



# Improvement of sludge dewaterability and removal of sludge-borne metals by bioleaching at optimum pH

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## ABSTRACT

Bio-acidification caused by bio-oxidation of energy substances during bioleaching is widely known to play an important role in improving sludge-borne metals removal. Here we report that bioleaching also drastically enhances sludge dewaterability in a suitable pH level. To obtain the optimum initial concentrations of energy substances and pH values for sludge dewaterability during bioleaching, bio-oxidation of  $\text{Fe}^{2+}$  and  $\text{S}^0$  under co-inoculation with *Acidithiobacillus thiooxidans* TS6 and *Acidithiobacillus ferrooxidans* LX5 and their effects on sludge dewaterability and metals removal during sludge bioleaching were investigated. Results indicated that the dosage of energy substances with 2 g/L  $\text{S}^0$  and 2 g/L  $\text{Fe}^{2+}$  could obtain bio-oxidation efficiencies of up to 100% for  $\text{Fe}^{2+}$  and 50% for  $\text{S}^0$  and were the optimal dosages for sludge bioleaching. The removal efficiencies of sludge-borne Cu and Cr could reach above 85% and 40%, respectively, and capillary suction time (CST) of bioleached sludge decreased to as low as  $\sim 10$  s from initial 48.9 s for fresh sludge when sludge pH declined to  $\sim 2.4$  through bioleaching. These results confirm the potential of bioleaching as a novel method for improving sludge dewaterability as well as removal of metals.

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## 1. Introduction

At present, there are more than 3080 municipal wastewater treatment plants working in China. The amount of wastewater treated by these plants exceeds 125,000,000  $\text{M}^3/\text{d}$ , which in turn generate about 6,000,000 dry tons of sewage sludge each year. Moreover, large amounts of municipal sewage sludge are expected to be generated in the next decade due to the increasing numbers of wastewater treatment facilities being constructed. Conventional dewatered sludge obtained through the addition of organic- or inorganic-flocculant followed by mechanical dewatering always contains high moisture (mostly 80% or more) and sometime high concentration of heavy metals depending on wastewater sources, both of which severely affect subsequent disposal and reutilization of sewage sludge such as incineration, landfilling, and composting [1–4]. Therefore, developing the technologies for high dewatering efficiency of waste activated sludge (WAS) or thickened sludge and for removing sludge-borne metals is of paramount significance.

Bioleaching is one of the microbial methods lately considered highly promising for the treatment of metal-containing sludge and other wastes. During bioleaching, bio-oxidation of  $\text{S}^0$  by *Acidithiobacillus thiooxidans* (*A. thiooxidans*) or the hydrolysis of

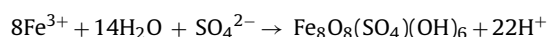
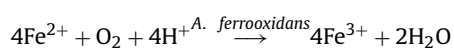
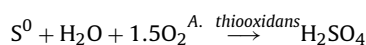
$\text{Fe}^{3+}$  from the bio-oxidation of  $\text{Fe}^{2+}$  by *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) produces  $\text{H}^+$  that causes a decrease in pH and leads to solubilization of sludge-borne metals. Since 1990s, the removal of sludge-borne metals using bioleaching has been extensively studied at the laboratory scale [2,5–7]. *A. ferrooxidans* and *A. thiooxidans* have been employed as the most significant microorganisms involved in bioleaching processes. However, in many previous studies, sludge bioleaching is performed mainly using a single strain of either *A. ferrooxidans* or *A. thiooxidans* and a single energy substance such as  $\text{Fe}^{2+}$  or  $\text{S}^0$  [6,8,9]. Indeed, elemental sulfur appears to be the most efficient substrate for bioleaching [10]. However, the excess sulfur remaining in the bioleached sludge will lead to acidification of soil when the resultant sludge is applied to farmland or produce sulfur dioxide when it is incinerated. Fortunately, it is found in our previous studies that the co-addition of  $\text{Fe}^{2+}$  and  $\text{S}^0$  in municipal sewage sludge bioleaching involving in *A. ferrooxidans* could accelerate pH decrease and enhance metal removal efficiency [10]. However, little information is available on the bio-oxidation efficiencies of  $\text{Fe}^{2+}$  and  $\text{S}^0$  during sludge bioleaching with co-inoculation of *A. thiooxidans* TS6 and *A. ferrooxidans* LX5 and the addition of  $\text{Fe}^{2+}$  and  $\text{S}^0$  at different ratio.

Among other things, many efforts have been devoted in recent decades to reduce the excess sludge in municipal waste treatment plants through sludge pre-treatment and subsequent mechanical dewatering. Thermal hydrolysis, freezing and thawing, chemical treatment using ozone, acids or alkali, mechanical disintegration

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using ultrasound, mills, homogenizers and other methods have been shown to give obvious improvement in the dewaterability of sludge. However, high operation cost or energy consumption makes their application in practical engineering difficult. Moreover, these pretreatment approaches as mentioned above will substantially reduce, to a great extent, organic matter content in dewatered sludge cakes or produce highly organic- and ammonia-loaded wastewater, which are difficult to be treated [11–14].

Strategies employing microbes are both efficient and cost-effective alternatives compared to chemical and physical methods because of its low demand for energy, materials and generation of fewer quantities of waste byproduct. It is noted that the bioleaching process can improve sludge dewaterability by 4–10 times with comparison to the control sludge without any pretreatment [15,16]. For example, the specific resistance to filtration (SRF) of municipal sewage sludge could be decreased from initial  $1.80 \times 10^{13}$  m/kg to final  $0.38 \times 10^{13}$  m/kg after bioleaching (the final sludge pH  $\approx 2.0$ ) [16]. This implies that the bio-acidification may play a critical role in sludge dewatering during bioleaching process. As mentioned above, the sludge bio-acidification is governed by the energy substances bio-oxidation during bioleaching as shown in the following equations:



Therefore, the degree of bio-oxidation of energy substances may affect the process of sludge dewatering during bioleaching. However, the optimal pH for higher sludge dewaterability and the influence of this optimum pH on the removal efficiency of sludge-borne metals in bioleaching process have not been reported.

