The influence of different sludge concentrations on its dewaterability during bioleaching

Wenfeng Yang, Liyuan Zeng, Weihao Zhang, Qi Yong Yang, Tianfeng Wang and Houfeng Xiong

ABSTRACT

Bioleaching, a technologically and economically feasible technology, is considered as the high efficiency method to improve dewaterability in sewage sludge. The objective of this study was to investigate the effect of different sludge concentrations on bioleaching dewaterability and understand the mechanism of the effect of bioleaching on sludge dewaterability. Variation in pH, oxidation-reduction potential (ORP), capillary suction time (CST), specific resistance to filtration (SRF) and different fractions of extracellular polymeric substances (EPS) including slime EPS (S-EPS), loosely bound EPS (LB-EPS), and tightly bound EPS (TB-EPS) were determined. Different sludge concentrations (5, 10, 15, 20 and 30 g·L⁻¹) were selected to investigate during bioleaching. Results indicated that sludge buffering capacity significantly inhibited bioleaching efficiency as sludge concentrations increased. Optimum enhancements in sludge dewaterability were observed during the 10 g·L⁻¹ sludge concentration treatment, and reached a maximum when the pH was 2.11. The variation of different fractions of EPS revealed that the ratio of S-EPS/TB-EPS significantly affected sludge dewaterability. Principal component analysis and Pearson’s correlation analysis both provided evidence that the higher TB-EPS followed by a very large reduction was positively correlated with sludge dewatering. However, the increase of protein and DNA in S-EPS content was negatively correlated with sludge dewaterability.

Key words | bioleaching, extracellular polymeric substances, sludge buffering capacity, sludge concentration, sludge dewaterability

HIGHLIGHTS

- Sludge buffering capacity significantly inhibited bioleaching efficiency as sludge concentrations increased.
- The higher protein in tightly bound EPS (TB-EPS) followed by a very large reduction was positively correlated with sludge dewatering.
- Increasing protein and DNA in slime EPS (S-EPS) was negatively correlated with sludge dewatering.
- The ratio of S-EPS/TB-EPS significantly affected sludge dewaterability.
INTRODUCTION

In recent years, due to rapid socioeconomic development, industrial and municipal wastewaters have increased enormously, resulting in large amounts of activated sludge being produced in wastewater treatment plants. Sludge management and disposal have gradually become the major aspect of waste treatment. For instance, Liu et al. (2021a) reported that the annual production of dry sewage sludge in 3,080 municipal wastewater treatment plants were approximately 6 million tons in China. Well-known sequential sludge disposal techniques include thickening, stabilization, conditioning and dewatering (Zhou et al. 2014). However, the limitation of mechanical dewatering is that the water content of sludge continues to be around 20 percent, thus leading to inefficiency, wasteful energy consumption and expensive disposal by incineration or landfilling (Chen et al. 2003; Nomeda et al. 2008). Therefore, there is considerable interest in improving the dewaterability of sludge and achieving an efficient, energy-saving and economical dewatering technology (Cai et al. 2018), which would be beneficial to reducing the problem of sludge disposal.

Bioremoval, using Acidithiobacillus species to treat sewage sludge, removes heavy metals from sludge and enhances sludge dewaterability. The heavy metals in sludge would have a terrible impact on human health if they entered the drinking water source (Dippong et al. 2017, 2019). Bioremoval is also used for sewage sludge dewatering. The bacteria A. thiooxidans and A. ferrooxidans are important in bioremoval. Specifically, elemental sulfur (S0) was bio-oxidized by A. thiooxidans, resulting in the plentiful production of H+ and rapidly decreasing in pH. Bioremoval does not significantly reduce the content of organic matter, total nitrogen, and total phosphorus in dewatering sludge cake, compared with other physical and chemical dewatering technologies (Liu et al. 2012b). However, bioremoval has the obvious advantage of economy, no secondary pollution and recyclability in dewatering sludge. Therefore, previous researchers have reported the positive prospects of bioremoval for improving sludge dewaterability. For instance, Song & Zhou (2008) found that the specific resistance to filtration (SRF) of sludge rapidly dropped from $1.83 \times 10^9 \text{ m} \cdot \text{kg}^{-1} \cdot \text{C}_0$ to $0.39 \times 10^9 \text{ m} \cdot \text{kg}^{-1} \cdot \text{C}_0$ during bioremoval. Furthermore, Kurade et al. (2016) also reported that the dewaterability of anaerobically digested sludge was significantly enhanced by using the biogenic flocculants produced by A. ferrooxidans; the maximum reduction of capillary suction time (CST) and SRF of sludge were 74 and 89%, respectively.

