Separation and Purification Technology 165 (2016) 53–59

Contents lists available at ScienceDirect

Separation and Purification Technology

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

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

He Liu [a,c], Shuo Yang [a], Jiasheng Shi [a], Xiaoyu Xu [b], Hongbo Liu [a,c,*], Bo Fu [a,c]

[a] School of Environmental and Civil Engineering, Jiangnan University, Wuxi 214122, China
[b] School of Pharmaceutical Sciences, Jiangnan University, Wuxi 214122, China
[c] Jiangsu Key Laboratory of Anaerobic Biotechnology, 214122 Wuxi, China

ARTICLE INFO

Article history:
Received 10 November 2015
Received in revised form 2 March 2016
Accepted 18 March 2016
Available online 22 March 2016

Keywords:
Activated sludge
Bioleaching
Dewaterability
Bio-substitution
Extracellular polymeric substances (EPS)
Microbial community

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.

© 2016 Elsevier B.V. All rights reserved.

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

* Corresponding author at: School of Environmental and Civil Engineering, Jiangnan University, Wuxi 214122, China.
E-mail address: liuhongbo@jiangnan.edu.cn (H. Liu).

http://dx.doi.org/10.1016/j.seppur.2016.03.037
1383-5866/© 2016 Elsevier B.V. All rights reserved.

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

phenol–sulfuric method [24], respectively. To measure soluble COD, soluble protein and soluble polysaccharides, the samples were first centrifuged at 10200*g* for 10 min, and then were filtered with 0.45 μm syringe filters.

3. Results and discussion

3.1. pH and zeta potential change during the bioleaching treatment

The pH of sludge is an important indicator reflecting the bioleaching progress and effects of dewaterability [25]. As shown in Fig. 1, pH considerably decreased from 6.03 to 3.92 in the first 12 h of bioleached sludge. Then it gradually decreased to 2.44 at 72 h. In the control sludge, the pH decreased slowly from 6.23 to 5.11 at 72 h, showing an apparent difference to the bioleached sludge.

The significant decrease of pH value of the bioleached sludge was attributed to microbial oxidation of elemental sulfur and ferrous iron by the inoculated sulfur- and iron-oxidizing bacteria [12]. The oxidation of Fe^{2+} follows the reactions [16,26]: $Fe^{2+} + O_2 + H^+ \rightarrow Fe^{3+} + H_2O$, $Fe^{3+} + H_2O \rightarrow Fe(OH)_3 + H^+$. Then Fe^{3+} tends to form jarosite according to the reaction: $Fe(OH)_3 + K^+ + SO_4^{2-} + 2H_2O \rightarrow KFe_3(OH)_6(SO_4)_2 + H^+$. The oxidation of S^0 follows the reaction: $S + 6Fe^{3+} + 4H_2O \rightarrow HSO_4^- + 6Fe^{2+} + 7H^+$. During these processes, the pH will decrease significantly due to the generation of SO_4^{2-} [12].

The zeta potential of raw sludge and bioleached sludge was measured to show the sludge surface charge, which affects the settling properties of the sludge [27] (Fig. 1b). Zeta potential represents the stability of the colloid substances. A higher zeta potential is benefited for the aggregation of the colloids in the sludge. Zeta potential of raw sludge supernatant was −40.00 mV, and maintained stably during the whole culture period, showing a negative surface charge and thus suggesting the presence of particles unlikely to aggregate. However, the zeta potential of bioleached sludge showed a significant increase, which rose from −36.98 mV to −8.36 mV at 72 h. The profile of zeta potential during the time is consistent with the pH change. This may be explained by the neutral reaction by the increase of H^+ from the pH decrease of the bioleached sludge. Additionally, we also observed that there was no significant difference of particle size of the raw sludge and bioleached sludge (data not shown), suggesting that the bioleaching treatment would not change the particle size of the sludge.

