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Towards understanding the dewatering mechanism of sewage sludge improved by bioleaching processing

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ABSTRACT

Bioleaching by sulfur oxidizing bacteria has been regarded as a novel dewatering process for the sludge treatment. Since the bioleaching process is a comprehensive biological and chemical process for sludge treatment, it is necessary to explore the dewatering mechanism of sewage sludge improved by bioleaching. The bioleached sludge showed a significant difference with the control sludge, mostly through a considerable reduction of pH (to 3.92) and an improved specific resistance to filtration (SRF), which reduced to 5.31×10^{10} m/kg after 72 h treatment. Separate sulfuric acid addition and Fe^{2+} addition did not result in significant decrease of sludge resistance filtration, indicating that the chemical acidification and Fe^{2+} addition are not the deciding factors for the improvement by bioleaching. The extracellular polymeric substances in bioleached sludge decreased considerably, with the protein and polysaccharide reduced by 97.42% and 76.00%, respectively. During the bioleaching process, the number of microbial genuses in the bioleached sludge gradually decreased and the dominant bacterial genus (*Acidimicrobium ferrooxidans*) shifted from 7.48% to 26.49% at the end of bioleaching. While many factors influence the dewaterability of the sludge, the bio-substitution which led to the decrease of EPS is the deciding factor for the dewaterability improvement of the bioleached sludge.

1. Introduction

During the last ten years, the resourceful utilizations of sludge is the most effective means of sludge disposal. However, a large amount of organic or inorganic-flocculant are used in the progress of dewatering by mechanical methods [1]. In order to replace the chemical flocculant (non-green), researchers have been trying to investigate sustainable, sound, and environmental friendly methods to enhance the dewaterability of sludge [2–4]. In recent years, Liu et al. and Song et al. reported that bioleaching by sulfur oxidizing bacteria can improve the sludge dewaterability significantly [5,6]. Fontmorin et al. also reported that the combination of bioleaching with Fenton-like reaction gave promising results for the treatment of sludge in terms of improving its dewaterability [7]. After bioleaching treatment, the moisture content of the sludge cake can decrease to as low as 60% during the diaphragm filter press while there is no or less flocculants are required. Actually, bioleaching was considered as a novel, economic and high efficient dewatering method due to the absence of chemical flocculants addition into the sludge [8,9].

Acidophilic sulfur- and iron-oxidizing bacteria are the most widely used microorganisms for bioleaching [6]. Chemoautotrophic bacterial species like *Acidithiobacillus thiooxidans* and *Acidothiobacillus ferrooxidans* have been used during bioleaching process [10]. Other acidophilic microorganisms were also applied in previous studies [11,12]. However, though the effective dewatering performance by the bioleaching has been demonstrated, the mechanisms are still unclear. Several studies have reported that extracellular polymeric substances (EPS) and cation concentration, pH, particle size, microbial composition jointly determined the sludge dewaterability [13–15]. Since the improved dewaterability is attributed to the EPS reduction of the sludge, but what caused the decrease of the EPS? Based on the characteristics of the chemoautotrophic bacterial species, we assume that it is attributed to the shift of microbial communities after bioleaching. Therefore, the objective of the present study is to explore the relationship between the dewatering characteristic of bioleached sludge and the shift of microbial communities. The findings of this study will shed a new insight into the mechanism of the biological

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dewatering method and be helpful for the novel dewatering process development for the industries.

2. Materials and methods

2.1. Sample collection and preparation

The raw sludge was collected from gravity thickener tank in waste water treatment plant, Wuxi, China and stored at 4 °C for subsequent use. The raw sludge was the mixture of primary sludge and secondary sludge, and the characteristics of the raw sludge are shown in Table.1. The required 20 g/L TS concentration of sludge was obtained by diluting the raw sludge with distilled water.

2.2. Preparation of inoculated sludge

On the basic of the raw sludge, inoculum sludge was obtained through laboratory acclimation. For preparing the inoculum, 50 mL raw sludge (40 g/L) was added in 500 mL Erlenmeyer flasks containing 250 mL of 9 K liquid culture medium, which contains $(NH_4)_2SO_4$ 3.0 g, KCl 0.1 g, K_2HPO_4 0.5 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, $Ca(NO_3)_2$ 0.01 g, $FeSO_4{\cdot}7H_2O$ 44.3 g in 1000 mL of distilled water [16]. Then the flasks were cultured in water bath shaker at 100 rpm (28 ± 2 °C) for 144 h with the addition of new K9 medium as substrate every three days. During this period, pH was measured to evaluate the process of acclimation.

In order to make the acclimated bacteria adapt to the sludge culturing environment, the bacteria was transferred from 9 K medium to 20 g/L sludge medium with 6.7% (w/w dry weight ratio) ferrous ion. The culture condition was as the same with the inoculum sludge. Iron-oxidizing bacteria *A. ferrooxidans* (CGMCC 1.6369) was stored in laboratory. When for the EPS measurement, the pure strain was cultured in 9 K medium to stationary phase and the biomass was harvested and washed by distilled water for the determination.