Sludge concentration is one of the major factors that affect bio-efficiency and sludge dewaterability in leaching. For example, Sreekrishnan et al. (1995) investigated the effect of sludge concentration on regulating acid production during bioremoval and observed that the rate of sludge pH reduction is directly related to sludge concentration of the system. Chen & Lin (2004) researched the relationship between sludge concentration and metal solubilization in bioremoval. They showed that the rate of pH reduction, oxidation-reduction potential (ORP) increase and metal solubilization were reduced with increasing sludge concentration dose. In addition, Song et al. (2012) found that there was a significant difference in SRF under different sludge concentrations during the same bioremoval period: SRF increased with higher sludge
concentration. Moreover, Yeneneh et al. (2016) investigated the relationship between sludge dewaterability and total solids concentration. The result indicated that lower total solids concentration positively improved sludge dewaterability.

Previous researchers have also paid much attention to the role of extracellular polymeric substance (EPS) on sludge dewaterability and have demonstrated that EPS had a significant impact on dewatering characteristics of sludge (Murugesan et al. 2016). It is common knowledge that EPS is mainly composed of slime EPS (S-EPS), loosely bound EPS (LB-EPS), and tightly bound EPS (TB-EPS) (Li et al. 2013). Yeneneh et al. (2016) investigated the influence of EPS on sludge dewaterability by adding or eliminating EPS during bio-leaching; their results revealed that EPS had a negative influence on sludge dewatering. Yang & Li (2009) found that excessive EPS, mainly LB-EPS, had a negative effect on sludge dewaterability. So this previous research indicates that the role of EPS on sludge dewaterability is inconsistent. Hence, this study focuses on investigating the influence of different sludge concentrations on its dewaterability and the role of different fractions of EPS on sludge dewaterability during bioleaching by determining the pH, ORP, CST, SRF, and the S-EPS, LB-EPS, and TB-EPS content. The outcome of this study is of significant to understanding the mechanism of the effect of bioleaching on sludge dewaterability.

**MATERIALS AND METHODS**

**Municipal sewage sludge sampling**

Fresh secondary sludge was collected from the sludge thickening tank of Hewen Lake Wastewater Treatment Plant, Jiujiang City, Jiangxi Province, China (Zhang et al. 2018). Most of the sludge in the plant was from domestic sewage, with some from some industrial wastewater. The sludge pH, SRF and CST were determined immediately after sampling. The sludge’s solid content was determined after oven drying at 105 °C for 24 hours. The measurement of dry weight organic matter content was as per APHA (2005). Table 1 presents the characteristics of fresh secondary sludge.

**Table 1 | Characteristics of the raw sludge**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.45 ± 0.03</td>
</tr>
<tr>
<td>Total solid content (%)</td>
<td>4.56 ± 0.04</td>
</tr>
<tr>
<td>CST (s)</td>
<td>32.25 ± 1.28</td>
</tr>
<tr>
<td>SRF (× 10¹¹ m/kg)</td>
<td>9.56 ± 0.89</td>
</tr>
<tr>
<td>Organic matter content (% dry weight)</td>
<td>27.30 ± 0.09</td>
</tr>
</tbody>
</table>

**Microorganisms and the preparation of bioleaching inoculum**

The A. thiooxidans JJU-1 (GenBank no. KM101109) employed in this research was isolated from municipal sewage sludge in our previous research (Yang et al. 2015) and cultivated by Waksman’s medium. The medium was autoclaved at 121 °C for 20 min, and the pH was adjusted to 3.6–4.0 using 9 M H₂SO₄. After this, 5 g L⁻¹ elemental sulfur was added to the medium as the energy source. The cultivation conditions of the inoculums were 500 mL Erlenmeyer flasks shaken at 180 rpm and kept at a constant temperature of 28 °C for 3–4 days. The original concentration of bacterial was 1 × 10⁷ cells·mL⁻¹.

