Use of polysorbate 20 and sodium thiosulfate to enhance sewage sludge dewaterability by bioleaching

Jie Zhao, Jingqing Gao and Junzhao Liu

ABSTRACT

Dewatering of sludge is a key problem that must be solved in the sewage sludge disposal industry. In this study, a series of process optimization tests were conducted to learn how to improve sludge treatment. The optimum process of sludge leaching treatment was studied in a specially designed 100-L reactor system. Four factors were investigated and nine batches of bioleaching tests were run at three levels of these factors. Orthogonal experiments showed that the effect of sludge return ratio and aeration rate on the sludge moisture content was significant and hydraulic retention time (HRT) had a clear effect, but nutrient types had a reduced effect on the moisture content of sludge. The primary and secondary order of each factor is reflux ratio > aeration rate > HRT > nutrient type. Under the optimal process, three batches of sludge were processed and the moisture content of the filter press cake was reduced to less than 60%, the organic matter content reduced to below 5%, and the concentration of heavy metals (Cu, Zn, Pb, and Cr) was much lower than the agricultural standard limit, which is suitable for landscaping, composting, and incineration power generation and other resource applications.

Key words | dewaterability, orthogonal analysis, polysorbate 20, sludge bioleaching, sodium thiosulfate

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HIGHLIGHTS

- Biological leaching can not only achieve better sludge dewatering, but also the dissolution of heavy metals.
- Polysorbate 20 can be used to enhance the dispersion of elemental sulfur in the bioleaching reaction.
- $Na_2S_2O_3$ can also be used as an energy source for *Thiobacillus* instead of sulfur powder.
- The sludge can be used for landscaping, composting, and other resource utilization after biological leaching.

INTRODUCTION

With increasing urbanization in China, construction of sewage treatment facilities is undergoing rapid development. A large amount of sludge is being produced, the disposal of which is a serious environmental concern (Pathak *et al.* 2009; Zhang *et al.* 2009a). The origin of sewage and its treatment in sewage treatment plants is variable, and sewage sludge contains high concentrations of toxic metals. Consequently, the disposal of untreated sludge, for example in landfills, is a potential hazard to human health and to the environment (Khanh Nguyen *et al.* 2021).

Activation of sludge is an effective method of treating waste, but it has a serious drawback in that it produces huge amounts of excess waste sludge. A subsequent dewatering step is usually needed to reduce the sludge volume and

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facilitate its transport and handling and to minimize the quantity of bulking agents added during composting or the energy needed to dry or incinerate the waste sludge (Chen *et al.* 2001a; Zhang *et al.* 2010). When the sludge is treated with mechanical dewatering methods, the moisture content can only be reduced to approximately 70% (Lo *et al.* 2001; Wójcik & Stachowicz 2019a) and the disposal of the excess sludge may account for 25–65% of the total operational cost of the entire wastewater treatment process (Lv *et al.* 2019). Consequently, effective and economical methods must be developed to treat the huge amount of sludge to enhance sludge dewaterability and reduce the degree of difficulty associated with removal of water from the sludge.

Deep dewatering of sludge (which is the chemical conditioning of sludge with higher moisture content, and then high-pressure squeezing and dehydration to below 60% moisture content) and the removal of harmful substances such as heavy metals are important steps and prerequisites for the safe disposal and recycling of sludge. Sludge conditioning methods to achieve sludge press filter dehydration include chemical agents, hydrothermal conditioning, microwave ultrasonic, and freeze–thaw conditioning (Diak & Örmeci 2018; Gao *et al.* 2019; Liu *et al.* 2020). These methods have problems including high moisture content of dewatered sludge (about 80%), high cost, increased inorganic content, and susceptibility to climatic conditions (Wójcik & Stachowicz 2019b). In order to solve the problems contained in the above-mentioned sludge treatment technology and the inability to dissolve heavy metals in sludge, based on the mechanism of biological hydrometallurgy, researchers have developed a new biological treatment technology of sludge–sludge biological leaching technology (bioleaching). Sludge dewaterability can be improved 4–10 times by bioleaching, and the moisture content of the resulting sludge cake can be as low as 60% after being pressed by a diaphragm filter (Liu *et al.* 2012; Huang *et al.* 2020). After bioleaching treatment, heavy metals in the sludge can be removed, pathogenic bacteria killed, and the odor eliminated (Fontmorin & Sillanpää 2015). With *Acidithiobacillus thiooxidans*, which oxidizes sulfur into sulfate ions, an acidic environment is produced. The heavy metals in the sludge are converted to heavy metal sulfates and, at the same time, in the acidic environment, the zeta potential of biological sludge is neutralized. The acidic environment improves the flocculation and settleability of the sludge, which in turn, improves its dewaterability (Chen *et al.* 2001b; Liu *et al.* 2016).

In the biological leaching system, it is necessary to add sulfur powder nutrient, which is a kind of powdered elemental sulfur. The direct contact reaction between *Thiobacillus* and elemental sulfur is an important prerequisite for bacterial biological sulfur oxidation. However, due to the hydrophobic nature of sulfur powder, it is difficult to disperse in the reactor, thus reducing contact with *Thiobacillus* and limiting the rate of the bioleaching reaction (Zhang *et al.* 2020). However, the addition of surfactants can enhance the hydrophilicity and dispersibility of elemental sulfur (Huo *et al.* 2014).

Polysorbate 20 is a commonly used surfactant with the advantages of solubilization, wetting, foaming, and emulsification. It can be used to enhance the dispersion of elemental sulfur in the bioleaching reaction. Sodium thiosulfate can also be used as an energy source for *Thiobacillus* instead of sulfur powder. It is easily soluble in water and is easily oxidized by organisms. In the early stage, the biological leaching technology was studied for the dewatering of sludge and the extraction of heavy metals in shake flask tests, which could not provide an accurate basis for actual project operation (Gao *et al.* 2018). The static amplification test carried out using the shake flask test is therefore a preliminary predictive test for actual engineering applications. However, the effectiveness of bioleaching is highly dependent on the physical, chemical, and biological characteristics of the system. The maximum yield of bioleaching is achieved when these parameters are considered and optimized collectively. In this study, the *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* were separated and purified first, and then polysorbate 20 and sodium thiosulfate were used to cultivate *Thiobacillus*. The cultivated bacteria was used to carry out sludge biological leaching research. An orthogonal design experiment was used to study the enhancement effect of polysorbate 20 and sodium thiosulfate on the biological leaching effect of sludge under the four reaction conditions: nutrient type, aeration rate, reflux ratio, and hydraulic retention time (HRT). The moisture content of the sludge cake dewatered by the plate and frame filter press were used to investigate the optimum sludge bioleaching reaction conditions for polysorbate 20 and sodium thiosulfate in order to provide important parameter support for the engineering application of sludge bioleaching.

MATERIALS AND METHODS

Materials

The sludge used in this study (Table 1) was obtained from the inlet of the sludge thickening tank from Zhengzhou Wulongkou Municipal Wastewater Treatment Plant in Henan, China. After 2 h settling, the supernatant was removed and the sludge was stored at 4 °C as the

Table 1 | Physicochemical characteristics of the tested sludge

Parameters	**Value**	**Parameters**	**Value**
pH	6.86	Cu (mg/kg)	513.5
Oxidation-reduction potential (ORP) (mV)	14	Zn (mg/kg)	986.3
Organic matter (%)	50.81	Pb (mg/kg)	103.9
Moisture content (%)	97.31	Cr (mg/kg)	206.6
Specific resistance to filtration (SRF) × 10^{13} (m/kg)	1.33		

experimental source material. Before conditioning and dewatering, the sludge sample was kept in a water bath at 20 °C for 30 min and some characteristics of the experimental sludge were analyzed.

