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Effects of sludge-reflux ratio and energy substrate dose on sludge bioleaching with a two-phase baffled flow reactor

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ABSTRACT

Although two-phase bioleaching can enhance the dual effects of sludge dewaterability improvement and heavy-metal removal due to a superior microbial synergistic effect caused by phase separation, it has not been widely applied because of the unclear optimal ranges of several essential parameters, such as sludge-reflux ratio and energy substrate dose. Therefore, we investigated how the sludge-reflux ratio (15%/10%, 25%/0%, 40%/0%, for the 1st/2nd compartment) and energy-substrate dose ($FeSO_4·7H_2O$: 2, 6, and 10 g/L) affected the two-phase bioleaching process of sewage sludge with a baffled flow bioreactor in this study. The results showed that increasing sludge reflux resulted in rapider acidification but no significant increase in bioleached sludge dewaterability, whereas increasing sludge reflux in 1st compartment enhanced the removal rates of Cu, As, and Pb but had no remarkable effects on Cd, Zn, Ni, and Cr. The acidification and removal rates of heavy metals increased when the $FeSO_4·7H_2O$ dose was increased from 2 to 10 g/L, whereas sludge dewaterability only increased from 2 to 6 g/L. Under optimal conditions (25% sludge-reflux ratio and 6 g/L $FeSO_4·7H_2O$), the specific resistance to filtration and capillary suction time of the sludge decreased by 96.14% and 75.88% after bioleaching, respectively, and the corresponding removal rates of heavy metals were 65.35% (Cd), 65.05% (Zn), 44.31% (Cu), 23.88% (As), 15.81% (Ni), 11.31% (Cr), and 8.15% (Pb).

Keywords: Sewage sludge; Bioleaching; Dewaterability; Heavy metal; Sludge-reflux ratio; Energy substrate dose

1. Introduction

A large amount of excess sludge is generated in the municipal sewage treatment process. For example, in 2019, China generated more than 60 million tons of excess sludge [1]. Excess sludge can cause environmental problems if not properly managed, [2]. However, the treatment and disposal of excess sludge is expensive, accounting for up to 50% of the total operation cost for a typical sewage treatment plant [3,4]. High-efficiency dewatering aids in reduction of expenditure on the subsequent treatment and disposal of excess sludge [2]. Nevertheless, the moisture content of the dewatered sludge remains high (>80%) after mechanical dewatering with flocculants [5]. Meanwhile, some excess sludge contains high total concentrations of heavy metals if the wastewater sources have these metals, which limits the land utilization of excess sludge [6]. Therefore, it is important to develop novel pre-treatment methods to promote sludge dewaterability and remove heavy metals from sewage sludge.

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In recent decades, several technologies have been developed to promote sludge dewaterability, including chemical, physical, and biological conditioning methods [4,5]. These methods have been proven to be able to improve sludge dewaterability [7–10]. However, the apparent disadvantage of chemical and physical conditioning methods is the high consumption of expensive chemicals or operational energy. For example, advanced oxidation processes (Fenton, persulfate oxidation, and potassium ferrate oxidation) are emerging chemical conditioning methods that require large amounts of oxidizer input [11–13]. Bioleaching is a promising approach when compared to chemical and physical procedures since it improves sludge dewaterability while also removing heavy metals [14,15]. During bioleaching, *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* can utilize S^0 or Fe^{2+} as electron donors to produce a high concentration of H^+ via the biological oxidation of S^0 or Fe^{3+} hydrolysis [5]. By charge neutralization the increase in H^+ reduces the zeta potential of the sludge floc to approximately zero, which is favorable for coagulation, settling, and subsequent mechanical dewatering of bioleached sludge [16]. Bioleached sludge shows lower values of specific resistance to filtration (SRF) and capillary suction time (CST) than un-bioleached sludge [17–19]. In addition, heavy metals can be removed from sewage sludge because they are transformed from carbonate-bound and organic-bound to dissolved ions in conditions having low pH and strongly oxidative environments [5,15,17,20]. Meanwhile, some studies have found that combining Fe^{2+} with sludge reflux could also accelerate the pH decrease and improve the removal rate of heavy metals in a bioleaching reactor [21]. Sludge reflux could supplement the bioleaching microbial flora lost during reactor operation. *Acidithiobacillus ferrooxidans* takes Fe^{2+} as an energy substrate and bio-oxidizes it, which generates H^+ because of the hydrolysis of Fe^{3+} [5,22].

However, several studies have shown that low-molecular-weight organic acids or dissolved organic matter (DOM) in the sludge can inhibit the growth of *Acidithiobacillus* species and hinder the bioleaching efficiency of the sludge [18,23]. To overcome this disadvantage, acid-tolerant heterotrophic microorganisms (*Brettanomyces* sp. and *Rhodotorula* sp.) have been isolated and introduced into the bioleaching system to degrade DOM and further enhance the activity of *Acidithiobacillus* species [19,23,24]. Despite the synergistic effect between *Acidithiobacillus* species and acid-tolerant heterotrophic microorganisms, the optimal pH values for their survival differ [22,25]. Pathak et al. [20] reviewed various studies with different modes of bioleaching operations and found that *Acidithiobacillus* species can grow at pH in the range of 1.0–4.5 with an optimum range of 2.0–2.3. The optimal pH value for acid-tolerant heterotrophic microorganisms is reported to be in the range of 5.0–7.0 [18,19].

The energy substrate is added to the same compartment of the reactor in which the bioleached sludge is refluxed in the single-phase bioleaching reactor, which causes the pH value (pH = 2.0) in the bioreactor to be around the optimal range of *Acidithiobacillus* species [21,26]. Nevertheless, this strongly acidic environment inhibits the growth of acid-tolerant heterotrophic microorganisms [9,22]. A feasible strategy is to modify single-phase bioleaching to two-phase baffled flow bioleaching, wherein two-phases with different pH values are produced to provide two types of suitable growth environments for each kind of microbe in the bioleaching reactor.

