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Supplementation of inorganic phosphate enhancing the removal efficiency of tannery sludge-borne Cr through bioleaching

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ABSTRACT

Four inorganic mineral nutrients including NH_4^+, K^+, Mg^{2+} and soluble inorganic phosphate (Pi) were investigated to reveal the potential limiting nutrients for tannery sludge bioleaching process driven by *Acidithiobacillus* species, and the feasibility of supplementing the limiting nutrients to accelerate tannery sludge bioleaching was studied in the present study. It was found that the concentration of Pi was lower than 3.5 mg/L throughout the whole bioleaching process, which is the most probable restricting nutrient for tannery sludge bioleaching. Further experiments revealed that the deficiency of Pi could seriously influence the growth of *Acidithiobacillus thiooxidans* and lower its oxidization capacity for S^0, and the limiting concentration of Pi for the growth of *A. thiooxidans* was 6 mg/L. The low concentration of soluble Pi in sludge matrix was resulted from the extremely strong sorbing/binding capacity of tannery sludge for phosphate. The supplementation of more than 1.6 g/L KH_2PO_4 into tannery sludge bioleaching system could effectively stimulate the growth of *Acidithiobacillus* species, enhance Cr removal rate and further shorten tannery sludge bioleaching period from 10 days to 7 days. Therefore, inorganic phosphate supplementation is an effective and feasible method to accelerate tannery sludge bioleaching process, and the optimum dosage of KH_2PO_4 was 1.6 g/L for tannery sludge with 5.1% of total solids.

1. Introduction

Cr(III) compounds are extensively used in tanning process to protect leather against microbial degradation, moisture, sweat and so on (Erdem, 2006; Zheng et al., 2009). However, a large amount of chromium still remains in the tannery effluent after tanning process because of low reaction efficiency of Cr(III) with hides and consequently goes into the tannery sludge during sewage treatment process (Esmaeili et al., 2005). As a result, tannery sludge usually contains high content of Cr(III) (1–4%, wt/wt) and is classified as a hazardous waste by many nations (Zheng et al., 2009). It is therefore urgent to find a suitable method to dispose tannery sludge economically and safely to avoid chromium accumulated being released to environment (Chuan and Liu, 1996) and thus threatening animal and human health (Bartlett, 1991).

It is found recently that bioleaching technique is a convincing way to remove Cr(III) from tannery sludge over other physical or chemical methods (Zhou et al., 2005, 2006; Fang and Zhou, 2007; Wang et al., 2007; Zheng et al., 2009).

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In this process, Cr(III) can be dissoluted through sludge acidification processes driven by *Acidithiobacillus* species (Sand et al., 2001; Rohwerder et al., 2003; Rawlings, 2005; Zheng et al., 2009; Wang et al., 2010), mainly *Acidithiobacillus ferrooxidans* and/or *Acidithiobacillus thiooxidans*, and the resulting sludge could perfectly maintain its soil conditioning and fertilizing properties for the subsequent land application (Couillard and Mercier, 1993; Fang and Zhou, 2007). To date, although many parameters of tannery sludge bioleaching have been extensively studied to optimize the growth conditions of applied bacteria and thus achieve the maximum efficiency of metal solubilization, such as type of substrates, sulfur concentration, solid content and dissolved carbon dioxide concentration (Zhou et al., 2005, 2006; Fang and Zhou, 2007; Wang et al., 2007; Zheng et al., 2009), litter information is available for the effect of inorganic mineral nutrients on the tannery sludge bioleaching process and the feasibility of enhancing bioleaching efficacy via adjusting inorganic mineral nutrients in sludge.

