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Enhanced Cr bioleaching efficiency from tannery sludge with *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*

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ABSTRACT

Bioleaching is a successful and cost-effective strategy with which to remove heavy metals from contaminated sludge. Most of the current studies on bioleaching are concentrated on municipal sludge, and bioleaching of tannery sludge has not been studied extensively. The present study investigated the bioleaching of chromium (Cr) from tannery sludge using the mixture of iron-oxidizing bacteria (*Acidithiobacillus ferrooxidans*) and sulfur-oxidizing bacteria (*Acidithiobacillus thiooxidans*) in batch flasks. Changes in the pH, ORP, Fe^{2+} and SO_4^{2-} concentrations and the efficiency of bioleaching of Cr from tannery sludge were analyzed. The results showed that the bioleaching efficiency of co-inoculation of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* was better than any of them. The co-inoculation with a mixture of iron-oxidizing and sulfur-oxidizing bacteria together with the addition of Fe^{2+} and elemental sulfur accelerated acid production and an increase in the oxidation-reduction potential originating from the bio-oxidation of Fe^{2+} and elemental sulfur. The bioleaching efficiency of co-inoculation of Cr increased as the pH of sludge decreased, reaching a maximum removal of 97.1%. The residual Cr concentration which is 531 mg/kg in bioleached sludge is below the thresholds for agriculture, according to China National Standard GB4284-2018 (<1000 mg/kg dry weight) for pollutants in sludges from agricultural use. The bioleaching efficiency was distinctly correlated with pH or ORP. The data presented showed that the bioleaching efficiency was negatively correlated ($R^2 = 0.9864$) with pH and positively ($R^2 = 0.9821$) with ORP, which implied the existence of a substantial indirect leaching mechanism responsible for Cr solubilization.

Keywords: Tannery sludge; Bioleaching; Chromium; *Acidithiobacillus ferrooxidans*; *Acidithiobacillus thiooxidans*; Correlation

1. Introduction

Cr(III) compounds have been widely used in the tanning process to protect leather from microbial degradation and perspiration [1,2]. The low reaction efficiency of Cr(III) results in large quantities of chromium remaining in tannery effluent, and consequently entering into the tannery sludge during the sewage treatment process [3]. Therefore, Cr(III) is an important source of environmental pollutants in tannery wastewater and sludge [4]. The primary problem associated with chromium is that, unlike many pollutants, it is not biodegradable. As a result, when present in the food chain it can eventually be metabolized by animals and plants, and ultimately bioaccumulated [5]. There are more than 2000 tanneries in China, and they generate a large amount of tannery sludge in the process of wastewater treatment. Tannery sludge is classified as hazardous waste in many countries due to its content of 1%–4% chromium [2]. Reduction of the content of Cr in tannery sludge to a standard and safe level is a challenge to sustainable development of the leather industry.

Conventional options for tannery sludge disposal currently include its use as a fertilizer [6], as a soil amendment material [7], in landfills [8], or its incineration [9]. At present, physicochemical technologies are the primary methods

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with which to remove Cr from tannery sludge. Although conventional physicochemical technologies have an obvious efficiency, in practice, many significant limitations still exist, including high consumption of acid, high investment and operation, and secondary pollution [10,11]. In recent years, bioleaching has been proved to be a successful and cost-effective strategy for removal of heavy metals from contaminated sludge [2,12,13]. Bioleaching is widely used to leach various ores [14], obsolete metal electronic products [15] and heavy metal-contaminated sludge [13,16,17]. Furthermore, bioleaching treatment can significantly improve the dewater ability of waste [18] or sludge [19]. Bioleaching is an environmentally cleaner process than physical and chemical processes [10,20] and it has advantages of low cost, easy operation, low energy requirement, and attaches minimal hazards to products [21,22].