The 1st and 2nd compartments were returned to bioleached sludge, and an energy substrate was added to the 2nd compartment. The 1st phase was referred to as the selection phase, while the 2nd phase was referred to as the acidification phase. A two-phase bioleaching reactor could reduce the DOM content and provide a more suitable growth environment for acid-tolerant heterotrophic microorganisms like *Acidithiobacillus* [9]. However, the reactor has not been extensively employed since several of its important process parameters, such as the sludge reflux ratio and amount of energy substrate, remain unknown. The current study evaluated how the sludge-reflux ratio and energy substrate dose influenced the sludge bioleaching process using a two-phase baffled flow reactor, including dewaterability based on CST and SRF and the removal rates of heavy metals from sewage sludge.

2. Materials and methods

2.1. Raw sewage sludge

Two types of sludge were collected from different wastewater treatment plants (WWTPs) in Guilin, China. The inoculated sludge came from the sludge thickening tank of the Yanshan WWTP (total solid content (TS) of 2%), whereas the conditioning sludge came from the dewatering plant of the Shangyao WWTP (TS of 16%). Tap water was used to dilute the sludge to 2%. After dilution, the sludge was passed through a 10-mesh (2 mm) nylon sieve to remove large particulates, such as sand and fibrous substances, to prevent the blocking of peristaltic pumps. Physico-chemical properties of the sludge from Yanshan/Shangyao WWTPs were as follows: pH 7.2 ± 0.2/7.0 ± 0.1, zeta potential −15.4 ± 1.2/−14.6 ± 0.6 mV, soluble chemical oxygen demand (SCOD) 619.0 ± 18.8/415.5 ± 3.2 mg/L, CST 31.5 ± 1.3/50.9 ± 7.8 s, and SRF 3.9 ± 0.3 × 10−13/2.5 ± 0.4 × 10−13 m/kg. Table 1 shows the concentrations of the heavy metals in the sludge.

2.2. Experimental bioreactor

The two-phase bioleaching system consisted of a mixing tank, a baffled-flow bioreactor with five compartments, a sludge storage tank, an air pump, and two peristaltic pumps (Fig. 1). The bioreactor was made of transparent Plexiglass with lengths, widths, and heights of 300, 200, and 300 mm, respectively, and was divided into four identical compartments. Each compartment was divided by a baffle into a downflow area and an upflow area, with lengths of 15 and 60 mm, respectively. The lower part of the baffle was equipped with a front 45° deflector with a length of 14 mm. The raw sludge was continuously supplied into the 1st compartment after being mixed in the mixing tank, and then passed through four compartments in sequence. The bioleached sludge was discarded into the sludge storage tank, a portion of which was refluxed into the 1st and 2nd compartments, and the remainder was discharged from the system daily. $FeSO_4{\cdot}7H_2O$ was added to the 2nd compartment as the energy substrate for two-phase bioleaching.

Table 1
Concentrations of heavy metals in the raw sewage sludge

Heavy metals	Zn	Cu	As	Ni	Cr	Pb	Cd
	(mg/kg)						
Group A	904.82 ± 3.68[a]	154.79 ± 15.08[a]	17.80 ± 0.14[a]	31.22 ± 2.94[a]	431.60 ± 2.58[a]	43.45 ± 1.47[a]	2.29 ± 0.03[a]
Group B	849.05 ± 17.07[a]	136.13 ± 1.74[a]	17.13 ± 0.46[a]	28.37 ± 1.08[a]	493.24 ± 5.17[a]	42.17 ± 1.81[a]	2.26 ± 0.44[a]

[a] indicates mean value ± standard error, $n = 4$.

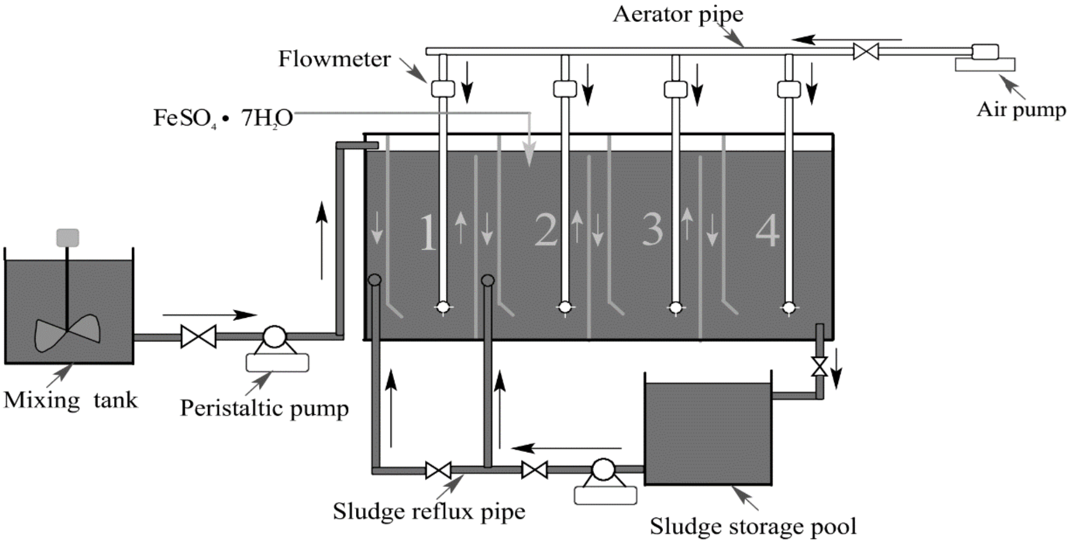

Fig. 1. Schematic diagram of baffled flow bioreactor for two-phase bioleaching: 1, 2, 3, and 4 for the 1st, 2nd, 3rd, 4th compartments, respectively.

2.3. Enrichment and acclimation of inoculum

A two-step procedure was applied to enrich and acclimate the inoculum to sludge bioleaching. Step one involved loading 3.3 L of raw sludge into the bioreactor for bioleaching with a $FeSO_4\cdot 7H_2O$ dose of 10 g/(L·d) and aeration at 3.8 L/min (aeration intensity, 60 $m^3/(m^3\cdot h)$). After the pH value of the bioleached sludge decreased to 2.0–3.0, bioleaching lasted for another 2–3 d. In step two, a portion of the bioleached sludge from step one remained in the bioreactor as the inoculum with a proportion of 25.0% (volume/volume) when the remaining bioleached sludge was discharged from the system. The above procedure was repeated three times, and the acclimated inoculum was used for the subsequent bioleaching trials.