Therefore, the objectives of the present study are (1) to investigate the oxidation efficiency of ferrous ion and element sulfur during sludge bioleaching with co-inoculation of *A. thiooxidans* TS6 and *A. ferrooxidans* LX5 and the addition of  $Fe^{2+}$  and  $S^0$  at different ratio, (2) to explore optimal pH value for sludge dewatering during the bioleaching process, and (3) to examine the removal efficiency of sludge-borne metal at optimal pH value in bioleaching system.

## 2. Materials and methods

### 2.1. Municipal sewage sludge sampling

The municipal sewage sludge used in this work was obtained from the First Wastewater Treatment Plant in High-tech Industrial Park, Suzhou City, Jiangsu Province, China. The plant receives mostly domestic sewage and partly industrial wastewater from metal surface treatment factories containing a high concentration of Cu. A mixed sludge from primary and secondary settling tanks was taken from sludge thickening pond for sludge bioleaching trials. Samples were collected in polypropylene bottles and kept at 4 °C before use. Sludge pH and capillary suction time (CST) were determined immediately after collection while sludge solid content was measured by oven-drying at 105 °C. The dried sludge sample was measured for Cu and Cr by ICP-atomic emission spectroscopy (ICP-AES) and for total N, P, K, and organic matter content according to APHA [17]. The physicochemical properties of the selected municipal sewage sludge are listed in Table 1. The Cu content in the collected sludge was found to be very high.

**Table 1**  
Physicochemical characteristics of the tested municipal sewage sludge.

Parameters	Value	Parameters	Value
pH	6.97	Total K (% dry weight)	0.59
Total solid (%)	3.14	Total Fe (% dry weight)	0.28
Organic matter (% dry weight)	50.4	CST(s)	48.9
Total N (% dry weight)	2.17	Cu (mg/kg, dry weight)	14,675
Total P (% dry weight)	1.88	Cr (mg/kg, dry weight)	105

### 2.2. Preparation of microorganisms and inoculum

*A. ferrooxidans* LX5 (CGMCC NO.0727) and *A. thiooxidans* TS6 (CGMCC NO.0759) obtained from China General Microbiological Culture Collection Center (CGMCC) were grown in modified 9K and SM liquor medium [18], respectively. Briefly, the modified 9K or SM medium was autoclaved at 121 °C for 15 min and adjusted to pH 2.5 or 3.0 with 9M  $H_2SO_4$  before being supplemented with either 44.2 g/L  $FeSO_4 \cdot 7H_2O$  or 10 g/L of elemental sulfur as the energy source. The culture inoculated with *A. ferrooxidans* LX5 or *A. thiooxidans* TS6 was incubated in 500 mL Erlenmeyer flasks shaken at 180 rpm and 28 °C for 3–4 days until bacterial cell density reached  $\sim 10^8$  cells/mL.

The inoculum was prepared by mixing 15 mL *A. ferrooxidans* and 15 mL *A. thiooxidans* cultures in 500 mL Erlenmeyer flasks containing 270 mL of municipal sewage sludge spiked with 4 g/L  $Fe^{2+}$  and 3 g/L  $S^0$ . The flasks were shaken at 180 rpm and 28 °C on a horizontal shaker. The pH of the sludge was periodically monitored during this process. When pH dropped to 2.0, 30 mL of this bio-acidified sludge was then transferred to 270 mL of fresh municipal sewage sludge mixed with 4 g/L  $Fe^{2+}$  and 3 g/L  $S^0$ . These mixtures containing fresh sludge, bio-acidified sludge, and microbial energy sources were shaken under the same conditions as described above until the pH of the sludge reached 2.0. The above procedures were repeated thrice, the acidified sludge gained in the last process was acted as the inoculum (pH  $\approx 2.0$ ) containing *A. ferrooxidans* and *A. thiooxidans*.

### 2.3. Bioleaching experiments

#### 2.3.1. Changes of $Fe^{2+}$ or $S^0$ oxidation efficiency under different concentrations

A series of 15 mL of the newly prepared inoculum was added to 250 mL Erlenmeyer flasks containing 135 mL fresh municipal sewage sludge followed by the addition of either (1) 2 g/L  $S^0$  and 0 g/L  $Fe^{2+}$ ; (2) 2 g/L  $S^0$  and 1 g/L  $Fe^{2+}$ ; (3) 2 g/L  $S^0$  and 2 g/L  $Fe^{2+}$ ; (4) 2 g/L  $S^0$  and 3 g/L  $Fe^{2+}$ ; (5) 2 g/L  $S^0$  and 4 g/L  $Fe^{2+}$  in the experiment series 1; or (6) 2 g/L  $Fe^{2+}$  and 0 g/L  $S^0$ ; (7) 2 g/L  $Fe^{2+}$  and 1 g/L  $S^0$ ; (8) 2 g/L  $Fe^{2+}$  and 2 g/L  $S^0$ ; (9) 2 g/L  $Fe^{2+}$  and 3 g/L  $S^0$ ; (10) 2 g/L  $Fe^{2+}$  and 4 g/L  $S^0$  in the experiment series 2. All treatments were carried out in triplicate. The flasks were shaken at 28 °C and 180 rpm to facilitate bioleaching and the sludge pH periodically monitored during the process. Then 8 mL sludge samples were withdrawn from each of the Erlenmeyer flasks and centrifuged at 12,000 rpm for 15 min in order to separate solid from liquid fractions. The liquid fractions were filtered through 0.45  $\mu m$  membrane filters, acidified with HCl to about pH 2.0 and then stored at 4 °C prior to determination of  $Fe^{2+}$ ,  $Fe^{3+}$ , total Fe, Cu, Cr, and  $SO_4^{2-}$  concentration.

