Effect of using different proportions of inoculum during bioleaching on sludge dewaterability

Qingfang Zhang, Wenfeng Yang, Qiyong Yang, Tianfeng Wang, Shenliang Chen, Moliang Zou and Zengye Yang

ABSTRACT

Bioleaching, the addition of bacteria to geological materials, has been applied to sludge to remove metals and improve upon sludge dewaterability. This paper investigates the effect of using different quantities of inoculum (bacteria) during bioleaching on sludge dewaterability. The analysis was based on bioleaching experiments conducted in a 20 L bio-reactor using different quantities of inoculum (20%, 10%, 5%, 2%, 0%). Changes in pH, oxidation reduction potential (ORP), capillary suction time (CST), specific resistance to filtration (SRF) and extracellular polymeric substances (EPS) were determined to gauge sludge dewatering. Results indicate that sludge dewaterability during the 2%, 10%, and 20% inoculum experiments declined through time. Decreased dewaterability is attributed to increases in the quantity of proteins and polysaccharides in slime EPS. Dewaterability improved during the 5% inoculum experiment, and reached a maximum when pH was 2.3. During this latter experiment, CST and SRF were reduced by 74% and 62%, respectively, in comparison to control conditions, while total EPS content decreased by 71%. The decrease in total EPS was primarily due to a decrease in proteins associated with tightly bound EPS (TB-EPS). Thus, changes in the amount of proteins in TB-EPS and sludge pH played a crucial role in sludge dewaterability.

Key words | bioleaching, extracellular polymeric substances, sludge dewaterability

Qingfang Zhang
Wenfeng Yang
College of Petrochemical Engineering,
Lanzhou University of Technology,
Lanzhou Gansu, 730050,
China

Qiyong Yang (corresponding author)
Poyang Lake Eco-economy Research Center,
Jiujiang University,
Jiujiang Jiangxi, 332005,
China
E-mail: *yqy46901@163.com*

Qiyong Yang
Tianfeng Wang
Jiujiang Key Laboratory of Basin Management and Ecological Protection,
Jiujiang University,
Jiujiang Jiangxi, 332005,
China

Wenfeng Yang
Qiyong Yang
Tianfeng Wang
Shenliang Chen
Moliang Zou
Zengye Yang
College of Chemistry and Environmental Engineering,
Jiujiang University,
Jiujiang Jiangxi, 332005,
China

INTRODUCTION

In response to the recent, rapid growth in industrialization and population, wastewater treatment plants have produced a large amount of activated sludge. Traditional mechanical dewatering methods can only reduce the moisture content of sludge by about 20%. The high water content of the processed sludge cake makes it difficult and expensive for disposal by incineration or landfilling. It also hinders its re-use as a composting material (Nomeda *et al.* 2008). Thus, there is considerable interest in developing technologies that can enhance the dewatering of sludge (i.e., its dewaterability), thereby reducing the costs of its disposal and/or reuse.

Bioleaching refers to the inoculation of ores, sludge, and other geological materials with microorganisms (bacteria) to remove selected metals. Recent studies have shown that bioleaching can efficiently remove metals from sewage sludge while improving its dewaterability. The bacteria *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* play a significant role in bioleaching processes. *A. thiooxidans* produces H^+ by the bio-oxidation of S^0, a process that leads to a decrease in pH. Bioleaching, in comparison to many other physical and chemical methods, will not dramatically decrease the organic matter (OM), total nitrogen or total phosphorus content of the dewatered sludge cake, enhancing its use as compost (fertilizer) (Liu *et al.* 2012a). The potential use of bioleaching to enhance sludge dewaterability has also been suggested by other studies. Song & Zhou (2008), for example, have observed that the specific resistance of sludge to filtration

doi: 10.2166/wst.2018.234

(SRF) could be decreased from 1.83×10^9 m/kg to 0.39×10^9 m/kg after bioleaching. Biogenic flocculants produced by *A. ferrooxidans* have been used to improve the dewaterability of anaerobically digested sludge; the capillary suction time (CST) and SRF of sludge were decreased by 74% and 89%, respectively (Kurade *et al.* 2016).

A potential disadvantage of using bioleaching is that dissolved organic matter (DOM) in activated sludge negatively impacts the growth of *A. thiooxidans* (Gu & Wong 2004; Ren *et al.* 2009). For instance, Fang and Zhou demonstrated that the presence of DOM was toxic to microorganisms used in bioleaching when its concentration was higher than 150 mg DOC/L (the dissolved organic carbon (DOC) as representative of sludge DOM was determined by using a total organic carbon analyzer) (Fang & Zhou 2006, 2007). Hence, to improve such negative effects and enhance the efficiency of bioleaching, many acid-tolerant yeast strains have been increasingly employed in bioleaching to reduce DOM in the processed sludge, thus significantly improving the activities of *Acidithiobacillus* species (Zhou *et al.* 2013). In addition, the use of the filamentous fungus *Mucor circinelloides* ZG-3, which is capable of dissolving DOM, positively improves sludge dewaterability; sludge SRF was reduced by 25.9% under its optimum sequential inoculation with *M. circinelloides* ZG-3 and *A. thiooxidans* LX5 (Zheng *et al.* 2016).

Previous studies aimed at determining the mechanisms controlling the dewatering of bioleached sludge have shown that many factors influence the efficiency of sludge dewatering, including particle size, the quantity of extracellular polymeric substance (EPS), water content, and pH (Karr & Keinath 1978; Mikkelsen & Keiding 2002; Neyens & Baeyens 2003). Chen *et al.* (2001) found that sludge dewaterability was improved with a reduction in sludge pH; water content of the sludge reached its lowest value at pH 2.5. In addition, a reduction in pH and the death of microorganisms in the sludge both influence the amount of EPS in the sludge (Chen *et al.* 2001; Raynaud *et al.* 2012), the latter of which is a major factor impacting sludge dewaterability (Neyens *et al.* 2004). Wong *et al.* (2015) found that the concentration of Fe^{2+} when the sludge was inoculated with *Acidithiobacillus* could enhance its dewaterability. At optimum Fe^{2+} concentrations, there was a 96% and 88% reduction in CST and SRF, respectively. Total EPS was also reduced. Similarly, Liu *et al.* (2016) reported that sludge dewaterability was improved by bioleaching because it decreased its EPS content; protein and polysaccharide concentrations were reduced by 97.42% and 76.00%, respectively. Huo *et al.* (2014a) found that the dominant factors impacting sludge dewaterability were changes in microbial, slime EPS (S-EPS), and bound water contents. Their contributions to improved dewaterability were 32.50%, 22.37% and 24.24%, respectively. Kang *et al.* (1989) investigated the effect of the EPS content of sludge on dewatering by removing or adding the EPS. The result indicated that EPS could exert a negative impact on the dewatering process. Yang & Li (2009) revealed that EPS in sludge flocs exerted a significant influence on the dewaterability of sludge. Excessive EPS in the form of loosely bound EPS (LB-EPS) had a negative influence on sludge dewaterability. Sun *et al.* (2017) researched the influence of the quantity of inoculum used in bioleaching on sludge dewaterability, and found that using 10% inoculum was optimum for the dewaterability of sludge; SRF decreased by 81.82%, whereas the tightly bound EPS (TB-EPS) content decreased. The TB-EPS content correlated better with sludge dewaterability than did LB-EPS.

