–	3	1.5	–	3
Crushed limestone	3				
SRB medium (mL)	5 mL	5 mL	5 mL	–	–

reactor R3 contained both SMC and RWW, to evaluate the performance of the combined use.

**Sampling and analysis**

Sampling was done every 3 days for 27 days. At every sampling event, two reactor bottles from each set were randomly picked and disposed of after measurements. The pH and the oxidation–reduction potential (ORP) of unfiltered samples were immediately measured (HQ-40D, HACH), after that the samples were filtered through a 0.45 μm membrane filter using a hand pump. To determine the concentration of cations in reactors, half of the filtered liquid was then immediately acidified to pH < 2.0 by adding HNO<sub>3</sub>. The other half was not acidified, and used for sulfate analysis. All samples were stored in a refrigerator at 4° C until analysis. Analysis of dissolved cations (Fe and Al) was performed using an inductively coupled plasma atomic emission spectrophotometer (ICP-AES; OPTIMA 7300 DV, PerkinElmer). Sulfate was determined using a spectrophotometer (DR 2800, HACH), following the sulfaVer 4 method.

**Results and discussion**

**pH and ORP**

The addition of crushed limestone caused a rapid increase in pH in all reactors during the initial period (Fig. 1a). The pH of R1–R5 increased sharply from 3 to 6.85, 6.32, 6.54, 6.71 and 6.53 in 3 days, respectively. And then, all reactors showed a gradual rise in pH, reaching values of 7.75, 6.86, 7.54, 7.4 and 7.4, respectively, by the end of the experiment. Low pH generally inhibits SRB activity, leading to poor sulfate reduction. Wu et al. (2010) reported that, as the initial pH increased from 3 to 7, the sulfate removal rate of rice straw increased from 13.93 to 21.77 mg L<sup>-1</sup> day<sup>-1</sup>. SRB requires an environmental pH between 5 and 8 for efficient metabolism (Willow and Cohen 2003). Therefore, a favorable environment for SRB in terms of pH seemed to be formed in R1–R3.

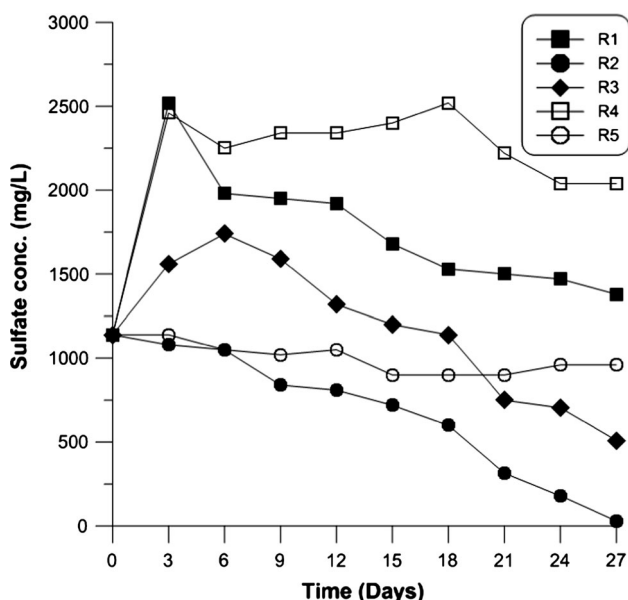
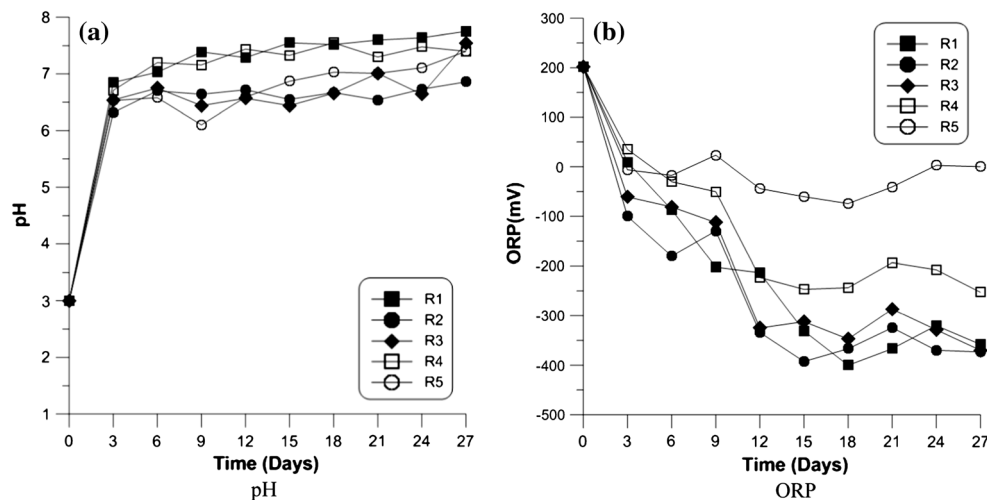
The development of reducing conditions for SRB was associated with a decrease in ORP. The ORP showed continuous decreases with time except for R5 (Fig. 1b). After 12–15 days, the ORP levels were below –300 mV, and these values were maintained until the end of the experiment in R1, R2, and also R3. In contrast, R4 showed ORP level of between –200 and –300 mV, while R5, which was used as the control of RWW, showed the lowest value (–74.3 mV) at day 18 and was about 0 mV at the end of the experiment. The required ORP for SRB activity was below –100 mV (Postgate 1984). Prasad et al. (1999) also reported that an ORP of –300 mV was suitable. Therefore, favorable conditions for SRB in terms of ORP were established in R1–R3.