Preparation of inocula and cultivation

Modified 9 K (Rubio & Garcı´a Frutos 2002; Murugesan *et al.* 2014) and Waksman (Zhou *et al.* 2013; Gao *et al.* 2018) liquor media were used to cultivate *Acidithiobacillus ferrooxidans* (ATCC 23270) and *Acidithiobacillus thiooxidans* (ATCC53990), respectively, both of which were obtained from American Type Culture Collection (ATCC). Before supplements of either 44.2 g/L $FeSO_4{\cdot}7H_2O$ (ferrous sulfate septihydrate) (Tianjin Guangfu Technology Co. Ltd) or 10 g/L elemental sulfur (Tianjin Shengao Chemical Reagent) were added as energy sources, the modified 9 K or SM medium was autoclaved at 121 °C for 15 min. The culture inoculated with *Acidithiobacillus ferrooxidans* or *Acidithiobacillus thiooxidans* was incubated at 28 °C and 3 Hz in a double-layer constant temperature culture oscillator is (ZHWY-2102, Shanghai Zhicheng) for 3–4 days until a level of 10^7–10^8 colony forming units (cfu)/mL was reached.

The sequencing batch reactor used in this experiment is composed of a number of 150-L plastic barrels and an aeration system. The aeration rate is controlled by the rotor flow meter. At the beginning of the reaction, 100 L of raw sludge, was placed in the reactor with 10 L of *Acidithiobacillus ferrooxidans* mixed with 10 L of *Acidithiobacillus thiooxidans* cultures. Elemental sulfur (Tianjin Shengao Chemical Reagent), sodium thiosulfate (Chemical reagents of Tianjin Denke), ferrous sulfate septihydrate, or polysorbate 20 (Chemical reagents of Sinopharm Group) were added, at a concentration of 2 g/L. Continuous aeration was applied until the sludge pH decreased to approximately 2. The reaction was carried out under natural conditions (15–20 °C) and an aeration level of 0.4 m^3/h, during which time the pH was monitored at 12-h intervals. The raw sludge was then inoculated with 10% (v/v) of the sludge slurry. After enriching the culture twice according to the above steps, *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* became the dominant strains. This mixed culture, called the returned sludge, was used as the inoculum in subsequent experiments. The test device is shown in Figure 1.

Orthogonal design

In this study, the types of nutrition, aeration rate, sludge return ratio, and HRT were used to test four factors, with each factor having three levels (Table 2). Nutrition X was 2 g/L elemental sulfur, 1 g/L polysorbate 20, and 8 g/L ferrous sulfate septihydrate. Nutrition Y was sodium

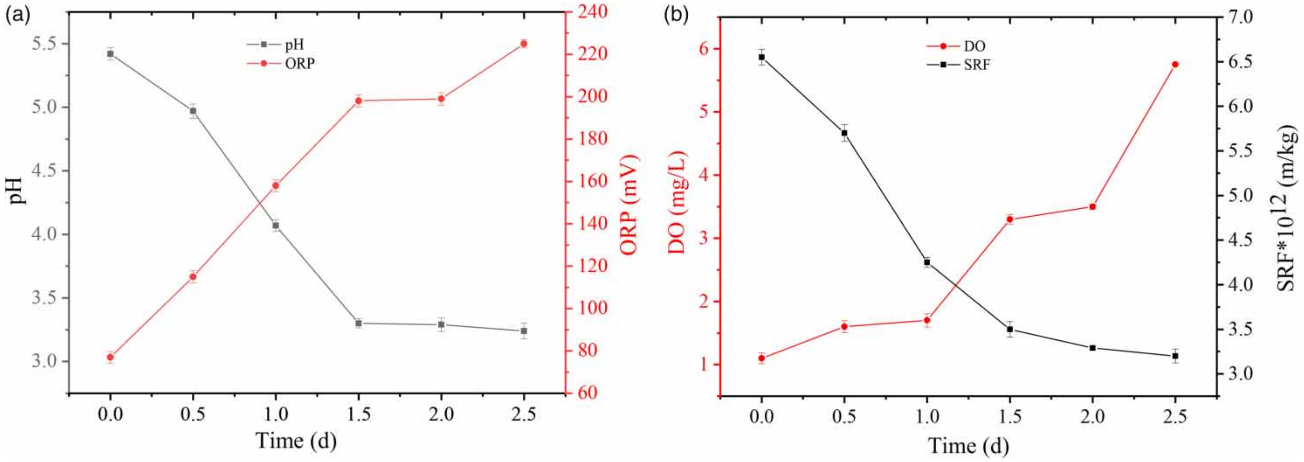

Figure 1 | Changes of pH and ORP (a), specific resistance to filtration (SRF) and dissolved oxygen (DO) (b) with time during the eighth batch of sludge bioleaching process.

Table 2 | The factors and levels of orthogonal test

	A Type of nutrition	B Aeration rate (m^3/h)	C Sludge return ratio (%)	D HRT (d)
1	X	0.8	50	1.5
2	Y	1.6	60	2
3	X + Y	2.4	70	2.5

thiosulfate 1.5 g/L and 8 g/L ferrous sulfate septihydrate. X + Y implies a 1:1 mixture of X and Y.

Analysis methods and statistical analysis

The pH and ORP were measured using a pHS-3C digital pH meter (pHS-3C, Shanghai INESA Scientific Instrument Co. Ltd, China), and dewaterability was determined by measuring the specific resistance to filtration (SRF), determined by the Buchner funnel vacuum suction method. Dissolved oxygen (DO) was measured with a Dissolved Oxygen Meter (OXi315i/SET, German WTW Company). The Pb, Zn, Cr, and Cu contents of the sludge were measured by inductively coupled plasma atomic emission spectrometry using the nitric acid–perchloric acid method (Velmuzhov *et al.* 2020) (iCAP6500DUO Spectrometer American Thermoelectric Company). All treatments were performed in triplicate, and the data presented graphically are the mean and standard deviation of three independent experiments. The single factor and general variance analysis of the test results were carried out using IBM SPSS Statistics 19. Differences were considered statistically significant when $p < 0.05$. Graphs were prepared using Origin 7.5.

RESULTS AND DISCUSSION

Range analysis of the results of the orthogonal test

The results of orthogonal experiments with these four influencing parameters are presented in Table 3. Judged by the R-value, the order of the influence on the moisture content of sludge is sludge return ratio > aeration rate > HRT > type of nutrition. The effect on the R-value of sludge return ratio and aeration rate were much higher than for HRT and the types of nutrition. According to the K-value, the optimum process conditions were sludge return ratio 70%; aeration rate 1.6 m^3/h; HRT 70%; the type of nutrition X + Y (elemental sulfur, polysorbate 20, sodium thiosulfate, and ferrous sulfate septihydrate).

Table 3 | Orthogonal experiment (L_9 (4^3))

	A Type of nutrition	B Aeration rate (m^3/h)	C Sludge return ratio (%)	D HRT (d)	Moisture content of sludge (%)
1	X	0.8	50	1.5	71.37
2	X	1.6	60	2	58.78
3	X	2.4	70	2.5	57.24
4	Y	0.8	60	2.5	66.86
5	Y	1.6	70	1.5	54.37
6	Y	2.4	50	2	61.87
7	X + Y	0.8	70	2	60.12
8	X + Y	1.6	50	2.5	63.01
9	X + Y	2.4	60	1.5	62.24
K1	62.46	66.12	65.42	62.66	
K2	61.79	58.72	62.63	60.26	
K3	61.03	60.45	57.24	62.37	
R	1.43	7.40	8.18	2.4	

General variance analysis of the results of orthogonal test

The intuitive analysis method only yields the optimal result; it does not distinguish between the test results caused by the fluctuation of the error and the test results caused by the change of the factor level, nor can it get an accurate quantitative estimate. Therefore, this experiment uses SPSS software to perform multiple single-factor analysis of variance and factor analysis of variance on the results.