3.2. Changes of SRF during bioleaching process

SRF of sludge was used to evaluate the dewaterability of sludge. The sludge with a high SRF usually cannot be dewatered easily [28]. In Fig. 2a, SRF decreased from 2.32×10^{12} at initial to 1.20×10^{12} m/kg after 72 h in the control sludge. Meanwhile, for the bioleached sludge, it declined significantly from 2.32×10^{12} m/kg to 5.31×10^{10} m/kg after 72 h treatment. It can be seen that the SRF of bioleached sludge and control sludge decreased by 97.72% and 48.40%, respectively, indicating a great difference between the two kinds of sludge. Fig. 2b and c showed the pictures of raw sludge and the bioleached sludge after filtrated through the filter with a vacuum pressure at 30 kPa. The color of the filtered cake for bioleached sludge turned yellow from black because the presence of elemental sulfur in the bioleached sludge cake. It also can be seen in Fig. 2c that the filtered sludge cake for bioleached sludge was cracked because of the lower water content (70%) under the pressure by vacuum pump filtration. It demonstrated the effectiveness of bioleaching treatment for the raw sludge. The filtration time of sludge is between 5 and 10 min for 100 ml sludge sample. The filtration time of bioleached sludge samples are shorter than the original sludge samples. It is obvious that the raw sludge contains more water content (85%) and the bioleached sludge with much less water content (70%) due to the improvement of the dewaterability by the bioleaching.

Based on the decease of SRF, it indicated the dewaterability of bioleached sludge was enhanced significantly. Song et al. also reported that the SRF of sludge decreased from 1.80×10^{13} m/kg to 0.38×10^{13} m/kg after bioleaching treatment [6]. The improvement of the dewaterability by bioleaching treatment can be partly attributed to the flocculation effect of Fe^{3+} transformed from the added Fe^{2+} in sludge and charge neutralization with the decrease of pH value. Therefore, it is necessary to further explore the mechanism of bioleaching treatment for the improvement of dewaterability.

3.3. Sludge filter resistance change by different chemical conditioning methods

Since the bioleaching is a comprehensive process, to investigate which deciding factors led to the improvement of dewaterability, three kinds of independent treatment, i.e., bioleaching, sulfuric acid addition and ferrous irons addition were conducted.

In Fig. 3, the SRF of the raw sludge is 2.32×10^{12} m/kg. After 72 h incubation, the SRF of control sludge declined to 1.20×10^{12} m/kg, which dropped by 48.40%. For the bioleached sludge, SRF changed from initial 2.32×10^{12} m/kg to 5.31×10^{10} m/kg after 72 h bioleaching treatment. With the addition of sulfuric acid, SRF declined to 2.09×10^{12} m/kg which dropped by 10.19%. In the case of Fe^{2+} addition, SRF declined to 8.20×10^{11} m/kg which declined by 64.73%. The results indicated that with the addition of acid or Fe^{2+}, the sludge dewaterability

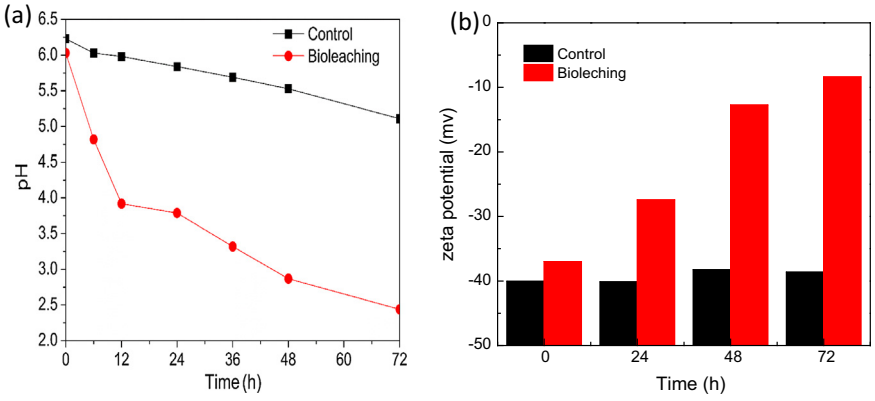

Fig. 1. The pH and zeta potential profiles of the bioleached and control sludge.