Despite the work conducted to date, bioleaching experiments remain in the laboratory stage. In addition, the results of previous studies defining the role of EPS on sludge dewaterability are often inconsistent, and need to be further understood. The objective of the present study was to investigate the effect of different proportions of inoculum on sludge dewaterability.

MATERIALS AND METHODS

Municipal sewage sludge sampling

Sewage sludge was collected from the Hewen Lake Wastewater Treatment Plant, Jiujiang City, Jiangxi Province, China. The plant receives mostly domestic sewage, although it also receives some industrial wastewater. Sludge pH, SRF and CST were determined immediately after sampling. Sludge solid content was measured after oven-drying at 105 °C for 2 h. The dry weight OM content of the sludge was measured according to APHA (2005). The characteristics of the raw sludge are shown in Table 1.

Table 1 | Characteristics of the raw sludge

Parameters	Value
pH	6.72 ± 0.02
Total solid content (%)	4.20 ± 0.02
CST (s)	40.10 ± 1.05
SRF ($\times 10^{11}$ m/kg)	8.47 ± 1.05
Organic matter content (%, dry weight)	28.00 ± 0.14

Microorganisms and the preparation of bioleaching inoculum

The *A. thiooxidans* JJU-1 (GenBank No. KM101109) used in this study was isolated from municipal sewage sludge in our previous study (Yang *et al.* 2015) and grown using Waksman medium. The medium was autoclaved at 121 °C for 15 min, and adjusted to pH 3.5–4.0 with 9 M H_2SO_4. It was then supplemented with 5 g/L elemental sulfur as the energy source. The inoculums were cultured in 500 mL Erlenmeyer flasks shaken at 180 rpm, and then held at a constant temperature of 28 °C for 3–4 d until the bacterial cell density reached ~10^8 cells/mL.

The *Rhodotorula mucilaginosa* JJU-2 bacterium was isolated from sludge during a previous study (Wang *et al.* 2010). *R. mucilaginosa* is capable of degrading sludge DOM and boosting the growth of A. *thiooxidans* JJU-1. The inoculums of R. *mucilaginosa* JJU-2 were grown in flasks containing potato dextrose agar at its optimal pH, which for this strain was 5 (although it can survive in the extremely low pH condition of 1) (Qiu *et al.* 2017). Flasks were shaken using a gyratory shaker at 28 °C and 120 rpm for 3–4 d until a cell density of 10^{12} cells/mL was reached.

Following completion of the above procedures, a volume of 2.1 L of A. *thiooxidans* JJU-1 inoculum and 700 mL of R. *mucilaginosa* JJU-2 inoculum were added to a 20 L automatic bioreactor containing 14.2 L of municipal sludge. The mixture was then supplemented with 8 g/L S^0, and an appropriate proportion of nutrients. The reaction was carried out at 28 °C, an agitator speed of 200 rpm and an aeration of 15 L/min until the sludge pH decreased to about 2.0. The above procedures were repeated three times. The bioleached sludge was then employed as bioleaching inoculum in the following bioleaching experiments.

Bioleaching experiments

Bioleaching using 20% inoculum was conducted in a 20 L automatic bioreactor containing 3.8 L acidified bioleaching inoculum, 12.2 L raw sludge, and 8 g/L S^0 as an energy source. The sludge was mixed by a continuous stirring system in the bioreactor at 200 rpm at a constant working temperature of 28 °C (maintained with an automated control system). The pH of the sludge was adjusted to about 4.8–5.0 with 2.0 mol/L H_2SO_4. During the incubation process, air was supplied at 15 L/min from the bottom of the bioreactor by an air compressor. After the sludge pH had dropped to about 2.0, the sludge was stored at 4 °C in a refrigerator for the next bioleaching experiments.

The 10%, 5%, and 2% inoculum experiments contained 1.6 L acidified bioleaching inoculum and 14.4 L raw sludge, 0.8 L acidified bioleaching inoculum and 15.2 L raw sludge, and 0.32 L acidified bioleaching inoculum and 15.68 L raw sludge, respectively. The other experimental conditions were identical to those used in the 20% experiment described above.

For the experimental control, 16 L of raw sludge and 8 g/L S^0 were added to the bioreactor; no acidified bioleaching inoculant was added. The other experimental conditions were identical to those used for the other experiments.

During each of the above bioleaching experiments, 600 mL of material was collected from the bioreactor at 24 h intervals, after which pH, oxidation reduction potential (ORP), CST, SRF, and SO_4^{2-} concentrations were determined along with S-EPS, LB-EPS, and TB-EPS contents according to the methods described below.

Analytical methods

Both pH and ORP were determined using a pHS-3C digital pH-meter with the Pt-Ag/AgCl electrode system. Dewaterability was determined through CST using a capillary suction timer (Model 304 M CST, Triton, UK), and SRF was determined using the Buchner funnel test (Lo *et al.* 2001). Moisture content of the filter cake was measure by oven-drying at 105 °C for 2 h. The dried digestate sample was then measured for OM content according to APHA (2005).

EPS extraction and chemical analysis

A heat extraction method was modified to extract different EPS fractions from the activated sludge (Li & Yang 2007). First, a 100 mL sludge sample was collected and placed into a flask. The samples were then washed twice with a 0.05% (w/w) NaCl solution brought to its original volume of 100 mL. Sixty millilitres of the sludge sample was subsequently centrifuged at 600 rpm for 15 min. The resulting sludge supernatant was collected as S-EPS; the precipitant remaining in the tube was used for additional EPS layer extractions. The solution of 0.05% NaCl was prepared. The sludge pellet in the tube was then resuspended into 60 mL of 0.05% NaCl to its original volume of 60 mL and treated by ultrasound at 20 kHz and 40 W for 2 min. The aliquot was centrifuged at 9,000 rpm for 20 min to separate solids and supernatant. The collected supernatant was regarded as the LB-EPS. Finally, the residual sludge pellet left in the centrifuge tube was re-suspended in a 0.05% NaCl solution to its original volume of 60 mL, then heated

at 80 °C for 30 min, and finally centrifuged at 20,000 rpm for 20 min to collect the TB-EPS. The supernatant samples were filtered through a 0.22 μm membrane before chemical analysis.

