Improvement of sewage sludge dewaterability by bioleaching in a continuous plug flow bioreactor: optimization of process parameters
Jingqing Gao, Na Ma, Linshuai Li, Songfeng Zhu, Yonghong Li, Jie Chen and Yong Chen

ABSTRACT
A novel process for sewage sludge bioleaching by mixed Thiobacilli (Acidithiobacillus thiooxidans and Acidithiobacillus ferrooxidans) using a 12-stage, 180 L working volume continuous plug-flow bioreactor, is presented. The objective of the present study was to assess the impact of some parameters on the sludge dewaterability and to improve the sludge dewaterability by optimization of these parameters. The parameters examined were sludge moisture content, nutrients dosage, aeration rate, and the number of reactors. The order of the influence of these factors on sludge dewaterability was found to be sludge moisture content > nutrients dosage > aeration rate > number of reactors. The optimized conditions were: sludge moisture content, 98.0%, nutrients dosage, 9 g/L, aeration rate, 8 m³/h, and 10 reactors. Confirmation experiments conducted under optimum conditions demonstrate the sludge dewaterability to be remarkably improved. After 2 days of bioleaching, the moisture of bioleached sludge cake was reduced to below 60%.

Key words | bioleaching, continuous plug flow bioreactor, mixed Thiobacilli inoculation, process optimization, sludge dewaterability

INTRODUCTION
Treatment of municipal wastewater often leads to the production of large amounts of sewage sludge whose disposal is a serious environmental problem (Huo et al. 2014). Sludge management has become the major challenge in sewage treatment process because it requires appropriate methods for volume reduction, dewatering and safe disposal (Wong et al. 2016). Bioleaching has been recognized as a promising microbial conditioning method (Pathak et al. 2017) with prominent advantages for enhancement of sludge dewaterability (Zheng et al. 2016). It has been proved to be an efficient and cost-effective alternative to other physical or chemical methods (Liu et al. 2012a).

The effectiveness of bioleaching depends on physical, chemical, and biological parameters, including initial hydraulic retention time (Li et al. 2015), temperature (Choi et al. 2013), pH (Liu et al. 2012b) and microbial levels (Huo et al. 2014). The present study mainly focused on four factors in continuous bioleaching system: sludge moisture content, nutrients dosage, aeration rate and the number of reactors. The sludge moisture content is one of the most critical parameters of the bioleaching process in terms of bioleaching efficiency, operational time and the impact on the size of the equipment required. Nutrients play important roles in regulating the growth of Acidithiobacillus species (Zheng et al. 2016) and determination of the nutrient levels is of great significance in developing an efficient bioleaching process. Ozturk et al. (2016) found that the aeration rate should be optimized...
because optimal aeration allows sufficient mixing in the sludge bioreactor and contributes to the overall efficiency of the treatment process. It is also necessary to determine the optimal number of reactors in a continuous plug flow bioreactor because more reactors than are required for the process will increase the overall operational cost. Consequently, identification of the appropriate optimum values of the effective parameters in bioleaching processes is a prerequisite for successful commercial operation.

In this paper, we present a novel process for sewage sludge bioleaching using a 12-stage, 180 L working volume plug-flow bioreactor. To date, a number of studies involving sludge bioleaching were carried out through shake flask experiments (Choi et al. 2013; Murugesan et al. 2014; Zhou et al. 2014; Zheng et al. 2016; Zhou et al. 2017). A continuous process can treat a larger volume of sludge in a shorter period and hence would appear to be more suited to use on a larger scale (Pathak et al. 2009). This process offers the advantage of the repeated use of the nutrients and microbial agents because of the added reflux system. Little information is available, however, on the feasibility of enhancing sludge dewaterability by optimization of process parameters in a continuous flow system for bioleaching.

The main objective was to assess the impact of these factors on the sludge dewaterability and to improve the sludge dewaterability by optimization of process parameters. The outcome may assist the development of bioleaching technology by optimization of dominant factors and provision of a basis for scaling up laboratory results for a full-scale operation.