2.4. Experimental design

The raw sludge was fed continuously into the 1st compartment with a semi-continuous reflux mode of the bioleached sludge after adding the inoculum prepared in the previous section, followed by the addition of $FeSO_4\cdot 7H_2O$. The bioreactor was fed with the same amount of refluxed sludge and $FeSO_4\cdot 7H_2O$ twice per day (09:00 and 21:00). Two groups of treatments were carried out to investigate the effects of the reflux ratio and substrate dose on two-phase sludge bioleaching of sludge, respectively. In group A, the reflux ratios of the sludge were the same as the inoculum ratios in the corresponding treatments, which were set as follows: A1, 15%/10% of the total bioreactor in the 1st and 2nd compartments, respectively; A2, 25% only in the 1st compartment; and A3, 40% only in the 1st compartment. The added dose of $FeSO_4\cdot 7H_2O$ for all the treatments in group A was 6 g/(L·d) for each treatment. In group B, the three treatments had the same inoculum ratios: 10%/15% of the total bioreactor in the 1st and 2nd compartments, respectively. In terms of sludge reflux ratio, there was a shift in the treatments of group B: 10.0%/15.0% during days 0–15 in the 1st and 2nd compartments, respectively, and 15.0%/10.0% during days 16–20 in the 1st and 2nd compartments, respectively. In group B, $FeSO_4\cdot 7H_2O$ was applied as an energy substrate at doses of 2, 6, and 10 g/(L·d) for B1, B2, and B3, respectively. For each treatment in both groups A and B, bioleaching was operated with a sludge influent of 7.5 L/d (retention time of 2 d) and a total aeration rate of 3.8 L/min. Before the bioleached sludge was semi-continuously refluxed, sludge samples were collected from each compartment to measure the pH value. The bioreactor was finished when the pH value was stable for 2–3 d. Subsequently, each bioreactor was operated for 7–10 d. During this period, samples of the bioleached sludge were collected from each compartment once in group A and twice in group B to determine the physico-chemical characteristics, heavy metal concentrations, and dewaterability properties.

2.5. Analytical method

The moisture content was measured using the gravimetric method, and the pH was measured using a pH meter (PB-10, Sartorius Group, Germany). The zeta potential was measured using a particle size and zeta potential analyzer (Nano ZS90, Malvern, UK). Sludge samples were centrifuged at 4,000 rpm for 10 min at room temperature, and then the supernatant was collected for the determination of SCOD using catalytic digestion and titration, where SCOD indicates the presence of DOM in the sludge slurry.

The SRF was measured by the vacuum-filtration method at –0.03–0.04 MPa in a 150 mm Buchner funnel with a Whatman Filter Paper Grade No. 1. The filtrate volume was recorded every 10 s until the sludge cake cracked or no filtrate was obtained. The filtration process of sludge on filter paper obeys the Kozeny–Carman equation [Eq. (1)] [27]:

$$\left(\frac{t}{v}\right) = \left[\frac{(\mu w r)V}{(2\mathrm{PA}^2)}\right] + \frac{(\mu R f)}{\mathrm{PA}} \qquad (1)$$

where t, V, μ, w, and A are the filtration time (s), volume of filtrate (m^3), dynamic viscosity coefficient of the filtrate (Pa·s), solid mass trapped in the sludge cake on the filter membrane per unit volume of filtrate (kg/m^3), and area of the filter membrane (m^2), respectively; and R_f is the resistance of the filter medium (L/m).

Based on Eq. (1), the SRF, labeled r, can be obtained using Eq. (2):

$$r = \left[\frac{(2\mathrm{PA}^2)}{\mu}\right]\left(\frac{b}{w}\right) \qquad (2)$$

where b is the slope obtained by the linear fitting of Eq. (1).

Sludge CST was determined using a CST Meter (304M, Triton, UK) with a 1.8 cm funnel diameter and 20 mL of sludge sample.

To determine the total concentrations of the heavy metals, the collected sludge samples were pretreated, digested, and filtered. Pretreatment included lyophilization and grinding to pass through a 100-mesh (0.15 mm) sieve. The sieved sample (0.1 g) was digested with boiling aqua regia ($HCl + HNO_3$, 3 + 1, volume) for 2 h and filtered through a Millipore membrane of 0.45 mm pore. The filtrate was used to determine the total concentrations of heavy metals using inductively coupled plasma mass spectrometry (NexION 350X, PerkinElmer, USA).

2.6. Statistical analysis

R software [28] and Microsoft Excel 2016 were used for statistical analysis and plot figures.

3. Results and discussion

3.1. Effects of sludge-reflux ratio on bioleaching

3.1.1. Physico-chemical properties

The bioleaching process was divided into two stages for three trials in group A: starting in the first 4–8 d and stabilization in the later 12–16 d (Fig. 2A–2D). Phase separation