It is important to strengthen bioleaching efficiency by addition of exogenous microorganisms during the bioleaching process. Single strains or artificial microbial consortia capable of oxidation of sulfur or iron are common choices [17,23,24]. During the bioleaching process, Cr(III) is released from tannery sludge, which is acidified by *Acidithiobacillus* species, mainly *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) or *Acidihtiobacillus thiooxidans* (*A. thiooxidans*) [2,12]. After bioleaching, sludge can maintain its soil conditioning capability and fertilizer efficiency for landfill or other land applications [25,26]. During bioleaching, bio-oxidation of elemental sulfur by *A. thiooxidans* or hydrolysis of Fe^{3+} formed by bio-oxidation of Fe^{2+} by *A. ferrooxidans* produces H^+ which causes a decrease in pH and leads to solubilization of heavy metals [4,27]. *A. ferrooxidans* and *A. thiooxidans* have been regarded as the most effective bacteria in the bioleaching processes, and they can handle tannery sludge containing higher concentrations of Cr^{3+} [16]. Sludge bio-acidification occurs as a result of bio-oxidation of different substances during bioleaching, as shown by the following equations:

$$S^0 + H_2O + 1.5O_2 \rightarrow H_2SO_4 \quad (1)$$

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \quad (2)$$

$$8Fe^{3+} + 14H_2O + SO_4{}^{2-} \rightarrow Fe_8O_8\left(SO_4\right)\left(OH\right)_6 + 22H^+ \quad (3)$$

Few studies have focused on the effect of a single inoculum of either *A. ferrooxidans* [12,14,17] or *A. thiooxidans* and a single material such as Fe^{2+} or S^0 [27,28]. Other studies have investigated the relationship between the removal efficiency and the concentration of ferrous iron or sulfur when using iron-oxidizing or sulfur-oxidizing bacteria [29,30]. Addition of inorganic ions, such as Ag^+ or $PO_4{}^{3-}$, can increase the efficiency of heavy metal bioleaching [31,32]. To date, most studies of bioleaching of *A. ferrooxidans* and *A. thiooxidans* have focused on the additives used, the bioleaching time, the pH and the added inorganic ions [24,33,34]. Many studies of bioleaching in municipal sludge have been reported, but tannery sludge has been addressed only infrequently. In addition, there is much information on the bioleaching efficiency of heavy metals, but little on the relationship of the bioleaching efficiency with factors that influence it, such as oxidation-reduction potentials (ORP) and pH. Therefore, the objectives of this present study are to (1) investigate the efficiency of bioleaching of Cr from tannery sludge using *A. ferrooxidans* and *A. thiooxidans* under various conditions, and (2) explore the relationship of the bioleaching efficiency with the influence of factors such as pH and ORP on the bioleaching process.

2. Materials and methods

2.1. Sampling and characterization of tannery sludge

The tannery sludge used in this work was obtained from a tannery factory in Xiamen City, Fujian Province, China. The sludge sample was taken to the laboratory and stored at 4°C prior to use. The pH and ORP of the tannery sludge were determined immediately after collection while the solid contents oven-dried at 105°C. The Fe and Cr content in a sample of dried sludge was measured by ICP-atomic emission spectroscopy and total N, P, K, and organic matter content were measured by standard methods [35]. The physicochemical characteristics of the sludge used are presented in Table 1.

2.2. Preparation of microorganisms and inoculum

A. ferrooxidans (CGMCC NO.0727) and *A. thiooxidans* (CGMCC NO.0759) obtained from China General Microbiological Culture Collection Center (CGMCC) were cultivated in improved 9K and SM liquor medium respectively. The improved 9K or SM liquor medium was sterilized with high pressure steam at 121°C for 15 min then the pH was adjusted to 2.5 or 3.0 with 9 M H_2SO_4. Finally, 44.2 g/L $FeSO_4 \cdot 7H_2O$ or 10 g/L elemental sulfur was added as an energy source. After inoculation with *A. ferrooxidans* or *A. thiooxidans*, the culture was incubated in 250 mL Erlenmeyer flasks shaken at 28°C and 180 rpm for 3–4 d until the bacterial cell density reached 10^7–10^8 cells/mL [28,36]. The inoculum was prepared by placing 15 mL *A. ferrooxidans* or 15 mL *A. thiooxidans* cultures separately in 250 mL Erlenmeyer flasks containing 115 mL of fresh sludge, which was then spiked with 4 g/L Fe^{2+} or 3 g/L sulfur respectively. The