#### 2.3.2. Determination of optimum pH for municipal sludge dewatering during bioleaching

Batch experiments were conducted through shaking 250 mL Erlenmeyer flasks each containing 30 mL of acidified bioleached sludge (pH  $\approx 2.0$ , collected from the above experiments with the addition of 2 g/L  $Fe^{2+}$  and 2 g/L  $S^0$ ) and 120 mL of the fresh municipal sewage sludge followed by the addition of 2 g/L  $Fe^{2+}$  and 2 g/L

S<sup>0</sup>. As described above, pH of sludge was periodically determined and 10 mL sludge samples were withdrawn from each flask for the determination of sludge CST and Zeta potential. The pH of bioleached sludge for highest dewaterability was determined based on CST where the optimum pH was the one that gave the shortest CST.

To observe the stability of bioleaching system, acidified bioleached sludge at optimum pH was reused as inoculum for subsequent three batch bioleaching trials under the same conditions as described above. All treatments were done in triplicate. pH and CST of bioleached sludge were also determined periodically.

#### 2.4. Analytical methods

pH was determined using a pHS-3C model digital pH-meter with the Pt-Ag/AgCl electrode system. Fe<sup>2+</sup> and total Fe concentrations were determined by a colorimetric procedure using 1,10-phenanthroline as described in standard methods [17]. Fe<sup>3+</sup> was taken as the difference between total Fe and Fe<sup>2+</sup>. SO<sub>4</sub><sup>2-</sup> was measured by ion chromatography (Dionex 320). The ferrous ion oxidation rate and sulfur oxidation rates were determined according to the method described by Fang and Zhou [19]. Cu and Cr were determined by using ICP-AES (Optima 2100DV, Perkin Elmer). Zeta potential was recorded on a micro-electrophoresis machine (Model JS94H, Shanghai, China) while CST values of municipal sewage sludge were determined by a capillary suction timer (Model 304M, Triton, Britain). In preliminary study, it was found that there was a significant positive linear correlation between CST and water content in dewatered sludge cake obtained by chamber filter press.

### 3. Results and discussion

#### 3.1. Sludge bio-acidification and removal of sludge-borne Cu and Cr during bioleaching

pH is widely known to be the most important parameter influencing sludge-borne heavy metals solubilization during bioleaching [20,21]. It is also well documented that pH decrease in bioleaching system is an indication of the higher activity of *Acidithiobacilli* bacteria [22,23]. For effective solubilization of metals, the maintenance of sludge pH around 2.0 as a result of bio-acidification during bioleaching is very important as most of the sludge-borne metals remain in the solution in this pH level. Therefore, a pH value of 2.0 is often chosen as the end point in sludge bioleaching studies [24,25].

In the experiment series 1, pH variations of sludge during bioleaching with addition of 2 g/L S<sup>0</sup> and 0–4 g/L Fe<sup>2+</sup> are given in Fig. 1a. pH sharply decline from initial ~5.7 to ~2.0 at 96 h and then decrease slightly to ~1.9 at 144 h for the treatments receiving >2 g/L Fe<sup>2+</sup>. Although addition of 1 g/L Fe<sup>2+</sup> led to rapid decline in pH, the rate of decrease is slower than the treatments receiving >2 g/L Fe<sup>2+</sup>. When no Fe<sup>2+</sup> is added, pH decreases from 7.6 to 5.8 in the first 24 h, and then maintains a stable ~5.7 until the completion of the experiment. These findings are consistent with the results obtained by Huang et al. [26], who found that sludge pH could not be sharply decreased with inoculation of *A. thiooxidans* TS6 if the initial pH in medium was 8.0. In the experiment series 2, pH variation during bioleaching with addition of 2 g/L Fe<sup>2+</sup> and 0–4 g/L S<sup>0</sup> is provided in Fig. 1b. It is noted that sludge pH decreases continuously from initial ~5.8 to ~2.4, ~2.1, ~2.0, and ~2.0 within 90 h of bioleaching for the treatments with the addition of 1, 2, 3, and 4 g/L S<sup>0</sup>, respectively. When only 2 g/L Fe<sup>2+</sup> but without S<sup>0</sup> is applied, sludge pH declines from initial ~5.8 to ~3.7 within the first 24 h of the bioleaching process, followed by a relative stable value ~3.3 until termination of bioleaching trial.

Obviously, it is difficult to completely finish sludge bioleaching process (pH drop to ~2.0) in 144 h with the addition of 2 g/L S<sup>0</sup> only (experiment series 1), or in 90 h with 2 g/L Fe<sup>2+</sup> alone (experiment series 2). However, the co-addition of Fe<sup>2+</sup> and S<sup>0</sup> sharply increases the acidified rate of the tested sludge to pH ~2.0 within the same reaction time. Therefore, it is clear that the reaction time for bioleaching may be sharply reduced by the co-addition of Fe<sup>2+</sup> and S<sup>0</sup>. The decrease of pH during bioleaching may be attributed to the generation of sulfuric acid due to bio-oxidation of S<sup>0</sup> by *A. thiooxidans* [27] and the release of H<sup>+</sup> resulting from the formation of iron oxyhydroxysulfate mineral due to the hydrolysis of Fe<sup>3+</sup> from the bio-oxidation of Fe<sup>2+</sup> by *A. ferrooxidans* [18,28,29].