**MATERIALS AND METHODS**

**Sludge samples**

The sewage sludge samples were collected from three different sewage treatment plants in Henan Province, China and are designated Sludge W (from Wulongkou sewage treatment plant, Zhengzhou City), Sludge H (from Hebi first sewage treatment plant, Hebi City) and Sludge C (from Chen Sanqiao sewage plant, Zhengzhou City). Samples were preserved in polypropylene bottles and kept at 4 °C prior to use. The sludge samples have a black appearance and an offensive odor. The selected physical and chemical properties of the sludge samples are shown in Table 1.

**Preparation of microorganisms and inoculums**

The *A. ferrooxidans* (ATCC 23270) and *A. thiooxidans* (ATCC 53990) obtained from American Type Culture Collection (ATCC) were grown in Modified 9 K (Rubio 2002) and SM media (Zhou et al. 2013), respectively. The modified 9 K (SM) medium was autoclaved at 121 °C for 15 min and individually spiked with 44.2 g/L FeSO4·7H2O and 10 g/L of elemental sulfur as microbial nutritional substances. The culture inoculated with *A. ferrooxidans* or *A. thiooxidans* was incubated at 180 rpm and 28 °C for 3–4 days until a bacterial cell density of 10^7–10^8 cfu/mL was attained (Li et al. 2018).

The sewage sludge was cultivated together with cultures of *A. ferrooxidans* and *A. thiooxidans* (1:1, v/v) in an inoculum proportion of 10% (v/v), and supplied with desired concentrations of nutrients for different batches. The reaction was carried out under natural conditions (15–20 °C), during which the pH of the sludge was monitored at 12 h intervals. For each batch of experiments, 18 L of activated sludge must be cultured. The activated sludge was used as inoculant after pH dropped to approximately 2.0 and the bacterial cell density reached 10^7–10^8 cfu/mL.

**Experimental configuration for bioleaching**

As shown in Figure 1, 12 plastic 20 L buckets were connected together with the 75% available volume to form a 12-stage plug flow bioreactor with total 180 L working volume. Each bucket is drilled at the 15 L level and

<table>
<thead>
<tr>
<th>Sludge</th>
<th>pH</th>
<th>Organic matter (%)</th>
<th>Moisture (w/w %)</th>
<th>CST (s)</th>
<th>SRF × 10^13 (m/kg)</th>
<th>Cu (mg/kg)</th>
<th>Zn (mg/kg)</th>
<th>Pb (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>6.86</td>
<td>47.8</td>
<td>97.32</td>
<td>85.2</td>
<td>1.32</td>
<td>236.3</td>
<td>473.6</td>
<td>125.1</td>
</tr>
<tr>
<td>H</td>
<td>7.08</td>
<td>52.9</td>
<td>97.82</td>
<td>76.5</td>
<td>3.23</td>
<td>175.1</td>
<td>473.8</td>
<td>159.4</td>
</tr>
<tr>
<td>C</td>
<td>7.18</td>
<td>47.6</td>
<td>97.50</td>
<td>97.5</td>
<td>1.13</td>
<td>180.9</td>
<td>454.2</td>
<td>132.3</td>
</tr>
</tbody>
</table>

W, H and C represent the sludge from three sewage treatment plants, respectively.
connected with plastic overflow pipes. In addition, draft tubes of each bucket were connected to a plastic overflow pipe, which reached the bottom of the reactor. An aeration head was placed in every reactor to aerate the reaction system. Reactor units were numbered from 1 (the starting unit) to 12 (the last unit).

Inoculation and operation of the bioreactor

The 18 L activated sludge was totally distributed amongst 12 reactors containing primary sludge (with the volume ratio of primary sludge to activated sludge at 10:1 for each reactor). Each reactor in the system contained 13.5 L of primary sludge and 1.5 L of activated sludge as inoculums, and was supplied with desired concentrations of nutrients for different batches. The pH of the sludge was periodically monitored during this process and found to drop to approximately 2.0 while the bacterial cell density reached $10^7 - 10^8$ cfu/mL, which is regarded as a stable state. Subsequently, bioleaching process for each batch was initiated under the conditions described in orthogonal experiment.