On the basis of previous research (Wang et al. 2010), Rhodotorula mucilaginosa JJU-2 bacteria were isolated from sludge in our work (Qiu et al. 2017). The strain JJU-2 has an excellent ability to degrade the dissolved organic matter (DOM) in sludge and promote the growth of A. thiooxidans JJU-1. The strain JJU-2 was cultivated in flasks with potato dextrose agar (PDA) under its optimal pH, which for this strain was 5, although strain can grow normally in the extremely low pH environment of 1 (Qiu et al. 2017). The flasks were kept in a gyratory shaker at 28 °C and 120 rpm for 2–5 days. The original concentration of bacteria used in this experiment was 1 × 10¹² cells·mL⁻¹.

In addition, an automatic bioreactor of 25 L was used in this bioleaching experiment, to which was added 2.1 L of A. thiooxidans JJU-1 inoculum, 700 mL of R. mucilaginosa JJU-2 inoculum and 14.2 L of municipal sludge, as per our previous research (Yang et al. 2017, 2018). Specific substances, including 10 g L⁻¹ S⁰ and set proportion of nutrients, were supplied for cultivation at a temperature of 28 ± 2 °C, agitator speed of 200 rpm, and an aeration intensity of 0.7 m³·min⁻¹·m⁻³, as per our previous research (Zhang et al. 2018). The bioleaching process was suspended until the solution pH dropped to around 2.0. The above steps were repeated three times. Finally, the acidified
bioleaching inoculum was selected for use as the bioleaching inoculum, which was used for following experiments.

**Bioleaching experiments**

Bioleaching, at various sludge concentrations (5 g·L⁻¹, 10 g·L⁻¹, 15 g·L⁻¹, 20 g·L⁻¹ and 30 g·L⁻¹, dry weight), were carried out in the 25 L automatic bioreactor containing 0.8 L acidified sludge, 15.2 L fresh secondary sludge, and 10 g·L⁻¹ S⁰ as an energy source, as per our previous study (Zhang et al. 2018). 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⁻¹ H₂SO₄ (Yang et al. 2018). Finally, when the pH value of inoculum sludge decreased to around 2.0, the rest of sludge was immediately refrigerated at 4°C, for use for the next bioleaching experiments.

**Analytical methods**

The pH and the ORP were measured using a pHS-3C digital pH-meter with the Pt-Ag/AgCl electrode system. Sludge dewaterability was represented by determining the values of CST and SRF, specifically, CST was determined using a capillary suction timer (Model 304 M CST, Triton, Britain), and SRF was measured by using the Buchner funnel test (Lo et al. 2001). The moisture content of the filter cake was determined by oven drying at a temperature of 105°C for about 2 hours. The organic matter content of the resulting dried sludge was measured according to APHA (2005).

**EPS extraction and chemical analysis**

A heat extraction method was modified to extract different EPS fractions from the activated sludge (Zhang et al. 2018). First, a 100 mL sludge sample was collected and placed in a flask. The sample was then washed twice with a 0.05% (w·w⁻¹) NaCl solution, which was used to make up the solution to its original volume of 100 mL. Next, 60 mL of the sludge sample was centrifuged at 600 rpm for 15 min. The supernatant obtained was defined as S-EPS; the precipitate remaining in the tube was used for additional EPS layer extractions. A solution of 0.05% NaCl was made up. The sludge pellet in the tube was re-suspended with 0.05% NaCl solution to 60 mL with a pipette and treated with ultrasound at 20 kHz and 30 W for 5 min. The aliquot was centrifuged at 9,000 rpm for 15 min to separate the solids from the supernatant. The collected supernatant was defined as LB-EPS. Finally, the 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 70°C for 20 min, then centrifuged at 20,000 rpm for 20 min to gather the TB-EPS. The supernatant samples were filtered through a 0.22 μm membrane, which is used for chemical analysis.

Polysaccharides were measured by the anthrone method (Gaudy 1962) that depends on glucose as the standard. Protein was measured by a modified Lowry method that relies on bovine serum albumin as the standard (Frolund et al. 1995). Finally, the DNA content was measured by the diphenylamine colorimetric method, which takes sodium DNA as the standard (Sun et al. 2009).