Previous studies focusing on the bioleaching of ores have revealed that other than energy sources and environmental factors including temperature, pH, redox potential and composition of the leaching medium inorganic mineral nutrients also play important roles in regulating the growth of *Acidithiobacillus* species. For instance, Niemelä et al. (1994) found that ammonium amendment (6 mM) could significantly enhance Fe^{2+} oxidation during bioleaching of a sulfidic black-schist ore, while it is reported by Hugues et al. (2008) that available ammonium ion was critical to both bacterial growth and bioleaching efficiency during continuous bioleaching of a pyrite concentrate in stirred reactors, which resulted from a combination of factors such as less precipitate formation and decreased bacterial attachment to the pyrite surface. Besides, the lack of inorganic phosphate (Pi) may also greatly influence the bioleaching of minerals (Rawlings, 2002), since phosphorus plays an essential part of cell structure and metabolism, forming part of nucleic acids, phospholipids, lipopolysaccharides, nucleotide cofactors, and some proteins, where it is incorporated through posttranslational modification (Vera et al., 2008). Other studies demonstrated that under phosphate starvation circumstances *A. ferrooxidans* reduced its growth rate and capacity of oxidizing ferrous iron and fixing CO_2 (Seeger and Jerez, 1993a, 1993b; Varela et al., 1998), and polyphosphate (poly P) which exists in *A. ferrooxidans* cells is involved in a functional heavy metal tolerance mechanism for the bacterium (Alvarez and Jezez, 2004). All these results illuminated that the major or minor nutrient requirements, e.g., N, P, K and Mg, have the possibilities of affecting bioleaching processes driven by *Acidithiobacillus* species, and nutrients amendment might be a feasible approach to enhance bioleaching efficiency.

There is a high content of nutrients including N, P and trace metals existing in sludge matrix (Couillard and Mercier, 1993), so it is usually deemed that *Acidithiobacillus* species could easily meet their nutrients requirement without any extra inorganic nutrients supplements during sludge bioleaching processes. However, not all nutrients in sludge can be readily utilized by *Acidithiobacillus* species since some nutrients are not present in dissolved inorganic form which can be used by the obligate chemolithotrophic autotrophs (Varela et al., 1998). In fact, Choi et al. (2009) have revealed that most phosphorus was either biologically bound to microorganisms or physicochemically bound to metals such as Fe and Al in sewage sludge, and soluble phosphorus was very less. Also, Maurer and Boller (1999) reported that very less inorganic phosphate was present in effluent of wastewater treatment plant since most of phosphorus consisting of particulate phosphorus, polyphosphates and organic phosphorus was removed as sludge. Therefore, it is presumed that the amount of some soluble nutrients, such as inorganic phosphate, in tannery sludge might be so low that restrict, to some extent, the growth of *Acidithiobacillus* species during tannery sludge bioleaching process.

Therefore, the objectives of the present study are to (1) investigate the changes of nutrients levels including Pi, NH_4^+, K^+ and Mg^{2+} during tannery sludge bioleaching processes to identify the potential restricting nutrients for the growth of *Acidithiobacillus* species, (2) study the behaviors of the restricting nutrients in tannery sludge matrix, and (3) explore the feasibility of enhancing tannery sludge bioleaching efficiency through supplementing the restricting inorganic nutrients into bioleaching systems.

2. Materials and methods

2.1. Sludge sample

The tannery sludge was collected from Tannery Sewage Treatment Plant from Fubang Leather Co. Ltd., Zhejing, China and stored at 4 °C until use. Freezed-dried sub-sample was first digested according to standard methods and then measured for heavy metals by inductively coupled plasma (ICP) method, total N and total P by persulfate method, total S by turbidimetric method, and organic matter content by high-temperature combustion method (APHA, 2005). Selected physicochemical properties are shown in Table 1.

2.2. Iron (sulfur)-oxidizing bacterium and bioleaching inoculum preparation

A. ferrooxidans LX5 (CGMCC No. 0727) and *A. thiooxidans* TS6 (CGMCC No.0759) obtained from China General Microbiological Culture Collection Center (CGMCC) were cultivated in modified 9 K medium (Silverman and Lundgren, 1959; Zhou et al., 2006; Vera et al., 2008) containing 3.0 g/L of $(NH_4)_2SO_4$, 0.1 g/L of KCl, 0.5 g/L of K_2HPO_4 and $MgSO_4 \cdot 7H_2O$, and

Table 1 – Selected physicochemical properties of tannery sludge.

pH	Solids content (%)	Organic matter (%)	Total N (%)	Total P (%)	Total S (%)	Total Fe (%)	Total Cr(III) (%)
7.92 (±0.06)	5.10 (±0.21)	43.11 (±1.94)	1.68 (±0.06)	0.47 (±0.02)	5.45 (±0.29)	2.11 (±0.13)	2.34 (±0.07)

The mean value of triplicate samples is shown; values within brackets denote standard deviation.