In the orthogonal experiment, the factor with the smallest sum of the square of the deviation was used as the error estimate. The sum of squares of the deviation of factor A is 3.071, which was the smallest among all factors (Table 4). Accordingly, the type of nutrient was used as the error estimate, and the effect of other factors on the sludge moisture content was tested.

As shown in Table 5, in the study on the effect of polysorbate 20 and sodium thiosulfate on the sludge bioleaching effect, the main effect of factor C is the most significant, that is, the sludge return ratio has a significant impact on the moisture content of the sludge cake

Table 4 | Analysis of variance for the orthogonal experiments

	A Type of nutrition	B Aeration rate	C Sludge return ratio	D HRT
Sum of squares of deviations (SS)	3.071	89.815	103.568	10.326

Table 5 | Analysis of variance for the orthogonal experiments after correction

Source of variance	Sum of deviation squares	Degree of freedom	Mean square	F-rate	Significance
Corrected model	203.709	6	33.951	22.112	0.044
Intercept	34,331.149	1	34,331.149	22,359.581	0.000
Aeration rate	89.815	2	44.907	29.248	0.033
Sludge return ratio	103.568	2	51.784	33.726	0.029
HRT	10.326	2	5.163	3.363	0.229
Error	3.071	2	1.535		
Total	34,537.928	9			
Corrected total	206.780	8			

$(0.01 < p < 0.05)$; followed by aeration volume $(0.01 < p < 0.05)$, and finally HRT $(0.1 < p < 0.5)$. According to Tables 4 and 5, the order of the influence on the moisture content of sludge was sludge return ratio > aeration rate > HRT > type of nutrition.

The sludge return ratio is the main influencing factor in the sludge biological leaching project. Since the pH of the acidified sludge after biological leaching is about 2.0 and the sludge contains a large number of acidophilic target microorganisms, the larger the return ratio, the faster the leaching reaction rate (Li *et al.* 2015). The amount of aeration also has a significant effect on the biological leaching of sludge, and the amount of aeration affects the concentration of O_2 and CO_2 in the sludge (Liao *et al.* 2009). HRT also has an effect on sludge biological leaching in that it affects the leaching effect and reaction efficiency of *Thiobacillus* sludge (Jain *et al.* 2010). Compared with other factors, the type of nutrient has less influence on the biological leaching of sludge, and mainly affects the number of dominant bacteria in the biological leaching process and the acidification process of sludge.

Single factor variance analysis of sludge return rate

It can be seen from Table 6 that when the sludge return ratio is 50%, the moisture content of the sludge cake is 65.417%. When the sludge reflux ratio is 60% and 70%, the moisture content of the sludge cake is 62.62% and 57.243%, respectively. Therefore, the average value of C_3 is the smallest (57.243), and $C_1 > C_2 > C_3$. The higher the sludge reflux ratio, the lower the moisture content of the sludge cake filtered by plate and frame filter press after bioleaching.

Table 6 | Statistics of single factor (return ratio)

			95% confidence interval	
Sludge return ratio	Mean	Standard error	Lower limit	Upper limit
50 (1)	65.417	0.715	62.339	68.495
60 (2)	62.627	0.715	59.549	65.705
70 (3)	57.243	0.715	54.165	60.321

In Figure 1(a) and 1(b) and Figure 3(e) and 3(f), the pH and SRF of the sludge decreased over time, while the SRF and pH of the sludge in Figure 2(c) and 2(d) decreased first, before finally showing a small increase. This may be because the nutrient is used up by *Thiobacillus*, and the original heterotrophic bacteria in the sludge continued to multiply, resulting in poor sludge dewatering performance.

The microorganisms (*Thiobacillus*) in the sludge bioleaching process are aerobic autotrophic microorganisms, and their growth process affects the DO in the system (Liu *et al.* 2007). As can be seen from Figures 1–3, as the reaction progresses, the pH of the sludge gradually decreases and the DO gradually increases. This indicates that the increase in DO during the biological reaction is not only affected by the aeration rate, but also by the pH of the sludge. In the initial stage of the reaction, the pH of the sludge is about 5.0, and the system contains a large number of aerobic heterotrophic microorganisms. The growth of heterotrophic bacteria requires a large amount of O_2. *Thiobacillus* relies on biological oxidation reactions to generate sulfuric acid, which reduces the pH value of the sludge and inhibits the activity of most neutral heterotrophic bacteria, resulting in a decrease in system oxygen consumption and a corresponding increase in DO (Li *et al.* 2018; Marchenko *et al.* 2018).

The eighth, second, and fifth batches of sludge treatment correspond to sludge return ratios of 50, 60, and 70%, respectively. The aeration rate is $1.6\ m^{3/}h$, and the initial pH values of the sludge are 5.24, 5.18, and 4.78, respectively. The greater the sludge return ratio, the lower the initial pH, which is mainly due to the acidified sludge returning to

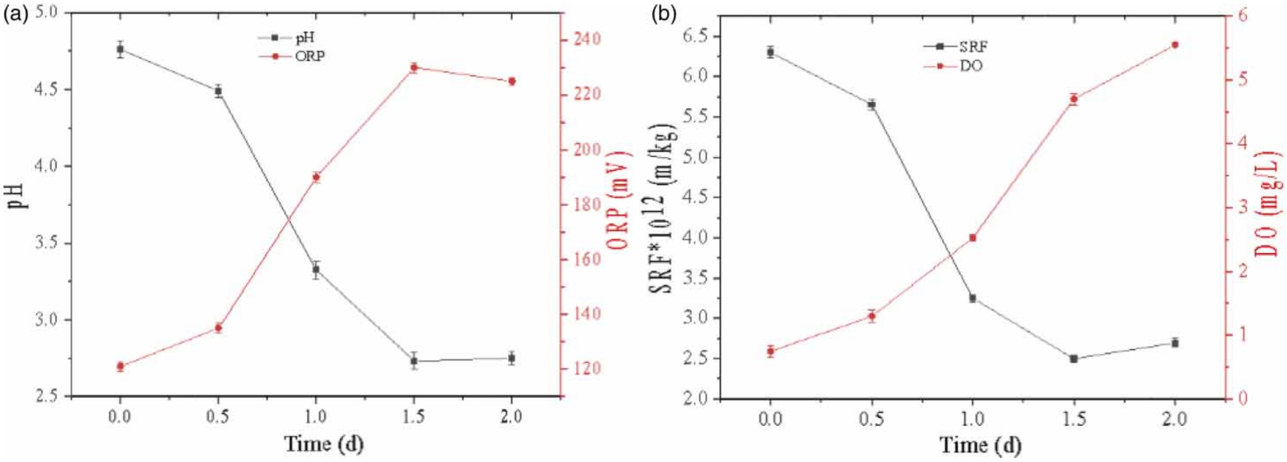

Figure 2 | Changes in pH and ORP (a), SFR and DO (b) over time during the second batch of sludge bioleaching process.