**Statistic analysis**

All results are the mean of three replicates ± standard deviation. To compare the effect of different sludge concentrations among all treatments, one-way analysis of variance (ANOVA) was carried out. Differences were supposed to be significant at p < 0.05. All statistical analyses, including principal component analysis (PCA) and Pearson’s correlation, were carried out using SPSS 19.0 software (IBM Inc., USA).

**RESULTS AND DISCUSSION**

**Variations of pH and ORP values as a function of treatment time**

The variation of pH and ORP is an indication of Acidithiobacillus activity and its oxidation of S⁰. As shown in Figure 1, the variations of pH and ORP under different sludge concentrations were different, indicating that sludge concentrations significantly affected bioleaching efficiency.

Figure 1(a) shows that the rate of pH reduction in 5 g·L⁻¹ and 10 g·L⁻¹ sludge concentrations were lower than in 15 g·L⁻¹ and 20 g·L⁻¹ sludge concentrations before 24 hours of incubation. However, during 24–48 hours of bioleaching, the rate of the former was higher than that of the latter. In the highest 30 g·L⁻¹ sludge concentration treatment, the pH remained unchanged at 12 hours and then decreased for the rest of the incubation. The above results showed that sludge concentration had no obvious dose effect on the rate of pH decrease, which indicated bioleaching efficiency.

Figure 1(b) presents the variations of ORP in different sludge concentrations, which showed the efficiency of sulfur oxidation and aeration during the bioleaching...
process. Clearly, ORP achieved the highest values (294.33 mV) in 10 g·L\(^{-1}\) sludge concentration treatment after 48 hours of bioleaching, which was closely related to the variations in pH. Furthermore, the rate of ORP increase in 5 g·L\(^{-1}\) and 10 g·L\(^{-1}\) sludge concentrations were lower than in 15 g·L\(^{-1}\) and 20 g·L\(^{-1}\) sludge concentrations before 24 hours of incubation. However, during 24–48 hours of bioleaching, the variation of the former was higher than that of the later. The rate of ORP increase in 30 g·L\(^{-1}\) sludge concentration was lower than in the other sludge concentrations. The above results indicated that sludge concentration had no obvious dose correlation with the rate of ORP increase.

In general, sludge concentration appears to play a significant role in bioleaching efficiency. It is common knowledge that the higher rate of pH reduction is a sign of a higher sulfate production rate (Liu et al. 2012b). The maximum pH reduction rate of 10 g·L\(^{-1}\) concentration sludge was achieved sooner than in the other sludge concentrations. It is worth noting that the bioleaching efficiency in higher sludge concentrations was slower than in the lower ones during the early stage of bioleaching, while it was the opposite during the later stage of bioleaching. In particular, the pH remained unchanged at 12 hours during the bioleaching of the 30 g·L\(^{-1}\) sludge concentration, suggesting that bioleaching efficiency was significantly inhibited. This phenomenon can be attributed to the sludge buffering capacity that mostly exists in the higher sludge concentrations (e.g. 20 g·L\(^{-1}\) and 30 g·L\(^{-1}\)). Furthermore, with the prolongation of bioleaching time, the sludge buffering capacity was becoming increasingly evident, as it required more time and acid for pH reduction and resulted in the reduction of the bio-acidification rate in later bioleaching. Therefore, bioleaching efficiency, shown by the pH decrease and ORP increase, could be hindered as sludge concentration increased. A similar conclusion was reported in previous research (Chen & Lin 2000; Chen et al. 2016).

**Variations in CST and SRF as a function of treatment time**

CST and SRF have been widely employed to characterize sludge dewaterability. Previous researchers revealed that lower CST or SRF values represent good sludge dewaterability (Feng et al. 2009). As shown in Figure 2, different sludge
concentrations have different effects on sludge dewaterability. Comparing the SRF values for different sludge concentrations, the rate of SRF reduction in the 10 g·L\(^{-1}\) treatment was faster than that in the others. Specifically, in the 10 g·L\(^{-1}\) treatment (Figure 2(b)), CST dropped from 24.5 seconds to 20.4 seconds at 48 hours and SRF decreased from \(5.50 \times 10^{12} \text{ m·kg}^{-1}\) to \(1.74 \times 10^{12} \text{ m·kg}^{-1}\) at 36 hours, indicating that the sludge dewaterability had improved. Subsequently, and unexpectedly, the SRF increased to \(2.71 \times 10^{12} \text{ m·kg}^{-1}\) at 48 hours, at which point the pH declined to below 2.0. The above results

