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Influence of ferrous ions on extracellular polymeric substances content and sludge dewaterability during bioleaching

Jonathan W.C. Wong [a,*], Jun Zhou [a,b], Mayur B. Kurade [a], Kumarasamy Murugesan [a]

[a] Sino Forest Applied Research Center for Pearl River Delta Environment & Department of Biology, Hong Kong Baptist University, Hong Kong Special Administrative Region
[b] College of Resources and Environment, Nanjing Agricultural University, Nanjing, PR China

HIGHLIGHTS

- Influence of pH and Fe^{2+} was investigated on sludge dewaterability.
- Acid treatment improved dewaterability at pH 2.66.
- Bioleaching treatment was superior in dewaterability compared to acid treatment.
- Bioleaching enhanced the dewaterability by lowering of the sludge EPS content.

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ABSTRACT

Pretreatment of activated sludge with sulfuric acid and bioleaching using *Acidithiobacillus ferrooxidans* along with addition of Fe^{2+} on sludge dewaterability was investigated. The sludge dewatering efficiency in terms of capillary suction time (CST) and specific resistant to filtration (SRF) was increased with a decrease in sludge pH. A pH of 2.67 was found to be optimum for dewatering, at which 81% and 63% reduction of CST and SRF were achieved, respectively. The dewaterability of sludge was enhanced after the addition of Fe^{2+} and *A. ferrooxidans*. Ideal concentration of Fe^{2+} was 2 g/L for sludge dewaterability, which showed 96% and 88% reduction in CST and SRF, respectively. In the control sludge, maximum part of the biopolymeric macromolecules was contributing by the tightly bound extracellular polymeric substances (TB-EPS). At optimum Fe^{2+} concentration, total EPS was reduced by 73%, enhancing sludge dewaterability. Bioleaching conducted by *A. ferrooxidans* could solubilized 88% Cu and 99% Zn within 120 h.

1. Introduction

Activated sludge process is the most commonly used method for municipal wastewater treatment. However, a large volume of excess sludge containing over 90% water is generated in this process which causes severe environmental pollution. Dewatering is immensely required as it can reduce the subsequent treatment and disposal operation cost (Niu et al., 2013). Conventional dewatered sludge obtained through the addition of organic or inorganic-flocculant followed by mechanical dewatering always contains high moisture content (>80%). In addition, it possesses high concentration of heavy metals depending on wastewater sources. These problems severely affect subsequent disposal and reutilization of sewage sludge such as incineration, landfilling, and composting. Developing the technologies for high dewatering efficiency of waste thickened sludge is of paramount significance.

Bioacidification or bioleaching approach has been recently found to be effective in improving dewatering of sewage sludge (Shi et al., 2013; Song and Zhou, 2008). This is a natural process that involves the interaction between several ionic species including iron and, sulfate, and microorganism such as *Acidithiobacillus ferrooxidans* and *A. thiooxidans*. The sulfur-oxidizing bacteria (*A. thiooxidans*) produce sulfuric acid by bio-oxidation of S^0 that lowers the sludge pH to <2. The presence of *A. thiooxidans* in sludge bioleaching causes flocs disintegration and deflocculation under extreme acidification that deteriorates the sludge dewaterability. Moreover, the inclusion of sulfur to the sludge system results in residual elemental sulfur and sulfate in sludge biosolids which would create environmental problems. Hence, *A. thiooxidans* mediated bioleaching may be suitable for metal leaching rather than sludge dewatering (Murugesan et al., 2014). In contrast, *A. ferrooxidans* culture is rich in Fe^{3+}, cell bound and/or released extracellular polymeric substances (EPS), and secondary minerals from iron

* Corresponding author at: Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong Special Administrative Region. Tel.: +852 3411 7056; fax: +852 3411 2355.
E-mail address: jwcwong@hkbu.edu.hk (J.W.C. Wong).

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oxidation (Kurade et al., 2014). *A. ferrooxidans* requires ferrous ion as an energy substrate and carries out hydrolysis of Fe^{3+} from the bio-oxidation of Fe^{2+}, which generates H^+ that causes a decrease in pH to 2.5. Such a low pH will facilitate metal leaching and enhance dewaterability of sewage sludge. Unlike metal leaching process that requires extremely low pH, bioleaching mediated sludge dewatering requires an optimum pH of 2.4 to achieve better dewaterability. *A. ferrooxidans* mediated bioleaching could be appropriate for sludge dewatering as it acidifies the sludge to the pH range suitable for better dewaterability (Wong and Gu, 2004; Murugesan et al., 2014).

Sludge acidification facilitates solubilization of sludge-borne metals and can also assist in dewaterability (Song and Zhou, 2008; Wong and Gu 2008; Wong et al., 2004, 2014). The sludge dewaterability is dependent on breakage of the flocs during acidification treatment. It neutralizes the negative charge on the sludge flocs and reduces the repulsive forces between sludge particles. This leads to the formation of bigger sludge flocs resulting in the release of bound water to surrounding. Acidification can improve dewatering corresponding with a more open structure and a smaller proportion of fine particles (Feitz et al., 2001). In addition, the acidification of sludge can break the EPS structure, which impedes the dewaterability and leads to an improved dewaterability. Microbial EPS are major components of the sludge flocs matrix. They are believed to act as the glue that binds cells together to form sludge flocs. Many investigations have been carried out on the role of EPS in bioflocculation and sludge settleability (Sheng et al., 2010; Wilen et al., 2003; Yang and Li, 2009). However, the results of previous studies are often inconsistent with their positive and negative impacts on dewaterability (Liao et al., 2001). Therefore, the exact role of EPS on bioflocculation still needs to be identified.