Table 1
Physicochemical characteristics of tannery sludge

Parameter	Value
pH	7.10
ORP (mv)	–130.9
Total solid (%)	12.88
Organic matter (%, dry weight)	43.6
Cr^{3+} (g/kg, dry weight)	15.890
Total Fe (%, dry weight)	3.01
Total S (%, dry weight)	1.88
Total P (%, dry weight)	1.30
Total N (%, dry weight)	3.21
Total K (%, dry weight)	0.55

sludge concentration in Erlenmeyer flasks was 2% (w/w). All incubations were performed on a rotary shaker at 28°C and 180 rpm. The pH of the sludge was measured at intervals in this process. When the pH decreased to 2.0, 15 mL of this bioacidified sludge was transferred to 135 mL of fresh sludge mixed with 4 g/L Fe^{2+} or 3 g/L S^0. These mixtures containing fresh sludge were shaken under the conditions described above until the pH of the sludge reached 2.0. The above procedures were repeated three times and the acidified sludge gained in the last process was used as the inoculum containing *A. ferrooxidans* or *A. thiooxidans* [37].

2.3. Bioleaching experiments

Batch experiments were carried out at 28°C and 180 rpm in 250 ml Erlenmeyer flasks containing 135 ml of tannery sludge. The tannery sludge was acidified to pH 6.50 with H_2SO_4 and then agitated at 28°C and 180 rpm for 12 h to adapt the sludge to the bioleaching temperature. This pH adjustment failed to solubilize the Cr in the sludge. An inoculum of 15% volume was added to the acidified sludge followed by addition of: (1) *A. ferrooxidans* and *A. thiooxidans* (v/v = 1:1) with 4.0 g/L $FeSO_4$ and 3.0 g/L S^0; (2) *A. ferrooxidans* and 4.0 g/L $FeSO_4$; (3) *A. thiooxidans* and 3.0 g/L S^0; (4) *A. ferrooxidans* and *A. thiooxidans* (v/v = 1:1); Control experiments without inoculum or with only 4.0 g/L $FeSO_4$ and 3.0 g/L S^0 were performed for comparison. Water loss due to evaporation was replenished with distilled water. All treatments were done in triplicate.

2.4. Analytical methods

Samples of 15 ml were withdrawn at 1 d intervals for pH and ORP measurement and subsequent centrifugation at 12000×g for 15 min to separate solids from the liquids. The pH and ORP values were determined with a pHS-3C digital pH meter (pHS-3C, Shanghai INESA Scientific Instrument, China) and an ORP-421 meter (ORP-421, Shanghai Kang-Yi Instruments, China), respectively. The supernatant was then filtered with 0.45 µm membrane filters prior to determination of Fe^{2+} by a standard colorimetric procedure using 1,10-phenanthroline (UV-2450, Shimadzu, Japan). The concentration of SO_4^{2-} was measured by ion chromatography (ICS-1500, Dionex, USA). Total Cr was determined using ICP-OES (iCAP-7000, Thermo, America). The Cr bioleaching efficiency was calculated with Eq. (4)

$$\text{Cr bioleaching efficiency } (\%) = \left(\frac{W_1}{W_2}\right)\cdot 100\% \qquad (4)$$

where W_1 represents the quantity of Cr in the aqueous phase (mg) after bioleaching and W_2 represents the quantity of Cr in the sludge (mg) at the beginning of the bioleaching. All analyses were performed in triplicate, and the mean values are reported.

3. Results

3.1. Changes in pH and ORP during bioleaching

It is well known that pH is a particularly significant parameter influencing solubilization of heavy metals during bioleaching [38]. There is evidence that the pH of sludge decreases in bioleaching systems and is dependent on the activity of *Acidithiobacillus* bacteria [33,39,40]. A large quantity of heavy metals was dissolved at a pH of 2.0, and sludge-borne metals remain in solution at this pH. Many bioleaching studies use a pH value of 2.0 as the end point during bioleaching [27,28]. As a result of the oxidization of ferrous iron and elemental sulfur, the sludge became acidified gradually and ORP peaks. The pH and ORP variations of sludge during bioleaching are shown in Fig. 1.