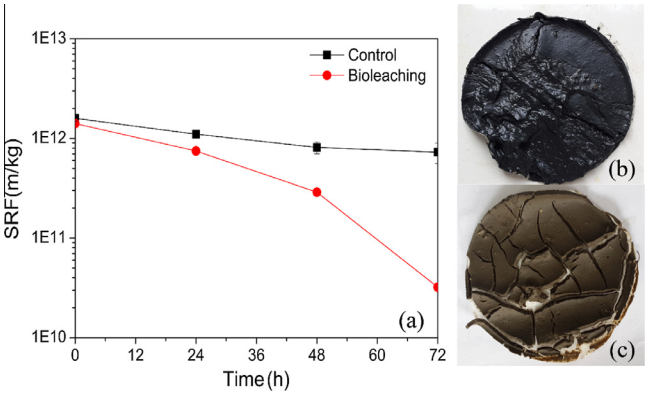

Fig. 2. SRF change of the bioleaching sludge (a), pictures of filtered raw sludge (b) and filtered bioleaching sludge (c).

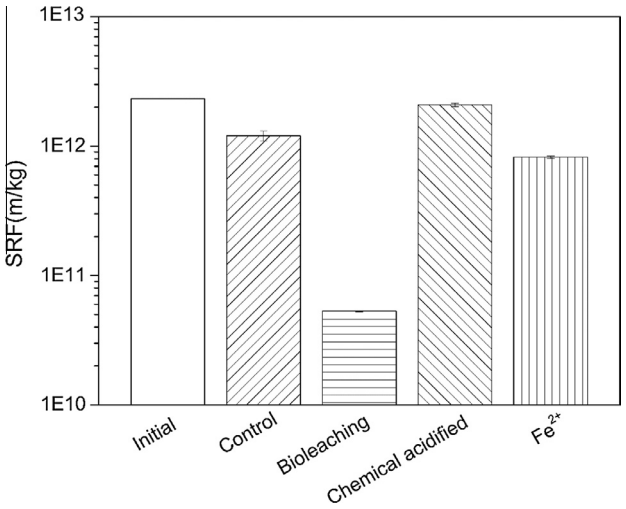

Fig. 3. SRF of sludge by different chemical conditioning methods.

was improved to some extent. Obviously, the bioleached sludge showed the lowest SRF of all the sludge and SRF decreased by 97.72% compared to the raw sludge.

Previous studies have demonstrated that many factors will influence the sludge dewaterability, such as pH decrease, ferrous addition [29,30]. The decrease of sludge pH value will cause cell lysis of microorganisms in sludge and result in the change of EPS content, then influence the sludge dewaterability [30,31]. The ferrous addition will change the charge and Zeta potential of the sludge. Since the bioleaching treatment is a complex biological process, in which the pH decrease and ferrous addition will involve in the process. Therefore, it is necessary to differentiate these factors and find the deciding factor for the improvement the sludge dewaterability.

In this study, to investigate the effect of chemical acidification on dewaterability, the sulfuric acid was added into the sludge to decrease the pH from 7.0 to 3.0. According to Fig. 3, the SRF of chemical acidified sludge only decreased by 10.19%, suggesting that the chemical acidification effect may not the deciding factor for the improvement of the dewaterability. The ferrous addition also indicated that the dewaterability improvement of bioleached sludge was not attributed to the ferrous addition.

3.4. Changes of EPS content and components during treatment

EPS is a kind of major component in sludge flocs and is regarded as one of the most important factors influencing the dewatering characteristics of sludge [32]. The EPS mainly consists of proteins and polysaccharides, with the water entrapped into the loosely bounded flocs. In order to investigate the effect of three kinds of treatment, the change of EPS content in the treated sludge was determined and the profiles are shown in Fig. 4.