---

**Figure 2** | The variations of CST and SRF values under different sludge concentrations during bioleaching: (a)–(e): 5 g·L\(^{-1}\), 10 g·L\(^{-1}\), 15 g·L\(^{-1}\), 20 g·L\(^{-1}\) and 30 g·L\(^{-1}\), respectively.
indicated that excessive bioleaching time had a negative impact on sludge dewaterability, which was consistent with previous researchers (Feng et al. 2009; Huo et al. 2014b).

It is worth noting that the CST value rose rapidly with sludge concentrations increasing from 10 g·L\(^{-1}\) to 30 g·L\(^{-1}\), which indicated that sludge dewaterability was deteriorating. However, sludge dewaterability in the 5 g·L\(^{-1}\) treatment was deteriorating faster than that in 10 g·L\(^{-1}\) treatment, because there was not enough substrate to consume during bioleaching. As shown in Figure 2(d), during the 20 g·L\(^{-1}\) treatment, CST and SRF decreased from 66.1 seconds and 1.75 \(\times\) \(10^{12}\) m·kg\(^{-1}\) to 37.7 seconds and 1.39 \(\times\) \(10^{12}\) m·kg\(^{-1}\), respectively, at 48 hours, at which point the pH was approximately 2.11 (Figure 1), indicating that sludge dewaterability had improved. A similar trend was also found in the 15 g·L\(^{-1}\) treatment (Figure 2(c)). However, from an economic point of view, the 10 g·L\(^{-1}\) sludge concentration was better for sludge dewaterability than the 15 and 20 g·L\(^{-1}\) sludge concentrations for industrial production. Moreover, there was an optimum pH (~2.11) for achieving the highest degree of dewaterability of the bioleached sludge. Figure 2(e) shows that CST and SRF drastically increased during the 30 g·L\(^{-1}\) sludge concentration treatment, suggesting that sludge dewaterability deteriorated.

The above results indicated that bioleaching using 10 g·L\(^{-1}\) sludge concentration was optimum for enhanced sludge dewaterability. The data suggested that the pH of 2.3 was optimal for improving the dewaterability of sludge. With increases in sludge concentration, the interactions between sludge floc structures becomes stronger. They change the colloidal and hydrodynamic forces between sludge particles, and thereby lead to the observed deterioration of sludge dewaterability (Baroutian et al. 2013; Markis et al. 2014). Song et al. (2012) also drew a similar conclusion: that with the higher sludge concentration, sludge particles increased, which is likely to generate bridging. It not only results in thickening of the filter cake layer, but decreases filtration speed and increases the SRF of the sludge.

**Variations in the content and fractions of sludge EPS with treatment time**

Previous researchers have extensively studied the role of sludge EPS in sludge dewatering and found that sludge EPS is a very important factor influencing sludge dewaterability (Houghton et al. 2001; Liu & Fang 2005). Sludge EPS contains many functional groups, such as hydroxyl, which has the obvious advantage of increasing the repulsion between flocs (Sanin & Vesilind 1994), absorbing a lot of bound water (Neyens et al. 2004), and improving sludge dewaterability (Houghton et al. 2001; Yang & Li 2009; Subramanian et al. 2010).

As shown in Figure 3, it was noticeable that from 5 g·L\(^{-1}\) to 20 g·L\(^{-1}\) treatment, with the increase of sludge concentration, the total EPS content gradually increased. This is due to the accumulation of carbon sources (Li et al. 2002). However, total EPS content in 30 g·L\(^{-1}\) sludge concentration treatment was lower than in the others. The sludge buffering capacity inhibited the production of EPS content under high sludge concentration. More concretely, all the carbon sources employed for cell synthesis in the system could not be completely used up by microbial cells, so excess amounts were converted into EPS, resulting in EPS levels increasing. The total EPS content in all sludge concentrations showed no significant variation with bioleaching time \((p > 0.05)\), suggesting that sludge dewaterability has an intimate relationship with sludge concentrations instead of changes in total EPS content.

