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Enhancement of the dewaterability of sludge during bioleaching mainly controlled by microbial quantity change and the decrease of slime extracellular polymeric substances content

Minbo Huo [a,1], Guanyu Zheng [a,b,1], Lixiang Zhou [a,*]

[a] Department of Environmental Engineering, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China
[b] Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing 210095, China

HIGHLIGHTS

- Microbial quantity change, bound water and slime EPS contents are dominant factors.
- Contribution rate of microbial quantity change on sludge dewaterability is 32.50%.
- Contribution rate of bound water content on sludge dewaterability is 24.24%.
- Contribution rate of slime EPS content on sludge dewaterability is 22.37%.

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ABSTRACT

Contribution rates of factors controlling sludge dewaterability during bioleaching, such as sludge pH, microbial quantity, extracellular polymeric substances (EPS), *etc.*, were investigated in this study. Results showed that the dewaterability of bioleached sludge was jointly enhanced by the growth of *Acidithiobacillus* sp., the increase of Fe^{3+} concentration, the decreases of sludge pH, heterotrophic microorganism quantity change, and the decreases of EPS and bound water contents. Ridge regression analysis further revealed that the contribution rates of microbial quantity change, bound water content and slime EPS content on sludge dewaterability enhancement were 32.50%, 24.24%, and 22.37%, respectively, all of which are dominant factors. Therefore, the enhancement of sludge dewaterability was mainly controlled by microbial quantity change and the decrease of bound water and slime EPS contents during bioleaching.

1. Introduction

The increasing demand for better water quality for human living, especially in Asian countries like China resulted in a large number of wastewater treatment plants being constructed in a short period of time, but it has also in the same time brought about an important shift in waste streams from the liquid phase to the semi-solid phase. As most municipal wastewater plants are operated by the activated sludge process, large amounts of waste activated sludge have been generated and require proper treatment before disposal. A subsequent dewatering step is usually needed to reduce the sludge volume for facilitating transport and handling, in which efficient sludge conditioning is crucial to improve sludge dewaterability and achieve a high solid content of sludge (Chen et al., 2001; Raynaud et al., 2012). However, the dewatered sludge still contains as high as 80% of moisture content when using conventional conditioning methods, such as adding organic or inorganic flocculants, followed by mechanical dewatering (Chen et al., 2001; Liu et al., 2012a). Therefore, more effective sewage sludge conditioning method should be developed to enhance the dewaterability of sewage sludge (Neyens et al., 2004; Liu et al., 2012b).

Bioleaching is reported recently as a microbial conditioning method which can improve sludge dewaterability by 4–10 times, and the moisture content of dewatered bioleached sludge cake is as low as 60% in commercial scale studies using bioleaching conditioning and diaphragm filter presses (Liu et al., 2012a,b). During bioleaching, energy substances including Fe^{2+} and S^0 are bio-oxidized by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*, respectively, and as a result the concentration of Fe^{3+}

* Corresponding author. Tel./fax: +86 25 84395160.
E-mail address: lxzhou@njau.edu.cn (L. Zhou).
[1] Both authors contributed equally to this work.

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in sludge increases rapidly and sludge pH decreases gradually (Tyagi et al., 1994; Liu et al., 2012b). In addition, it was found in previous studies that bioleaching process is effective in killing bacterial cells or lysing cells, as shown that the counts of neutrophilic bacteria decreased from an initial level of 4.33×10^6 CFU/mL to below 1×10^3 CFU/mL during the bioleaching of anaerobically digested sludge (Gu and Wong, 2007). However, the detailed mechanisms responsible for the sludge dewaterability improvement by bioleaching have not been clarified.

Previous studies have extensively studied factors influencing the dewaterability of sludge. For instances, Chen et al. (2001) and Neyens et al. (2003) reported that the dewaterability of activated sludge was improved when sludge pH decreased, and Fe^{3+} can improve the sludge dewaterability through altering small sludge particles to large flocs (Li et al., 2012). In addition, the decrease of sludge pH and the death or cell lysis of microorganisms in sludge potentially result in the change of extracellular polymeric substances (EPS) (Chen et al., 2001; Raynaud et al., 2012), which is widely considered as one of most important factors affecting sludge dewaterability (Chen et al., 2001; Liu and Fang, 2003; Neyens et al., 2004). Indeed, Houghton et al. (2001) and Bala Subramanian et al. (2010) have found that the decrease of sludge EPS content could make sludge to be more easily dewatered, and Yang and Li (2009) revealed that the EPS in sludge flocs determines the dewaterability of sludge and excessive EPS in the form of loosely bound EPS (LB-EPS) would deteriorate the sludge dewaterability and result in poor biosolid–water separation. In addition, bound water content is also an important factor influencing sludge dewatering (Vaxelaire and Cézac, 2004; Lee et al., 2006), and a lower bound water content in sludge usually means better sludge dewaterability (Lee et al., 2006).