**Change in sulfate concentrations**

Figure 2 shows the changes in sulfate concentrations within all reactors during the experiment. Sulfate concentrations increased in the beginning of the experiment in reactors with SMC. In R1 and R4 (without SRB), the sulfate increased from 1,140 to 2,520 and 2,460 mg L<sup>-1</sup> in 3 days, respectively. The sulfate then continuously decreased in R1, while only a slight drop was observed in R4 by the end of the experiment. The average amount of sulfate released from SMC was 1,350 ± 15 mg L<sup>-1</sup> based on the sulfate in R1 and R4. In contrast to SMC, no increase in sulfate was observed in R2 or R5 (without SRB) containing RWW. The sulfate of R2 continued to drop after day 6, while that of R5 remained steady throughout the experiment. In R3, which contained a mixture of SMC and RWW, an increase in sulfate was also recorded. However, the increase in sulfate from the mixture was lower than that from SMC. The sulfate in R3 reached its peak value at day 6 and then continued to drop.

The increase of sulfate in R1, R3 and R4 with SMC in the beginning of the experiment could have been caused by SMC itself. Similar trends have been reported by other researchers (Ji and Kim 2008; Robinson-Lora and Brennan 2010; Neculita et al. 2011; Song et al. 2012). Potential sources of the sulfate derived from SMC could include cow manure or gypsum (CaSO<sub>4</sub>·2H<sub>2</sub>O), which are the major ingredients of SMC (Bhattacharya et al. 2008; Song et al. 2012). Neculita et al. (2011) reported that cow manure released high concentrations of sulfate (22.5 g SO<sub>4</sub><sup>-2</sup> kg<sup>-1</sup> of cow manure). The dissolution of the gypsum can be enhanced through an ion-pair mechanism and a common ion effect (Truesdale 2011). In addition, rice straw, which can be used as the main substrate for mushroom cultivation (Food and Fertilizer Technology Center (FFTC) 2013), could also cause sulfate to be released. Indeed, rice straw was reported to release sulfate for 5 days in a column test by Wu et al. (2010).