Starkey's medium (Suzuki et al., 1990; Takeuchi and Suzuki, 1994; Fang and Zhou, 2006; Zhou et al., 2006) containing 0.3 g/L of $(NH_4)_2SO_4$, 3.0 g/L of KH_2PO_4, 0.5 g/L of $MgSO_4 \cdot 7H_2O$ and 0.25 g/L of $CaCl_2$, respectively. The modified 9 K medium and Starkey's medium autoclaved at 121 °C for 15 min were adjusted to pH 2.5 and 3.0 with sulfuric acid, and then spiked with 44.2 g/L of 0.22 μm membrane-filtered $FeSO_4 \cdot 7H_2O$ or 10 g/L of elemental sulfur as the energy source, respectively. The inoculums were prepared by culturing these bacteria in 500 mL conical flasks each containing 250 mL of these modified 9 K or Starkey's medium on a gyratory shaker at 200 rpm and 28 °C.

2.3. *Nutrients changes during the bioleaching process of tannery sludge*

Bioleaching of tannery sludge was conducted in three parallel 500 mL conical flasks, each containing 285 mL of tannery sludge, 1.2 g of S^0 (Zhou et al., 2005; Zheng et al., 2009), and 15 mL of viable cultures of *A. ferrooxidans* LX5 and *A. thiooxidans* TS6 (1:1, v/v) (Zhou et al., 2006). These flasks were incubated in a gyratory shaker at 28 °C and 180 rpm. During the incubation, 10 mL of sludge samples were withdrawn from each flask at two days intervals, centrifuged at 19,784 × g for 15 min and filtered through 0.45 μm membrane filter. The filtrates were analyzed for Mg^{2+} and K^+ using inductively coupled plasma-atomic emission spectrometry (ICP-AES, Optival200, USA); inorganic phosphate (Pi) and NH_4^+ in the filtrates were also determined using molybdenum blue method and Nessler's reagent method, respectively. All experiments were performed in triplicate throughout the present study unless otherwise noted, and the data presented are the mean values of the triplicate samples with standard deviation.

2.4. *Effect of inorganic phosphate on the growth of A. thiooxidans TS6 in liquid medium*

Previous studies have revealed that phosphate starvation could seriously restrict the growth of *A. ferrooxidans* (Seeger and Jerez, 1993a, 1993b; Varela et al., 1998), while litter information is available for its influence on the growth of *A. thiooxidans*. Therefore, the present study focuses only on investigating Pi starvation on the growth of *A. thiooxidans* TS6 in liquid medium. 50 ml of logarithmic growth phase *A. thiooxidans* TS6 cells cultivated as described above was filtered through 0.45 μm membrane filter to remove elemental sulfur particles and the filtrate was subsequently centrifuged at 8793 × g for 10 min to collect bacterial cells which was then resuspend in 100 mL of acidified distilled water (pH = 3.0). This washing procedure was repeated continuously for three times to remove any inorganic phosphate in the medium, after which cells were stored at 4 °C for less than 2 h before inoculation. Effect of Pi on the growth of *A. thiooxidans* TS6 was studied in 250 mL conical flasks containing 0.4 mL (0.4%, v/v) of *A. thiooxidans* TS6 cells prepared above, 1 g (1%, w/w) of elemental sulfur and 97.6 mL of Pi-deficient Starkey's medium. Then, 2 mL of autoclaved phosphate stock solutions prepared with KH_2PO_4 and with the concentration of Pi at 100, 200, 300, 500, 4000 and 8000 mg/L were added to these flasks to make the final concentration of Pi in the medium at 2, 4, 6, 10, 80, 160 mg/L, respectively. The controls were also performed through adding autoclaved distilled water instead of phosphate stock solutions to make the medium without phosphate. All flasks were adjusted to pH 3.0 with sulfuric acid and then incubated in a gyratory shaker at 28 °C and 180 rpm. The loss of water in each flask due to the evaporation was compensated by adding distilled water based on weight loss. During the incubation, samples were withdrawn everyday from flasks and measured for pH and SO_4^{2-} concentration according to the standard methods (APHA, 2005). The S^0 oxidation rate was calculated as the ratio of oxidized S^0 in the form of SO_4^{2-} to the total S (10 g/L) present in the medium.