Our investigation into the fractions of sludge EPS in different treatments showed that the rate of TB-EPS content decrease and S-EPS content increase was higher as the sludge concentrations decreased. For instance, in the 10 g·L\(^{-1}\) sludge concentration treatment (Figure 2(b)), representing the improvement of sludge dewaterability, the total EPS content slightly fluctuated within the range of 18 to 24 mg·g\(^{-1}\) dry sludge (SS) in the whole treatment; TB-EPS, as the predominant component of that, rapidly dropped from 14.2 mg·g\(^{-1}\) SS to 0.5 mg·g\(^{-1}\) SS at 48 hours. Conversely, S-EPS increased in the whole treatment at 48 hours; meanwhile, the TB-EPS content was higher than the S-EPS content during most of the bioleaching time. In contrast to the above trend, in the 20 and 30 g·L\(^{-1}\) sludge concentrations (Figure 3(d) and 3(e)), as a sign of the deterioration of sludge dewaterability, the S-EPS content was much higher than the TB-EPS content during the whole bioleaching time, and this could be one of factors affecting the deterioration dewaterability of sludge.

In general, the above results indicated that there was no direct relationship between sludge dewaterability and total EPS content. However, the reduction in TB-EPS content exerted a positive influence on sludge dewaterability. It can be attributed to the bio-acidification of *A. thiooxidans* leading to extensive transfer of protein and polysaccharides from TB-EPS to S-EPS during the bioleaching process (He et al. 2008). Furthermore, the ratio of S-EPS/TE-P content played a key role in contributing to the deterioration of sludge dewaterability. Specifically, the higher the S-EPS content the less the sludge was dewatered, which coincides with our previous study (Zhang et al. 2018).
Variations in different EPS fractions with treatment time

To investigate the influence of different fractions of EPS on sludge dewaterability, protein, polysaccharides and DNA were determined every 12 hours during bioleaching under different sludge concentrations. Figure 4(b) presents the protein content in the 10 g·L⁻¹ sludge concentration treatment, showing optimum enhancements in sludge.

Figure 3 | The variations of the total and various fractions of sludge EPS content during bioleaching: (a)–(e) 5 g·L⁻¹, 10 g·L⁻¹, 15 g·L⁻¹, 20 g·L⁻¹ and 30 g·L⁻¹, respectively.
Figure 4 | The variations of different EPS fractions under different sludge concentrations during bioleaching: (a) 5 g·L$^{-1}$, (b) 10 g·L$^{-1}$, (c) 15 g·L$^{-1}$, (d) 20 g·L$^{-1}$ and (e) 30 g·L$^{-1}$, respectively. (Continued.)
sludge dewaterability. This protein content, as a chief component of TB-EPS, showed a very large reduction from 15.17 mg·g⁻¹ SS to 0.63 mg·g⁻¹ SS at 48 hours. And this reduction rate was faster than in the other treatments. Therefore, this decline of protein in TB-EPS content was the major factor in relieving the improvement of sludge dewatering. It could be due to the bio-acidification by A. thiooxidans in the bioleaching system, the decomposition of sludge EPS components by enzymes, such as protease, amylase, DNase, and RNase (Esakki et al. 2013; Kavitha et al. 2013), and the consumption and biodegradation of EPS by acid-tolerant microorganisms (Rani et al. 2012; Yang et al. 2015).

The 5 g·L⁻¹ sludge concentration treatment also showed similar result (Figure 4(a)); more specifically, the content of protein in TB-EPS in the 10 g·L⁻¹ sludge concentration treatment was higher than in the 5 g·L⁻¹ sludge concentration treatment. However, during the 20 and 30 g·L⁻¹ sludge concentration treatments (Figure 4(d) and 4(e)), the sludge dewaterability was worse, and the protein and DNA, as the major components of S-EPS, were higher than TB-EPS. Meanwhile, the decrease of the protein content in TB-EPS was lower than in the 10 g·L⁻¹ sludge concentration treatment. Therefore, the higher protein and DNA in the S-EPS content had an important effect on the deterioration of sludge dewaterability, as compared with TB-EPS content.