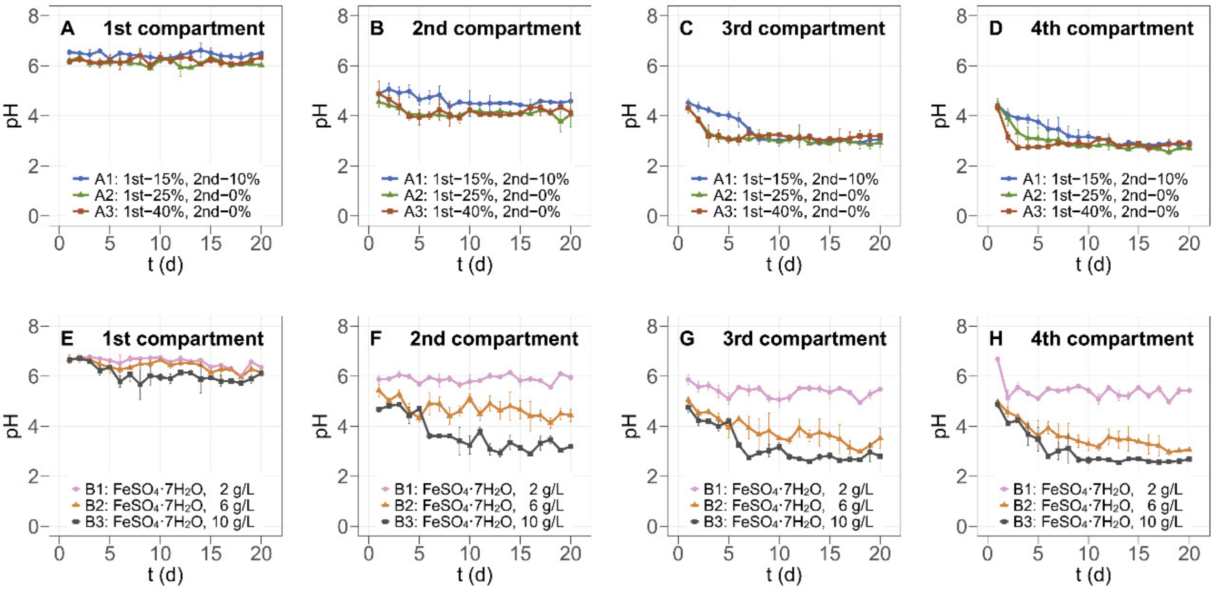

Fig. 2. pH values in all the compartments during bioleaching in two groups of three trials: A, B, C, D for the 1st–4th compartments in group A, E, F, G, and H for the 1st–4th compartments in group B. In group A, the reflux ratios for the 1st and 2nd compartments were 15% and 10% (A1), 25% and 0% (A2), 40% and 0% (A3), respectively; in group B, adding $FeSO_4·7H_2O$ with the doses of 2 g/L (B1), 6 g/L (B2), and 10 g/L (B3).

was observed during both the start and stabilization stages. During the initial stage, acidification occurred in each compartment (2nd, 3rd, 4th) of the acidification phase, indicated by the decrease in pH. The pH values gradually decreased along the sludge-flow direction from the 2nd to 4th compartments, while no pronounced acidification was observed in the selection phase (the 1st compartment). A previous study also reported a similar acidification process during the initial stage of single-phase bioleaching [29]. During the stabilization process, the pH values in each compartment were kept approximately constant, and the pH value in the 1st compartment was substantially higher than the values in the other three compartments. Meanwhile, the increase in the sludge-reflux ratio (for the 1st compartment) accelerated sludge acidification to different extents in the other three compartments (the 2nd, 3rd, 4th) of the acidification phase during the start stage. Compared to A1, both A2 and A3 had a shorter period of 4 d for the start of the bioreactor than A1 (8 d), which was considered to be due to the supplementation of *Acidithiobacillus* from the refluxed sludge, which was observed in another study [9]. The increase in sludge reflux (for the 1st compartment) brought more *Acidithiobacillus* into the bioreactor, which was beneficial for the bio-oxidization of Fe^{2+} and resulted in rapider acidification [5,19].

Both zeta potential and SCOD were investigated only during the stabilization stage (Fig. 3A and B). The zeta potential increased sharply along the sludge-flow direction from the 1st to 3rd compartments in each treatment (group A), with no pronounced difference between the 3rd and 4th compartments. Except for the 2nd compartment, increasing the sludge reflux had no effect on the zeta potentials of each compartment, which was attributed to the similar pH values of the same compartments among the three treatments (group A). During bioleaching, the zeta potential is determined by the neutralization of negatively charged groups with free H^+ [5,9,19], which is related to the pH value in each compartment. The SCOD of the sludge decreased by approximately 80% in the 1st compartment, which meant that the selection phase enhanced the removal rate of SCOD caused by acid-tolerant heterotrophic bacteria (Fig. 3B). However, SCOD increased in the other compartments, which may be due to over-lysis of microorganisms at low pH [5,18]. There was no obvious difference in SCOD in the same compartments between treatments of sludge reflux in the range of