In bioleached sludge, EPS_P and EPS_{PS} contents decreased from 4.63 mg/g VS and 12.34 mg/g VS of the raw sludge to 0.12 mg/g VS and 2.96 mg/g VS, respectively. Meanwhile, EPS_P and EPS_{PS} contents in the control sludge decreased, dropped by 43.60% and 14.76%, respectively (Fig. 4). After 72 h treatment, EPS_P and EPS_{PS} in the controlling group declined to 2.61 mg/g VS and 10.51 mg/g VS. In the sludge with sulfuric acid addition, EPS_P and EPS_{PS} contents declined to 2.59 mg/g VS and 7.23 mg/g VS, with 43.92% and 37.36% decrease, respectively. In the Fe^{2+} addition sludge, EPS_P and EPS_{PS} contents dropped to 1.21 mg/g VS and 5.25 mg/g VS, decreased by 73.85% and 57.44%, respectively. The most significant decrease of the EPS was observed in bioleached sludge, in which EPS_P and EPS_{PS} contents dropped by 97.42% and 76.00%, respectively. This change of EPS in the sludge is consistent with the SRF in Fig. 3. As a comparison with the sludge, the EPS_P and EPS_{PS} contents of pure *A. ferrooxidans* strain were only 0.10 mg/g VS and 0.04 mg/g VS, respectively.

It is obvious that the bioleaching treatment improves the dewaterability of the sludge indicated by the SRF and EPS change in the flocs. Ye et al. reported that high EPS concentrations increased the viscosity of sludge and decreased its filterability, because polysaccharides and proteins can entrap the water, and then cause high viscosity of sludge [28]. Houghton et al. and Bala Subramanian et al. found that the decrease of EPS content in sludge could make sludge more easily dewatered [33,34]. Furthermore, Yang and Li revealed that the EPS in sludge flocs determined 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 separation of biosolids and water [35].

3.5. Changes of microbial community during bioleaching

Based on the results in Figs. 3 and 4, it can be deduced that the chemical acidification and ferrous addition are not the deciding factors to improve the dewaterability of sludge. In addition, the EPS content of the pure *A. ferrooxidans* is much lower than the raw sludge. Huo et al. observed that the counting number of

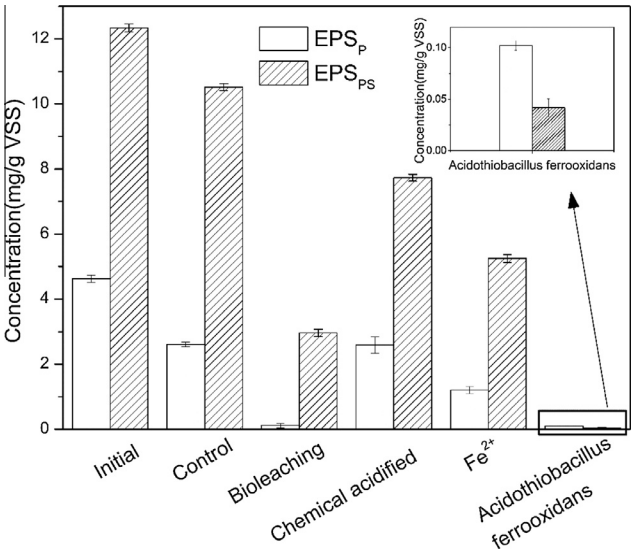

Fig. 4. Variation of protein and polysaccharide contents in EPS of sludge by different treatments.

Acidithiobacillus ferrooxidans LX5 increased rapidly from 2.12×10^7 CFU/g dry weight to 5.51×10^8 CFU/g dry weight sludge in the first 48 h of bioleaching treatment [36]. Therefore, it is reasonable to deduce that the EPS significant decrease of the bioleached sludge is attributed to the shift of the microbial community, e.g. bio-substitution of heterotrophic bacteria by autotrophic acidophilic bacteria with less EPS content.

The shift of the microbial community of the bioleached sludge was conducted by T-RFLP analysis and the results are shown in Fig. 5.