2.5. *Sorption behavior of inorganic phosphate in tannery sludge and effect of inorganic phosphate supplementation on the removal of Cr during the bioleaching of tannery sludge*

The sorption behavior of Pi on the tannery sludge was studied in a series of 250 mL conical flasks, each containing 100 mL of tannery sludge. Inorganic phosphate in the form of KH_2PO_4 covering a wide range from 0.4 to 3.2 g/L was added into the flasks. 0.05 g of NaN_3 was added to the mixture as microbial growth inhibitor. Then, all flasks were incubated on gyratory shaker at 25 °C and 250 rpm for an equilibration period of 24 h as determined from a preliminary study, after which 10 mL of sludge sample was collected from each flask and centrifuged at 13,739 × g for 10 min. The supernatant was filtered through 0.22 μm membrane filter and subjected to determine inorganic phosphate using molybdenum blue method.

Bioleaching of tannery sludge was also conducted in 500 mL conical flasks as described above. Except 285 mL of tannery sludge, 1.2 g of S^0 and 15 mL of viable cultures of *A. ferrooxidans* LX5 and *A. thiooxidans* TS6 (1:1, v/v), KH_2PO_4 in the concentration range of 0.4–3.2 g/L was also added into the flasks. Then all flasks were incubated in a gyratory shaker at 28 °C and 180 rpm as described before, during which 7.5 mL of sludge samples were collected at 24 h intervals, centrifuged at 19,784 × g for 15 min and filtered through 0.45 μm membrane filter. The filtrates were used to determine pH value and solubilized Cr concentration using inductively coupled plasma-atomic emission spectrometry (ICP-AES, Optival200, USA). Cr solubilization efficiency was calculated as the ratio of the solubilized Cr in the sludge to the total Cr present in sludge.

3. Results and discussion

3.1. *Changes of inorganic nutrients during the bioleaching process of tannery sludge*

Previous studies have revealed that the essential inorganic nutrients for the growth of *A. ferrooxidans* and *A. thiooxidans* included ammonium-nitrogen, phosphate, potassium, magnesium and sulfate, while excess nitrate and chloride could inhibit the growth of the two obligate chemolithotrophic autotrophs (Tuovinen et al., 1971; Niemelä et al., 1994; Suzuki et al., 1999). Since elemental sulfur was employed as the energy source in tannery sludge bioleaching process, sulfate concentration in the system is thus much higher than the limiting concentration (Zhou et al., 2005; Zheng et al., 2009; Wang et al., 2010). Therefore, mainly four ions including NH_4^+, K^+, Mg^{2+} and soluble inorganic phosphate (Pi) were studied to reveal the potential limiting nutrients for tannery sludge bioleaching process. As shown in Table 2, the concentration of NH_4^+ changed in the range from 216.8 mg/L to 392.9 mg/L, higher than 84.8 mg/L which is its concentration in Starkey's medium, indicating that NH_4^+ in sludge is enough to support the growth of *Acidithiobacillus* species during tannery sludge bioleaching. The concentrations of K^+ and Mg^{2+} were 82.4–90.5 mg/L and 112.2–124.7 mg/L, respectively, within the incubation period, both of which are higher than either the limiting concentrations for *A. ferrooxidans* (Tuovinen et al., 1971) or their concentrations in Starkey's medium. Among the four inorganic nutrients investigated, the concentration of soluble Pi is the least, which was always less than 0.94 mg/L during the initial 6 days of incubation. Although it slightly increased to 3.50 mg/L in the rest period of bioleaching process probably due to the decrease of sludge pH, its concentration was still far lower than both the concentration present in Starkey's medium and the concentration range (0.2–4.9 mM) during bioleaching of Black-Schist ore (Niemelä et al., 1994). Therefore, inorganic phosphate (Pi) is most probably to be the restricting nutrient for the growth of *Acidithiobacillus* species during tannery sludge bioleaching process.