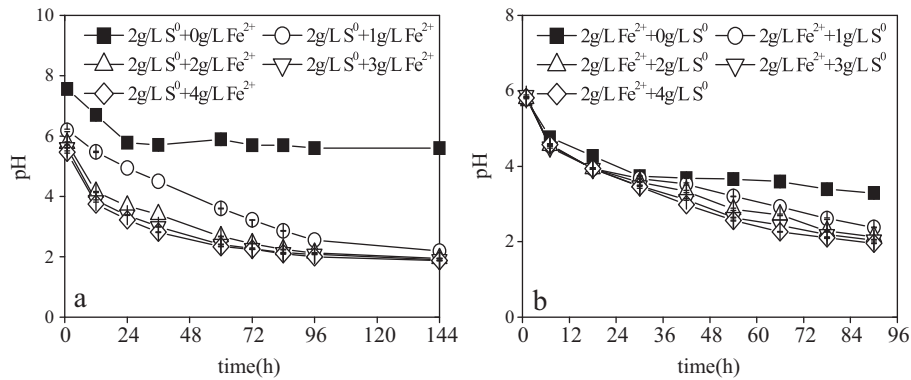
Sludge-borne Cu is dissolved gradually with a continuous decrease of pH in bioleaching systems. As shown in Fig. 2a, the removal efficiency of Cu increases from initial 13.2%, 15.5%, 17.3%, 17.8%, and 17.7% to 19.3%, 76.9%, 84.3%, 86.8% and 89.0% at termination of bioleaching while pH decreasing from ~7.6, ~6.2, ~5.7, ~5.6, and ~5.5 to ~5.8, ~2.2, ~1.9, ~1.9, and ~1.9 for these sludge bioleaching system with co-addition of 2 g/L S<sup>0</sup> and 0, 1, 2, 3, and 4 g/L Fe<sup>2+</sup> in the experiment series 1, respectively (Fig. 2a). Similar removal efficiencies of Cu were also observed in the experiment series 2 (Fig. 2b). In fact, Zhou et al. [10] found that the removal efficiency of Cu could reach nearly 100% when pH values of municipal sewage sludge leaching systems were around 2.0. The results obtained in the study also showed that there was a threshold pH of between 4.0 and 3.0 in which >50% of sludge-borne Cu was dissolved. For example, Cu removal efficiency increases from 28.5% to 59.6% when pH decreases from ~4.2 to ~3.7, respectively, for the treatments with the co-addition of 2 g/L S<sup>0</sup> and 2 g/L Fe<sup>2+</sup> in the experiment series 1. This is in agreement with the results obtained by Li et al. [30] who found that the threshold pH was 4.0 for Cu removal from sewage sludge.

However, the removal of Cr was not as effective as that of Cu (Fig. 2c and d). It was noted that sludge-borne Cr required pH ~3.0 or below to begin solubilization. At pH ~2.6 or below, Cr solubilization took place very rapidly, which is consistent with the conclusions obtained by Villar and Garcia [24], who reported that Cr required pH of 2–3 to start their dissolution from the sludge in bioleaching process. The results also indicated that more than 60% of Cr in the solid phase was solubilized when the sludge pH decreased to ~2.0 in bioleaching system.

#### 3.2. Bio-oxidation of Fe<sup>2+</sup> during bioleaching

Fig. 3 illustrates dynamics of Fe<sup>2+</sup>, Fe<sup>3+</sup> and total Fe concentrations in sludge during bioleaching. Fe<sup>2+</sup> is readily oxidized into Fe<sup>3+</sup> by *A. ferrooxidans* LX5 in all treatments apart from the treatment with addition of 2 g/L Fe<sup>2+</sup> and 0 g/L S<sup>0</sup>. After 1 h of reaction, the actual concentrations of Fe<sup>2+</sup> in liquid fractions were much lower than the added Fe<sup>2+</sup> concentrations in experiment series 1 (see Fig. 3a). For example, the actual concentration of Fe<sup>2+</sup> after 1 h of bioleaching was only 875 mg/L for the treatment with addition of 2 g/L S<sup>0</sup> and 2 g/L Fe<sup>2+</sup>. This reduction could be attributed to the adsorption of Fe<sup>2+</sup> on the surface of sludge granules as shown in the results of the preliminary study and as explained in other studies [16,31]. Furthermore, bio-oxidation of Fe<sup>2+</sup> in all treatments was finished in 96 h and the oxidation of Fe<sup>2+</sup> is coupled with Fe<sup>3+</sup> generation. During the first 60 h, total soluble Fe concentration in the treatments with addition of Fe<sup>2+</sup> gradually decreased with the increase of reaction time owing to the formation of ferric precipitates such as iron oxyhydroxysulfate mineral [32].

In experiment series 2, the variation of Fe<sup>2+</sup>, Fe<sup>3+</sup> and total Fe concentrations in liquid fractions of municipal sludge during bioleaching are given in Fig. 3b. It is found that Fe<sup>2+</sup> oxidation rate just increases to 67% after 96 h in the treatment with addition of

