Minerals Engineering 69 (2014) 81–90

Contents lists available at ScienceDirect

Minerals Engineering

journal homepage: www.elsevier.com/locate/mineng

Organic wastes as carbon sources to promote sulfate reducing bacterial activity for biological remediation of acid mine drainage

Mingliang Zhang *, Haixia Wang

School of Resources and Environment, University of Jinan, Jinan 250022, China

ARTICLE INFO

Article history:
Received 23 February 2014
Accepted 16 July 2014

Keywords:
Carbon source
Sulfate reduction
Acid mine drainage
Heavy metal

ABSTRACT

Chicken manure, dairy manure and sawdust were evaluated as carbon sources in promoting sulfate reduction, and the mechanism of heavy metals removal in sulfidogenic bioreactor was revealed. The sulfate reduction reached 79.04% for chicken manure, 64.78% for dairy manure, and 50.27% for sawdust on 35th day, which showed that chicken manure could promote sulfate reducing bacteria (SRB) activity, followed by dairy manure and sawdust. In batch experiment, although chicken and dairy manure bioreactors showed sulfidogenic activity, it demonstrated less than 5% contribution from sulfide precipitation and over 95% from other removal mechanisms (sorption to manure particles and hydroxides precipitation, etc.). Column bioreactor showed satisfactory performance in biological remediation of acid mine drainage, evidenced by effluent Eh and pH, high removal efficiencies of sulfate and metals, and a considerable SRB counts. SEM–EDS analysis of the formed precipitate showed metal sulfides were formed. The results indicated that organic waste played an important role in sulfidogenic activity, which could not only provide reducing condition and carbon source for sulfate reduction process, but also reduce the adverse effect of heavy metal and strong acidity on SRB activity owning to metals sorption and acidity buffer of organic waste.

1. Introduction

Acid mine drainage (AMD) is one of the most serious environmental threat to the mining area because of its strong acidity and high concentrations of sulfate, dissolved metals and other toxic elements. Many methods have been widely applied for AMD treatment, among which microbial treatment by sulfate reducing bacteria (SRB) has been considered as one promising biological process (Kieu et al., 2011). The biological process can generate alkalinity by reducing sulfate to sulfide when supplied with liable organic carbon under anaerobic conditions, and cause metal precipitation as sulfides in low redox condition. It is clear that effectiveness of the biological treatment can be affected by organic carbon supply.

The concentration of dissolved organic carbon of AMD is usually lower than 10 mg/L (Kolmert and Johnson, 2001), thus addition of a suitable carbon source for SRB is necessary to promote bacterial sulfate reduction process. Considering the operation cost, many organic wastes such as poultry manure, wood chip, sawdust, leaf, wine and cheese industry waste, rice straw and other organic materials have been applied as carbon sources of SRB in lab or field-scale AMD treatment (Waybrant et al., 1998; Chang et al., 2000; Benner et al., 2002; Cocos et al., 2002; Gibert et al., 2004; Zagury et al., 2006; Pagnanelli et al., 2009; McCullough and Lund, 2011; Neculita et al., 2011; Battaglia-Brunet et al., 2012).

At present, most of the literature is more concerned about the overall performance of microbial remediation, omitting the identification of metal removal mechanisms besides sulfide precipitation. Many studies have concluded that many organic wastes usually had good sorption capacity for heavy metals. However, the significant contribution of sorption onto organic materials was not isolated from the whole removal efficiency in SRB treatment system when organic materials were used as carbon source for SRB. It is necessary to distinguish between the metal removal mechanisms in order to avoid an overestimation of the SRB's performance. Otherwise, it may lead to misleading results in the field design.

In this study, three organic wastes (chicken manure, dairy manure and sawdust) were assessed as carbon sources to promote sulfate reducing activity. The role of organic waste playing in the SRB treatment system and the metals removal mechanisms were studied by batch and column bioreactors in order to avoid overestimating the sulfidogenic capacity of the SRB treatment system.

* Corresponding author. Tel.: +86 531 82769233.
E-mail addresses: mlzhangsd@126.com, minewastestudy@gmail.com (M. Zhang).

http://dx.doi.org/10.1016/j.mineng.2014.07.010
0892-6875/

2. Materials and methods

2.1. Growth medium and isolation of sulfate-reducing bacteria

The SRB culture was isolated from the urban soil (10–15 cm depth) in Jinan city, Shandong, China. The growth medium used for SRB growth was modified Postgate C medium with the following compositions (Postgate, 1984): KH_2PO4 0.5 g/L; NH_4Cl 1 g/L; Na_2SO_4 4.5 g/L; $CaCl_2{\cdot}6H_2O$ 0.06 g/L; $MgSO_4{\cdot}7H_2O$ 0.06 g/L; sodium lactate 6 g/L; yeast extract 1 g/L; $FeSO_4{\cdot}7H_2O$ 0.5 g/L; sodium citrate$\cdot 6H_2O$ 0.3 g/L. The pH of the medium was not adjusted at about 5.5.

The effect of heavy metals on SRB activity was studied by the following batch test. Modified Postgate C medium described above was used as control medium for SRB's tolerance toward different concentrations of heavy metal ions. The experiments were conducted in anaerobic conditions at 30 °C in a biochemical incubator for 14 days, using 150 mL Erlenmeyer flask with stopper containing 100 mL of growth medium with 5 mL of SRB inoculum of log phase cells (pH around 5.5, not adjusted). The pH, Eh and sulfate concentration of solution were measured to study the tolerance of SRB toward heavy metals. The effect of pH and temperature on SRB activity was also studied by similar batch tests.