As can be seen from Fig. 1a, the pH of the inoculated sludge continued to fall, while ORP (Fig. 1b) increased continually during the bioleaching process. In the control treatment, the pH increased from 6.50 to 7.50 and then decreased

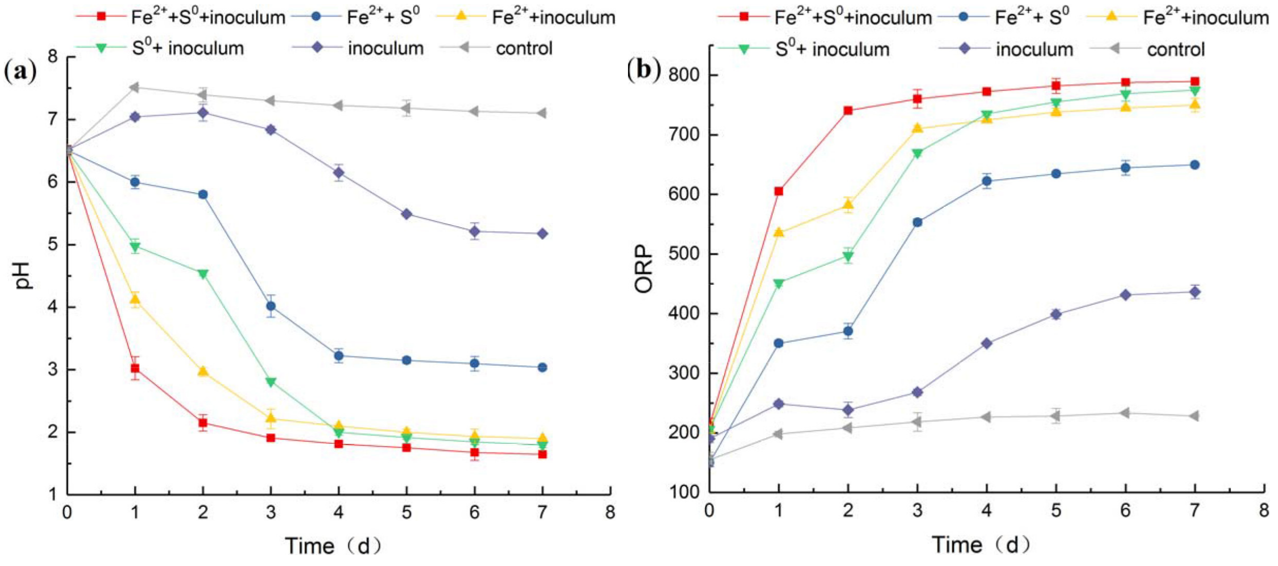

Fig. 1. Variation of pH (a) and ORP (b) in tannery sludge over time during bioleaching (sludge concentration, 2% (w/w); $FeSO_4$ concentration, 4 g/L, S_0 concentration, 3 g/L; inoculum concentration, 10 % (v/v); temperature, 28°C; agitation speed, 180 rpm).

slightly. In the uninoculated samples with added Fe^{2+} and S^0, the pH decreased from 6.50 to 3.20 and maintained this level till the end of the bioleaching. This pH decrease was attributed to the hydrolysis of Fe^{2+} and to Fe^{3+} generated by chemical oxidation of Fe^{2+} at pH > 4.50. The pH in inoculated treatments decreased at a different rate. The relative rates of acid production were Fe^{2+} + S^0 + inoculum > S^0 + inoculum > Fe^{2+} + inoculum > inoculum alone. The final pH values were 1.65, 1.80, 1.90 and 5.18, respectively. The reason why the pH in Fe^{2+} + S^0 + inocu1um was lower than in S^0 + inoculum or Fe^{2+} + inoculum was that a rapid decrease of pH initiated by the chemical oxidation of Fe^{2+} favored the growth of acidophilic iron-oxidizing and sulfur-oxidizing bacteria, which in turn further accelerates the fall in pH. The oxidation of elemental sulfur and Fe^{2+} provides sufficient energy for the growth of bacteria. In addition, Fe^{3+} is an oxidizing agent, and it is speculated that the oxidation of S^0 was not only achieved by bacteria but enhanced by the presence of Fe^{3+}. In contrast to the trend of changes in pH, the ORP of different treatments increased at a different rate: Fe^{2+} + S^0 + inoculum >S^0 + inoculum >Fe^{2+} + inoculum > Fe^{2+} + S^0 > inoculum > control.