Polysaccharide was measured by the anthrone method (Gaudy 1962) using glucose as the standard. The protein content was determined with a modified Lowry method using bovine serum albumin as a standard (Frolund *et al.* 1995). Lastly, the DNA content was determined with the diphenylamine colorimetric method using sodium DNA as a standard (Sun *et al.* 2009).

RESULTS AND DISCUSSION

Variations in pH and ORP as a function of treatment time

As shown in Figure 1, pH decreased and ORP increased at different rates when different proportions of inoculum were used during the bioleaching experiments. More specifically, the data indicate that *Acidithiobacilli* bacteria have a higher activity, which is consistent with previous research (Liu *et al.* 2012b).

In the control experiment (Figure 1(a)), pH decreased slowly from 4.97 to 3.36 and ORP increased from 110 mV to 198 mV at day 4. These trends probably resulted from the oxidation of S^0 by oxygen in the circulating air and the residual *Acidithiobacilli* bacteria in the sludge. In the 20% inoculum experiment (Figure 1(b)), pH rapidly decreased from 4.8 to 0.76, while the value of ORP increased from 125 mV to 346 mV during the 4 d bioleaching treatment. When the proportion of inoculum was reduced to 10%, 5%, and 2%, the rate of bioleaching slowed. For instance, during the 10% inoculum experiment (Figure 1(c)), pH declined from 4.92 to 1.56, during which the value of ORP increased from 118 mV to 300 mV at day 4. In the 5% inoculum experiment (Figure 1(d)), pH and ORP changed from 5.05 and 108 mV to 1.74 and 291 mV, respectively, after 4 d of bioleaching. During the 2% inoculum experiment (Figure 1(e)), pH and ORP changed from 4.97 and 110 mV to 2.52 and 253 mV, respectively, after 4 days of bioleaching.

With increases in the proportions of inoculum, the amount of *A. thiooxidans* was significantly increased, and the rate at which pH decreased and ORP increased was drastically accelerated. These differences may be attributed to the generation of sulfuric acid that varied in response to

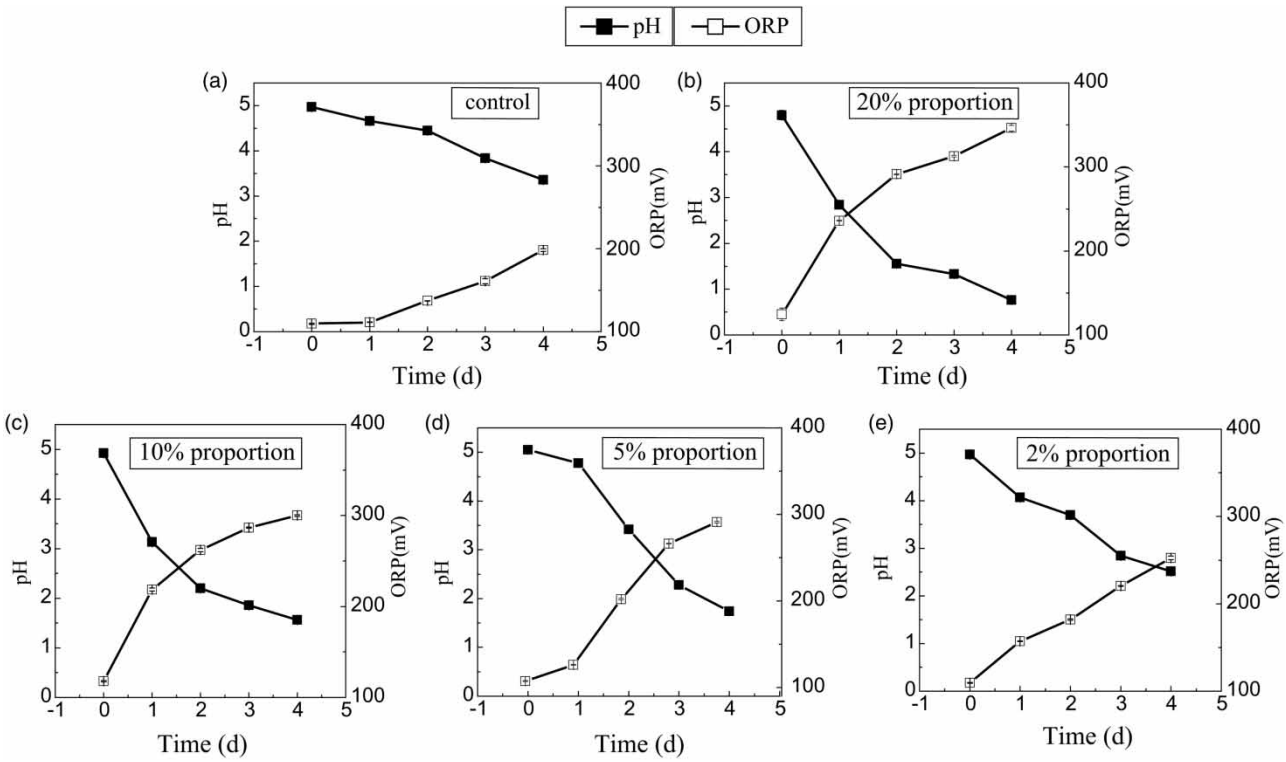

Figure 1 | Comparison of variations in pH and ORP with treatment time between control experiment and experiments using different proportions of inoculum during bioleaching.

differences in the rate of microbial oxidation of elemental sulfur (Pathak *et al.* 2009).

Variations in CST and SRF as a function of treatment time

CST and SRF are widely used parameters to monitor the dewaterability of sludge. Many studies show that longer CST or higher SRF values are indicative of poor sludge dewaterability (Feng *et al.* 2009).