The fresh sludge was stored in a sludge storage tank and slowly agitated in order to avoid sedimentation of sludge solids. The fresh sludge was continuously pumped from sludge storage tank into the system by a metering pump, along with microbial nutrients (FeSO$_4$·7H$_2$O and S$^0$). Microbial nutrients comprised FeSO$_4$·7H$_2$O and S$^0$ at 6:1 (m/m) proportion were added to obtain concentrations of 3, 6, and 9 g/L. The fresh sludge flowed from reactor 1 through reactors 2–12 in turn, then flowed back into reactor 1 with a sludge reflux ratio of 30%. Treated sludge flowed out from the last reactor through an outlet. By controlling the flow of the metering pump, the sludge retention time was set to 2 d.

Orthogonal experiment design

A three-level, four-factor Taguchi orthogonal experimental array design L$_0$(5$^4$) was constructed to determine the optimal conditions for bioleaching using *A. ferrooxidans* and *A. thiooxidans* with the filter cake moisture as the target index (Tables 2 and 3). The sludge sample used in the orthogonal experiments is Sludge W obtained from Wulong-kou sewage treatment plant.

Analytical methods

After 2 d of bioleaching, in a specific operating condition, samples were collected from each reactor. The reactor's pH and oxidation reduction potential (ORP) were monitored using a pH meter (pHS-3C, Shanghai INESA Scientific Instrument, China). Sludge dewaterability was assessed using the SRF (specific resistance to filtration) and CST (capillary suction time). SRF was determined by the Buchner funnel vacuum suction method, while CST values of sludge were determined by a capillary suction timer (Type 294–50, OFITE, USA). Fe$^{2+}$ concentrations were determined by a colorimetric procedure using 1,10-phenanthroline as described in *Standard Methods* (APHA © 1981 J. Gao et al. | Optimization of process parameters for sewage sludge bioleaching Water Science & Technology | 77.8 | 2018). SO$_4^{2-}$ concentrations

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Factors and levels of the orthogonal test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levels</td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
</tr>
</tbody>
</table>
| A: number of reactors; B: nutrients dosage; C: aeration rate; D: sludge moisture content.
were measured by ion chromatography (ICS-90, Dionex). The moisture and organic matter content of the sludge cake were analyzed according to APHA (2005) after filter pressing by a plate and frame filter (model XMG10/800-UK, working pressure 4.5 MPa). The counts of A. thiooxidans and A. ferrooxidans were determined by using a double layer plate method (Li et al. 2015). The contents of Cu, Zn, and Pb were determined on a dry basis with an inductively coupled plasma emission spectrometer (ICP, iCAP6500DUO). All measurements were made in triplicate and the data presented are the mean and standard deviation of three independent samples. The collected data were then analyzed by Origin Pro 7.5 software and IBM SPSS Statistics (version 19.0).

RESULTS AND DISCUSSION

Results of orthogonal experiments

In all the experiments, the minimum cake moisture (53.01%) was obtained using 12 reactors, a nutrients dosage level of 6 g/L, an aeration rate of 8 m$^3$/h and a sludge moisture content of 98.0% (Table 3). According to the $R$-value, the order of the influence on cake moisture was: sludge moisture content > nutrients dosage > aeration rate > reactor number. According to the $K$-value, the best co-optimization combination of these parameters for bioleaching is D3B3C1A2 and, consequently, the orthogonal experiment suggests a sludge moisture content of 98.0%, a nutrients dosage level of 9 g/L, an aeration rate of 8 m$^3$/h and eight reactors to be recommended for optimal selective bioleaching conditions.

Judging by the sum of squares (SS) in Table 4, the influence of the various factors on the cake moisture is the order: D > B > C > A. The SS of the A (reactor number) was minimal, indicating that it had the least influence on the moisture content of sludge cake. Therefore, with the reactor number set as error evaluation, the results of a statistical analysis of variance (ANOVA) are listed in Table 5. The ANOVA was performed to determine which process parameters are statistically significant and the results are listed in Tables 5. The F-value for each process parameter is simply a ratio of the mean of the squared deviations to the mean of the squared error (Tzeng et al. 2009). Usually, the larger the F-value, the greater the effect on the dissolution value due to the change of the process parameter. The results showed that the sludge moisture content had an appreciable impact on the sludge dewaterability (0.01 < $p$ < 0.05), while the nutrient dosage also had a significant impact (0.05 < $p$ < 0.1) and the aeration rate also had a certain impact, while the reactor amount was the least consequential.