3.2. *Effect of inorganic phosphate on the growth of A. thiooxidans in Pi-deficient Starkey's medium*

The changes of medium pH and S^0 oxidation rate during the growth of *A. thiooxidans* in Starkey's medium supplemented with different concentration of Pi are displayed in Fig. 1 and Fig. 2, respectively. It was found that when Pi was lower than 6 mg/L the growth of *A. thiooxidans* was severely and negatively influenced, as exhibiting that both medium pH decrease and S^0 oxidation rate were lowered by Pi deficiency. For example, when Pi concentration in the Starkey's medium was decreased from 6 mg/L to 4 mg/L, S^0 oxidation rate achieved by *A. thiooxidans* within 72 h of incubation was lowered from 25.6% to 18.6%, and the final medium pH correspondingly increased from 0.71 to 1.06. When Pi was completely removed from the Starkey's medium, the growth of *A. thiooxidans* was not totally inhibited probably due to the fact that *Acidithiobacillus* species could assimilate some phosphonates to meet their phosphorus requirements (Vera et al., 2008). However, S^0 oxidization rate within the same incubation period was only 4.2% which is less than 16% of that achieved when more than 6 mg/L of Pi was incorporated into the medium, indicating that the oxidizing capacity of *A. thiooxidans* for S^0 was decreased by more than 84% by the Pi deficiency, and the medium pH was only 1.74 at the end of incubation. On the other hand, the insignificant differences between the treatments amended with more than 6 mg/L of Pi implied that even more Pi supplementation could not further enhance the growth of *A. thiooxidans*. All these results revealed that the limiting concentration of Pi for the growth of *A. thiooxidans* was 6 mg/L, below which the bacterial growth would be significantly influenced. Combining the present results with Pi change during tannery sludge bioleaching, it is reasonable to presume that inorganic phosphate is the restricting nutrient for the growth of *Acidithiobacillus* species during tannery sludge bioleaching process, and the supplementation of Pi might have the potential of effectively enhancing the overall bioleaching efficiency.

3.3. *Sorption behavior of inorganic phosphate in tannery sludge matrix*

Soluble Pi concentration as a function of KH_2PO_4 added into sludge matrix was plotted in Fig. 3. Obviously, soluble Pi detected in sludge was not linearly increased with the increase of KH_2PO_4 added until the amount of KH_2PO_4 exceeded 1.2 g/L, below which no Pi could be detected in sludge supernatant. The batch adsorption isotherm of Pi in tannery sludge matrix was given in Fig. 4. It was found that the amount of Pi adsorbed on tannery sludge increased with an increase in equilibrium Pi concentration and eventually attained a plateau value at high equilibrium Pi concentrations. The adsorption data were plotted according to the Langmuir isotherm model as demonstrated in Fig. 4. Obviously the Pi adsorption isotherms conformed better to the Langmuir equation ($R^2 = 0.9977$), and the maximum binding/sorping capacity of tannery sludge for Pi was 11,177 mg/kg dry sludge. It should also be noted that sludge solid content could influence the equilibrium sorption amount of Pi onto tannery sludge, and the equilibrium sorption amount of Pi would decrease with the reduction of sludge solid content or even increase with the increase of sludge solid content.

Previous studies have found that chemical removal of P during wastewater treatment processes results from both precipitation of phosphates with Fe(II), Fe (III), Al(III) and Ca(II) –salts in the forms of $Fe(PO_4)\cdot 2H_2O$ (strengite), $Al(PO_4)\cdot 2H_2O$ (variscite) and $Ca_x(PO_4)_y(OH)_z$ (apatite), and the adsorption of phosphates on metal-hydroxide/oxide precipitates

Table 2 – Changes of inorganic mineral nutrients during tannery sludge bioleaching.

Time (D)	pH	Mg^{2+} (mg/L)	K^+ (mg/L)	NH_4^+–N (mg/L)	Pi (mg/L)
0	6.92 (±0.06)	114.37 (±3.87)	86.66 (±2.95)	216.83 (±11.47)	0.34 (±0.02)
2	4.90 (±0.11)	122.91 (±2.14)	90.91 (±4.10)	392.86 (±17.89)	0.55 (±0.04)
4	3.78 (±0.07)	123.34 (±5.69)	89.92 (±4.21)	364.24 (±15.63)	0.69 (±0.04)
6	2.57 (±0.09)	112.21 (±1.37)	91.34 (±3.95)	320.95 (±18.17)	0.94 (±0.03)
8	2.16 (±0.02)	127.35 (±2.72)	91.51 (±2.78)	344.21 (±16.24)	1.68 (±0.05)
10	1.81 (±0.04)	115.34 (±3.18)	92.19 (±2.67)	308.79 (±15.38)	3.50 (±0.16)

The mean value of triplicate samples is shown; values within brackets denote standard deviation.