As shown in Figure 2(a), CST and SRF increased from 46.5 s and 0.78×10^{12} m/kg to 290.0 s and 3.79×10^{12} m/kg, respectively, after 4 d in the control experiment, indicating that sludge dewaterability deteriorated. For the bioleached sludge, the proportion of inoculum used exerted an important influence on the dewaterability of sludge. More specifically, during the 20% inoculum experiment (Figure 2(b)), CST and SRF rapidly increased from 153.2 s and 1.19×10^{12} m/kg to 885.4 s and 10.64×10^{12} m/kg, respectively, at day 4. Similarly, during the 10% inoculum experiment (Figure 2(c)), CST and SRF drastically increased from 90.9 s and 1.22×10^{12} m/kg to 364.9 s and 4.08×10^{12} m/kg, respectively, at day 4. During both of these experiments, CST and SRF unexpectedly increased, indicating that sludge dewaterability deteriorated. In addition, the rate of CST and SRF increase during the 20% inoculum experiment was faster than during the 10% experiment.

As shown in Figure 2(d), during the 5% inoculum experiment, CST and SRF decreased from 151.0 s and 2.60×10^{12} m/kg to 65.0 s and 1.00×10^{12} m/kg, respectively, at 48 h. Subsequently, and unexpectedly, CST and SRF increased to 82.8 s and 5.79×10^{12} m/kg, respectively, at 24 h. The lowest value of CST and SRF was 65.0 s and 1.00×10^{12} m/kg, respectively, at which the corresponding pH was approximately 2.3 (Figure 1(d)). The observed patterns demonstrate that bioleaching using 5% inoculum was optimal for improving the dewaterability of sludge. Moreover, there was an optimum pH (~2.3) to achieve the highest degree of dewaterability of the bioleached sludge. Sludge characterized by the highest degree of dewaterability exhibited a 74% and 62% reduction in CST and SRF, respectively, as compared to the control within 3 days of bioleaching.

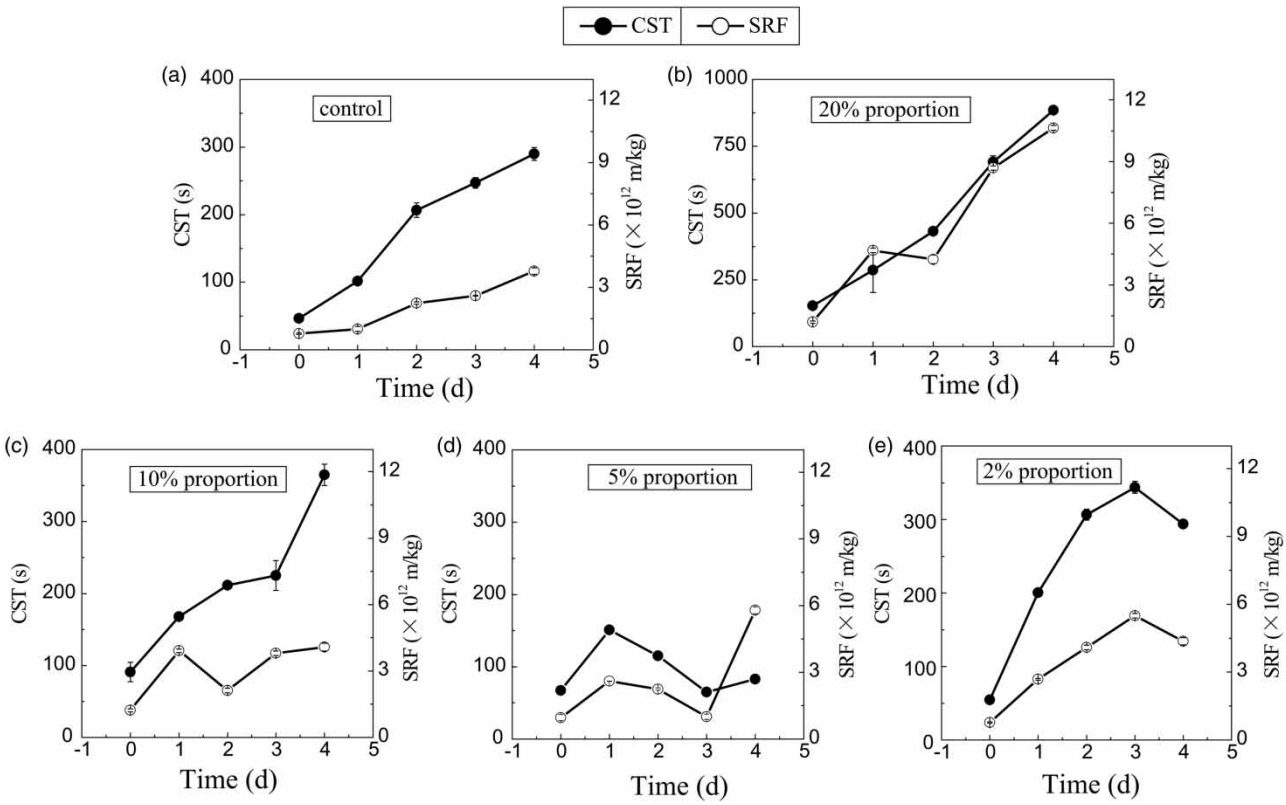

Figure 2 | Comparison of the variations in CST and SRF with treatment time between the control experiment and the experiments using different proportions of inoculum during bioleaching.

Figure 2(e) shows that during the 2% inoculum experiment, CST and SRF increased from 54.6 s and 0.78×10^{12} m/kg to 343.8 s and 5.50×10^{12} m/kg, respectively, at day 3. During this time, pH declined from 4.97 to 2.87 (Figure 1(e)). CST and SRF then rapidly decreased to 293.7 s and 4.37×10^{12} m/kg at 24 h, respectively, at which time the pH was 2.5 (Figure 1(e)). Data collected during the 2% inoculum experiment show that the dewaterability of the sludge was inferior to the sludge treated with 5% inoculum.

The combined results indicate that a significant difference exists between the control and the bioleached sludge. Bioleaching using 5% inoculum achieved optimum results with regards to sludge dewaterability; CST and SRF were reduced by 74% and 62%, respectively, as compared with the control within a period of 3 d. The data also demonstrated that a pH of 2.3 was optimum for enhanced dewaterability of sludge. When sludge pH declined to below 2.3, sludge dewaterability deteriorated, suggesting that excessive bioleaching time negatively affected sludge dewatering, a finding consistent with previous analyses (Feng *et al.* 2009; Huo *et al.* 2014b).