As shown in Fig. 5, the number of terminal restriction fragments (TRFs) in the raw sludge is 11 and the TRF 214.85 bp is the predominant TRF with the percentage of 17.50%, but this TRF disappeared in the bioleached sludge. At 24 h, 48 h and 72 h, the number of TRF is 10, 7 and 6, respectively, suggesting that the number of TRF decreased gradually with the bioleaching. After 72 h bioleaching treatment, the TRFs 37.24 bp, 68.79 bp, 73.44 bp, 261.62 bp, 184.85 bp, 188.61 bp, 193.86 bp and 214.85 bp disappeared. However, a new TRF 250.48 bp appeared and the TRF 212.00 bp became the predominant fragment with the percentage increased from 7.48% to 26.67%. The decrease of the TRFs means that the number of the microbial species is declining with bioleaching processing and this is consistent with the pH dramatic decrease since less and less microbial species can exist in extreme acidic pH condition except the acidophilic bacteria like sulfur oxidizing bacteria. The different possible genuses of microorganisms represented by the T-RFs are listed in Table 2.

In the raw sludge, the TRF 214.85 bp is the predominant and was probably affiliated to one of the genuses of *Carnococcus allantoicus*, *Eubacterium aerofaciens*, *Geobacter metallireducens*, *Leptothrix cholodnii*, *Oxobacter pfennigii* and *Trichococcus flocculiformis*. The TRF 23.25 bp and 24.25 bp are also the major microbial components of the microbial community in raw sludge. The TRF 24.25 bp is affiliated to *Brevibacillus brevis*. It should be noted that these two genuses occurred in the whole bioleaching process, suggesting that they can stand the extreme acidic pH environment. The TRF 88.64 bp appeared from 24 h and its percentage increased gradually from 14.51% to 16.82% (72 h). The dominant TRF 212.00 bp may represent the *Acidimicrobium ferrooxidans*. Since the percentage of *A. ferrooxidans* increased gradually with bioleaching process and accounted for 26.49% at the end of bioleaching, this trend was consistent with the fact of pH rapid decrease (Fig. 1a). With all the changes of the TRFs, it is clear that the microbial community shift occurred during the bioleaching process. Considering the two facts that the EPS content of the *A. ferrooxidans* is much lower than the raw sludge and the bioleached sludge was dominated by *A. ferrooxidans*, it explains well that the bioleached sludge shows a much lower EPS content and improved dewaterability after bioleaching treatment. Since the chemical acid addition and Fe^{2+} cannot result in the significant decrease of EPS and improvement of dewaterability (Figs. 3 and 4), it is reasonable to deduce that the shift of microbial community and the dominance of *A. ferrooxidans* in the bioleached sludge are the deciding factors for the improvement of dewaterability by bioleaching treatment. This finding is very helpful for the parameters selection and bioleaching process design in the future industrial application.

The bioleaching treatment has demonstrated as a feasible biotechnology to improve the dewaterability of the sludge and remove the heavy metals at the same time. The water content of sludge cake after bioleaching treatment can easily be lower than 60% by subsequent mechanic dewatering method. Currently, the key techniques for the bioleaching treatment is the cost of the sulfur and ferrous addition and how to accelerate the growth of the sulfur oxidizing bacteria, which is a kind of slow-growing