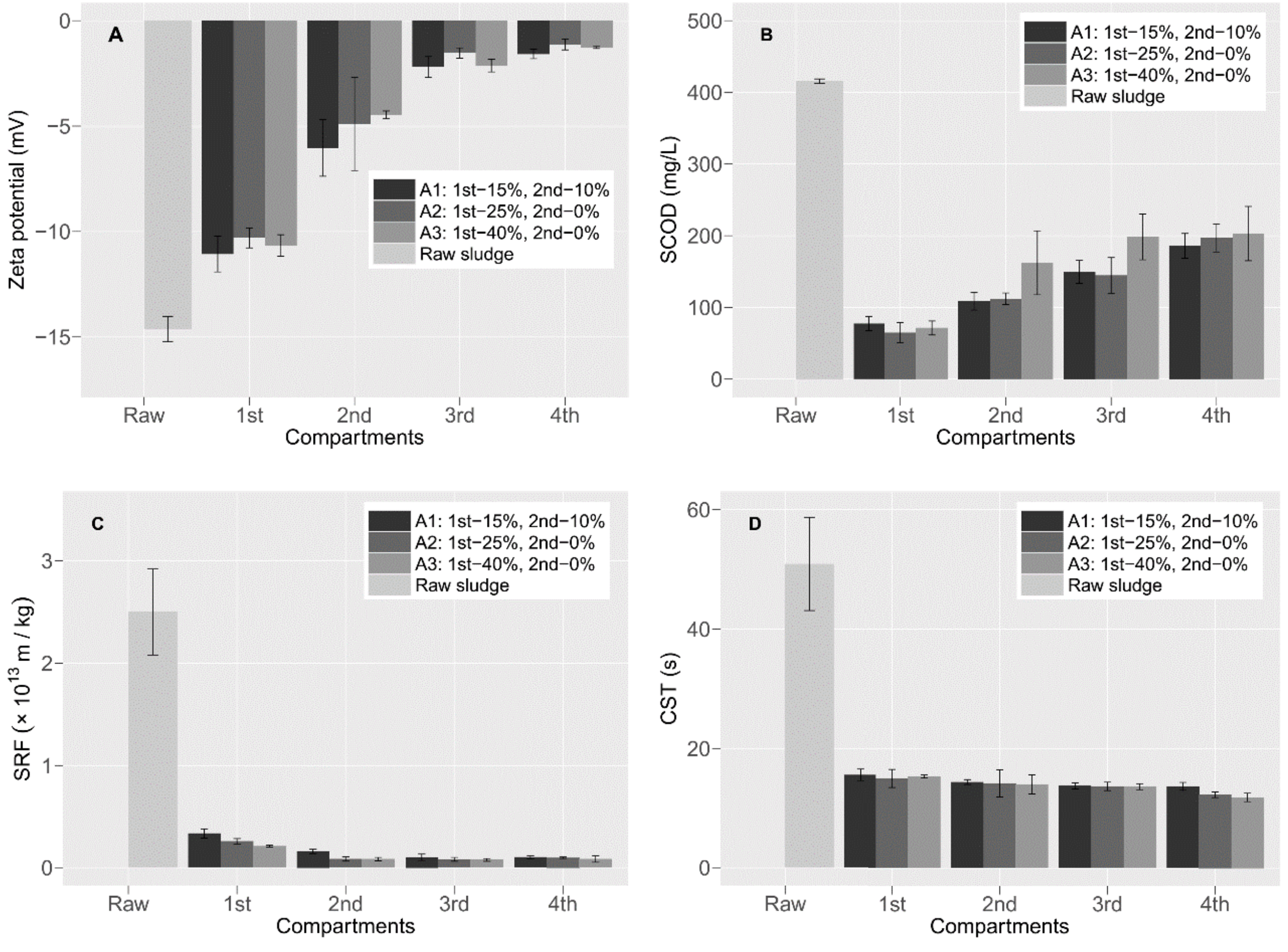

Fig. 3. Zeta potentials (A), SCOD (B), SRF (C) and CST (D) of bioleached sludge in the 1st–4th compartments of three trials in group A. In group A, the reflux ratios for the 1st and 2nd compartments were 15% and 10% (A1), 25% and 0% (A2), 40% and 0% (A3), respectively.

15%–25% for the selection phase, while more SCOD was seen in the 2nd and 3rd compartments when the sludge reflux ratio increased to 40%. The variation in these compartments may be attributed to more *Acidithiobacillus* in A3 resulting from higher sludge reflux [19].

3.1.2. Dewaterability

Fig. 3 shows the evolution of the SRF and CST of sludge in the stabilization stage during the bioleaching process at different sludge reflux ratios (Fig. 3C and D). Based on the raw sludge, the SRF removal rates in the final bioleached sludge were reduced by 89.56% (A1), 96.14% (A2), and 96.69% (A3). With a similar change trend in the SRF through the bioleaching treatment, the sludge CST was reduced by 73.13% (A1), 75.88% (A2), and 76.77% (A3). The high reduction in SRF and CST implied that sludge dewaterability was obviously promoted during two-phase bioleaching. The changes in SRF and CST in the three groups were correlated with the zeta potentials (Fig. 3A). In general, a zeta potential around zero provides less repelling force to sludge particles, facilitating sludge dewatering [30,31]. For these three treatments, the absolute values of the zeta potential in the 4th compartment changed to less than 2 mV after two-phase bioleaching when the corresponding pH values were less than 3, indicating that sludge dewaterability was significantly improved in the three treatments. However, there were only slight differences in the SRF and CST among the groups with different sludge-reflux ratios, which was consistent with the small variation in zeta potentials in these groups. As a result, increasing the sludge-reflux ratio had no effect on the degree of sludge dewaterability improvement during two-phase bioleaching.

3.1.3. Removal of heavy metals

Generally, increasing sludge-reflux ratio in the 1st compartment enhanced the removal of Cu, As, and Pb but had no remarkable effects on Cd, Zn, Ni, and Cr. Fig. 4A and C show the residual concentration and removal rates of heavy metals in the sludge after the two-phase bioleaching treatment with different sludge reflux ratios. The removal rates of heavy metals of sludge with the different sludge reflux ratios throughout the bioleaching were 64.68%/66.13%/62.09% (A1/A2/A3, Zn), 58.08%/65.50%/58.52% (A1/A2/A3, Cd), 37.60%/47.67%/42.49%(A1/A2/A3,Cu),17.75%/25.56%/22.70% (A1/A2/A3, As), 20.37%/19.63%/23.29% (A1/A2/A3, Ni), 7.56%/11.31%/8.54% (A1/A2/A3, Cr), 0.23%/8.15%/6.56% (A1/A2/A3, Pb) (Fig. 4C). During the sludge bioleaching process, microorganisms oxidize insoluble metal oxides to soluble metal sulfates [30,32,33]. Moreover, many studies have found that more heavy metals are leached at low pH [2,31,34,35]. Heavy metal removal rate is highly dependent on the form of metal binding in the sludge [36]. The higher removal rates of Zn, Cd, and Cu were attributed to the dominant chemical forms of the metals in the raw sludge, which were the unstable exchangeable and carbonate fractions [9,37]. The relatively low removal rates of Cr may be due to the reduction of soluble Cr^{6+} to insoluble Cr^{3+} by Fe^{3+}, although some microorganisms may oxidize Cr^{3+} to Cr^{6+} [31]. The lowest removal rate of Pb may be due to a high concentration of SO_4^{2-}, which could bond with Pb^{2+} to form an insoluble sediment, $PbSO_4$ [21,38].