Variations in the content and fractionations of sludge EPS with treatment time

Sludge EPS is composed of functional groups, such as hydroxyl, that add to the repulsion between flocs (Merrylin *et al.* 2013a) and collect a massive amount of bound water (Raynaud *et al.* 2012). The role of EPS on sludge dewaterability has been widely studied and has been found to significantly impact the dewatering characteristics of sludge (Murugesan *et al.* 2016). However, the relationship between EPS content and sludge dewatering remains controversial (Huo *et al.* 2014a; Murugesan *et al.* 2016). In order to investigate the effect of EPS content on sludge dewaterability, changes in the quantity of total EPS and different EPS fractions were determined for the control experiment as well as for the experiments using different proportions of inoculum during bioleaching.

As shown in Figure 3(a), in the control experiment, sludge dewaterability deteriorated as both the S-EPS and the LB-EPS content slightly increased. In contrast, the TB-EPS content decreased. During the 20% inoculum

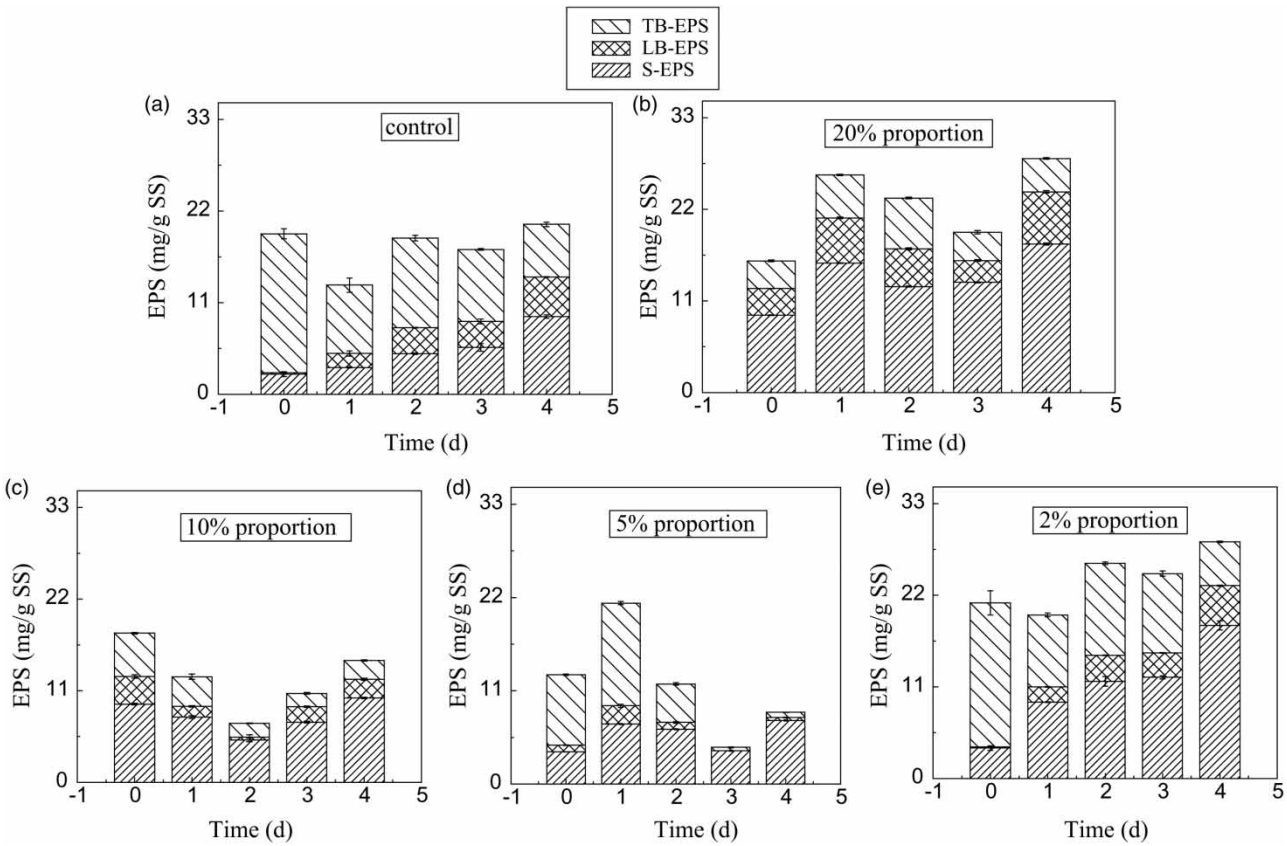

Figure 3 | Comparison of the variations in the total and fractionation of sludge EPS with treatment time between the control experiment and the experiments using different proportions of inoculum during bioleaching.

experiment, sludge dewaterability deteriorated, the total EPS content increased, and S-EPS rapidly increased from 9.29 mg/g SS to 17.83 mg/g SS at day 4 (Figure 3(b)). During the 10% inoculum experiment, sludge dewaterability decreased further, the S-EPS content, which comprised most of the total EPS, dropped from 9.39 mg/g SS to 5.1 mg/g SS at day 2, before increasing to 10.13 mg/g SS (Figure 3(c)). Given the above, it appears that S-EPS content was the dominant factor controlling the deterioration of sludge dewaterability. It is likely that acid-tolerant microorganisms generated large amounts of stress resistance protein to protect themselves from the low pH environment (Cabiscol *et al.* 2010), thus leading to the following increase of total EPS content.

It was remarkable that variations in different fractions of EPS during the 5% inoculation experiment differed from the 20% and 10% inoculation experiments. Interestingly, total EPS content during the 5% experiment decreased from 21.78 mg/g SS to 6.27 mg/g SS at 3 d, and then increased to 8.37 mg/g SS at 4 d (Figure 3(d)). The lowest value corresponds to a pH of approximately 2.3 (Figure 1(d)). Variations in total EPS content appear to have been synchronized with CST and SRF (Figure 2(d)). Notably, at 48 h of leaching, the content of TB-EPS comprised the majority of the total EPS, the latter of which dropped from 12.10 mg/g SS to 0.38 mg/g SS. Then, when sludge pH dropped below 2.3, sludge dewaterability deteriorated as the content of S-EPS increased and become the predominant component of total EPS (end of day 3). Thus, the decrease in TB-EPS content was the dominant factor controlling the improvement of sludge dewaterability. It is possible that the decrease is due to abscission and decomposition of EPS from the surface of the sludge as it is affected by acidification (Chen *et al.* 2001). The observation that S-EPS increased at end of day 3 and led to the deterioration of sludge dewaterability during the 5% inoculum experiment was consistent with the 20% and 10% inoculum experiments. The observed changes are probably due to the secretion of EPS by *Acidithiobacillus* sp. to protect themselves against the exceedingly low pH environment (pH <2.3).