2.3. Experimental procedure

In the bioleaching experiments, ten milliliters of inoculum, 6.7% (w/w dry weight ratio) ferrous ion and 2 g/L elemental sulfur were added into 240 mL raw sludge in the 500 mL Erlenmeyer flasks. Then the flasks with sludge were cultured in water bath shaker at 100 rpm and 28 ± 2 °C) for 72 h. For the control experiment, there is no inoculum sludge added into the raw sludge and the other conditions are as same as the bioleached sludge. For each treatment, five parallel 500 mL flasks were carried out to provide enough volume of sludge for measurement. Samples were collected periodically and analyzed.

2.4. EPS extraction and analysis

EPS was extracted using a modified thermal extraction method [17]. 30 mL of sludge was first centrifuged at 12240*g* for 15 min. The organic matter in the supernatant was regarded as soluble microbial products (SMP). The sludge pellet in the centrifuge tube was resuspended and diluted to its original volume with 0.05% NaCl solution. Then the sludge mixture was shaken by a vortex mixer for 1 min. The tube was heated in a water bath with temperature of 80 °C for 30 min, followed by centrifugation at 12240*g* for 15 min. The organic matter in the supernatant was regarded as EPS. The supernatant was filtered through a 0.45 μm syringe-driven filter. The extracted EPS was analyzed for the concentration of protein (EPS_P) and polysaccharide (EPS_{PS}).

Table 1
Characteristics of the raw sludge.

The total solids (TS)	40 g/L
Volatile solids (VS)	19.97 g/L
pH	6.44
Specific resistance to filtration (SRF)	2.86×10^{12} m/kg

2.5. Dewaterability measurement

The dewaterability of sludge was measured by the Buchner funnel test [18]. In each test, 100 mL of sludge sample was filtered through a filter paper (12.5 cm Whatman No. 1). After 1 min of gravitational drainage, a vacuum of 30 kPa was applied. Then the filtrate volume (V) collected at different times was recorded until no additional water flowed through the filter paper. The SRF of sludge was calculated by using the following equation, according to Arhan et al. [19]:

$$SRF = \frac{2bPA^2}{\mu c} \quad (1)$$

where P is the pressure applied, N/m^2; A is the filtration area, m^2; μ is the filtrate viscosity, $N(s)/m^2$; c is the weight of solids/unit volume of filtrate, $kg/m^3 = 1/Ci/(100 - Ci) - C_f/(100 - C_f)$; C_i is the initial moisture content, %; C_f is the final moisture content, %; b is the slope of the curve determined from the t/v vs v plot; v is the volume of filtrate, m^3; and t is the filtration time, s.

2.6. Shift of microbial communities

To measure the changes of microbial communities, terminal restriction fragment length polymorphism (T-RFLP) method was used and followed the protocols described elsewhere [20]. DNA was extracted from the sludge samples by using a MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA). For terminal restriction fragment (T-RF) analysis, bacterial 16S rRNA genes were amplified with 5′ fluorescently labeled forward primer (27F labeled with 6-carboxyfluorescein, 5′-AGAGTTTGATCCTGGCTCAG-3′) and a universal reverse primer (1492R, 5′-GGTTACCTTGTTACGACTT-3′). The reaction conditions were carried out with a program consisting of an initial denaturation at 95 °C for 10 min; 30 cycles of 95 °C for 1 min, 60 °C for 50 s, and 72 °C for 1 min; and a final elongation cycle at 72 °C for 10 min. PCR products were purified and digested with HaeIII for 3 h at 37 °C followed by 10 min at 65 °C. The digested amplicons were mixed with GeneScan 1000 ROX size standards (Applied Biosystems Inc., USA) and analyzed by capillary electrophoresis with GeneScan software (Applied Biosystems Inc., USA). Signals with a peak area that was less than 1000 relative fluorescence units were regarded as background noise and excluded from the analysis. The relative abundance of a detected T-RF within a given terminal restriction fragment length polymorphism (T-RFLP) pattern was calculated as the respective signal area of the peak divided by the peak area of all peaks of the T-RFLP pattern. The size of each bacterial T-RFLP species peak corresponded to the value for that species determined by in silico analysis of clone library with Lasergene (DNAStar Co., USA). The digestion products were analyzed by Shanghai Gene Core BioTechnologies Co., Ltd, China. The T-RFLP results were uploaded to the network database (http://mica.ibest.uidaho.edu/pat.php), and obtained each gene fragment corresponding microorganisms [21].

2.7. Analytical methods

The total solids of the sludge (TS), volatile solids (VS), chemical oxygen demand (SCOD) and pH were determined according to Standard Methods [22]. The concentrations of proteins and polysaccharides were measured using the Lowry–Folin [23] and