**Fig. 1** Variations in a pH and b ORP in each reactor with time



**Fig. 2** Variations in sulfate concentrations in each reactor with time

The released sulfate could adversely impact the sulfate removal ratio in passive treatment systems. In a study by Cocos et al. (2002), the use of the poultry manure as a carbon source resulted in continuous release of sulfate from the carbon source itself and adversely affected SRB activity and bioreactor efficiency. In this study, the sulfate removal ratios based on the initial sulfate concentrations of AMD ( $1,140 \text{ mg L}^{-1}$ ) were  $-21 \%$  (SMC),  $97 \%$  (RWW) and  $55 \%$  (mixture of SMC and RWW) for 27 days. Sulfate reduction by SRB occurred in R1–R3 after 6 days, which was supported by the presence of black precipitates. Nevertheless, the estimated sulfate removal ratio of R1 was negative, indicating that the sulfate concentration at day 27 was higher than that of the initial AMD due to the release of sulfate. Because of the sulfate released from SMC, the

sulfate removal ratio based on the initial sulfate concentrations of AMD was not appropriate to evaluate the efficiency of sulfate removal. Accordingly, it may be appropriate to estimate the sulfate removal ratios and sulfate reduction rates based on changes in sulfate concentrations between days 6 and 27. The estimated sulfate removal ratios were  $30 \%$  (R1),  $97 \%$  (R2) and  $70 \%$  (R3) based on this period.

Sulfate reduction rates were estimated using linear least squares regression analysis while disregarding early-time data because they may have been affected by acclimation periods and adsorption of sulfate onto ferric (oxy)hydroxides, as well as late-time data because they may have been sulfate limited (Waybrant et al. 1998; Cocos et al. 2002). R1, containing SMC, had the lowest sulfate reduction rate of  $-31.8 \pm 3.5 \text{ mg L}^{-1} \text{ day}^{-1}$  ( $n = 8$ ), whereas R3, which contained a mixture of carbon sources, showed the greatest reduction rate of  $-58.8 \pm 3.6 \text{ mg L}^{-1} \text{ day}^{-1}$  ( $n = 8$ ). The sulfate reduction rate of RWW was  $-46.1 \pm 4.6 \text{ mg L}^{-1} \text{ day}^{-1}$  ( $n = 7$ ) (Table 3). In terms of sulfate removal, RWW was more efficient than SMC, while no release of sulfate from RWW was observed. Ji et al. (2008) reported that one of the major shortcomings of field-scale bioreactors employing mushroom compost as the sole substrate was excessive release of sulfate from the substrates causing various problems during the long-term operation of bioreactors. Thus, RWW seems to be an alternative carbon source for SMC or an ingredient of mixed carbon sources for SRB in this regard. Although R3, which contained a mixture of SMC and RWW, had a lower sulfate removal ratio than that of RWW, it showed the greatest sulfate removal (Table 3). Moreover, R3 showed the highest sulfate reduction rates. According to the results of this study, the removal efficiency of sulfate occurred in the order of the R3 (RWW and SMC) > R2 (RWW) > R1 (SMC). These findings are concurrent with those of