Influence of controllable factors on sludge dewaterability

Influence of sludge moisture content

As shown in Figure 2(a), at higher sludge moisture levels (D3 and D6 treatments), the pH values fell drastically from the initial values of 5.15 and 5.56 in reactor No. 1 to a final 3.65 in the No. 8 and 3.02 in the No. 10 reactor at the end of experiment. However, at lower sludge moisture levels (D9 treatment), the pH showed a lesser reduction. During bioleaching, A. thiooxidans oxidizes sulfur to sulfuric acid, which causes the pH to fall below 3.5 (Figure 2(a)). Ferric ions generated by bio-oxidation of Fe$^{2+}$ by A. ferrooxidans by a sequence of hydrolysis,

### Table 3 | Orthogonal experiment (L$_9$($3^4$)) arrangement and results

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>A</th>
<th>B (g/L)</th>
<th>C (m$^3$/h)</th>
<th>D (%)</th>
<th>Cake moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>3</td>
<td>8</td>
<td>95.0</td>
<td>76.39</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>6</td>
<td>16</td>
<td>96.5</td>
<td>64.20</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>9</td>
<td>24</td>
<td>98.0</td>
<td>55.14</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>3</td>
<td>16</td>
<td>98.0</td>
<td>64.14</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>6</td>
<td>24</td>
<td>95.0</td>
<td>69.41</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>9</td>
<td>8</td>
<td>96.5</td>
<td>54.96</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>3</td>
<td>24</td>
<td>96.5</td>
<td>68.45</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>6</td>
<td>8</td>
<td>98.0</td>
<td>53.01</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>9</td>
<td>16</td>
<td>95.0</td>
<td>70.03</td>
</tr>
<tr>
<td>$K_1$</td>
<td>65.24</td>
<td>69.66</td>
<td>61.45</td>
<td>71.94</td>
<td></td>
</tr>
<tr>
<td>$K_2$</td>
<td>62.84</td>
<td>62.21</td>
<td>66.12</td>
<td>62.54</td>
<td></td>
</tr>
<tr>
<td>$K_3$</td>
<td>63.83</td>
<td>60.04</td>
<td>64.33</td>
<td>57.43</td>
<td></td>
</tr>
<tr>
<td>$R$</td>
<td>2.40</td>
<td>9.62</td>
<td>4.67</td>
<td>14.52</td>
<td></td>
</tr>
</tbody>
</table>

A: number of reactors; B: nutrients dosage; C: aeration rate; D: sludge moisture content. $R$ is the difference between the maximum value and the minimum value of K, of any column.

### Table 4 | The sum of squares (SS) of four factors

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of squares (SS)</td>
<td>8.776</td>
<td>152.712</td>
<td>33.307</td>
<td>325.200</td>
</tr>
</tbody>
</table>

A: number of reactors; B: nutrients dosage; C: aeration rate; D: sludge moisture content.
adsorption and precipitation reactions, forming ferric hydroxide species and protons. This also leads to a decrease in the pH.

At higher sludge moisture levels (D3 and D6 treatments), the sludge ORP clearly increased and this increase may be associated with the biooxidation of Fe$^{2+}$ by *A. ferrooxidans* and precipitation of Fe(OH)$_3$. The increase in ORP could also be attributed to the production of sulfuric acid, from the oxidation of added elemental sulfur by *A. thiooxidans*. As shown in Figure 2(a), a decrease in sludge moisture content in bioleaching process resulted in a slow rate of pH decrease. Previous studies have shown that sludge with a lower moisture content has a higher buffering capacity and leads on average, to a slower sludge acidification during the bioleaching process (Tyagi et al. 1997). These reports focused principally on the buffering capacity of sludge to explain this phenomenon. However, it is necessary to obtain a better understanding of the mechanisms involved concerning the effects of sludge moisture content on bioleaching.