As shown in Figure 3(e), during the 2% inoculum experiment, total EPS content increased from 22.50 mg/g SS to 28.43 mg/g SS at 4 d, decreasing the dewaterability of sludge. The increase in total EPS was mainly due to an increase in S-EPS content from an initial of 3.66 mg/g SS to 18.38 mg/g SS after 4 d of treatment. S-EPS was the predominant component of total EPS. It is noteworthy that the content of TB-EPS rapidly dropped from 9.49 mg/g SS to 5.29 mg/g SS at 3–4 d, during which the dewaterability of sludge improved and pH was 2.5 (Figure 2(e)).

These results indicate that a decrease in TB-EPS content and sludge pH exerted a significant impact on sludge dewaterability as demonstrated by a decrease in CST and SRF. Moreover, the amount of S-EPS present was the dominant factor controlling the deterioration of sludge dewaterability: the higher S-EPS content, the less the sludge could be dewatered.

Variations in different EPS fractionations with treatment time

To compare the effect of different fractions of EPS on the dewaterability of sludge, protein, polysaccharides and DNA were measured every 24 h during the control experiment and experiments using different proportions of inoculum. As shown in Figure 4(a), during the control treatment, the quantity of proteins, polysaccharides and DNA in S-EPS increased slightly, whereas the amount of proteins in TB-EPS had decreased by day 4. During the 20% inoculum experiment, the amount of proteins, polysaccharides and DNA in S-EPS increased from 2.31 mg/g SS, 1.36 mg/g SS and 5.61 mg/g SS to 4.79 mg/g SS, 5.18 mg/g SS and 7.83 mg/g SS by day 4, respectively (Figure 4(b)). Changes in DNA are linked to the death and lysis of bacteria, processes that release DNA to the sludge. It was apparent that the increase of proteins and polysaccharides in S-EPS had a significant impact on the deterioration of sludge dewaterability. Figure 4(c) shows that during the 10% inoculum experiment, the results had some similarities to the 20% inoculum experiment; more specifically, increases in protein and polysaccharide content within S-EPS were major contributors to the deterioration in sludge dewaterability. In fact, these results demonstrate that the increase of proteins and polysaccharides in S-EPS was the dominant factor controlling the deterioration of sludge dewaterability, which was mainly due to the death and lysis of bacteria that probably released intracellular materials such as protein, carbohydrate, lipids, DNA and RNA (Merrylin *et al.* 2013b).

During the 5% inoculum experiment, protein content, as a major component of TB-EPS, exhibited a tremendous reduction of 97%, declining from 10.35 mg/g SS to 0.32 mg/g SS (Figure 4(d)). This decline exerted an important control on improvements of sludge dewaterability. It could be due to the decomposition of sludge EPS components by enzymes, such as protease, amylase, DNase, and RNase (Esakki *et al.* 2013; Kavitha *et al.* 2013), and

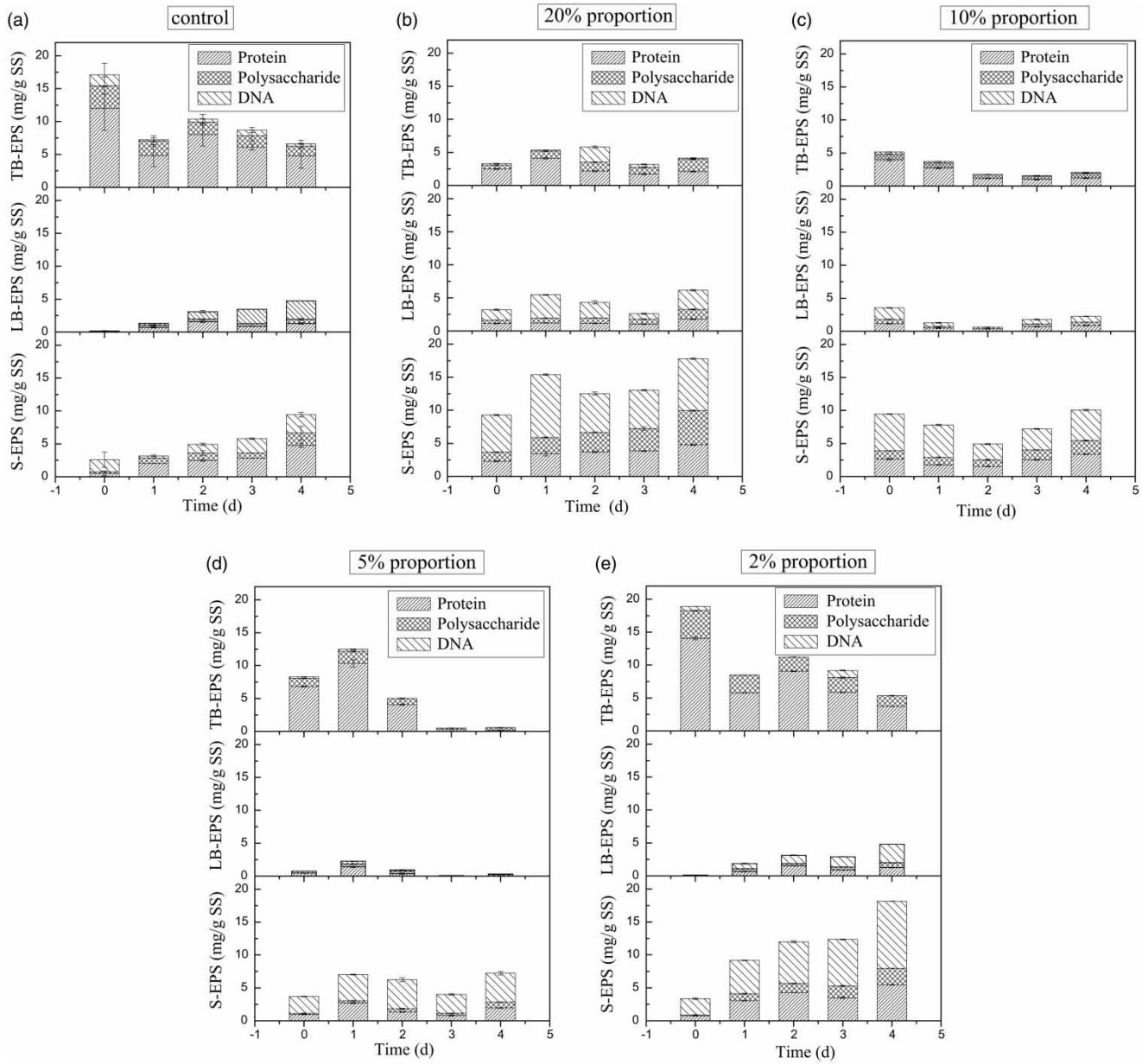

Figure 4 | Variations in different EPS fractions (protein, polysaccharides and DNA) with treatment time during the control experiment and the experiments using different proportions of inoculum during bioleaching.