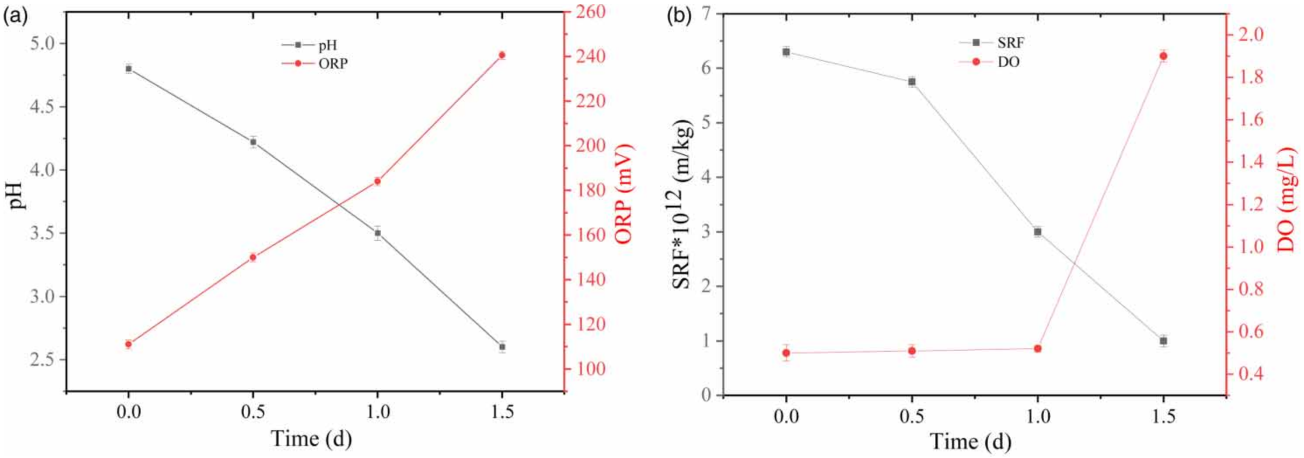

Figure 3 | Changes in pH and ORP (a), SFR and DO (b) over time in the fifth batch of sludge bioleaching process.

pre-acidification. The decrease in sludge pH and the increase in ORP value are mainly due to the sulfuric acid and ferrous ions of *Thiobacillus* biological redox sulfides and ferrous ions, which can indirectly reflect the growth and reproduction of *Thiobacillus* (Lee *et al.* 2020). The SRF of the sludge reflects its dewaterability (Peng *et al.* 2011). The smaller the SRF, the better the sludge dewaterability. The changes of pH significantly affect the SRF. The increase in H^+ ion concentration caused by the oxidation of nutrients can neutralize the negative charge on the surface of the sludge particles, which can reduce the repulsive force between them and improve the dewaterability of the sludge. In addition, the decrease of sludge pH may lead to heterotrophic bacteria dying in captivity, releasing combined water, and increasing the sludge compression coefficient. It can be seen from Figure 4 that the moisture content of sludge with 50% sludge return ratio was about 70%, with poor formability (that is, the ability of sludge to be compressed into a certain shape and maintain this shape in subsequent processes). However, the sludge with 60% and 70% sludge return ratio can be pressed into complete sludge cake, and the moisture was reduced to approximately 55%.

From the paired comparison table of sludge return ratio in Table 7, it can be concluded that C_3 is significantly different from C_2 and C_1 ($0.1 > p > 0.05$), while the difference between C_2 and C_1 is not significant ($0.01 < p < 0.05$). In this experiment, the sludge return ratio of 70% had a significant effect on the moisture content of the sludge cake. Considering the lowest moisture content of the sludge cake, the best return ratio obtained in this test was 70%. The water content of the filter press sludge cake is below 60% to meet the engineering application. Although the leaching reaction time can be shortened, a larger sludge

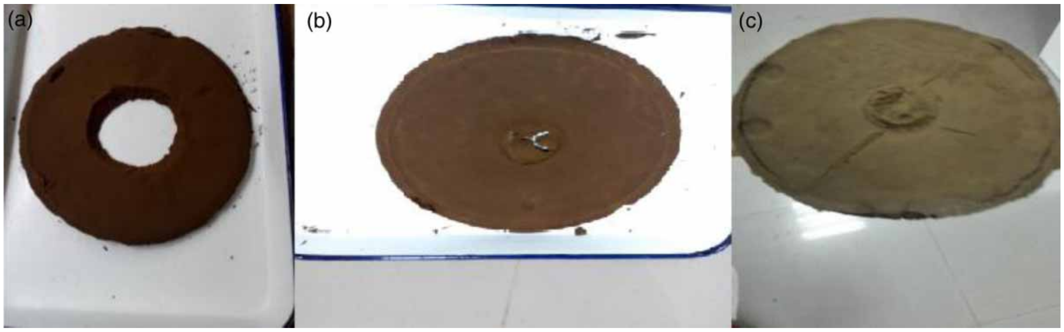

Figure 4 | Pictures of the eighth (a), second (b) and fifth (c) batches of sludge press filter cake.

Table 7 | Paired comparison table of return ratio

					95% confidence interval of difference	
(I) Return ratio	**(J) Return ratio**	**Mean difference (I−J)**	**Standard error**	**Sig.**	**Lower limit**	**Upper limit**
50 (1)	60	2.790	1.012	0.110	−1.563	7.143
	70	8.173*	1.012	0.015	3.820	12.526
60 (2)	50	−2.790	1.012	0.110	−7.143	1.563
	70	5.383*	1.012	0.034	1.030	9.736
70 (3)	50	−8.173*	1.012	0.015	−12.526	−3.820
	60	−5.383*	1.012	0.034	−9.736	−1.030

*The mean difference is more significant at the 0.05 level.

return ratio it may cause a decrease in the volume load or treatment efficiency of the reactor. According to the relationship between the sludge return ratio (E) and the sludge cake moisture content (F), a straight line is fitted. The linear equation is $F = 86.284–0.4087E$. Substituting $F = 60$ into the equation, the sludge return ratio is calculated to be approximately 64.3%. Therefore, combining the treatment effect and treatment efficiency, the best return ratio obtained in this experiment is about 64%.

Aeration rate single factor variance analysis

It can be seen from Table 8 that when the aeration rate is 0.8 m^3/h, the average water content of the sludge cake is 66.117%. When the aeration rate increased to 1.6 m^3/h and 2.4 m^3/h, the average water content of the sludge cake was 58.72% and 60.45%, respectively. By comparison, B_2 has the smallest mean value, $B_1 > B_3 > B_2$.

As shown in Figures 5–9, when the aeration rate is less than 1.6 m^3/h, as the aeration rate increases, the water content of the sludge cake gradually decreases, mainly due to O_2 and CO_2 in the air that can be used as an electron acceptor and carbon source in energy metabolism of

Table 8 | Single factor (aeration volume) statistics

			95% confidence interval	
Aeration rate	**Mean**	**Standard error**	**Lower limit**	**Upper limit**
0.80 (1)	66.117	0.715	63.039	69.195
1.60 (2)	58.720	0.715	55.642	61.798
2.40 (3)	60.450	0.715	57.372	63.528

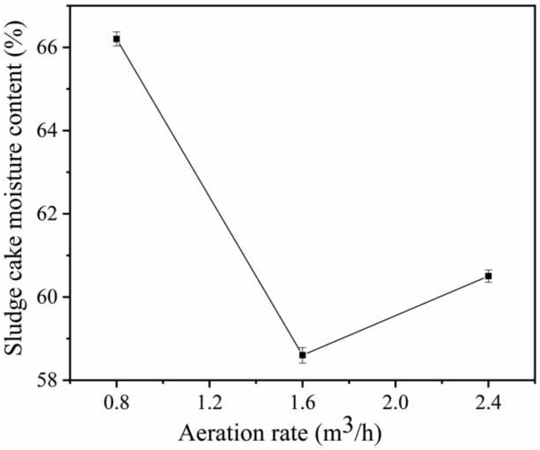

Figure 5 | The relationship between the aeration rate and the moisture content of the sludge cake after biological leaching.