3.2. Oxidization of ferrous iron and elemental sulfur

Details of the oxidization of ferrous iron and elemental sulfur are shown in Figs. 2a and 2b. In 2 d, the concentration of Fe^{2+} of the inoculated treatments with Fe^{2+} + S^0 and Fe^{2+} proceeded from 1120 mg/L and 1115 mg/L to 50 mg/L and 80 mg/L, and the oxidation rates reached 95.5% and 92.8%, respectively. The oxidation rate of Fe^{2+} of the inoculated treatment with Fe^{2+} + S^0 was faster than the rate with Fe^{2+} alone. The probable explanation for the acceleration in the rate of Fe^{2+} oxidation can be found in the added S^0 energy substance. This may create favorable conditions for the growth and activity of *A. ferrooxidans* as a result of the decrease in pH and the increase in sulfate concentration [25].

$$2Fe^{2+}+\frac{1}{2O_2}+2H^{+}\rightarrow 2Fe^{3+}+H_2O \tag{5}$$

It is known that Fe^{2+} at neutral pH is chemically unstable and tends to be oxidized to Fe^{3+}. This leads to precipitation of iron hydroxides or ferric hydroxy-sulfates, lowering the pH.

$$Fe^{3+}+3H_2O\rightarrow Fe(OH)_3+3H^{+} \tag{6}$$

$$4FeSO_4+O_2+2H_2O\rightarrow 4Fe(OH)SO_4 \tag{7}$$

This is the reason that the Fe^{2+} concentration in the uninoculated treatment with addition of Fe^{2+} + S^0 decreased in 3 d from 1110 mg/L to 700 mg/L.

Fig. 2b shows the oxidation of added elemental sulfur with concomitant sulfate production.

$$2S^0+3O_2+2H_2O\rightarrow 2H_2SO_4 \tag{8}$$

As can be seen from Fig. 2b, the initial sulfate concentration in tannery sludge was approximately 1500 mg/L. It remained unchanged in the control and other uninoculated treatments during bioleaching, indicating that elemental sulfur was chemically stable. Due to biooxidation of added S^0, there was a net increase in sulfate for the inoculated treatment with co-addition of Fe^{2+} and S^0 or with the addition of S^0 alone. The initial average sulfate concentration of 1500 mg/L and 1520 mg/L increased to 4455 mg/L and 4255 mg/L in the inoculated treatments with additional Fe^{2+} + S^0 and with additional S^0 respectively. At the end of the process, the sulfate concentration tended to be stable, which indicated that the elemental sulfur energy had been adequately used.

3.3. Removal of chromium from tannery sludge.

As shown in Fig. 3, solubilization of Cr seems to be dependent on the presence of bacteria and energy-rich substrates as indicated by the low Cr solubilization efficiencies for the all uninoculated treatments and for inoculated treatment in the absence of energy substrates, throughout