the consumption and biodegradation of EPS by acid-tolerant microorganisms (Rani *et al.* 2012; Yang *et al.* 2015). Furthermore, the increase in DNA content in S-EPS demonstrated that the death and lysis of bacteria occurred during bioleaching, which may also help improve sludge dewaterability. In addition, as sludge dewaterability decreased during later leaching as sludge pH declined below 2.3 (Figures 1(e) and 2(e)), the increase in S-EPS content became the major component of total EPS. This phenomenon was likely due to the generation of stress resistance proteins by some acid-tolerant microorganisms present within the extremely low pH environment (Cabiscol *et al.* 2010; Zhu *et al.* 2012).

As shown in Figure 4(e), during the 2% inoculum experiment, the increase of DNA demonstrated that death and lysis of bacteria occurred during bioleaching. In addition, protein and polysaccharide contents in S-EPS also increased, and exerted an important influence on the deterioration of sludge dewaterability. This was consistent with results from the 20% and 10% inoculum treatments.

Given the above, sludge pH and decreases in protein content within TB-EPS had a significant impact on improvements in sludge dewaterability. Increases in proteins and polysaccharides in S-EPS were the dominant factors controlling the deterioration of sludge dewaterability. Change in EPS is mainly controlled by changes in microbial quantity.

CONCLUSION

Different proportions of inoculum have a significant impact on the bio-oxidation rates of S^0 and bio-acidification during bioleaching. Optimum enhancements in sludge dewaterability were observed during the 5% inoculum experiment during which CST and SRF significantly declined during bioleaching. The enhancement of sludge dewaterability was related to sludge pH; a pH of 2.3 resulted in the largest improvements in sludge dewaterability. Enhancements in sludge dewaterability were also linked to a reduction in proteins within TB-EPS. Furthermore, an increase in proteins and polysaccharides in S-EPS exerted a significant impact on the deterioration of sludge dewaterability. Changes in the composition of EPS were linked to the quantity (proportion) of inoculum used in bioleaching.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (21367014, 51741805), Natural Science Foundation of Jiangxi Province, China (20151BAB203026), and Science and Technology Program of Education Department of Jiangxi Province, China (GJJ14732).

REFERENCES

APHA 2005 *Standard Methods for the Examination of Water and Wastewater*, 21st edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.

Cabiscol, E., Tamarit, J. & Ros, J. 2010 Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int. Microbiol.* **3** (1), 3–8.

Chen, Y., Yang, H. & Gu, G. 2001 Effect of acid and surfactant treatment on activated sludge dewatering and settling. *Water Res.* **35** (11), 2615–2620.

Esakki, R. S., Rajesh, B. J., Kaliappan, S., Yeom, I. T. & Adish, K. S. 2013 Effects of side-stream, low temperature phosphorus recovery on the performance of anaerobic/anoxic/oxic systems integrated with sludge pretreatment. *Bioresour. Technol.* **140**, 376–394.

Fang, D. & Zhou, L. X. 2006 Effect of sludge dissolved organic matter on oxidation of ferrous iron and sulfur by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. *Water Air Soil Pollut.* **171** (1–4), 81–94.

Fang, D. & Zhou, L. X. 2007 Enhanced Cr bioleaching efficiency from tannery sludge with coinoculation of *Acidithiobacillus thiooxidans* TS6 and *Brettanomyces* B65 in an air-lift reactor. *Chemosphere* **69** (2), 303–310.

Feng, X., Deng, J. C., Lei, H. Y., Bai, T., Fan, Q. J. & Li, Z. X. 2009 Dewaterability of waste activated sludge with ultrasound conditioning. *Bioresour. Technol.* **100** (3), 1074–1081.

Frolund, B., Griebe, T. & Nielsen, P. H. 1995 Enzymatic-activity in the activated sludge floc matrix. *Appl. Microbiol. Biotechnol.* **43** (4), 755–761.

Gaudy, A. 1962 Colorimetric determination of protein and carbohydrate. *Ind. Water Wastes* **7**, 17–22.

Gu, X. & Wong, J. W. 2004 Identification of inhibitory substances affecting bioleaching of heavy metals from anaerobically digested sewage sludge. *Environ. Sci. Technol.* **38** (10), 2934–2939.

Huo, M. B., Zheng, G. & Zhou, L. X. 2014a 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**, 190–197.

Huo, M. B., Zheng, G. Y. & Liang, J. R. 2014b Influence of microbial flora and extracellular plyometric substances on municipal sewage sludge dewaterability enhanced by bioleaching process. *J. Acta Sci. Circumstantiae* **34** (9), 2199–2204 (in Chinese).

Kang, S. M., Kishimoto, M., Shioya, S., Yoshida, T., Suga, K. & Taguchi, H. 1989 Dewatering characteristics of activated sludge and effect of extracellular polymer. *J. Ferment. Bioeng.* **68** (2), 117–122.

Karr, P. R. & Keinath, T. M. 1978 Influence of particle size on sludge dewaterability. *J. Water Pollut. Control Fed.* **50** (8), 1911–1930.

Kavitha, S., Kumar, S. A., Yogalakshmi, K. N., Kaliappan, S. & Rajesh, B. J. 2013 Effect of enzyme secreting bacterial pretreatment on enhancement of aerobic digestion potential of waste activated sludge interceded through EDTA. *Bioresour. Technol.* **150**, 210–219.

Kurade, M. B., Murugesan, K., Selvam, A., Yu, S. M. & Wong, J. W. C. 2016 Sludge conditioning using biogenic flocculant produced by *Acidithiobacillus ferrooxidans* for enhancement in dewaterability. *Bioresour. Technol.* **217**, 179–185.

Li, X. Y. & Yang, S. F. 2007 Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Res.* **41** (5), 1022–1030.

Liu, F. W., Zhou, J., Wang, D. Z. & Zhou, L. X. 2012a Enhancing sewage sludge dewaterability by bioleaching approach with comparison to other physical and chemical conditioning methods. *J. Environ. Sci.* **24** (8), 1403–1410.