Despite of few reports on the role of bioacidification in dewaterability, effect of different sludge pHs and different concentrations of Fe^{2+} as an energy source during bioleaching process and its impacts on EPS contents of the sludge still need detailed investigations. In the present study, the effects of acid at different pHs and Fe^{2+} on dewaterability and EPS content of the sludge were studied. The dewaterability was monitored using dewatering parameters including CST and SRF. At optimized conditions, bioleaching mediated heavy-metal solubilization (Cu and Zn) was also determined.

2. Methods

2.1. Sludge sampling

Thickened activated sludge was collected from Shek Wu Hui Wastewater Treatment Plant, Hong Kong, in clean 10 L polyethylene containers, which was quickly transferred to laboratory and stored at 4 °C for further use. When the sludge was collected, the initial pH of the sludge was 7.41, total solids content was 2.72% and organic matter was 76%. The Cu and Zn content were 311 and 653 mg/kg, respectively.

2.2. Microorganisms and inoculum

A. ferrooxidans LX5 obtained from the China General Microbiological Culture Collection Center were cultivated in a modified 9K medium. [g/L of $(NH_4)_2$ SO_4-3.5, KCl-0.119, K_2HPO_4-0.058, $Ca(NO_3)_2.4H_2O$-0.0168, $MgSO_4.7H_2O$-0.583, H_2O-1000 mL, pH 2.5]. The modified 9K medium was supplied with 44.2 g/L $FeSO_4.7H_2O$ as the energy source. The pH of the medium after addition of $FeSO_4.7H_2O$ was readjusted to 2.5. The culture was grown in 500 mL Erlenmeyer flasks containing 250 mL of 9K at 30 °C and 180 rpm on a rotating shaker. The inoculum was prepared by transferring 10% v/v of 72 h old culture to fresh medium. *A. ferrooxidans* cell numbers were about 10^8 cells/mL after 72 h of growth.

2.3. Effect of acid treatment on dewaterability of sludge

Experiments were carried out in 500 ml Erlenmeyer flasks. Sulphuric acid (14.08 mol/L) at a volume of 0, 0.24, 0.52, 0.72, 0.96, 1.4, or 1.8 mL was added separately to each flask containing 200 mL sludge and then the sludge mixtures were kept at 30 °C and 180 rpm for 1 h on a rotary shaker. After 1 h of shaking, the pHs of sludge changed to 7.41, 5.94, 4.54, 3.84, 2.67, 1.82 and 1.58 respectively with increasing concentration of acid. The dewaterability of sludge was monitored using CST and SRF as dewaterability parameters.

2.4. Dewaterability using bioleaching at different concentrations of Fe^{2+}

Experiments were carried out in 500 ml Erlenmeyer flasks and *A. ferrooxidans* inoculum (10%) was added to each flask containing 270 mL of sludge. The energy substrate, Fe^{2+} was added at different concentrations ranging from 0 to 6 g/L. Three replicates were tested for each treatment. Flasks were incubated at 30 °C and 180 rpm on a rotary shaker for 24 h. Samples were collected from each treatment at regular time intervals to determine the dewaterability.

2.5. Bioleaching of heavy metals

Experiments were carried out in 500 ml Erlenmeyer flasks. *A. ferrooxidans* inoculum was added (10%) to each flask containing 270 mL sludge and flaks were incubated at 30 °C and 180 rpm on a rotary shaker for 120 h. At regular time interval, the sludge samples were centrifuged at 12,000 g for 15 min, filtered through 0.45 μm membrane filter and the filtrate was used to determine Zn and Cu solubilization efficiency using atomic absorption spectrometry (AAS).

2.6. Analyses

At each sampling point, pH levels were measured using an Orion 920A pH meter. To assess dewaterability, CST was measured using a capillary suction timer (Triton Electronics Type 304 M) using 18 mm reservoir (sludge sample holding capacity – 6 mL) resting on standard CST filter paper (7 × 9 cm). SRF was determined using 30 mL of well mixed sludge sample by filtration through a Buchner funnel (Lo et al., 2001). Briefly, the sample was added into a Buchner funnel placed with a Advantec No. 1 filter paper and suction filtration was performed using a vacuum pump with 0.07 MPa constant pressures. The SRF was calculated according to the method of Lo et al. (2001). Samples were centrifuged at 12,000 g for 15 min, filtered through 0.45 μm membrane filter and used for the determination of Fe content using the 1,10-phenanthroline method as per the standard methods (APHA, 2005). All the data presented are the means and standard error of three independent samples.

2.7. Extraction of EPS

The loosely bound-extracellular polymeric substances (LB-EPS) and tightly bound-extracellular polymeric substances (TB-EPS) of the sludge samples were extracted using the ultrasound methods followed by the centrifugal methods by modifying earlier reported protocol (Domínguez et al., 2010). For sludge samples (50 mL), the flocs were harvested by centrifugation at 550 g for 15 min. The supernatant collected after first centrifugation was used to deter-