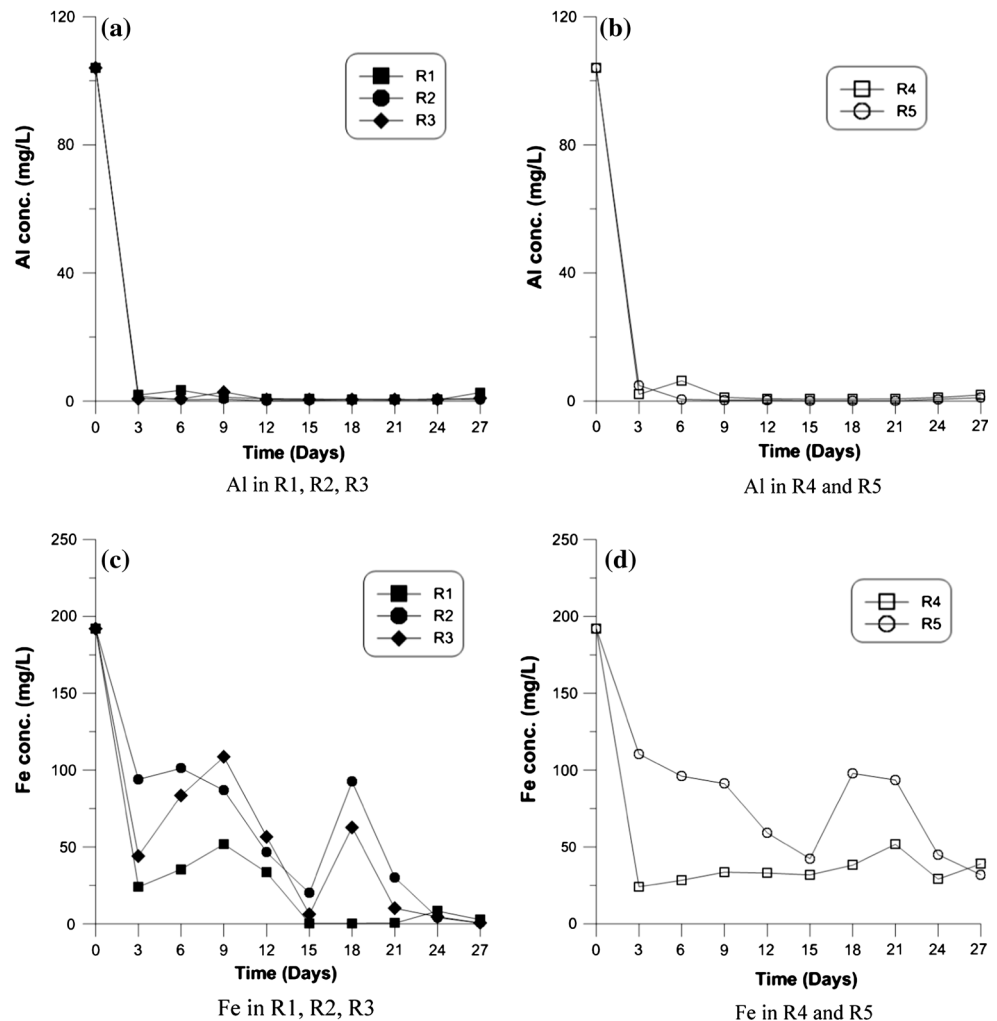
**Table 3** Summary of changes in sulfate

Reactor	Sulfate concentration (mg L <sup>-1</sup> )			Sulfate removal ratio (%)	Amount of sulfate removed (mg L <sup>-1</sup> )	Sulfate reduction rate <sup>a</sup> (mg L <sup>-1</sup> day <sup>-1</sup> )	Sample size <sup>b</sup>	R <sup>2</sup>
	Initial	At day 6	At day 27					
R1	1,140	1,980	1,380	30	600	-31.8 ± 3.5	8	0.93
R2	1,140	1,050	30	97	1,020	-46.1 ± 4.6	7	0.95
R3	1,140	1,740	510	70	1,230	-58.8 ± 3.6	8	0.98

<sup>a</sup> Regression coefficient ± standard error

<sup>b</sup> Sample size for estimating sulfate reduction rates

**Fig. 3** Variations in **a, b** Al and **c, d** Fe concentrations with time. **a, c** Correspond to reactors with SRB and **b, d** to the controls. **a** Al in R1, R2, R3, **b** Al in R4 and R5, **c** Fe in R1, R2, R3, **d** Fe in R4 and R5



previous studies (Cheong et al. 1998; Waybrant et al. 1998; Cocos et al. 2002; Neculita and Zagury 2008; Neculita et al. 2011).

**Change in metal concentrations**

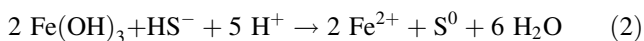
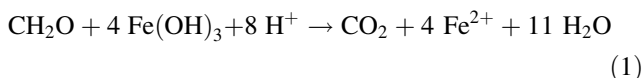
The results of previous studies generally indicate that early decreases in metal concentration can be attributed to adsorption or precipitation of (oxy)hydroxides and carbonates (Neculita et al. 2007). Once sulfate-reducing