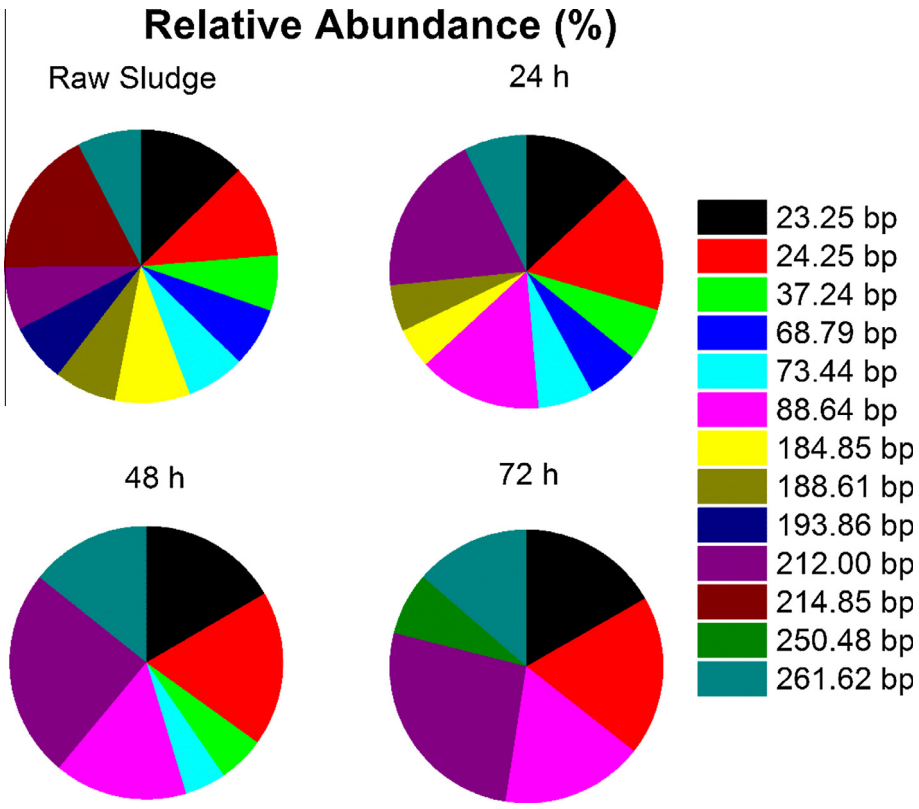

Fig. 5. Genus variation of microbial community of bioleaching sludge at different times.

Table 2
PAT outputs for raw sludge and bioleaching samples at different times.

TRFs	Species or clone
24.25	*Brevibacillus brevis*
37.24	*Escherichia coli*
68.79	*Ectothiorhodospira marina*; *Methylobacter psychrophilus*; *Methylococcus capsulatus*; *Methylomicrobium agile*; *Methylomonas aurantiaca*; *Rubrivivax gelatinosus*
73.44	Clone SJA-170
184.85	*Halochromatium glycolicum*; *Treponema* sp.
188.61	*Arhodomonas aquaeolei*; *Beggiatoa Monterey Canyon*; *Brucella melitensis*; *Methylarcula terricola*; *Rhizobium* sp.
193.86	*Afipia clevelandensis*; *Amaricoccus macauensis*; *Amoebobacter purpureus*; *Erythrobacter longus*; *Eubacterium lentum*; *Methylocystis* sp.; *Mycoplasma salivarium*; *Oceanospirillum jannaschii*; *Piscirickettsia salmonis*; *Porphyrobacter neustonensis*
212.00	*Acidimicrobium ferrooxidans*; *Buchnera aphidicola*; *Clostridium halophilum*; *Desulfitobacterium dehalogenans*; *Frankia* sp.; *Methylophilus methylotrophus*; *Sporolacto bacillusinulinus*; *Treponema phagedenis*
214.85	*Carnococcus allantoicus*; *Eubacterium aerofaciens*; *Geobacter metallireducens*; *Leptothrix cholodnii*; *Oxobacter pfennigii*; *Trichococcus flocculiformis*
250.48	*Acinetobacter calcoaceticus*; *Haliscomenobacter hydrossis*; *Methylomicrobium buryaticum*; *Methylophaga marina*; *Mycoplasma agalactiae*; *Oceanospirillum kriegii*; *Ruminobacter amylophilus*; *Spiroplas mamirum*; *Thermomonospora chromogena*
261.62	*Actinobacillus succinogenes*; *Bacteroides distasonis*; *Bifidobacterium inoinatum*; *Clostridium estertheticum*; *Flavobacterium ferrugineum*; *Haemophilus* sp.; *Pasteurella volantium*; *Phytoplasma* sp.; *Prevotella heparinolytica*; *Spirochaeta smaragdinae*; *Streptobacillus moniliformis*; *Streptococcus mitis*

autotrophic bacterium, during the bioleaching process. With the optimization of the process parameters, the bioleaching approach will show a more extensive application prospect in the future.