The present study shows that another important factor in the bioleaching process is that the growth of *A. ferrooxidans* and *A. thiooxidans* are strongly inhibited at the lower sludge moisture content (95.0%) because of the higher pH of the sludge which is 6.09 at the end point for bioleaching (Figure 2(a)). *Acidithiobacillus* can tolerate a wide pH range (1.3–4.5 for *A. ferrooxidans*; 0.5–5.5 for *A. thiooxidans*), but the optimal pH range for the growth of *A. ferrooxidans* and *A. thiooxidans* is 2.0–3.0 (Kelly & Wood 2000). In addition, the pH associated with the D3 treatment decreases slowly and does not effectively kill heterotrophic bacteria in the sludge, and this might hinder the growth of *Acidophilic thiobacillus* (Li et al. 2015). For enhancement of sludge dewaterability, the rapid growth and high activity of *Acidithiobacillus* species are essential for the success of sludge bioleaching (Zheng et al. 2016). Nonetheless, at a lower sludge moisture content, restriction

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>152.712</td>
<td>2</td>
<td>76.356</td>
<td>17.401</td>
<td>0.054</td>
</tr>
<tr>
<td>C</td>
<td>33.307</td>
<td>2</td>
<td>16.654</td>
<td>3.795</td>
<td>0.209</td>
</tr>
<tr>
<td>D</td>
<td>325.200</td>
<td>2</td>
<td>162.600</td>
<td>37.055</td>
<td>0.026</td>
</tr>
<tr>
<td>Pure error</td>
<td>8.776</td>
<td>2</td>
<td>4.388</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>519.995</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B: nutrient dosage; C: aeration rate; D: sludge moisture content, SS: sum of squares, DF: degrees of freedom, MS: mean square.

Figure 2 | Variations of (a) pH, (b) ORP, (c) SRF and (d) CST at different sections of system after 2 d of bioleaching. Sludge moisture content: D3: 98.0%, D6: 96.5%, D9: 95.0%.
of mass transfer in the gas phase involving O$_2$ or CO$_2$ might result in partial inhibition of the bacterial activity. Generally, the increase of ORP should be faster at lower moisture concentrations due to the increased availability of nutrients in the sludge but, as shown in Figure 2(b), the increase of ORP becomes slower at lower sludge moisture concentration (D3 treatment). Moreover, such a slow increase in the ORP combined with the high pH for D3 treatment, (Figure 2(b)), is an indicator of low activity of both *A. ferrooxidans* and *A. thiooxidans*. Although a high dosage (9 g/L) of nutrients in the D3 treatment could enhance microbial activity, the growth of microbes was still restricted due to low system pH resulting from the low sludge moisture content (Figure 2(a)), and as a result, the bioleaching performance of sludge deteriorated. These results also confirmed that, as mentioned in the ‘Results of orthogonal experiments’ section, the sludge moisture had a larger influence than nutrients on bioleaching.

Generally, a lower SRF value or shorter CST indicates better sludge dewaterability (Murugesan et al. 2014). As shown in Figure 2(c) and 2(d), it was found that the dewaterability of sludge was improved after bioleaching treatment with *A. ferrooxidans* and *A. thiooxidans* because of decreases in SRF and CST. The results also indicate that, as the sludge moisture content increases, the system becomes more acidic and as a result, the CST and SRF values decrease dramatically (Figure 2(c) and 2(d)). The dewaterability of sludge is dependent on breakup of the flocs during the acidification treatment. Acidification of sludge leads to the breakup of the sludge flocs because it causes the zeta potential of the sludge particles to approach neutrality (Kurade et al. 2016). The neutralization of the negative charges on the sludge flocs reduces the repulsive force between sludge particles and this leads to the formation of larger sludge flocs, resulting in the release of bound water to the surroundings (Wong et al. 2016), thus causing a remarkable improvement in the sludge dewaterability.

The cake moisture with sludge moisture contents of 98.0% and 96.5% can be reduced to about 55% after the filter press step (Table 6), and the resulting cake was more intact with a better shape (Figure 3(a) and 3(b)). When the sludge moisture content is 95.0%, the molding effect of filter cake is relatively poor (Figure 3(c)) and the cake moisture levels were as high as 70% (Table 6), implying that the dewaterability of sludge had deteriorated severely.