3.2. Effects of energy substrate dose

3.2.1. Physico-chemical properties

Even though there was a larger fluctuation in pH values for each trial in group B than that in group A, the bioleaching process in group B was also divided into two stages: starting on the first 3–9 d and stabilizing in the later 11–17 d (Fig. 2E–H). For each compartment of B2 and B3, there was a similar trend of acidification and phase separation to the corresponding compartment in the trials of group A during the whole bioleaching process, while both acidification and phase separation were not observed in B1 (Fig. 2). Moreover, the pH values of sludge in the compartments of B3 were higher than those in B2 and B1, which meant that adding more energy substrate strengthened the acidification of sewage sludge during two-phase bioleaching. This can be explained by the greater bio-oxidation of Fe^{2+} and increased hydrolysis of Fe^{3+}, which have been observed in previous research on single-phase bioleaching [5,15]. In addition, the pH values in the 3rd, 4th compartments slightly decreased in B3 when the reflux ratio changed from 10%/15% (the 1st/2nd compartment) to 15%/10% (the 1st/2nd compartment), whereas no marked difference was observed for either B1 or B2.

The zeta potential increased sharply along the sludge-flow direction from the 1st to 4th compartments in B2 and B3 (Fig. 5A and B), only a large increase was observed between the 1st and 2nd compartments, and there was little increase from the 2nd to 4th compartments in B1. A larger dose of $FeSO_4{\cdot}7H_2O$ caused higher zeta potentials in both the 3rd and 4th compartments, while no obvious change was observed in both the 1st and 2nd compartments (Fig. 5A and B). The variation in zeta potential was attributed to the variation in the pH value in each compartment among the three treatments with different additions of $FeSO_4{\cdot}7H_2O$ (Fig. 2E–H). The zeta potential increased further in the 2nd compartment for B3 when the reflux ratio was changed from 10%/15% (the 1st/2nd compartment) to 15%/10% (the 1st/2nd compartment), which could be related to more extracellular polymeric substance (EPS) biodegradation by acid-tolerant heterotrophic bacteria [16]. The activity of acid-tolerant heterotrophic bacteria was speculated to recover better when more sludge was refluxed into the selection phase, which was beneficial for biodegrading more EPS and reducing the negative charges outside the surface of the sludge flocs during bioleaching [39]. In group B, the SCOD of the sludge showed a similar change along the sludge-flow direction in each trial of group B to that in group A (Fig. 5C and D), which meant that phase separation effectively recovered the bioactivity of acid-tolerant heterotrophic bacteria under the $FeSO_4{\cdot}7H_2O$ dose of 2–10 mg/L. An increase in the $FeSO_4{\cdot}7H_2O$ dose resulted in higher SCOD in almost four compartments for each trial in group B, which was due to increased cell lysis at a lower pH (Fig. 2E–H). When the reflux ratio varied from 10%/15% (the 1st/2nd compartment) to 15%/10% (the 1st/2nd compartment), B2 had a lower value of bioleached-sludge SCOD in the 3rd, 4th compartments, while higher values were observed for B3, and no distinct difference was observed for B1. These results showed that the biodegradation of SCOD could be enhanced by increasing the reflux proportion of bioleached sludge in the 1st compartment only when the $FeSO_4·7H_2O$ dose was within a limited range.

3.2.2. Dewaterability

Sludge dewaterability was promoted only when the $FeSO_4·7H_2O$ dose was increased from 2 to 6 g/L, whereas no pronounced enhancement was observed from 6 to 10 g/L. Fig. 5 shows the SRF and CST of the raw sludge and bioleached sludge in each compartment during the stabilization stage in three trials of group B. After bioleaching with different energy substrate doses, the SRF of the sewage sludge decreased by 89.40%–95.60%, and most of the reduction occurred in the selection phase, which was similar to the results from group A (Fig. 3C). When the $FeSO_4·7H_2O$ dose was increased from 2 to 6 g/L, more decline occurred in the SRF of the bioleached sludge in the 3rd, 4th compartments, whereas no marked difference was observed between the SRF in these two compartments at doses of 6 and 10 g/L. In addition, the 2nd compartment in B2 had a lower SRF value than the values in B1 and B3, which indicated that adding an energy substrate dose accelerated the decrease in SRF with the dose of $FeSO_4·7H_2O$ in the range of 2–10 g/L. When there was a higher reflux ratio in the 1st compartment, only slight variation occurred in the SRF of the bioleached sludge in the 3rd, 4th compartments, which meant that the SRF change throughout the bioleaching was independent of the reflux portion. The zeta potential is considered a key factor for SRF during sludge bioleaching, and the decrease in SRF becomes larger when the zeta potential is closer to zero [2,31]. However, in our study, a large decrease in SRF occurred in the 2nd compartment of B3, even though there was a low value of zeta potential, which could be explained by the flocculation enhancement caused by a high concentration of Fe^{3+} in this trial. For the trials of group B with reflux ratios of 10%/15% (the 1st/2nd compartment), the decrease in sludge CST occurred mainly in the first two compartments, and there was no obvious difference between the 3rd and 4th compartments (Fig. 5G and H). Under the conditions of sludge reflux, the increase in the $FeSO_4·7H_2O$ dose caused a similar trend in CST variation to that of the SRF (Fig. 5E–H). Generally, there was a better promotion of sludge dewaterability during bioleaching with a high dose of $FeSO_4·7H_2O$ (6–10 g/L),