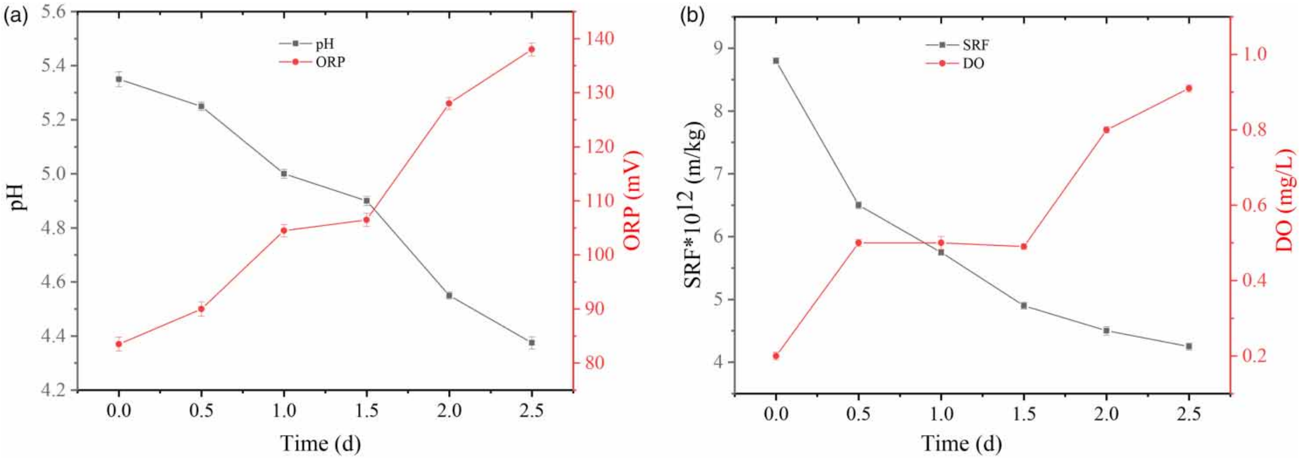

Figure 6 | pH and ORP (a), SFR and DO (b) changes with time during the fourth batch of sludge bioleaching process.

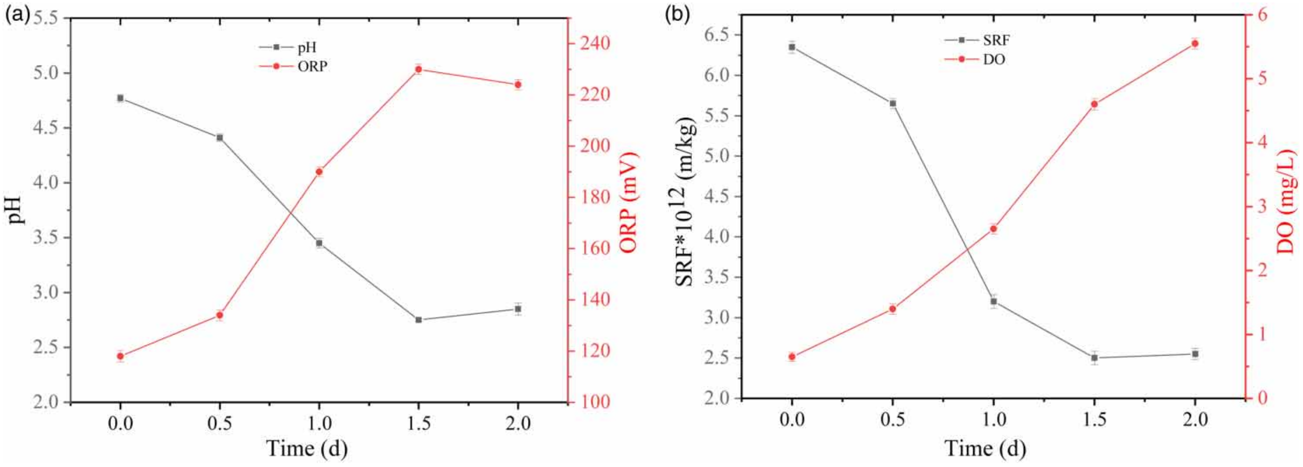

Figure 7 | Changes in pH and ORP (a), SFR and DO (b) with time during the second batch of sludge bioleaching process.

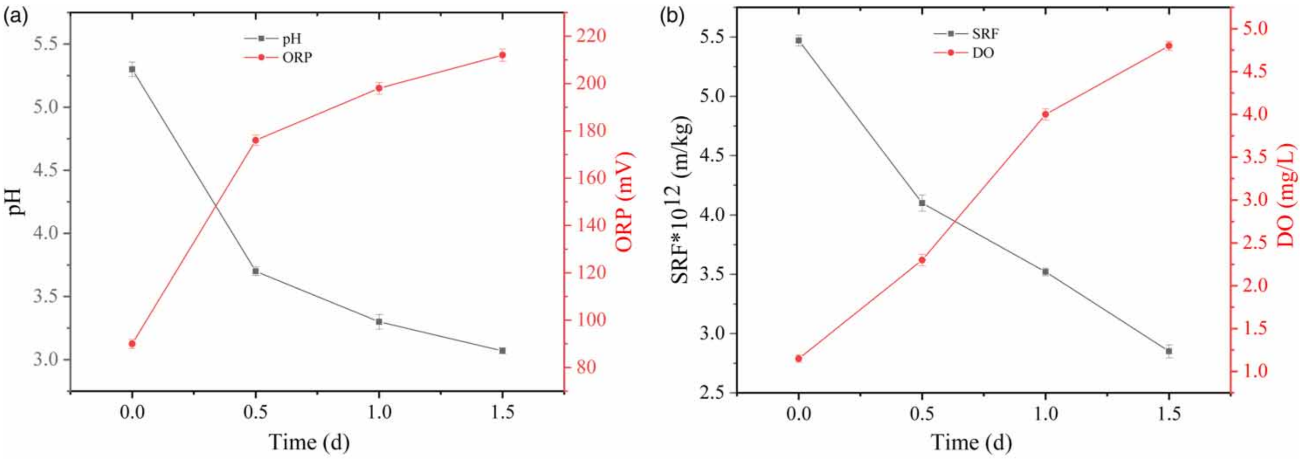

Figure 8 | pH and ORP (a), SFR and DO (b) changes with time during the ninth batch of sludge bioleaching process.

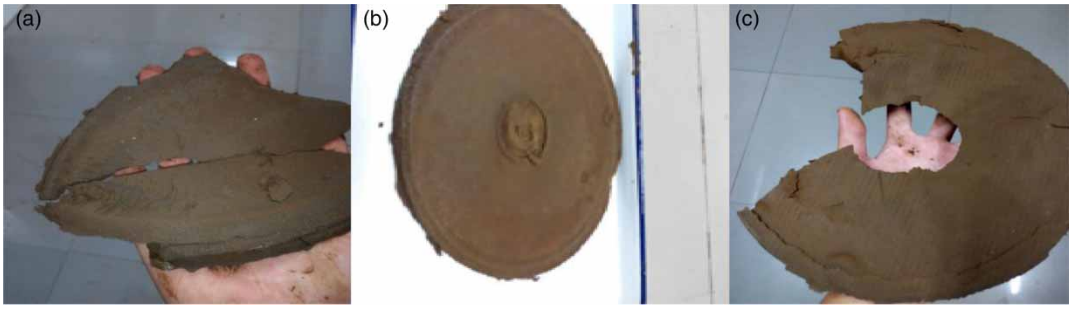

Figure 9 | The pictures of the fourth (a), second (b) and ninth (c) batches of sludge filter cake.