According to the results of the orthogonal experiments, a moisture content of sludge of 98% was found to be optimum. Generally, at higher level of sludge moisture, improved bioleaching efficiency could be achieved as a result of the decrease in buffering capacity. On the other hand, it is apparently desirable to operate the bioleaching system at a lower sludge moisture content in order to increase the sludge throughput in the bioreactor. Although sludge with higher moisture content incurred higher costs resulting from the increase in the volume of sludge and an increased hydraulic charge of leachate (Tyagi et al. 1997), sewage sludge generally contains high levels of moisture, approximately >95% water in practical treatment (Murugesan et al. 2014). As discussed above, considering the integrated treatment effect and practical application, our experiment showed that the optimum sludge moisture content was 98.0%.

### Influence of nutrient levels

After 2 d of bioleaching treatment, the pH decreased from the initial value of 6.86 (Table 1) to 5.06, 5.29 and 3.01 (Figure 4(a)) for nutrients dosage of 3 g/L, 6 g/L and 9 g/L, respectively. The pH of B6 treatment (9 g/L) from reactor 1 to reactor 10 changes rapidly, while the pH of B7 treatment (3 g/L) showed no significant reduction in pH (Figure 4(a)). This indicates that the level of nutrients present in sludge is insufficient to support the growth of *A. thiooxidans* and *A. ferrooxidans* and also sludge bioacidification. In contrast to the trends with pH, the ORP showed an increased trend.
for three treatments. The high values of ORP, together with the low pH, are responsible for improvement of sludge dewaterability in the more oxidative environment.

In the bioleaching process, *Acidithiobacillus* species trigger the oxidation of substances including Fe$^{2+}$ and S$^{0}$, and, consequently, the concentration of Fe$^{2+}$ decreases rapidly, while that of SO$_4^{2-}$ increases gradually, which is in agreement with the results shown in Figure 4(c) and 4(d). The sludge bio-acidification is governed by the bio-oxidation of energy substances and the degree of bio-oxidation may affect the process of sludge dewatering during bioleaching (Liu et al. 2012b). At high levels of nutrients, the high surface area of Fe$^{2+}$ and S$^{0}$ particles available to bacterial adsorption leads to rapid oxidation of Fe$^{2+}$ and S$^{0}$ and production of acid. Meanwhile, the rapid oxidation of Fe$^{2+}$ and S$^{0}$ by bacteria leads to increased sulfate concentration and decreased Fe$^{2+}$ concentration followed by rapid increases in ORP (Figure 4(c), 4(d) and 4(b)). For instance, the ORP of B6 treatment (9 g/L) increased rapidly from 79 mV to 213 mV after 2 d of bioleaching (Figure 4(b)).
As expected, the CST and SRF decreased upon the addition of the tested conditioners (Table 6). At levels of nutrients of 9 g/L (B6), sludge CST was drastically reduced from 85.2 s for fresh sludge to 29.3 s after 2 d of treatment and the SRF value was $0.87 \times 10^{12}$ m/kg, corresponding to a 93% reduction. However, the B7 and B2 treatments showed slight reduction in CST and SRF, indicating poor improvement in dewaterability compared with the B6 treatment. This reveals that the dosage of energy substrate is insufficient for the growth of *A. thiooxidans* and *A. ferrooxidans*. As shown in Table 6, the cake moisture was 68.45%, 64.20% and 54.96% at 3, 6 and 9 g/L, respectively, of nutrient dosage. These results demonstrate that compared with the moisture content of fresh sludge of 96.5%, the dewaterability of the conditioned sludge was significantly improved in the B6 treatment with 9 g/L of nutrient dosage.

In conclusion, supplementation of bioleaching system with 9 g/L of nutrients can effectively stimulate the growth of *A. thiooxidans* and *A. ferrooxidans* and further enhance sludge dewaterability. Additionally, the cake is yellow, solid and moldable and lacks any obvious odor (Figure 5(c)). Compared with B7 treatment (9 g/L), the molding effects of filter cakes were not as good for sludge in the B2 (3 g/L) and B6 treatments (6 g/L) (Figure 5). Therefore, it could be concluded that the optimum dosage of nutrients is 9 g/L for sewage sludge with 96.5% of moisture content.