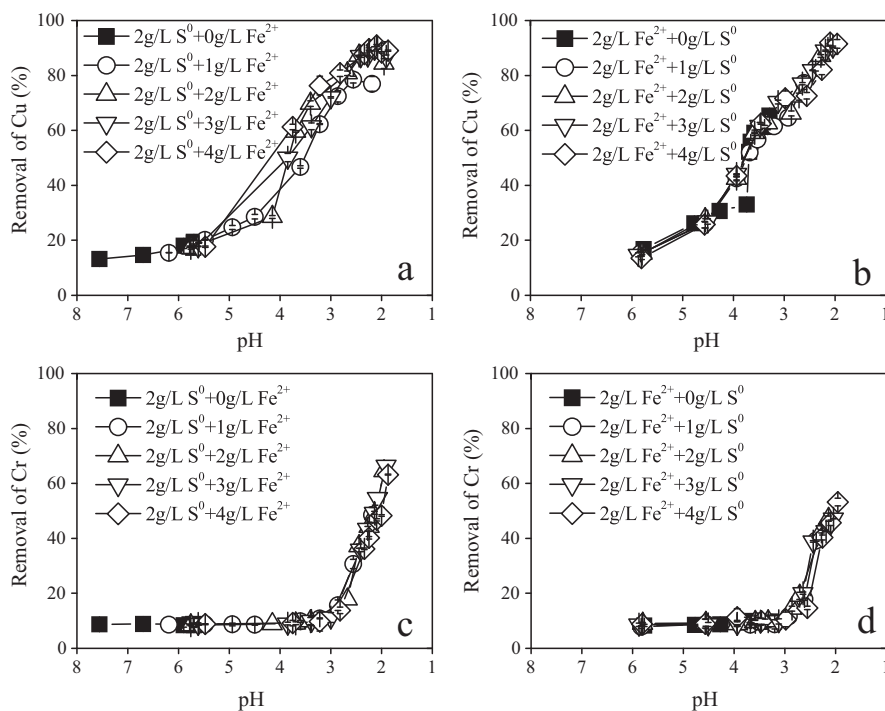


**Fig. 1.** Variation in sludge pH with time during bioleaching with co-inoculation of *A. thiooxidans* TS6 and *A. ferrooxidans* LX5 and co-addition of  $\text{Fe}^{2+}$  and  $\text{S}^0$  at different ratio.

2 g/L  $\text{Fe}^{2+}$  and 0 g/L  $\text{S}^0$ . However, the ferrous oxidation rate could be accelerated by adding  $\text{S}^0$  as energy substance in the bioleaching systems (see Fig. 3b) where ferrous oxidation rates at 66 h are 64.9% and 100% with addition of 1 g/L  $\text{S}^0$  and  $\geq 2$  g/L  $\text{S}^0$ , respectively. The probable explanation for acceleration in the rate of ferrous oxidation in the presence of added  $\text{S}^0$  may be the creation of favorable conditions for the growth and activity of *A. ferrooxidans* as a result of the decrease in pH and the increase in sulfate concentration due to bio-oxidation of  $\text{S}^0$ . The increased growth and activity of *A. ferrooxidans* in turn accelerates further fall of pH through the bio-oxidation of ferrous. This deduction is supported by the findings of Zhou et al. [10] who found a positive linear correlation between sulfate originating from bio-oxidation of  $\text{S}^0$  and the oxidation of  $\text{Fe}^{2+}$  in the bioleaching ( $r^2 = 0.820$ ,  $n = 24$ ). Steiner and Lazaroff [33] and Lazaroff [34] also confirmed that sulfate is a specific anion required for the biooxidation of  $\text{Fe}^{2+}$  by *A. ferrooxidans* and they also found that the rate of  $\text{Fe}^{2+}$  oxidation was maximal when sulfate concentration was about twice as high as  $\text{Fe}^{2+}$  concentration.

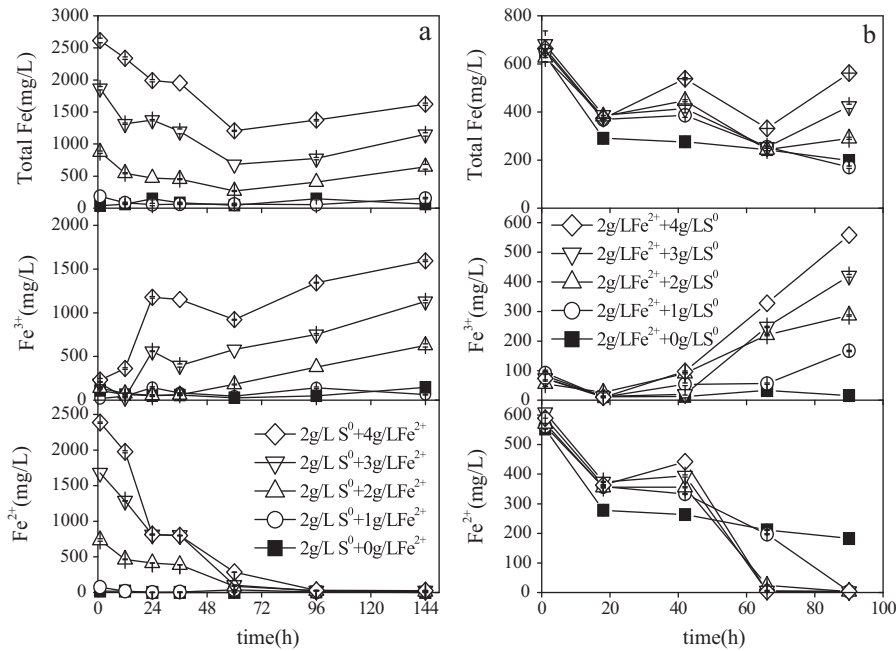
### 3.3. Bio-oxidation of $\text{S}^0$ during bioleaching

Oxidation of sulfur as measured by sulfate production during bioleaching is an important step in the process as it results in pH fall in systems. Production of sulfate during bioleaching is presented in Fig. 4. Fig. 4a indicates the dynamics of sulfate in the liquid fractions of municipal sludge during 144 h bioleaching in the experiment series 1. In the first 24 h where sludge pH is  $>3$ , the sulfate concentrations maintain a constant value similar to initial concentration for all treatments. This suggests that *A. thiooxidans* TS6 does not grow well or is of low activity under pH  $>3$  of bioleaching environment conditions (Fig. 4c). It is consistent with the results reported by Zhou et al. [35] who found that the bio-oxidation of  $\text{S}^0$  was extremely slow when medium pH was more than 3 during tannery sludge bioleaching with co-inoculation of *A. thiooxidans* TS6 and *A. ferrooxidans* LX5 and the co-addition of  $\text{Fe}^{2+}$  and  $\text{S}^0$  as energy substrates. As shown in Fig. 4a, the bio-oxidation of  $\text{S}^0$  is initiated when pH of bioleaching systems drop to  $\leq 3.0$ . After 24 h of reaction, the sulfate concentrations gradually increase until the end of



**Fig. 2.** Removal of sludge-borne Cu and Cr as a function of pH during bioleaching with co-inoculation of *A. thiooxidans* TS6 and *A. ferrooxidans* LX5 and co-addition of  $\text{Fe}^{2+}$  and  $\text{S}^0$  at different ratio.