Given the above, the ratio of S-EPS/TB-EPS significantly affected sludge dewaterability. The higher S-EPS content, consisting of protein and DNA, had a significant effect on the deterioration of sludge dewaterability. By contrast, the higher TB-EPS followed by a very large reduction had a positive impact on the improvement of sludge dewaterability. Furthermore, the sludge pH decreased due to the bio-acidification of A. thiooxidans, thereby leading to the transfer of protein and polysaccharides from TB-EPS to S-EPS during bioleaching. It exerts a significant influence on the enhancement of sludge dewaterability, and He et al. (2008) reached a similar conclusion.

**PCA analyses on the correlation between EPS content and sludge dewaterability**

PCA was used to investigate the relationship between the sludge dewaterability and the different fractions of EPS. The results of the 10% sludge concentration during the whole of bioleaching were chosen to calculate the PCA model. Figure 5 shows the two principal components describing 83.69% of the total variance. PC1 and PC2 described 61.55% and 22.14%, respectively, of the overall variance. The results indicated that the variation of SRF was very similar to that of the protein in TB-EPS, suggesting a close connection between sludge dewaterability and the protein in TB-EPS. Moreover, the results showed that the variation of protein and DNA in the S-EPS content had an inverse relationship with CST and SRF, thus demonstrating that S-EPS had a negative effect on sludge dewaterability. Hence, the PCA analyses revealed the close relationship between sludge dewaterability and different fractions of EPS, and they showed that the reduction of protein in TB-EPS was the dominant factor contributing the enhancement of sludge dewaterability. In addition, the increase of protein and DNA in S-EPS content had a negative impact on sludge dewaterability. Table 2 also provides evidence that the variation of protein in TB-EPS has a positive relationship with that of CST and SRF, while the change of protein in S-EPS has a negative relationship with that of CST and SRF.
However, our present study is not in complete agreement with previous studies. For instance, Murugesan et al. (2016) also found that the decrease in TB-EPS content had a positive correlation with sludge dewaterability (p < 0.01) during bioleaching with A. ferrooxidans inoculation. By contrast, Wang et al. (2015) found that the variation of sludge SRF has a positive correction with the polysaccharide content in S-EPS, and a negative correction with the protein content in TB-EPS. Our present study showed that the ratio of S-EPS/TB-EPS significantly affected sludge dewaterability, which has rarely been reported by most of previous research. The higher S-EPS content, contributed by protein and DNA, had an effect on the deterioration of sludge dewaterability. In contrast, the higher protein in TB-EPS followed by a very large reduction contributed a significant impact on improvement of sludge dewaterability.

CONCLUSION

The sludge concentration, and the important ingredients influence the bioleaching process and sludge dewaterability, were investigated in this research. The results indicated that different sludge concentrations have different influences on the bio-oxidation rates of $S^0$ and bio-acidiﬁcation during bioleaching. It was due to the sludge buffering capacity, indicating that the rate of pH reduction and ORP increase during bioleaching were clearly hindered when sludge concentration was increased. From an economic point of view, optimum enhancements in sludge dewaterability were observed during the 10 g·L⁻¹ sludge concentration treatment; a pH of 2.11 was suggested as the optimal improvement in sludge dewaterability. And very importantly, due to the bio-acidiﬁcation by A. thiooxidans in the bioleaching system, most of the protein and polysaccharides in TB-EPS were initially converted to S-EPS. In addition, the ratio of S-EPS/TB-EPS exerted a signiﬁcant inﬂuence on sludge dewaterability. PCA and Pearson’s correlation analysis also revealed that the higher TB-EPS followed by a very large reduction had a positive connection with the improvement of sludge dewaterability. However, the increases of protein and DNA in S-EPS had a negative connection with sludge dewaterability.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (21367014), Key research and development program of science and technology department of Jiangxi province, China (20181BBG70043), Natural Science Foundation of Jiangxi province, China (20181BAB216032), and a grant from China Scholarship Council (No. 201808360306).
The Pearson’s correlation among indicators in the 10 g L\(^{-1}\) sludge concentration bioleaching experiment