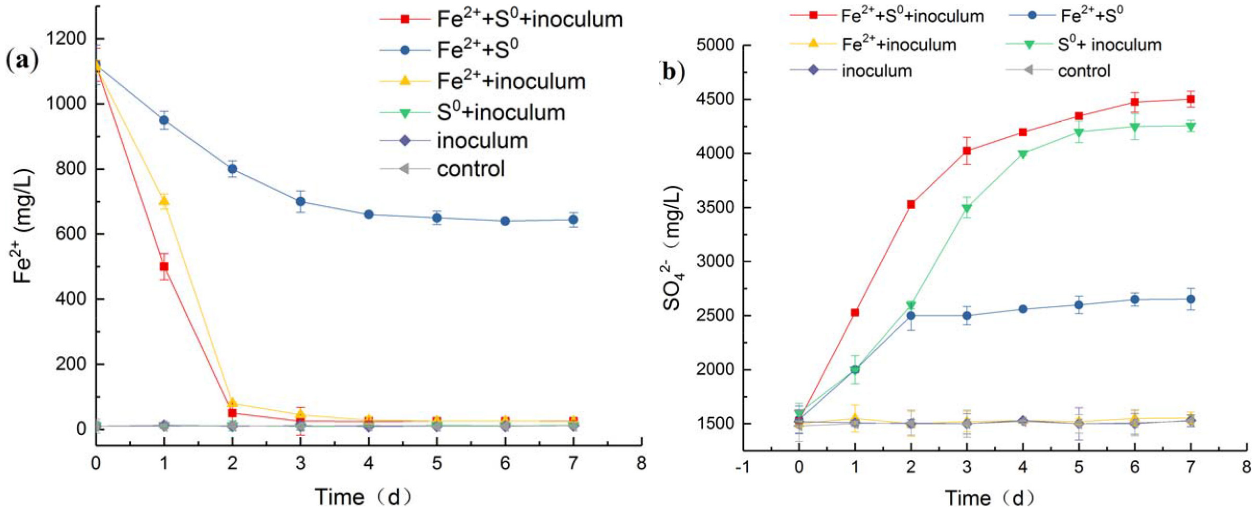

Fig. 2. Variations of Fe^{2+} and SO_4^{2-} in tannery sludge over time during bioleaching (sludge concentration, 2% (w/w); $FeSO_4$ concentration, 4 g/L, S0 concentration, 3 g/L; inoculum concentration, 10% (v/v); temperature, 28°C; agitation speed, 180 rpm).

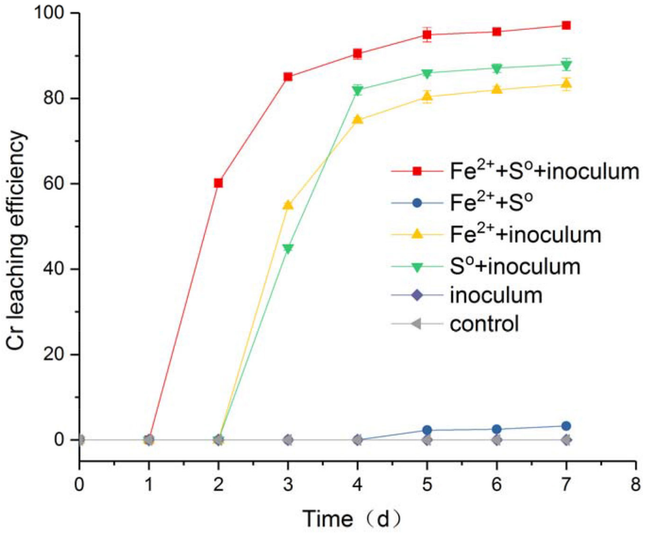

Fig. 3. Removal of Cr from tannery sludge by bioleaching.

the bioleaching period. Cr solubilization also depends on pH because it improved only when the pH in sludge was below 3.0. Therefore, Cr was dissolved largely from the sludge when the inoculated treatments received energy-providing substrates for the next few days. From day 3, the dissolved Cr concentration increased sharply as the pH decreased. Many studies have reported that the metal solubilization efficiency is closely associated with metal speciation [13,16,27]. An earlier study reported that 10%~20% of Cr(III) in tannery sludge was bound to organic matter and 80%~90% existed as mixed oxides of Cr-Fe co-precipitates [41]. A large fraction of heavy metals is mainly transformed to comparatively stable species after bioleaching [33]. It has reported that dissolved organic matters have a negative influence on the growth and activity of chemolithoautotrophic microbes [20]. The highest bioleaching efficiency is found in the treatment of Fe^{2+} + S^0 + inoculum. A possible interpretation of this is that microbiological assimilation and the highly oxidizing condition facilitates breakdown of the organic fraction, and as a result, bioleaching removes Cr more efficiently.