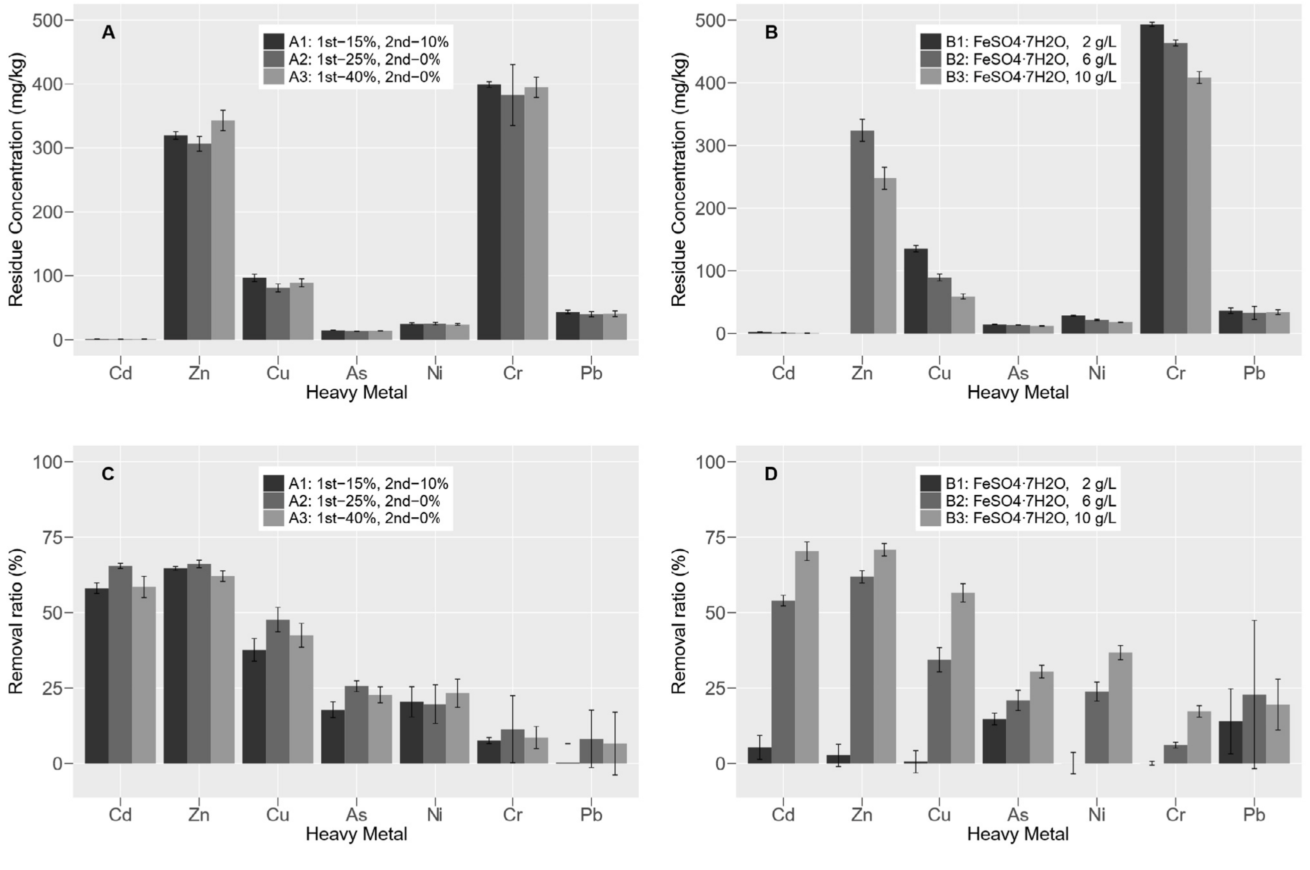

Fig. 4. Residue concentration and removal rates of heavy metals of bioleached sludge in the 1st–4th compartments from raw sludge. In graphs A and C, three trials with different sludge reflux ratios in group A, the reflux ratios for the 1st and 2nd compartments were 15% and 10% (A1), 25% and 0% (A2), 40% and 0% (A3), respectively. In graphs B and D, three trials with different energy substrate doses in group B, adding $FeSO_4·7H_2O$ with the doses of 2 g/L (B1), 6 g/L (B2) and 10 g/L (B3).