### Influence of aeration rate

According to Table 6, the pH values of the bioleached sludge in the three batches of the experiment are all below 4.0, the CSTs and SRFs are largely reduced, and sludge dewaterability is improved significantly. In the C8 treatment, the SRF and CST were significantly lower than in the C4 and C3 treatments, and the moisture content of the filter cake was 53.01% (C8), lower than that in C4 (64.14%) or C3 (55.14%). Consequently, an aeration rate of 8 m$^3$/h could meet the required amount of aeration in the reaction system.

The aeration rate can affect the concentration of O$_2$ and CO$_2$ in bioleaching systems and influence the growth of microorganisms. *A. ferrooxidans* and *A. thiooxidans* are obligate chemolithotrophic autotrophs that can grow in inorganic media with low pH values (Lee & Pandey 2012). Furthermore, these acidophilic, chemoautotrophic microorganisms require atmospheric CO$_2$ as a carbon source. But the process should meet with some limitations of oxygen supply due to the increased O$_2$ demand associated with the growth of heterotrophs as opposed to autotrophs. It follows that the activity of heterotrophs may be enhanced by an increase of the aeration rate. Consequently, heterotrophs rather than autotrophs become the dominant species in the bioleached sludge. As evidenced by Table 6, the organic matter of cake, at an aeration rate of 16 m$^3$/h (C4), was less than that in cake produced by the other two treatments. This may be partly explained by the strong activity of heterotrophs. However, heterotrophic microorganisms probably produce more extracellular polymeric substances (EPS) than these two autotrophic bacteria (Zhou et al. 2014). Yang & Li (2009) revealed that the EPS in sludge flocs determines the dewaterability of sludge and excessive EPS would cause the sludge dewaterability to deteriorate, resulting in a poor treatment effect of bioleaching.

The aeration rate in simultaneous sludge dewatering plays a critical role in the overall economy of the treatment process. Too high an aeration rate will inhibit the bacterial attachment to the solid substrate and result in a large energy burden associated with the compression of air. This study suggests that 8 m$^3$/h, balanced between practical need and cost, can be used as the optimal aeration rate.

### Influence of number of reactors

As pointed out in the section Results of orthogonal experiments, the number of reactors had the least impact on sludge dewaterability compared to the other three factors. According to K-value from Table 3, the cake moisture
content was 65.24% at eight reactors, 63.83% at 12 reactors and 62.84% at 10 reactors with the same sludge retention time of 2 d. In the continuous plug flow bioleaching system with more reactors, the growth of target microorganisms might be inhibited in the flowing reactors since the nutrients in the previous reactors have been depleted, thus causing poor dewatering performance. As the number of reactors increases, the easier the bioleaching system reaches stability and the larger the volume of sludge that could be treated. Thus, balancing the treatment effect of bioleaching with the cost of engineering, 10 appears in this study to be the optimum number of reactors in a continuous plug flow bioreactor.

**Confirmation experiments**

In this study, three confirmation experiments were performed to confirm the validity and adequacy of the optimized conditions suggested by orthogonal testing. Sludge samples (W, H and C) from three different wastewater treatment plants were selected to verify the sewage sludge treatment effect under the optimal conditions.

The pH of each of the W, H and C sludge was reduced from 6.86, 7.08 and 7.18 to 2.45, 2.53 and 2.83, respectively (Table 1 and 7), indicating that *A. thiooxidans* and *A. ferrooxidans* grow well in these confirmation experiments. The CSTs were decreased to 20.8, 17.1 and 19.3 s from the initial 85.2, 76.5 and 97.5 s, respectively, measured with raw sludge from three different plants. Meanwhile, the results showed that the SRFs of W, H and C sludge were decreased by 82%, 86% and 83%, respectively. This significant reduction of CST and SRF demonstrates that sludge dewaterability had improved significantly. After filter pressing the moisture contents of bioleached cake were decreased to 55.50, 54.23 and 56.47% from the initial 97.32, 97.82 and 97.50%, respectively, measured with raw sludge from three different plants. Importantly, the moisture content of dewatered bioleached sludge cake should be below 60% after the filter press in order to meet engineering requirements (Liu et al. 2012b; Huo et al. 2014). Thus, the moisture contents of bioleached cake in three confirmation experiments meet the objectives and requirements for engineering applications. According to Figure 6, the cake was complete and retained good shape for three treatments after 2 d of bioleaching treatment. In addition, the odor of the original sludge disappeared and the sludge changed from the initial black to a yellow-brown (Figure 6).