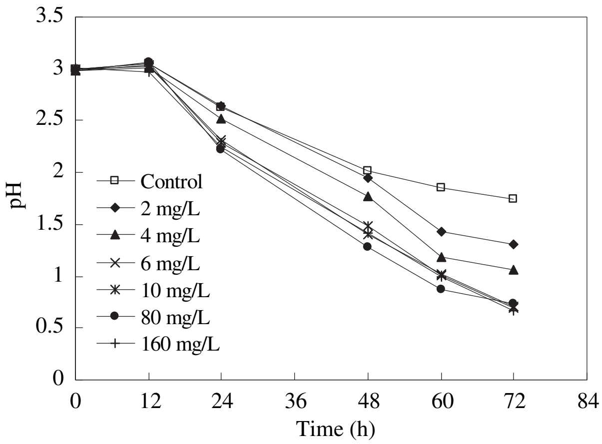

Fig. 1 – Change of medium pH with the time during the growth of *A. thiooxidans* in synthetic Starkey's medium with the presence of different concentration of inorganic phosphate (Pi).

mechanisms (Luedecke et al., 1989). Iron precipitation is extensively used in tannery sewage treatment processes (Esmaeili et al., 2005; Erdem, 2006), thus there is usually high content of iron-hydroxide/oxide and iron sulfide precipitates which possess high binding/sorping capacity for Pi in tannery sludge (Smolders et al., 2001; Liao et al., 2009; Choi et al., 2009; Wang et al., 2010), which might be responsible for the strong sorption behavior of Pi onto tannery sludge particles in the present sorption study. However, during tannery sludge bioleaching process, Fe^{2+} was steadily solubilized out from sludge particles because of the decreased sludge pH values and oxidization effect of *Acidithiobacillus* species. Then the resulting Fe^{2+} was oxidized to Fe^{3+} readily by *A. ferrooxidans*, which could form iron hydroxysulfate precipitates such as schwertmannite very easily in bioleach solutions (Liao et al., 2009; Wang et al., 2010). Therefore, the soluble phosphates in tannery sludge matrix might be both precipitated with Fe^{2+}

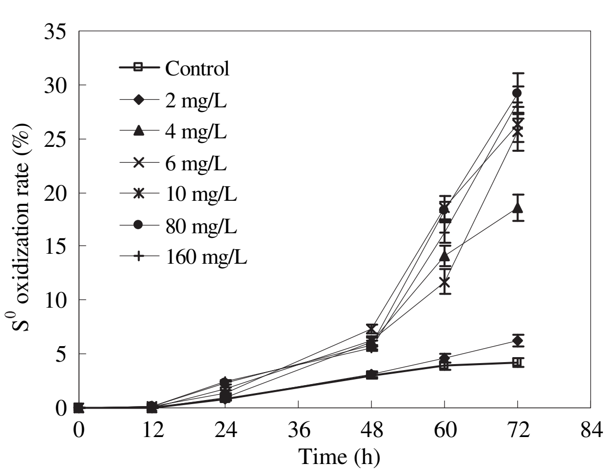

Fig. 2 – Change of S^0 oxidization rate achieved by *A. thiooxidans* grown in synthetic Starkey's medium with the presence of different concentration of inorganic phosphate (Pi).

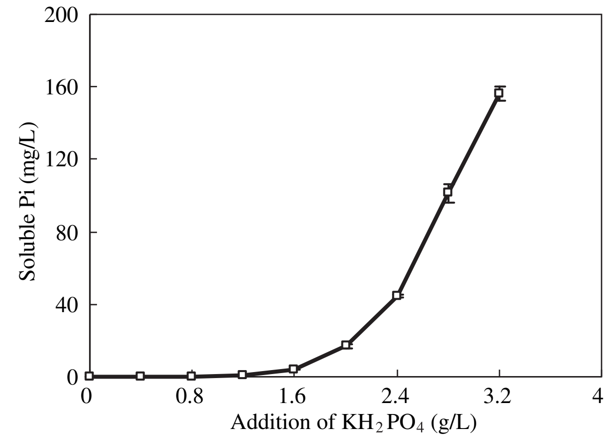

Fig. 3 – Change of soluble Pi in the sludge after supplementing different amounts of KH_2PO_4 into tannery sludge.

and/or Fe^{3+} and sorbed onto the surface of these already-formed secondary iron precipitates, resulting in the low concentration of soluble Pi during tannery sludge bioleaching processes as revealed previously. Furthermore, when the sludge pH value was below 2, the solubility of $Fe(PO_4)\cdot 2H_2O$ steadily increased with pH decrease (Maurer and Boller, 1999), which probably resulted in the increased Pi concentration in sludge matrix at the last several days of tannery sludge bioleaching.