4. Conclusions

The treatment of bioleaching can improve the dewaterability of the sludge significantly. The pH of bioleached sludge drastically decreased to 3.92 and the sludge SRF declined to 5.31×10^{10} m/kg after 72 h treatment, showing a significant difference to the control sludge. The process of bioleaching is a comprehensive biological and chemical process. Sulfuric acid addition and Fe^{2+} addition will not result in the decrease of SRF, indicating that the chemical acidification and Fe^{2+} addition are not the deciding factors for the improvement by bioleaching. The EPS content in bioleached sludge decreased dramatically, in which the protein and polysaccharide dropped by 97.42% and 76.00%, respectively. The reduction of EPS content in bioleached sludge is consistent with the SRF change. But the root cause of the change of EPS is the dominant bacterial genus (*A. ferrooxidans*) shift. During the bioleaching process, the number of microbial genuses in the bioleached sludge decreased gradually with the process of bioleaching. The dominant bacterial genus shifted from 7.48% to 26.49% at the end of bioleaching. The bio-substitution which lead to the reduction of EPS is the deciding factor for the improvement of dewaterability by bioleaching treatment.

Acknowledgement

This work was financially supported by Joint innovative R&D program of University and Industry (BY2014023-03), National Key Technology R&D Program of the Ministry of Science and Technology (2014BAD24B03-02), Fundamental Research Funds for the Central Universities in China (JUSRP51633B) and National Natural Science Foundation of China (51208231).

References

[1] M.T. Nguyen, N.H. Mohd Yasin, T. Miyazaki, et al., Enhancement of sludge reduction and methane production by removing extracellular polymeric substances from waste activated sludge, Chemosphere 117 (2014) 552–558.
[2] X. Feng, J. Deng, H. Lei, Dewaterability of waste activated sludge with ultrasound conditioning, Bioresour. Technol. 100 (2009) 1074–1081.
[3] A.H. Molla, A. Fakhru'l-Razi, Mycoremediation-a prospective environmental friendly technique of bioseparation and dewatering of domestic wastewater sludge, Environ. Sci. Pollut. Res. 19 (2012) 1612–1619.
[4] M.B. Huo, G.Y. Zheng, L.X. Zhou, Enhancement of the dewaterability of sludge during bioleaching mainly controlled by microbial quantity change and the decrease of slime extracellular polymeric substances content, Bioresour. Technol. 168 (2014) 190–197.
[5] F. Liu, L. Zhou, J. Zhou, Improvement of sludge dewaterability and removal of sludge-borne metals by bioleaching at optimum pH, J. Hazard. Mater. 221 (2012) 170–177.
[6] X. Song, L. Zhou, The influence of bioleaching on dewaterability of municipal sewage sludge, Acta Sci. Circumst. 28 (2008) 2012–2017.
[7] J.M. Fontmorin, M. Sillanpää, Bioleaching and combined bioleaching/Fenton-like processes for the treatment of urban anaerobically digested sludge: removal of heavy metals and improvement of the sludge dewaterability, Sep. Purif. Technol. 156 (2015) 655–664.
[8] G. Mercier, M. Chartier, D. Couillard, Strategies to maximize the microbial leaching of lead from metal-contaminated aquatic sediments, Water Res. 30 (1996) 2452–2464.
[9] N. Duan, C. Zhou, B. Chen, Bioleaching of Mn from manganese residues by the mixed culture of *Acidithiobacillus* and mechanism, J. Chem. Technol. Biotechnol. 86 (2011) 832–837.
[10] I.S. Kim, J.U. Lee, A. Jang, Bioleaching of heavy metals from dewatered sludge by *Acidithiobacillus ferrooxidans*, J. Chem. Technol. Biotechnol. 80 (2005) 1339–1348.
[11] D.A. Clark, P.R. Norris, *Acidimicrobium ferrooxidans* gen. nov., sp. nov.: mixed-culture ferrous iron oxidation with Sulfobacillus species, Microbiology 142 (1996) 785–790.
[12] D.B. Johnson, Biodiversity and ecology of acidophilic microorganisms, FEMS Microbiol. Ecol. 27 (1998) 307–317.
[13] J. Li, Effects of Fe(III) on floc characteristics of activated sludge, J. Chem. Technol. Biotechnol. 80 (2005) 313–319.
[14] L.H. Mikkelsen, K. Keiding, Physico-chemical characteristics of full scale sewage sludges with implications to dewatering, Water Res. 36 (2002) 2451–2462.
[15] X.Y. Li, S.F. Yang, Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge, Water Res. 41 (2007) 1022–1030.
[16] H. Nie, C. Yang, N. Zhu, Isolation of *Acidithiobacillus ferrooxidans* strain Z1 and its mechanism of bioleaching copper from waste printed circuit boards, J. Chem. Technol. Biotechnol. 90 (2014) 714–721.
[17] J.W. Morgan, C.F. Forster, L. Evison, A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges, Water Res. 24 (1990) 743–750.
[18] C. Wisniewski, A. Grasmick, Floc size distribution in a membrane bioreactor and consequences for membrane fouling, Colloids Surf., A 138 (1998) 403–411.
[19] Y. Arhan, I. Öztürk, T. Cifci, Settling and dewatering characteristics of sludge from baker's yeast production wastewater treatment, Water. Sci. Technol. 34 (1996) 459–467.
[20] K. Xu, H. Liu, J. Chen, Effect of classic methanogenic inhibitors on the quantity and diversity of archaeal community and the reductive homoacetogenic activity during the process of anaerobic sludge digestion, Bioresour. Technol. 101 (2010) 2600–2607.
[21] A.D. Kent, D.J. Smith, B.J. Benson, Web-based phylogenetic assignment tool for analysis of terminal restriction fragment length polymorphism profiles of microbial communities, Appl. Environ. Microbiol. 69 (2003) 6768–6776.
[22] A.P.H.A, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association/Water Environment Federation, Washington DC, USA, 1998.
[23] O.H. Lowry, N.J. Rosebrough, A.L. Farr, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[24] M. Dubois, K.A. Gilles, J.K. Hamilton, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356.
[25] A. Pathak, M. Dastidar, T. Sreekrishnan, Bioleaching of heavy metals from sewage sludge: a review, J. Environ. Manage. 90 (2009) 2343–2353.
[26] Y. Xiang, P. Wu, N. Zhu, Bioleaching of copper from waste printed circuit boards by bacterial consortium enriched from acid mine drainage, J. Hazard. Mater. 184 (2010) 812–818.
[27] F. Ye, X. Liu, Y. Li, Effects of potassium ferrate on extracellular polymeric substances (EPS) and physicochemical properties of excess activated sludge, J. Hazard. Mater. 199 (2012) 158–163.
[28] L. Wang, F. He, Q. Tong, Characterization of dewatering process of activated sludge assisted by cationic surfactants, Biochem. Eng. J. 91 (2014) 174–178.