<table>
<thead>
<tr>
<th></th>
<th>CST</th>
<th>SRF</th>
<th>S-EPS (protein)</th>
<th>S-EPS (polysaccharides)</th>
<th>S-EPS (DNA)</th>
<th>LB-EPS (protein)</th>
<th>LB-EPS (polysaccharides)</th>
<th>LB-EPS (DNA)</th>
<th>TB-EPS (protein)</th>
<th>TB-EPS (polysaccharides)</th>
<th>TB-EPS (DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CST</td>
<td>1</td>
<td></td>
<td>-0.338</td>
<td>-0.426</td>
<td>.676**</td>
<td>.795**</td>
<td>-.549**</td>
<td>.046</td>
<td>.061</td>
<td>-.658**</td>
<td>-.606*</td>
</tr>
<tr>
<td>SRF</td>
<td>-0.338</td>
<td>1</td>
<td>-0.342</td>
<td>-0.088</td>
<td>-0.113</td>
<td>-0.091</td>
<td>0.382</td>
<td>0.441</td>
<td>0.418</td>
<td>0.043</td>
<td>-0.177</td>
</tr>
<tr>
<td>S-EPS (protein)</td>
<td>-0.426</td>
<td>-0.342</td>
<td>1</td>
<td>-.670**</td>
<td>-.672**</td>
<td>-.578**</td>
<td>0.435</td>
<td>-.065</td>
<td>-.070</td>
<td>.758**</td>
<td>.956**</td>
</tr>
<tr>
<td>S-EPS (polysaccharides)</td>
<td>.676**</td>
<td>-0.088</td>
<td>-.670**</td>
<td>1</td>
<td>.792**</td>
<td>.962**</td>
<td>-.049</td>
<td>0.133</td>
<td>0.140</td>
<td>-.960**</td>
<td>-.836**</td>
</tr>
<tr>
<td>S-EPS (DNA)</td>
<td>-.795**</td>
<td>-0.113</td>
<td>-.672**</td>
<td>.792**</td>
<td>1</td>
<td>.839**</td>
<td>-.785**</td>
<td>-.038</td>
<td>-.030</td>
<td>-.879**</td>
<td>-.865**</td>
</tr>
<tr>
<td>LB-EPS (protein)</td>
<td>.673**</td>
<td>-.091</td>
<td>-.578**</td>
<td>.962**</td>
<td>.839**</td>
<td>1</td>
<td>-.526**</td>
<td>0.191</td>
<td>0.205</td>
<td>-.938**</td>
<td>-.802**</td>
</tr>
<tr>
<td>LB-EPS (polysaccharides)</td>
<td>-.549**</td>
<td>.382</td>
<td>.043</td>
<td>-.490</td>
<td>-.785**</td>
<td>-.526**</td>
<td>1</td>
<td>.626*</td>
<td>.606*</td>
<td>.673**</td>
<td>.593*</td>
</tr>
<tr>
<td>LB-EPS (DNA)</td>
<td>0.046</td>
<td>.441</td>
<td>-.063</td>
<td>.133</td>
<td>-.038</td>
<td>0.191</td>
<td>.626*</td>
<td>1</td>
<td>.994*</td>
<td>.041</td>
<td>-.077</td>
</tr>
<tr>
<td>TB-EPS (protein)</td>
<td>.061</td>
<td>.418</td>
<td>-.070</td>
<td>.140</td>
<td>-.030</td>
<td>0.205</td>
<td>.606*</td>
<td>.994*</td>
<td>1</td>
<td>.033</td>
<td>-.085</td>
</tr>
<tr>
<td>TB-EPS (polysaccharides)</td>
<td>-.658**</td>
<td>.043</td>
<td>.758**</td>
<td>-.960**</td>
<td>-.879**</td>
<td>-.938**</td>
<td>.673**</td>
<td>.041</td>
<td>0.033</td>
<td>1</td>
<td>.914**</td>
</tr>
<tr>
<td>TB-EPS (DNA)</td>
<td>-.606*</td>
<td>-.177</td>
<td>.936**</td>
<td>-.836**</td>
<td>-.865**</td>
<td>-.802**</td>
<td>.593*</td>
<td>-.077</td>
<td>-.085</td>
<td>.914**</td>
<td>1</td>
</tr>
</tbody>
</table>

*P < 0.05.
**P < 0.01.


First received 6 May 2020; accepted in revised form 23 June 2020. Available online 9 July 2020.