Acidithiobacillus (Murugesan *et al.* 2014). *Acidithiobacillus*, nutrients, and sludge equalized during sludge aeration. The greater the aeration rate, the stronger the turbulence degree and the smaller the viscosity, which are conducive to the transfer of O_2 and enhance the activity of the microbial flora (Li *et al.* 2019). Increasing the aeration rate supports increasing the ORP of the sludge and accelerating the acidification rate of the sludge. Therefore, compared with the group with 0.8 m^3/h aeration rate, the 1.6 m^3/h aeration rate group had the lower pH and SRF. But when the aeration rate was larger than 1.6 m^3/h, the moisture content of the sludge gradually increased with an increase in the aeration rate. Because the native heterotrophic bacteria in the sludge decomposes the organic matter in the growth process, the oxygen consumption is larger than with *Acidithiobacillus*. In the later bioleaching, due to *Acidithiobacillus* rather than heterotrophic bacteria becoming the dominant bacteria, the DO content of the system increased significantly (Zhang *et al.* 2009b). When the aeration rate was high, the acidophilic heterotrophic bacteria in the sludge continued to reproduce, inhibiting the growth and reproduction of autotrophic bacteria. Compared with the autotrophic bacteria, the heterotrophic bacteria are more hydrophilic. Because of the strongly hydrophilic characteristic of extracellular polymeric substances (EPS), excessive EPS leads to poor sludge dewaterability (Zhou *et al.* 2015).

In each group, the DO increased with a decrease of pH in the bioleaching process. Therefore, controlling a 2 mg/L aeration rate in the late leaching reaction can reach the growth demand of *Acidithiobacillus*, and costs can be reduced. It can also be seen from the paired comparison in Table 9 that the difference between B_1 and B_2 and B_3 is significant ($0.01 < p < 0.1$), and the difference between B_2 and B_3 is not significant ($0.5 > p > 0.1$). In summary, the optimal aeration rate required in this test is 1.6 m^3/h.

HRT single factor variance analysis

It can be seen from Table 10 that when the HRT is 1.5 d, the water content of the sludge cake is 62.660%; when the HRT is 2.0 d and 2.5 d, the water content of the sludge cake is 60.257% and 62.37%, respectively. Therefore, the average value of D_3 is the smallest (60.257%), and $D_1 > D_3 > D_2$.

When the HRT is less than 2 d, as the HRT increases, the water content of the sludge cake gradually decreases;

Table 9 | Paired comparison of aeration rate

					95% confidence interval of difference	
(I) Aeration rate	**(J) Aeration rate**	**Mean difference (I–J)**	**Standard error**	**Sig.**	**Lower limit**	**Upper limit**
0.80 (1)	1.60	7.397*	1.012	0.018	3.044	11.750
	2.40	5.667*	1.012	0.030	1.314	10.020
1.60 (2)	0.80	−7.397*	1.012	0.018	−11.750	−3.044
	2.40	−1.730	1.012	0.229	−6.083	2.623
2.40 (3)	0.80	−5.667*	1.012	0.030	−10.020	−1.314
	1.60	1.730	1.012	0.229	−2.623	6.083

*The mean difference is more significant at the 0.05 level.

Table 10 | Single factor (HRT) statistics

			95% confidence interval	
HRT	**Mean**	**Standard error**	**Lower limit**	**Upper limit**
1.5 (1)	62.660	0.715	59.582	65.738
2.0 (2)	60.257	0.715	57.179	63.335
2.5 (3)	62.370	0.715	59.292	65.448

but when the HRT is greater than 2 d, as the HRT increases, the water content of the sludge cake gradually increases.

Microbial growth and reproduction need a certain period to adapt in order to enter the logarithmic growth period. This dictates that the HRT in the biological leaching system cannot be shortened without limitation. When the HRT is relatively short, the *Acidithiobacillus* fails to make full use of nutrients. Utilization of the nutrients will produce H^+ and so the higher the rate of use of nutrients, the lower the pH of the system and the sludge dewaterability is improved (Wong *et al.* 2015). With the sludge bioleaching, however, nutrients will be exhausted and acidophilic heterotrophic bacteria will begin to reproduce, gradually replace the autotrophic bacteria, and become the predominant bacteria. Heterotrophic bacteria produce more EPS than autotrophic bacteria and because of the strongly hydrophilic characteristics of EPS, excess EPS may lead to poorer dewaterability of sludge (Zhu *et al.* 2012). When the HRT was 2 d, the pH and SRF of the treated sludge was the lowest, and the ORP the highest (Figures 10–12). As shown in Figure 12(a) and (b) with the increase of reaction time, the pH decreased first and then increased, and there was a small increase in SRF at the later stage. This result is similar to that reported for the dewaterability of sludge: it deteriorates later in the bioleaching process.

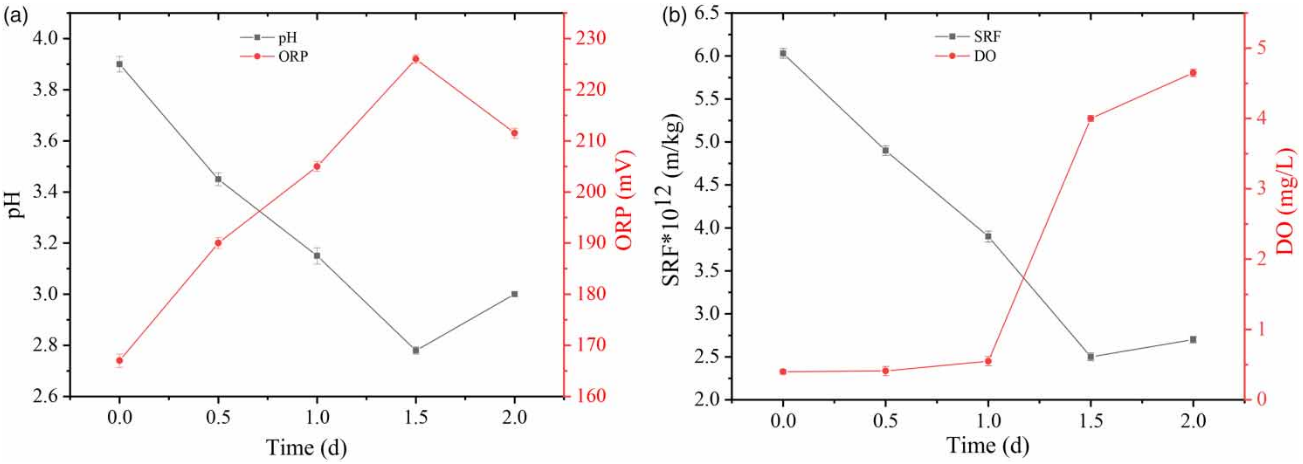

Figure 10 | Changes of pH and ORP (a), SFR and DO (b) with time during the seventh batch of sludge bioleaching process.