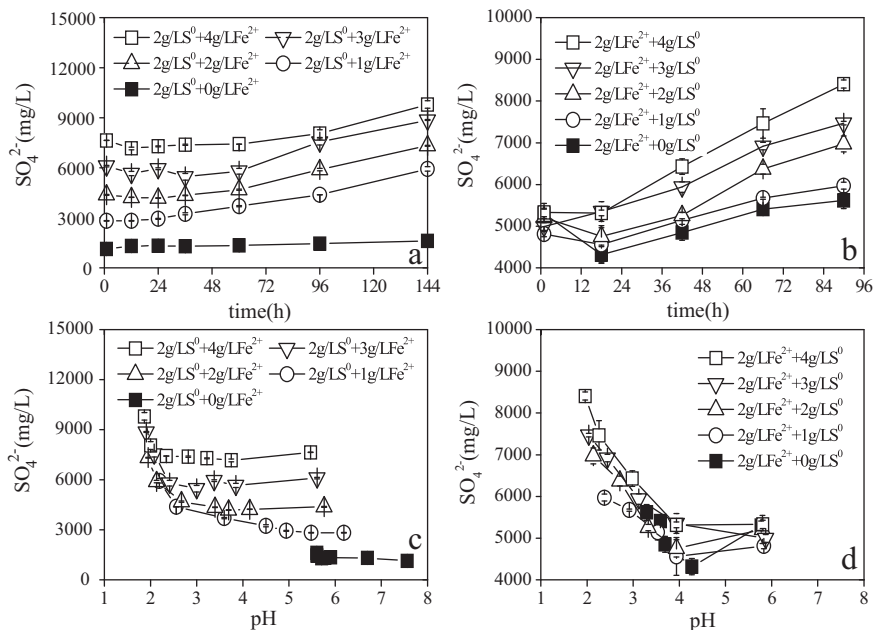


**Fig. 3.** Variations of  $Fe^{2+}$ ,  $Fe^{3+}$  and total Fe with time during sludge bioleaching with co-inoculation of *A. thiooxidans* TS6 and *A. ferrooxidans* LX5 and co-addition of  $Fe^{2+}$  and  $S^0$  at different ratio.

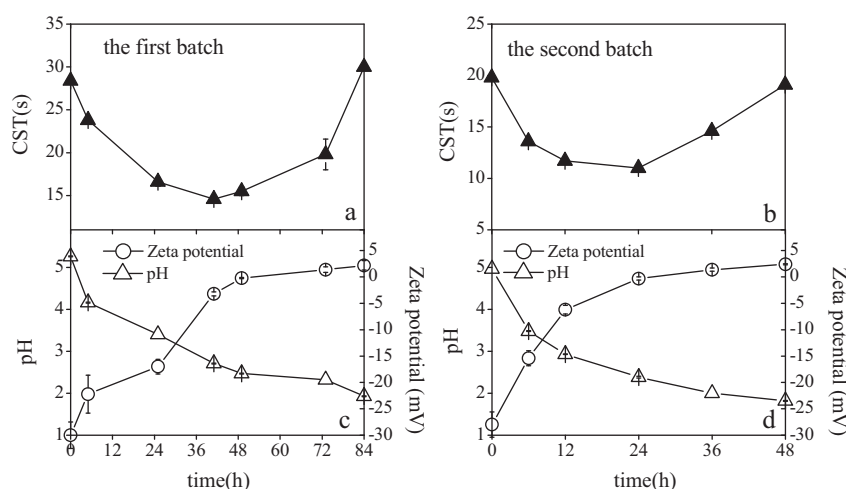
bioleaching for all but the treatment with 2 g/ $LS^0$  and 0 g/L  $Fe^{2+}$ . Sulfur oxidation percentages are calculated as 8.3%, 51.8%, 49.1%, 46.1%, and 35.9% for the treatments with 2 g/L  $S^0$  and 0, 1, 2, 3, and 4 g/L  $Fe^{2+}$ , respectively. It is evident that the oxidation of elemental sulfur can be increased from 8.3% for the treatment with only 2 g/L  $S^0$  to about 50% for these treatments with 1–2 g/L  $Fe^{2+}$  together with 2 g/L  $S^0$  as energy substances. This indicates that addition of  $Fe^{2+}$  as energy substance is helpful in enhancing the activities of *A. ferrooxidans* which in turn favors the bio-oxidation of added  $S^0$ . As regards the apparent decrease of elemental sulfur oxidation from about 50% for the treatments with 2 g/L  $S^0$  + 1–2 g/L  $Fe^{2+}$  to 46.1% and to 35.9% for the treatments with 2 g/L  $S^0$  + 3 g/L or 4 g/L  $Fe^{2+}$ ,

respectively, it may be attributed to the involvement or incorporation of sulfate from bio-oxidation of  $S^0$  in the formation of iron oxyhydroxysulfate mineral because higher  $Fe^{2+}$  concentration and  $SO_4^{2-}$ -rich acidic environments in these treatments facilitate the formation of biogenic iron oxyhydroxysulfate precipitates including schwertmannite and jarosite [18].