conditions develop, sulfide precipitation becomes the main metal removal mechanism (Machemer and Wildeman 1992). In this study, Al was almost completely removed from all reactors (>97 %) (Fig. 3a, b). Specifically, the concentration of Al dropped from 104 to 1–4 mg L<sup>-1</sup> in all reactors after day 3, then steadily decreased for the remainder of the experiment. Because sulfate reduction processes by SRB did not occur during this period, the removal of this metal was likely due to precipitation in the hydroxides (Robinson-Lora and Brennan 2010). It was

assumed that Al decreased to about  $1 \text{ mg L}^{-1}$  in R4 and R5, which did not have SRB. Al concentrations are governed solely by pH, with high values remaining at pH 3.5 or less, but values lower than  $1 \text{ mg L}^{-1}$  being observed at pH values between 5 and 8 (Hedin et al. 1994).

Similar to Al, Fe was also rapidly removed from all reactors in 3 days. However, reactors containing SMC showed lower concentrations than those containing RWW. During this period, Fe concentrations decreased by an average of 87 % in reactors with SMC and 47 % in those with RWW. In addition, 77 % of Fe was removed from R3 (Fig. 3c). Except for R4, a decrease was observed in R1–R3 during the experiment. Fe in R4 reached the lowest concentration at day 3, after which a steady increase was observed (Fig. 3d). After 27 days, the Fe removal ratios were almost 100 % in R1–R3, while R4–R5 showed removal ratios of 80 and 83 %. Overall, SMC and RWW showed similar Fe and Al removal efficiency.

During the experimental period, Fe was partially released back to solution (Fig. 3c, d). This phenomenon was similar to that reported by Robinson-Lora and Brennan (2010), who used chitin and SMC as carbon sources in batch experiments. This release of Fe was most likely caused by the reduction of precipitated Fe(III) (hydr)oxides (Robinson-Lora and Brennan 2010). In addition, iron-reducing bacteria (IRB) may lead to the release of Fe into solution (1). IRB are capable of coupling the reduction of solid Fe(III) (hydr)oxide phases to Fe(II), while oxidizing  $\text{H}_2$  or organic carbon to  $\text{H}_2\text{O}$  or  $\text{CO}_2$ . The biogenic sulfide generated by SRB may also react with Fe(III) (hydr)oxide phases, producing Fe(II) (2) (Senko et al. 2008; Bertel 2011).



The abnormal release of Fe observed for R2, R3 and R5 on day 18 might be an analytical error, since the continuous decrease of sulfate caused by SRB activity in R2 and R3 should reflect a continuous decrease of dissolved Fe. In addition, the release of Fe from the carbon source was not likely due to the near neutral pH of the solution.

The adsorption of dissolved metals onto organic sites in the substrate material is an important process during startup of passive bioreactors (Machemer and Wildeman 1992). Thus, differences in Fe concentration on day 3 (Fig. 3d) between carbon sources may be related to the characteristics of adsorption by SMC and RWW. Generally, the adsorption performance varies with the characteristics of a material and the pH of solution (Machemer and Wildeman 1992). However, additional research is needed to accurately reveal this phenomenon.

## Conclusions

The efficiency of RWW as a carbon source for treating synthetic acid mine drainage, compared with that of SMC, was evaluated in a batch experiment for 27 days. The test results showed that RWW could be more efficient for promoting sulfate reduction than SMC. Also, as previously shown in other studies, a higher sulfate reduction rate was observed with a mixture of carbon sources (R3), compared with the cases of sole carbon sources. A considerable sulfate release in reactors with SMC was observed in the beginning of the experiment, while no such release was observed in reactors only containing RWW. Although SMC is one of the common carbon sources for SRB in passive treatment systems, this release can adversely influence the efficiency of the passive treatment systems during the initial phase of operation. Similar results for Fe and Al removal were observed in R1–R3; however, SMC showed lower Fe concentrations in the early stage of the experiment, which was likely due to differences in the characteristics of carbon sources. Additional studies are currently underway to elucidate the mechanisms responsible for these differences. Overall, the results of this study indicate that RWW can be used as an alternative carbon source for SMC or an ingredient of mixed carbon sources in treatment systems that utilize SRB.

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