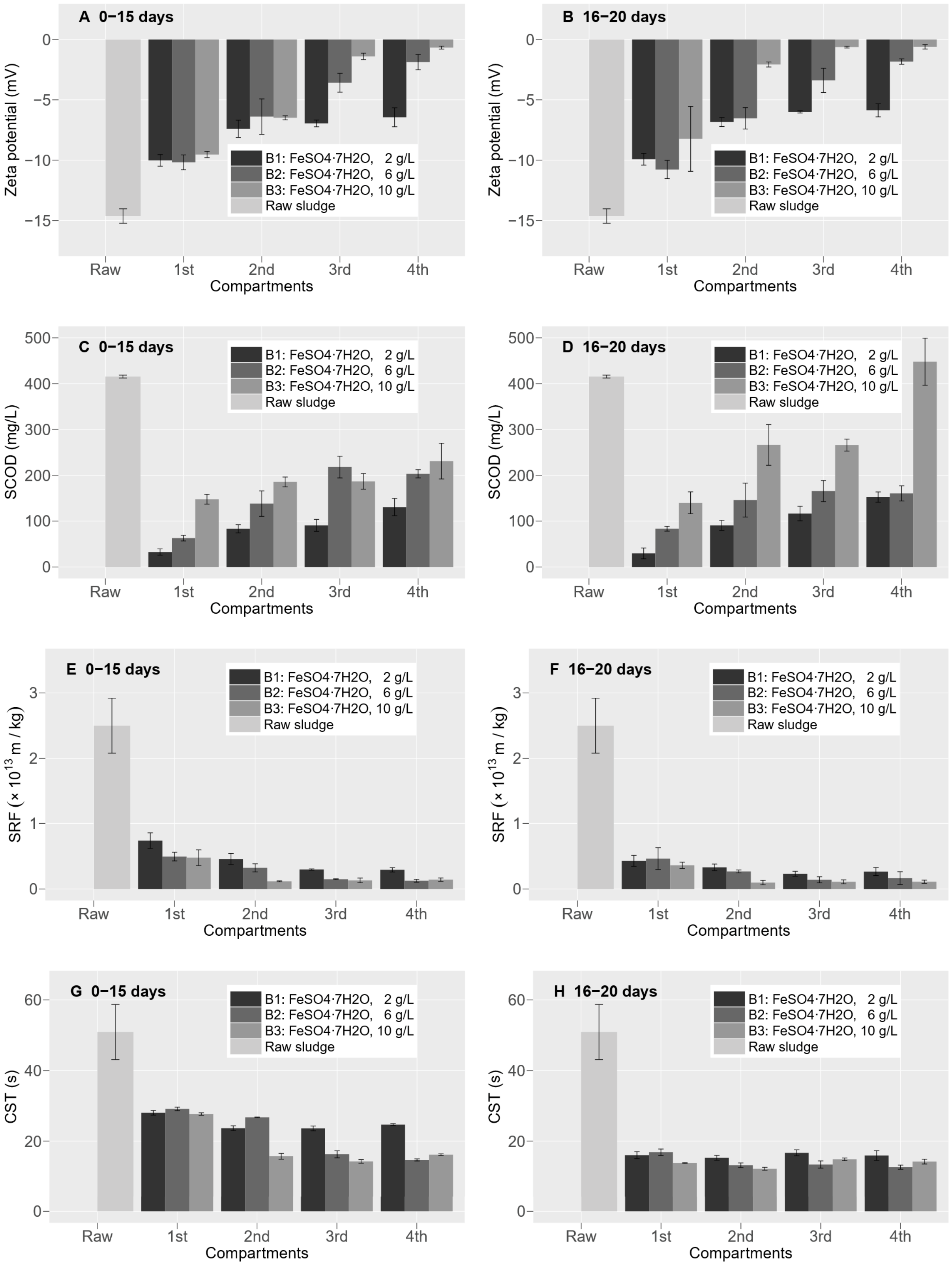

Fig. 5. Zeta potentials, SCOD, SRF, and CST of bioleached sludge in the 1st–4th compartments of three trials in group B, adding $FeSO_4·7H_2O$ with the doses of 2 g/L (B1), 6 g/L (B2) and 10 g/L (B3): A and B for zeta potential; C and D for SCOD; E and F for SRF; G and H for CST; A, C, E, and G with reflux ratios of 10%/15% for the 1st/2nd compartments during days 0–15; B, D, F, and H with reflux ratios of 15%/10% for the 1st/2nd compartments during days 15–20.

while increasing the sludge-reflux portion only in 1st compartment enhanced the promotion of sludge dewaterability with a low dose of $FeSO_4{\cdot}7H_2O$ (2 g/L).

3.2.3. Removal of heavy metals

The removal of heavy metals was strengthened when the $FeSO_4{\cdot}7H_2O$ dose was increased from 2 to 10 g/L, and the residual concentration and removal rates of heavy metals in the sludge after two-phase bioleaching treatment with different energy substrate doses are shown in Fig. 4B and C. In group B, the removal rates of heavy metals from sludge with different energy substrate doses throughout the bioleaching were 2.67%/61.85%/70.83% (B1/B2/B3, Zn), 5.31%/53.98%/70.35% (B1/B2/B3, Cd), 0.53%/34.39%/56.52% (B1/B2/B3, Cu), 14.71%/20.90%/30.41% (B1/B2/B3, As), 0.04%/23.83%/36.73% (B1/B2/B3, Ni), 0.02%/6.00%/17.19% (B1/B2/B3, Cr), and 13.94%/22.84%/19.49% (B1/B2/B3, Pb); the removal rates of heavy metals gradually increased with increasing energy substrate dose, which is consistent with previous studies [31]. The increase in $FeSO_4{\cdot}7H_2O$ accelerated the bio-oxidization of Fe^{2+} and resulted in rapider acidification, which allowed more heavy metals to leach. The removal of heavy metals is related to the pH value, chemical fraction, and ion components in the bulk solution of the bioleached sludge [18,19,31]. A more acidic environment causes more dissolution of heavy metals in the form of carbonate, exchangeable, and sulfide fractions, whereas AsO_4^{3-} and Pb^{2+} can be bonded with Fe^{3+} and SO_4^{2-}, respectively, to produce insoluble sediment [38,40].

3.3. Engineering applicability and economic feasibility

When processing per m^3 of concentrated sludge (TS 2%), 6.00 kg $FeSO_4{\cdot}7H_2O$ and 0.34 kWh electric energy should be consumed under ideal conditions. According to the market information in China, the unit price of $FeSO_4{\cdot}7H_2O$ and electrical energy were 0.25 $/kg (www.alibaba.com) and 0.11 $/kWh, respectively. The operation cost is approximately 64.68 $/ton of dry sludge (DS) for two-phase bioleaching and one-phase bioleaching.

The approximate costs of the one-phase bioleaching, $FeCl_3$/CaO, Fenton, and microwave treatment were 74.32 $/ton DS, 79.25 $/ton DS, 71.1 $/ton DS, and 112.5 $/ton DS, respectively [41–44]. The cost of sludge treatment and disposal of bioleaching with a two-phase baffled flow reactor was lower than that of the other treatments because of the lower volume of bioleached sludge and the dual effects of sludge dewaterability improvement and heavy metal removal. Generally, bioleaching with a two-phase baffled flow reactor is a more practical and economical technique for achieving deep sludge dewatering and removing heavy metals from sludge.

4. Conclusion

- For two-phase bioleaching, increasing sludge reflux in the 1st compartment accelerated acidification and enhanced the dissolution of Cu, As, and Pb but had no pronounced effect on the dewaterability of the final bioleached sludge and removal of Cd, Zn, Ni, and Cr.
- Adding more energy substrate gradually strengthened sludge acidification during the two-phase bioleaching. A better promotion of sludge dewaterability was obtained in bioleaching with a high dose of $FeSO_4{\cdot}7H_2O$ (6–10 g/L), and increasing the energy substrate dose gradually increased the removal rates of seven heavy metals.
- Under optimal conditions (25% sludge reflux ratio and 6 g/L $FeSO_4{\cdot}7H_2O$), the SRF and CST of the sludge decreased by 96.14% and 75.88%, respectively, after bioleaching, and the corresponding removal rates of heavy metals were 65.35% (Cd), 65.05% (Zn), 44.31% (Cu), 23.88% (As), 15.81% (Ni), 11.31% (Cr), and 8.15% (Pb).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

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Authors' contributions

Jiajing Pan: Investigation, Formal analysis, Writing of the original draft. Yu Dai: Methodology, Investigation, Formal analysis. Rongjun Wu: Data curation and visualization. Jun Zhang: Conceptualization, Supervision, Project administration, Writing – review & editing. Hongtao Liu: Conceptualization, Writing – review & editing. Yulan Lu: Visualization and writing (review).

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