Obviously, many factors including sludge pH, Fe^{3+} concentration, the death or cell lysis of sludge microorganisms or bioleaching bacteria, the contents of sludge EPS and bound water may jointly control sludge dewaterability during sludge bioleaching treatment, but previous studies have not studied the contribution of these factors on the sludge dewaterability enhancement during bioleaching treatment. Therefore, the objectives of the present study are to systematically investigate the changes of sludge pH, Fe^{3+} concentration, the counts of heterotrophic bacteria and the two *Acidithiobacillus* species, the contents of sludge EPS and bound water during sludge bioleaching treatment, and further find the dominant factors among these factors influencing the sludge dewaterability during sludge bioleaching. The outcome of the present study may be helpful in exploring the possibility of combining some physical and/or chemical techniques with bioleaching to optimize dominant factors found to further enhance sludge dewaterability in much shorter bioleaching periods.

2. Methods

2.1. Municipal sewage sludge sample

The municipal sewage sludge used in this study was collected from the sludge thickening pond of Taihu New City Wastewater Treatment Plant in Wuxi City, Jiangsu Province, China. Sludge pH, solid content and organic matter content in sludge were determined immediately after collection according to their respective Standard methods (APHA, 2005), and the values are 6.95, 3.78%, and 50.84%, respectively. The capillary suction time (CST) of the sludge was measured by using a capillary suction timer (Model 304M, Triton), and its value is 20.50 s. Municipal sewage sludge was then stored in polypropylene bottles and kept at 4 °C before use.

2.2. Microorganisms and bioleaching inoculum preparation

Acidophilic chemoautotrophic bacteria *A. ferrooxidans* LX5 (CGMCC No.0727) and *A. thiooxidans* TS6 (CGMCC No.0759) obtained from China General Microbiological Culture Collection Center (CGMCC) were cultured in modified 9K and SM medium (Zheng et al., 2009), respectively. The modified 9K and SM medium autoclaved at 121°C for 15 min were adjusted to pH 2.5 and 3.0 with sulfuric acid, and then spiked with 44.2 g/L of 0.22 μm membrane-filtered $FeSO_4{\cdot}7H_2O$ and 10 g/L of elemental sulfur (S^0) as the energy source, respectively. The inoculums were cultured in 500 mL Erlenmeyer flasks shaken at 180 rpm and 28 °C until their respective densities reached approximately 10^8 cells/mL.

Firstly, 75 mL *A. ferrooxidans* LX5, 75 mL *A. thiooxidans* TS6 and 150 mL sludge were mixed in a 500 mL Erlenmeyer flask and then supplemented with 2 g/L Fe^{2+} and 2 g/L S^0, and subsequently the flask was incubated in a rotary shaker at 28 °C and 180 rpm, during which sludge pH was monitored at 12 h intervals. When the sludge pH dropped to about 2.0, 150 mL acidified sludge was transferred to another flask containing 150 mL fresh municipal sewage sludge, 2 g/L Fe^{2+} and S^0. Then the mixture was incubated at the same conditions as described above till the sludge pH dropped to about 2.0 again. The above procedures were repeated twice, and the bioleached sludge was employed as the bioleaching inoculum in the following experiments (Wang et al., 2010; Liu et al., 2012b). The counts of *A. ferrooxidans* LX5 and *A. thiooxidans* TS6 in this bioleaching inoculum were 2.12×10^8 CFU/g dw and 4.76×10^8 CFU/g dw, respectively.

2.3. Bioleaching experiments

The bioleaching experiments were conducted in 2 L Erlenmeyer flasks each containing 120 mL bioleaching inoculum and 1080 mL sludge, which was supplemented with 2 g/L Fe^{2+} and 2 g/L S^0 (Liu et al., 2012b). Meanwhile, one control treatment was conducted in 2 L Erlenmeyer flasks each containing 1200 mL sludge only, and one energy substances treatment was conducted in 2 L Erlenmeyer flasks each containing 1200 mL sludge supplemented with 2 g/L Fe^{2+} and 2 g/L S^0. Each treatment was carried out in triplicate, and all these flasks were incubated in a rotary shaker at 28 °C and 180 rpm (Wang et al., 2010; Liu et al., 2012b). During the incubation, 120 mL sludge sample was collected from each flask at 0, 1, 24, 48, 72, and 96 h and then determined for the counts of total heterotrophic bacteria, *A. ferrooxidans* LX5 and *A. thiooxidans* TS6, deoxyribonucleic acid (DNA) concentration in sludge supernatant, Fe^{2+} concentrations, sludge pH, slime EPS content, loosely bound EPS (LB-EPS) content, tightly bound EPS (TB-EPS) content, bound water content and sludge CST, respectively.

2.4. Analytical methods

After collecting sludge samples from these flasks, sludge pH was determined immediately, and sludge CST was measured by using a CST analyzer (Model 304M, Triton). Then, a volume of 5 mL sludge sample was filtered through qualitative filter paper, and the filtrate was dialyzed with 3500 Dalton dialysis bag to remove iron ions. The concentration of DNA in the resulting solution was measured by using diphenylamine colorimetric method, in which DNA of *Escherichia coli* was used as a standard solution of DNA (Kavitha et al., 2013). Another 3 mL sludge sample was filtrated through 0.22 μm cellulose nitrate membrane, and the concentration of Fe^{2+} in the filtrate was determined by using 1, 10-phenanthroline method (APHA, 2005). Oxidation rate of $Fe^{2+}(\%) = [(C_0 - C_t)/C_0] \times 100\%$ (where C_0 was the initial Fe^{2+} concentration, C_t was the Fe^{2+} concentration at different times after treatments). The bound water content in sludge samples was determined by