Liu, F. W., Zhou, L. X., Zhou, J., Song, X. W. & Wang, D. Z. 2012b Improvement of sludge dewaterability and removal of sludge-borne metals by bioleaching at optimum pH. *J. Hazard. Mater.* **221–222** (30), 170–177.

Liu, H., Yang, S. & Shi, J. S. 2016 Towards understanding the dewatering mechanism of sewage sludge improved by bioleaching processing. *Sep. Purif. Technol.* **165** (13), 53–59.

Lo, I. M. C., Lai, K. C. K. & Chen, G. H. 2001 Salinity effect on mechanical dewatering of sludge with and without chemical conditioning. *Environ. Sci. Technol.* **35** (23), 4691–4696.

Merrylin, J., Kumar, S. A., Kaliappan, S., Yeom, I. T. & Banu, J. 2013a Biological pretreatment of non-flocculated sludge augments the biogas production in the anaerobic digestion of the pretreated waste activated sludge. *Environ. Technol.* **34**, 2113–2123.

Merrylin, J., Kumar, S. A., Kaliappan, S., Yeom, I. T. & Banu, J. R. 2013b Biological pretreatment of non-flocculated sludge augments the biogas production in the anaerobic digestion of the pretreated waste activated sludge. *Environ. Technol.* **34** (13–14), 2113–2123.

Mikkelsen, L. H. & Keiding, K. 2002 Physico-chemical characteristics of full scale sewage sludges with implications to dewatering. *Water Res.* **36** (10), 2451–2462.

Murugesan, K., Ravindran, B., Selvam, A., Kurade, M. B., Yu, S. & Wong, J. W. C. 2016 Fate of extracellular polymeric substances of anaerobically digested sewage sludge during pre-dewatering conditioning with *Acidithiobacillus ferrooxidans* culture. *Bioresour. Technol.* **217**, 173–178.

Neyens, E. & Baeyens, J. 2003 A review of thermal sludge pre-treatment processes to improve dewaterability. *J. Hazard. Mater.* **98** (1–3), 51–67.

Neyens, E., Baeyens, J. & Dewil, R. 2004 Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering. *J. Hazard. Mater.* **106** (2–3), 83–92.

Nomeda, S., Valdas, P., Chen, S. Y. & Lin, J. G. 2008 Variations of metal distribution in sewage sludge composting. *Waste Manage.* **28** (9), 1637–1644.

Pathak, A., Dastidar, M. & Sreekrishnan, T. 2009 Bioleaching of heavy metals from sewage sludge: a review. *J. Environ. Manage.* **90** (8), 2343–2353.

Qiu, X. W., Zhou, G. X. & Yang, Q. Y. 2017 Isolation, identification and heavy metal tolerance of an acidophilic heterotrophic microorganism. *Environ. Sci. Technol.* **40** (1), 64–69 (in Chinese).

Rani, R. U., Kumar, S. A., Kaliappan, S., Yeom, I. T. & Rajesh, B. J. 2012 Low temperature thermo-chemical pretreatment of dairy waste activated sludge for anaerobic digestion process. *Bioresour. Technol.* **103**, 415–424.

Raynaud, M., Vaxelaire, J., Olivier, J., Dieude-Fauvel, E. & Baudez, J. C. 2012 Compression dewatering of municipal activated sludge: effects of salt and pH. *Water Res.* **46** (14), 4448–4456.

Ren, W. X., Li, P. J., Zheng, L., Fan, S. X. & Verhozina, V. A. 2009 Effects of dissolved low molecular weight organic acid on oxidation of ferrous iron by *Acidithiobacillus ferrooxidans*. *J. Hazard. Mater.* **162** (1), 17–22.

Song, X. W. & Zhou, L. X. 2008 The influence of bioleaching on dewaterability of municipal sewage sludge. *Acta Sci. Circumstantiae* **28**, 2012–2017.

Sun, X., Wang, S., Zhang, X., Paul, C. J., Li, X., Gao, B. & Ma, Y. 2009 Spectroscopic study of Zn^{2+} and Co^{2+} binding to extracellular polymeric substances (EPS) from aerobic granules. *J. Colloid Interf. Sci.* **335** (1), 11–17.

Sun, Q., Shi, M. Y., Liu, H. F., Jian, G. D., Huang, F. D. & Huang, L. F. 2017 Influence of bioleaching in the amounts of inoculum on dewaterability of the channel sediment. *J. Shanxi Univ. (Nat. Sci. Ed.)* **40** (1), 195–200 (in Chinese).

Wang, S., Zheng, G. & Zhou, L. 2010 Heterotrophic microorganism *Rhodotorula mucilaginosa* R30 improves tannery sludge bioleaching through elevating dissolved CO_2 and extracellular polymeric substances levels in bioleach solution as well as scavenging toxic DOM to *Acidithiobacillus* species. *Water Res.* **44** (18), 5423–5431.

Wong, J. W. C., Zhou, J. & Kurade, M. B. 2015 Influence of ferrous ions on extracellular polymeric substances content and sludge dewaterability during bioleaching. *Bioresour. Technol.* **179**, 78–83.

Yang, S. F. & Li, X. Y. 2009 Influences of extracellular polymeric substances (EPS) on the characteristics of activated sludge under non-steady-state conditions. *Process Biochem.* **44** (1), 91–96.

Yang, Q. Y., Qiu, Q. W. & Cheng, P. F. 2015 Isolation, identification of an *Acidithiobacillus thiooxidan* strain and its characteristic of acid production. *J. Ecol. Environ. Sci.* **24** (8), 1366–1374 (in Chinese).

Zheng, G. Y., Wang, Z. Y., Wang, D. Z. & Zhou, L. X. 2016 Enhancement of sludge dewaterability by sequential inoculation of filamentous fungus *Mucor circinelloides* ZG-3 and *Acidithiobacillus ferrooxidans* LX5. *Chem. Eng. J.* **284**, 216–223.

Zhou, J., Zheng, G. Y., Wong, J. W. C. & Zhou, L. X. 2013 Degradation of inhibitory substances in sludge by *Galactomyces* sp. Z3 and the role of its extracellular polymeric substances in improving bioleaching. *Bioresour. Technol.* **132**, 217–223.

Zhu, H. F., Zhou, L. X. & Wang, D. Z. 2012 Effect of acidification on the dewaterability of sewage sludge in bioleaching. *Environ. Sci.* **33** (3), 916–921 (in Chinese).

First received 4 January 2018; accepted in revised form 14 May 2018. Available online 5 June 2018