2.2. Organic substrate and synthetic AMD

Chicken manure, dairy manure, and sawdust used as potential carbon sources of SRB in this study were collected from one farm in Linqu County, Shandong Province, China. The samples were dried at 75 °C and passed through 2 mm sieve for the following experiments.

Dissolved organic carbon (DOC) and total bound nitrogen (TN_b) was analyzed after stirring 10 g of a particular sample with 100 ml of deionised water (shaking for 24 h at room temperature). Afterwards the mixture were centrifuged and filtered (0.45 um) and then determined by TOC analyzer (Vario Toc cube, Elementar). COD was measured by Spectroquant Pharo300 (Merck). Easily available substances (EAS) is usually considered as the estimation of biodegradable fraction of subtract, mainly including soluble sugars, amino acids, proteins and some other biodegradable portions. The analytical method of EAS content was described by Prasad et al. (1999). Lignin content is very important indicator to affect the biodegradation of subtracts, which was determined by the successive extractions method described by Rahn et al. (1999) and Gibert et al. (2004). The pH of organic waste samples was measured by pH meter (PHS-3C, Leici) with 1:5 (w/v) solid/water suspensions after shaking 24 h.

The components of synthetic acid mine drainage was listed in Table 1. The metal solution was prepared by using sulfate salts (analytical reagent). The pH was maintained at 3.0–3.5.

2.3. Effect of carbon source on sulfate reduction

The ability of the three organic substrates (chicken manure, dairy manure, and sawdust) as potential carbon sources to promote microbial sulfate reduction was evaluated in batch experiment at 30 °C in a biochemical incubator for 35 days in a series of 150 mL anaerobic plastics bottles. 10 g of each organic substrate as sole carbon source was added into 100 mL of modified Postgate C medium (without lactate, yeast extract and sodium citrate, initial pH 5.0–5.5), and 5 mL of SRB inoculum of log phase cells was added. The effect of carbon sources to promote SRB growth was assessed by measuring sulfate reduction levels. The pH and Eh of samples were measured, and the formation of black precipitation (FeS) and H_2S odour was also monitored.

Table 1
The components of synthetic acid mine drainage.

Component	Concentration (mg/L)	Source
Fe^{2+}	586.4–598.8	$FeSO_4{\cdot}7H_2O$
Mn^{2+}	29.3–29.6	$MnSO_4{\cdot}H_2O$
Cu^{2+}	28.6–30.0	$CuSO_4{\cdot}5H_2O$
Zn^{2+}	49.3–50.4	$ZnSO_4{\cdot}7H_2O$
Cd^{2+}	11.2–12.2	$3CdSsO_4{\cdot}8H_2O$
Ni^{2+}	15.2–16.0	$NiSO_4{\cdot}6H_2O$
SO_4^{2-}	2570–2830	–
pH	3.0–3.5	–

In order to determine the effect of solid–liquid ration (organic substrate mass/AMD volume) on SRB treatment, the different organic substrate mass (1, 3, 5, 7, 10 g chicken manure) was added into 100 mL of modified Postgate C medium (without lactate, yeast extract and sodium citrate, initial pH 5.0–5.5), and 5 mL of SRB inoculum of log phase cells was added. The test was conducted for 20 days at 30 °C in a biochemical incubator.

2.4. Batch experiments for AMD treatment

The batch treatment experiment was conducted through 150 mL anaerobic plastics bottles at 30 °C in a biochemical incubator. 10 g of each organic substrate was added into 100 mL of synthetic AMD solution, and then 5 mL of SRB inoculum of log phase cells was added. The solution pH of different treatments was adjusted to 5.5 in the beginning. The samples were collected after a period of 1, 3, 5, 7, 10 and 15 days. Each organic substrate without SRB inoculums was conducted as control.

2.5. Column experiments for AMD treatment

The removal of sulfate and metal ions from AMD by SRB with organic waste as carbon source in dynamic leaching condition was assessed using column bioreactor (diameter 5.0 cm, height 30 cm) at 30 °C in a biochemical incubator. The column bioreactor was packed with the mixture of chicken manure (45 g), sawdust (5 g) and silica sand (45 g), because of chicken manure as good carbon source in preliminary test and silica sand improving permeability to facilitate drainage. The column bioreactor was inoculated with 10 mL of SRB of log phase cells under anaerobic conditions for 2 days to promote SRB growth before feeding with the AMD. Then the column bioreactor was fed with synthetic AMD influent (80 mL/d) at the top of the column and effluent samples were collected from a port at the base of column every day.

2.6. Analytical methods

The pH (PHS-3C meter) and Eh (501 ORP electrode) of samples were measured immediately after collection. The samples was filtered through 0.45 um filter paper, and sulfate concentration was determined using the turbidimetric method (US EPA 1986: Method 9038) by by UV–VIS spectrophotometer (Shimadzu). The filtered samples were acidified with one drop of nitric acid and the concentrations of Fe, Mn, Cu, Cd, Ni, Zn were measured by Atomic Absorption Spectroscopy (AAS) (AA-7000 model spectrometer, Shimadzu).

The formed black precipitates in the sulfidogenic column bioreactor were collected at the end of AMD treatment. Micro-morphology of the precipitates was examined using field emission scanning electron microscope (SEM, FEI Quanta FEG 250). Energy dispersion spectrometer (EDS, INCA Energy X-MAX-50) coupled with the SEM was used to the elemental analysis of the precipitates. SRB populations were enumerated by the three-tube Most Probable Number (MPN) method with serial dilutions in modified Postgate