Some researchers have reported that the bioleaching efficiency of heavy metals is negatively correlated with sludge pH, but positively correlated with ORP [42,43]. Fang [25] reported that the bioleaching efficiency was positively correlated with the pH/ORP. However, the lower correlations fail to explain the dissolution of Cr. As can be seen in Figs. 4a and 4b, a high correlation was found between the bioleaching efficiency of Cr and pH, or ORP. The data presented showed that the bioleaching efficiency of Cr had a significant negative correlation (R^2 = 0.9864) with pH and a positive correlation (R^2 = 0.9821) with ORP, implying a substantial indirect leaching mechanism responsible for Cr solubilization. Bioleaching of Cr from tannery sludge does not depend on these two chemical parameters alone. Future studies are needed to investigate detailed chemical speciation changes and the bio-oxidation of *thiobacillus* during the bioleaching process.

4. Discussion

Low pH coupled with a high ORP value has been recognized as characteristic of the existing population of iron-oxidizing and sulfur-oxidizing bacteria [44]. The decrease in the pH of sludge during bioleaching has been ascribed to the energy associated with the bio-oxidation of Fe^{2+} and S^0 by *A. ferrooxidans* and *A. thiooxidans* [4,28,45]. The lower pH and higher ORP of the sludge indicated that *A. ferrooxidans* and *A. thiooxidans* adapted to the sludge environment and grew, which played an important role in sludge acidification. Therefore, this bioleaching treatment could obtain high bioleaching efficiency. It should be noted that the pH of inoculated treatment with addition of Fe^{2+} was lower than that following addition of S^0 in 3 d, but finally, the latter was lower. *A. ferrooxidans* was the dominant species in the early stages, while the ratio of sulfur-oxidizing bacteria increased later. This is not consistent with previous studies [33]. Acid is necessary when Fe^{2+} is oxidized to Fe^{3+}, and the decrease in pH depends

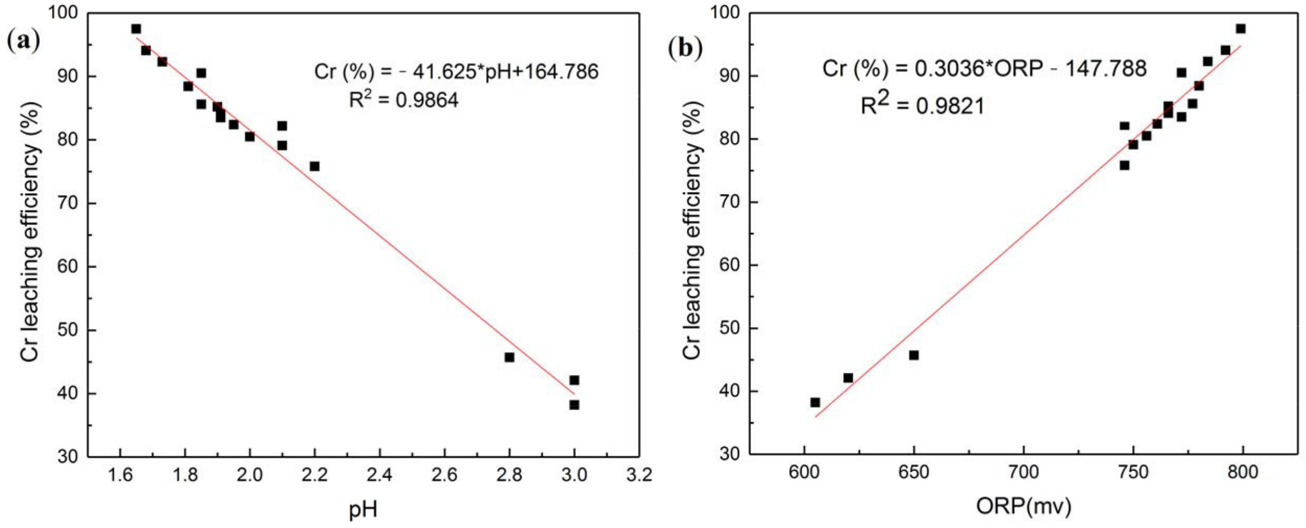

Fig. 4. Relationship between Cr solubilization and pH (a) or oxido-reduction potential (b) ratio.

on hydrolysis of Fe^{3+} salts. Reducible sulfur is a better substrate than Fe^{2+} with respect to acid production, and a previous study reported that elemental sulfur is more efficient than $FeSO_4·7H_2O$ [42,46].