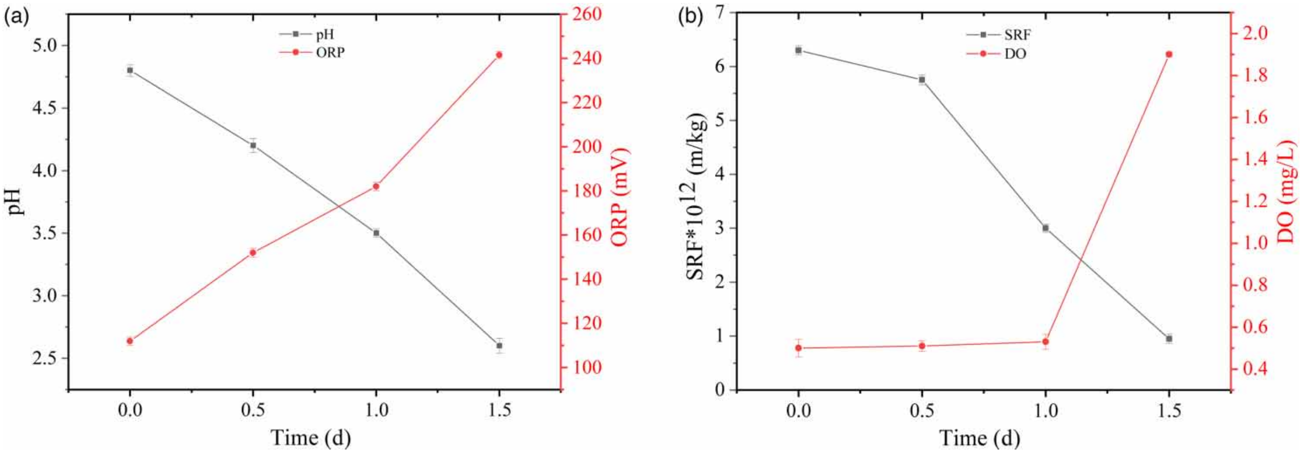

Figure 11 | Changes of pH and ORP (a), SFR and DO (b) with time during the fifth batch of sludge bioleaching process.

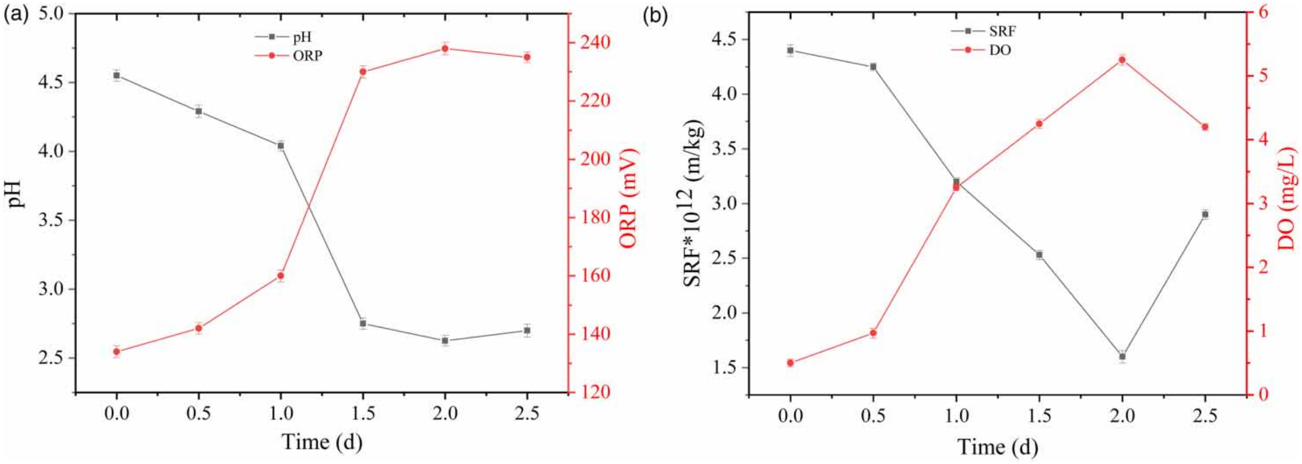

Figure 12 | Changes in pH and ORP (a), SFR and DO (b) with time during the third batch of sludge bioleaching process.

It can be seen from the paired comparison in Table 11 that D_3 is not significantly different from D_1 and D_2 ($0.5 > p > 0.1$). When the HRT was 2 d, the mean value of sludge moisture content was at a minimum. In practical engineering applications, the shorter the time the sludge was in the reactor, the greater the required amount of sludge treatment. The HRT required in this experiment is 2 d.

The type of nutrition single factor variance analysis

Compared with sludge return ratio, aeration rate, and HRT, the type of nutrient had less effect on the moisture content of sludge. As shown in Table 3, when the nutrient agent was X + Y, the moisture content of sludge is the lowest. Using elemental sulfur as a nutrient for bioleaching, because sulfur is strongly hydrophobic, the acidification rate and heavy metal leaching rate are slightly lower. If too much elemental sulfur is added, a large amount of elemental sulfur will remain in the sludge after the reaction, which will affect subsequent resource utilization (Seidel *et al.* 2006). Due to the wetting and dispersion of polysorbate 20, when the concentration of polysorbate 20 was appropriate, the solubility of elemental sulfur was increased, promoting its oxidation. Sodium thiosulfate is extremely soluble in water, and can be used as a sulfur-containing a solution in sludge bioleaching. The nutrient type mainly affects the dominant bacterial population, the acidification process of sludge, the removal efficiency of heavy metals, and the running cost (Liu *et al.* 2015). However, in this experiment, microbial species can grow and propagate after all nutrients have been used. As shown in Table 3, when the nutrient agent was X + Y, the moisture content of sludge was the lowest. It was concluded, therefore, that the best nutrient for this experiment is X + Y.

Verification test

In summary, the optimum conditions of this orthogonal experiment were sludge return ratio 64%, the aeration rate 1.6 m^3/h, HRT of 2 d, nutritional agent elemental sulfur

Table 11 | HRT pair comparison

					95% confidence interval of difference	
(I) HRT	**(J) HRT**	**Mean difference (I−J)**	**Standard error**	**Sig.**	**Lower limit**	**Upper limit**
1.5 (1)	2.0	2.403	1.012	0.141	−1.950	6.756
	2.5	0.290	1.012	0.801	−4.063	4.643
2.0 (2)	1.5	−2.403	1.012	0.141	−6.756	1.950
	2.5	−2.113	1.012	0.172	−6.466	2.240
2.5 (3)	1.5	−0.290	1.012	0.801	−4.643	4.063
	2.0	2.113	1.012	0.172	−2.240	6.466

(1 g/L), polysorbate 20 (0.5 g/L), sodium thiosulfate (0.75 g/L), and ferrous sulfate septihydrate (6 g/L). Under these conditions, three batches of verification tests were carried out to verify the effect of the sludge bioleaching. The indicators before and after the sludge bioleaching are shown in Table 12.

As shown in Table 12, after bioleaching, the pH of three batches decreased from about pH 7 to less than 3, and SRF of the sludge was greatly reduced. This indicated that in the process of bioleaching, the sludge was gradually acidified with the growth and proliferation of the *Acidithiobacillus*, and the dewaterability of the sludge was improved (Jin *et al.* 2019). After bioleaching, the sludge was passed directly through the filter press for 1 h at a pressure of 0.5 MPa without any added flocculant to give a completely formed sludge cake (Figures 13 and 14). The sludge almost fails to stick to the filter cloth, thereby reducing the cloth washing process. The organic matter content of the sludge cake decreased by less than 5%. The level of heavy metals (Cu, Zn, Pb, Cr) in the sludge cake is lower than the value cited in 'control standards for pollutants in agricultural sludge' (GB 4284-1984) (Figure 15). Therefore, the sludge is suitable for landscaping, compost, and other resource utilization. Determining whether the low pH value of the sludge after biological leaching (2.35–2.57) and the leachate containing heavy metals during the leaching process are both harmful is the next step.