The contents of organic matter in cake were decreased by less than 10% (Table 1 and 7). Although sewage sludge that contains nutrients can be used on land as a soil fertilizer, the presence of toxic heavy metals in sludge restricts its use as a fertilizer (Pathak et al. 2009). In the present study, the heavy metal (Cu, Pb, Zn) contents in the filter cake (Table 7) were significantly lower than in fresh sludge (Table 1). The heavy metal contents of treated sludge were

**Table 7 | Characteristics of sludge after 2 d of bioleaching under the optimal conditions**

<table>
<thead>
<tr>
<th>Sludge</th>
<th>pH</th>
<th>Organic matter (%)</th>
<th>Cake moisture (%)</th>
<th>SRF x 10^12 (m/kg)</th>
<th>CST (s)</th>
<th>Cu</th>
<th>Zn</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>2.45 ± 0.01</td>
<td>43.94 ± 0.72</td>
<td>55.50 ± 1.06</td>
<td>2.4 ± 0.10</td>
<td>20.8 ± 0.5</td>
<td>26 ± 0.91</td>
<td>90 ± 1.33</td>
<td>31 ± 0.98</td>
</tr>
<tr>
<td>H</td>
<td>2.53 ± 0.02</td>
<td>47.10 ± 0.93</td>
<td>54.23 ± 1.14</td>
<td>4.5 ± 0.15</td>
<td>17.1 ± 0.6</td>
<td>23 ± 0.87</td>
<td>128 ± 1.50</td>
<td>19 ± 0.64</td>
</tr>
<tr>
<td>C</td>
<td>2.83 ± 0.05</td>
<td>41.77 ± 0.56</td>
<td>56.47 ± 1.05</td>
<td>1.9 ± 0.08</td>
<td>19.3 ± 0.5</td>
<td>20 ± 0.96</td>
<td>82 ± 1.04</td>
<td>16 ± 0.79</td>
</tr>
</tbody>
</table>

W, H and C represent the sludge from three sewage treatment plants, respectively.

**Figure 6 | Bioleached sludge cakes by filter press:** (a) W sludge; (b) H sludge; (c) C sludge. W, H and C represent the sludge from three sewage treatment plants, respectively.
far lower than required by the GB4284-84 regulations, formulated by China in 1985, for farmland application of sewage sludge. It is favorable for subsequent disposal or reutilization of the sludge cake if sludge is either incinerated or used for land application.

CONCLUSION

In continuous plug-flow bioreactor, the order of the importance for sludge dewaterability, is the sludge moisture content, followed by the nutrients dosage, the aeration rate, and finally, the number of reactors according to orthogonal biocoating experiments. Balanced between practical need and cost, the optimum biocoating conditions in this study include a sludge moisture content range of 98.0%, a nutrient dosage of 9 g/L, an aeration rate of 8 m³/h and 10 reactors. The confirmation experiments showed that the continuous plug flow bioreactor could achieve significant enhancement of sludge dewaterability due to notable reduction in CST and SRF under the optimized conditions. The findings presented here may serve as a starting point towards the realization of large-scale biological based treatment of sewage sludge.

ACKNOWLEDGEMENTS

The present study was supported jointly by Production-Study-Research Cooperation Project of Henan Province Science and Technology Department of China (162107000018), the Basic and Frontier Technology Research Project of Henan Province Science and Technology Department of China (1152300410036), and the Key Research Projects of Henan Province Education Department of China (15A610020).

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First received 4 September 2017; accepted in revised form 12 February 2018. Available online 15 March 2018