[29] E. Neyens, J. Baeyens, M. Weemaes, Hot acid hydrolysis as a potential treatment of thickened sewage sludge, J. Hazard. Mater. 98 (2003) 275–293.
[30] Y. Chen, H. Yang, G. Gu, Effect of acid and surfactant treatment on activated sludge dewatering and settling, Water Res. 35 (2001) 2615–2620.
[31] M. Raynaud, J. Vaxelaire, J. Olivier, Compression dewatering of municipal activated sludge: effects of salt and pH, Water Res. 46 (2012) 4448–4456.
[32] Q. Yu, H. Lei, G. Yu, Influence of microwave irradiation on sludge dewaterability, Chem. Eng. J. 155 (2009) 88–93.
[33] J.I. Houghton, J. Quarmby, T. Stephenson, Municipal wastewater sludge dewaterability and the presence of microbial extracellular polymer, Water. Sci. Technol. 44 (2001) 373–379.
[34] S.S. Bala, S. Yan, R.D. Tyagi, R.Y. Surampalli, Extracellular polymeric substances (EPS) producing bacterial strains of municipal wastewater sludge: isolation, molecular identification, EPS characterization and performance for sludge settling and dewatering, Water Res. 44 (2010) 2253–2266.
[35] S. Yang, X. Li, Influences of extracellular polymeric substances (EPS) on the characteristics of activated sludge under non-steady-state conditions, Process Biochem. 44 (2009) 91–96.
[36] M. Huo, G. Zheng, L. Zhou, Enhancement of the dewaterability of sludge during bioleaching mainly controlled by microbial quantity change and the decrease of slime extracellular polymeric substances content, Bioresour. Technol. 168 (2014) 190–197.