Table 12 | Various indexes of sludge before and after leaching

	Raw sludge	First batch	Second batch	Third batch
pH	6.86	2.35	2.57	2.43
SRF (10^{13} m/kg)	1.33	0.15	0.23	0.19
Moisture content of sludge cake (%)	97.31	54.69	56.66	48.94
Organic matter content (%)	50.81	47.39	45.03	46.58

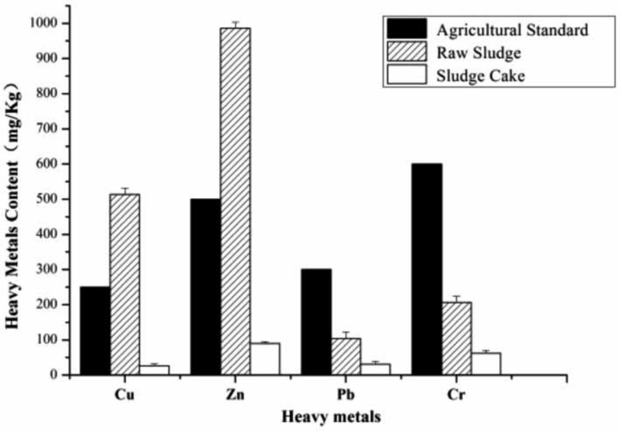

Figure 15 | Contents of heavy metals in the raw sludge and sludge cake.

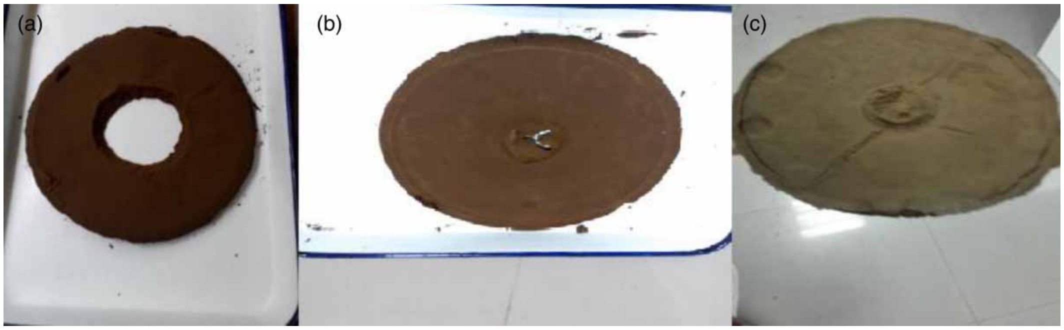

Figure 13 | The pictures of the seventh (a), fifth (b) and third (c) batches of sludge filter cake.

Figure 14 | Sludge filter cakes by filter press in verification test (a: batch 1, b: batch 2, c: batch 3).

Mechanism of bioleaching enhanced sludge dewatering and leaching of heavy metals

Bioleaching enhanced sludge dewatering mechanism

The binding states of heavy metals in the sludge mainly include metallic sulfides, phosphates, carbonates, and organic matter.

Thiobacillus removes heavy metals in the sludge by relying on its biological oxidation reaction to generate sulfuric acid, which reduces the pH of the sludge and increases the ORP. The heavy metals in the sludge are transferred from the original solid phase to the liquid phase. Solid–liquid separation is carried out to remove heavy metals and is generally divided into two leaching mechanisms: direct mechanism and indirect mechanism (Xu *et al.* 2017; Bolton *et al.* 2019).

(1) Direct mechanism

Thiobacillus is directly adsorbed on the sludge particles. After the bacterial EPS reacts with heavy metals on the surface of the sludge, the metals in the sludge are dissolved in an ion state, and the sulfur element is oxidized to SO_4^{2-} (Zeng *et al.* 2015):

$$MS + 2O_2 \rightarrow M^{2+} + SO_4^{2-} \ (\textit{Thiobacillus})$$

(2) Indirect mechanism

Thiobacillus ferrooxidans first oxidize Fe^{2+} to Fe^{3+}. Fe^{3+} can oxidize metal sulfur compounds with a lower valence state to form elemental sulfur and dissolve metal ions. At the same time, elemental sulfur is oxidized by *Thiobacillus thiooxidans* to sulfuric acid, and Fe^{2+} is oxidized to Fe^{3+} by bacteria, forming an oxidation–reduction cycle system. The pH of the sludge drops to about 2.0 and the ORP increases, which accelerates the dissolution of heavy metal ions. The reaction equation is (Wiśniowska & Włodarczyk-Makuła 2018; Zheng *et al.* 2019):

$$2Fe^{2+} + 1/2O_2 + 2H^+ = 2Fe^{3+} + H_2O \ (\textit{Thiobacillus})$$

$$MS + 2Fe^{3+} = 2Fe^{2+} + M^{2+} + S^0 \ (\text{chemical oxidation})$$

$$2S + 3O_2 + 2H_2O = 2H_2SO_4 \ (\textit{Thiobacillus})$$

$$H_2SO_4 + \text{M-sludge} = 2\text{H-sludge} + M^{2+} + SO_4^{2-}$$

Bioleaching enhanced sludge dewatering mechanism

Biological leaching technology can significantly improve the dewatering performance of sludge, and its mechanism of action is more complicated. It can be related to the following factors:

(1) Biological acidification effect

After leaching, the pH value of the sludge can be reduced to below 3.0. The content of H^+ in the sludge is greatly increased. Because H^+ is positively charged, it offsets the negative charge on the sludge particles and reduces the repulsive force between the particles, which makes it easier to coagulate and promote sludge dewatering (Kurade *et al.* 2014).

(2) Microbial substitution effect

In the biological leaching reaction of sludge, the autotrophic *Thiobacillus acidophilus* will completely replace the complex bacteria (mainly heterotrophic bacteria) in the original activated sludge. The *Thiobacillus* form is smaller than the heterotrophic bacteria in the original mud. After the reaction, the heterotrophic bacteria are inhibited or killed, and so the bound water of the sludge will flow out and become free water (Wu *et al.* 2018). Studies have shown that the amount of EPS secreted by *Thiobacillus* is much smaller than that of heterotrophic bacteria, but EPS is more hydrophilic, and the less content, the more beneficial it is for sludge dewatering.

(3) Other effects

Bioleaching microorganisms can oxidize Fe^{2+} to Fe^{3+} at low pH. Fe^{3+} has a certain flocculation effect and can also hydrolyze to form some secondary minerals. The minerals can combine with sludge particles to increase the density of sludge, which is beneficial to compaction and sedimentation of sludge. Minerals can also become the frame support of sludge flocs, changing the compression performance of sludge, and thereby promoting sludge particle coagulation and sludge dewatering.

$$8Fe^{3+} + 14H_2O + SO_4^{2-} = Fe_8O_8(SO_4)(OH)_6 + 22H^+$$

$$M^+ + 3Fe^{3+} + 2SO_4^{2-} + 6H_2O = MFe_3(SO_4)_2(OH)_6 + 6H^+ (M = K, Na, NH_4 \text{ or } H_3O)$$

CONCLUSIONS

The optimal test combination was sludge return ratio 64%, aeration rate 1.6 m^3/h, HRT of 2 d and the type of nutrient

(X + Y elemental sulfur 1 g/L, polysorbate 20 0.5 g/L, sodium thiosulfate 0.75 g/L, and ferrous sulfate septihydrate 8 g/L). Under this condition of bioleaching, the water content of the sludge cake is reduced to below 60%, the decrease in the content of organic matter is no more than 5%, and the content of heavy metals is lower than the limit value of relevant agricultural standards. Therefore, the sludge prepared in this way is suitable for landscaping, compost, and other resource utilization.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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