In the first 2 d, the oxidization of Fe^{2+} in the mixture of Fe^{2+} + S^0 + inocu1um treatment was more rapid than in the Fe^{2+} + inoculum mixture and the sulfate concentration in Fe^{2+} + S^0 + inoculum increased faster than in the S^0 + inoculum with identical ferrous or elemental sulfur energy. This suggested that *A. ferrooxidans* or *A. thiooxidans* alone did not grow well when compared to their combination. The probable explanation for the acceleration in the rate of ferrous oxidation or the increase in sulfate concentration is attributed to the synergism of *A. ferrooxidans* and *A. thiooxidans*. The added elemental sulfur may create conditions favorable for the growth and activity of *A. ferrooxidans* as a result of the decrease in pH and the increase in sulfate concentration due to the bio-oxidation of S^0 [28]. The indigenous bacteria existing in tannery sludge, which also utilizes elemental sulfur or ferrous as sources of energy, resulting in low pH and high ORP, facilitates leaching of heavy metals sludge [13]. Furthermore, the concentration of Fe^{2+} is reduced and that of sulfate is increased slightly as a result of treatment with Fe^{2+} and S^0, which failed to inoculate the system. This phenomenon suggests the existence of iron-oxidizing and sulfur-oxidizing bacteria in tannery sludge, which make a minor contribution to sludge acidification and solubilization of chromium [13]. The contribution of heterotrophic microbes to bioleaching requires further investigation.

The presence of toxic metals is one of the main factors limiting the application of sewage sludge as a fertilizer [47]. After 7 d of bioleaching, the treatment of the co-addition of Fe^{2+} and S^0 reached the maximum removal efficiency of 97.1%. The residual Cr concentration in the bioleached sludge is 531 mg/kg, which is lower than the Chinese national standard GB4284-2018 (<1000 mg/kg dry weight) for pollutants in sludges from agricultural use. The removal efficiency of inoculated treatments with added either Fe^{2+} or S^0 is about 10% lower than with added Fe^{2+} together with S^0 and it can be attributed to higher pH and lower ORP. The inoculated treatments with added Fe^{2+} or S^0 had a similar bioleaching efficiency, and this should be explored in future studies using an inexpensive source of energy such as S^0 to enhance the bioleaching efficiency of Cr [48,49]. It has been reported that the pH of sludge can decrease to 2.0 or lower, and Cr solubilization can be deeply influenced by sludge pH. This conclusion is consistent with our results: the removal efficiency of Cr increases sharply (>80%) when the pH drops to 2.0, it is also indicated that pH of 2.0 is the threshold value for Cr dissolution.

5. Conclusion

This study has demonstrated that the iron-oxidizing and sulfur-oxidizing bacteria of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* readily acclimate to tannery sludge and are feasible for bioleaching Cr from tannery sludge. The bioleaching efficiency of Cr of *Acidithiobacillus thiooxidans* with added S^0 was better than *Acidithiobacillus ferrooxidans* with added Fe^{2+}, but both were inferior to the combination of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. The bioleaching efficiency of Cr increased as the pH of sludge decreased and reached a maximum removal of 97.1%. The residual Cr concentration in bioleached sludge was below the thresholds for agriculture or landfill dispose, according to China National Standard GB4284-2018. The data presented shows that the bioleaching efficiency has a significant negative correlation with pH and a positive correlation with ORP, which implies a substantial indirect leaching mechanism responsible for Cr solubilization. Removal of Cr from tannery sludge does not depend only on these two parameters, and the detailed chemical speciation changes and the bio-oxidation of *Acidithiobacillus* species during the bioleaching process need be investigated in future studies.

Conflict of interests

The authors declare that they have no competing interests.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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