Fig. 4b and d exhibits dynamic changes of sulfate concentration and pH in liquid fractions of sludge in a 90 h bioleaching period in the experiment series 2. The oxidation of sulfur in sludge mainly occurs after 42 h when the medium pH has declined below 3.0 in the treatments with addition of 1–4 g/L  $S^0$ , while sulfate concentrations in the treatment without  $S^0$  hardly changes throughout



**Fig. 4.** Variation of  $SO_4^{2-}$  with time and pH during sludge bioleaching with co-inoculation of *A. thiooxidans* TS6 and *A. ferrooxidans* LX5 and co-addition of  $Fe^{2+}$  and  $S^0$  at different ratio.



**Fig. 5.** Dynamic changes of sludge pH, Zeta potential, and CST value during two batch bioleaching processes with co-inoculation of *A. thiooxidans* TS6 and *A. ferrooxidans* LX5 and addition of 2 g/L  $\text{Fe}^{2+}$  and 2 g/L  $\text{S}^0$ .

experiment. The sulfur oxidation rates are 38.6%, 29.4%, 27.5% and 25.6% for those treatments containing a fixed 2 g/L  $\text{Fe}^{2+}$  and 1, 2, 3 and 4 g/L  $\text{S}^0$ , respectively. It indicates that the decrease in the ratio of added  $\text{Fe}^{2+}$  to added  $\text{S}^0$  is unfavorable to bio-oxidation of  $\text{S}^0$  during sludge bioleaching. Consequently, the addition of excess  $\text{S}^0$  into sludge before bioleaching will lead to the presence of residual  $\text{S}^0$  in bioleached sludge, probably resulting in soil acidification if this bioleached sludge is applied to land.

### 3.4. Optimum pH for dewatering of municipal sewage sludge during bioleaching

The dewaterability of bioleached sludge of various pH levels collected at different bioleaching times was measured (Fig. 5). The treatment with the addition of 2 g/L  $\text{Fe}^{2+}$  and 2 g/L  $\text{S}^0$  was selected for the determination of bioleached sludge dewaterability in the present study since (1) pH of leaching systems easily decreased to  $\sim 2.0$  with addition of 2 g/L  $\text{Fe}^{2+}$  and 2 g/L  $\text{S}^0$  and the rate of these decreases is similar to those under addition of  $\text{Fe}^{2+} > 2$  g/L and  $\text{S}^0 > 2$  g/L, (2) the bio-oxidation rate of  $\text{Fe}^{2+}$  reached 100% if more than 2 g/L  $\text{S}^0$  was simultaneously added, and (3) more than 50% of sulfur oxidation efficiency can be achieved under this condition.

As shown in Fig. 5, sludge CST is shortened from 48.9 s for fresh sludge (Table 1) to 28.4 s in batch 1 and 19.8 s in batch 2 when the acidified bioleached sludge is recycled to fresh sludge at the beginning of bioleaching (0 h). The decreases in CST at the beginning may result from coagulation effects of ferric and ferrous ions in the acidified bioleached sludge because ferric and ferrous ions, acting as coagulants, can adsorb onto the negatively charged sludge granules and consequently facilitate aggregation of sludge granules through charge neutralization, adsorption bridging and precipitation catching. In addition, the coefficient of compressibility of municipal sludge may be decreased due to the introduction of oxyhydroxysulfate mineral particles from recycled bioleached sludge [16]. In the first batch test, sludge CST is decreased from initial 28.4 s to 14.6 s at 40 h, and then unexpectedly increased to 30.0 s at 84 h. The lowest point of CST is 14.6 s, in which the corresponding pH value is around 2.7 (Fig. 5a). Likewise, in the second batch test, the shortest CST is 11.0 s, and its corresponding pH value is around 2.4 (Fig. 5b). This demonstrates that there is an optimum pH (2.4–2.7) for the highest degree of dewaterability of bioleached sludge. During the initial stage of bioleaching, the gradual increase of  $\text{Fe}^{3+}$  concentration and potentially biogenic iron oxyhydroxysulfate mineral particles are

probably responsible for the corresponding decrease in CST over the period. During the final stage of bioleaching, the adsorption between  $\text{Fe}^{3+}$  and sludge granules may be weakened since sludge pH decreases to 2 or below. In addition, it is also observed that Zeta potential in the second batch test gradually increased from  $-28$  mV at the initial stage to close to the point of zero charge (0 mV) when sludge pH is  $\sim 2.4$  and continued to lift with further pH drops until  $+2.4$  mV at the end of bioleaching trial when sludge pH is 1.82 (Fig. 5d).

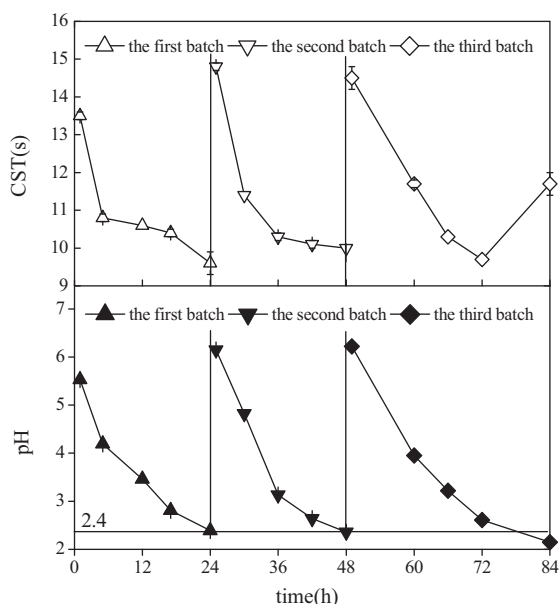
It has been well documented that particles in suspension can aggregate easily and settle down when Zeta potential is close to 0 because of no charge in sludge particle surfaces. Mahmood and Elliott [36] also found that chemically acidifying sludge from the pulp and paper industry enhanced the sludge dewaterability due to the Zeta potential being at or close to 0 mV when it was modified by chemical acids.

Besides, there is a gradual shift from heterotrophic microorganisms in waste activated sludge to autotrophic microorganisms in sludge bioleaching system. The type and feature of microbial extracellular polymeric substances (EPS) differ significantly between these two microbial systems. Since sludge dewaterability is related to the structure of microbial extracellular polymeric substances in the sludge system [37], the shift of microbial systems could potentially be one of reasons for improving sludge dewaterability by bioleaching and this will need to be studied in the future.

### 3.5. The termination and stability of municipal sludge bioleaching

Considering dewaterability of bioleached sludge, along with the efficiency of heavy metal removal in the system, optimum termination of bioleaching for the selected sludge should be at the point when bioleached sludge pH is  $\sim 2.4$  where the removal efficiencies of sludge-borne Cu and Cr could also reach more than 85% and 40%, respectively (Fig. 2). It takes at most 45 h of sludge retention time (SRT) for bioleaching to reach this termination.

The stability of bioleaching system is also studied through successive three batch trials by recycling the acidified bioleached sludge (pH  $\sim 2.4$ ) in the second and the third batch tests, using a recycle ratio of 1:4 ( $V_{\text{bioleached}}:V_{\text{fresh sludge}}$ ) and addition of 2 g/L  $\text{Fe}^{2+}$  and 2 g/L  $\text{S}^0$  for fresh sludge. Changes in sludge pH and CST values during three successive batch bioleaching trials are given in Fig. 6. Results showed that the bioleaching process could be finished in 24–36 h (pH  $\approx 2.40$ ). In the first batch test, sludge CST decreased



**Fig. 6.** Changes in sludge pH and CST values during bioleaching in three successive batch trials with co-inoculation of *A. thiooxidans* TS6 and *A. ferrooxidans* LX5 and addition of 2 g/L  $\text{Fe}^{2+}$  and 2 g/L  $\text{S}^0$  (the pH of recycled bioleached sludge is around 2.4).

from an initial 13.5 s to a final 9.6 s as the pH decreased from 5.6 to 2.4. Similar results were obtained in the second batch test. In the third batch, pH of the system reduced from initial 6.2 to 2.2 within 36 h. CST of sludge gradually decreased from initial 14.5 s to 9.7 s at 24 h and then unexpectedly increased to 11.7 s at 36 h in which sludge pH had declined below 2.4. Therefore, SRT is calculated as 36–45 h according to the 1:4 recycle ratio of bioleached sludge to fresh sludge. The results from these three batch trials exhibit that the sludge bioleaching system employed in this study is experimentally stable because the bioleaching process could be finished in 24–36 h when the recycled bioleached sludge pH is around 2.4. Sludge pH of ~2.4 can therefore be taken as the optimum pH value to achieve the highest degree of dewaterability of municipal sewage sludge through bioleaching with 2 g/L  $\text{Fe}^{2+}$  and 2 g/L  $\text{S}^0$  as energy substances.

To assess directly sludge dewatering efficiency after bioleaching process, concentrated sludge before and after bioleaching were dewatered for 30 min using a small chamber filter press with 0.5 m<sup>2</sup> of filtering area and 0.35 MPa of working filter pressure for 30 min in the study. The moistures of the dewatered sludge cakes from both the original and bioleached sludge samples (pH 2.4–2.7) were 84% and 58%, respectively. Correspondingly, the specific resistance to filtration (SRF) of the tested sludge before and after bioleaching was also measured. It was found that SRF of sludge was drastically decreased to  $9 \times 10^{11}$  m/kg after bioleaching from  $1.00 \times 10^{13}$  m/kg of original sludge before bioleaching. It is well known that higher SRF value indicates the worse sludge dewaterability [38]. Indeed, the moisture of dewatered sludge cakes can also be reduced to below 60% in pilot-scale studies [39,40]. Furthermore, similar reduction of dewatered sludge moisture is also observed in a bioleaching pilot plant with 100 M<sup>3</sup>/d of concentrated sludge (97% moisture) treatment capacity (data not shown). The sludge bioleaching plant is located at Wuxi Taihu Sewage Treatment Plant, Jiangsu, China, and operated since August, 24, 2010. According to the results as mentioned above, it is clear that sewage sludge pre-treatment or conditioning through bioleaching prior to mechanical dewatering is very effective to obtain sludge cake of high solid content.

In our previous studies, it was found that the dewaterability of sewage sludge could be enhanced with inoculation of *A. thiooxidans* TS6 and *A. ferrooxidans* LX5 during municipal sewage sludge bioleaching [15,16]. But the end point (pH ≈ 2.0) of bioleaching process in these studies was proposed only by considering the removal of sludge-borne heavy metals and did not take into account the optimum point for dewatering of municipal sewage sludge. This has been adequately addressed in the present study for establishing a new endpoint of pH 2.4.

#### 4. Conclusions

Concentration and ratio of  $\text{Fe}^{2+}$  and  $\text{S}^0$  added to sewage sludge have a significant impact on the bio-oxidation rates of  $\text{Fe}^{2+}$  and  $\text{S}^0$  and sludge bio-acidification during bioleaching involving in co-inoculated *A. ferrooxidans* LX5 and *A. thiooxidans* TS6. 2 g/L  $\text{Fe}^{2+}$  and 2 g/L  $\text{S}^0$  are considered to be the ideal initial concentrations for sludge bioleaching systems. The present study demonstrates that sludge-borne metals in bioleaching system are dissolved gradually as sludge pH declines. Although sludge dewaterability is improved at first, unexpectedly it tends to deteriorate due to excess bio-acidification with the increase of bioleaching time. To achieve optimal the efficiencies of sludge dewatering and sludge-borne metals removal, pH ~ 2.4 is suggested as the optimum end point for bioleaching. These results have profound implications for sludge bioleaching treatment operator because optimum operational conditions will need a balance among the energy substance bio-oxidation, desired sludge-borne metals removal, and sludge dewaterability.

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