Considering the limiting concentration of Pi for the growth of *A. thiooxidans* was 6 mg/L, the amount of KH_2PO_4 added to enhance bioleaching efficiency during tannery bioleaching process was calculated to be higher than 1.6 g/L, above which the concentration of Pi in tannery sludge could be higher than the limiting concentration for *A. thiooxidans* growth. In other words, the dosage of KH_2PO_4 has to exceed 64% of the maximum binding/sorping capacity of tannery sludge for Pi.

3.4. *Effect of inorganic phosphate supplementation on the tannery sludge bioleaching process*

As shown in Fig. 5 and Fig. 6, it took about 10 days by bioleaching systems either without inorganic phosphate

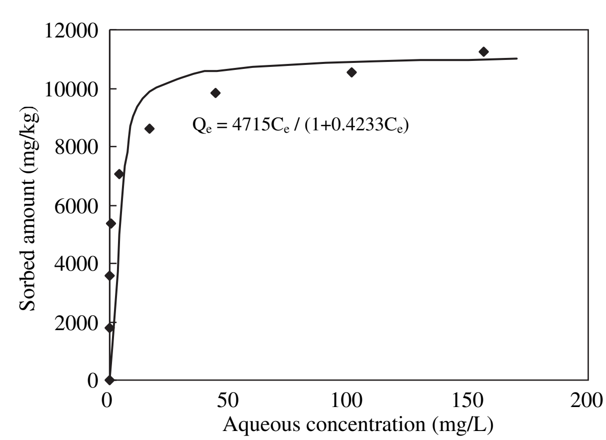

Fig. 4 – Fit of adsorption of Pi onto tannery sludge with Langmuir isotherm.

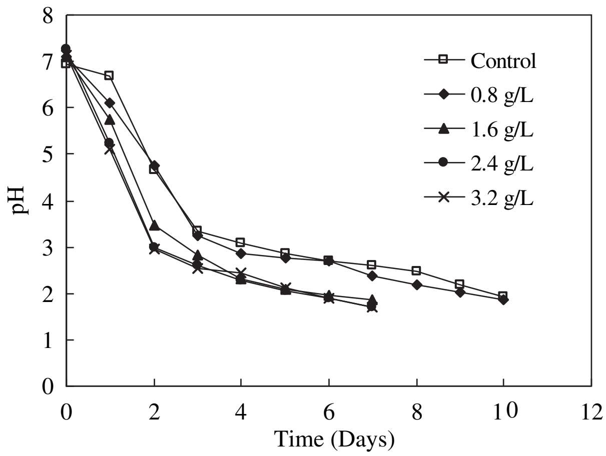

Fig. 5 – Change of pH during tannery sludge (with 2.34% of total Cr present) bioleaching process with/without the supplementation of KH_2PO_4 at 0.8, 1.6, 2.4 and 3.2 g/L.

amendment or with 0.8 g/L KH_2PO_4 supplementation to decrease sludge pH from 6.94–7.10 to 1.86–1.94 and solubilize more than 90% of tannery sludge-borne Cr, which is usually considered as the end of tannery sludge bioleaching (Zhou et al., 2005). These results were consistent with our previous studies that the sludge-borne Cr would be released or removed drastically when sludge pH declines to 2 or below (Zhou et al., 2005; Fang and Zhou, 2007; Zheng et al., 2009). However, the supplementation of more than 1.6 g/L of KH_2PO_4 to bioleaching system could significantly enhance bioleaching efficiency, as exhibiting that only 7 days were needed by the three treatments amended with more than 1.6 g/L of KH_2PO_4 to achieve pH decrease from initial 7.1–7.2 to 1.69–1.86 and more than 91.6% of Cr solubilization. Undoubtedly, three days of bioleaching time was shorten by more than 1.6 g/L of KH_2PO_4 supplementation in comparison with systems without inorganic phosphate amendment or with only 0.8 g/L of KH_2PO_4 supplementation, although the differences among

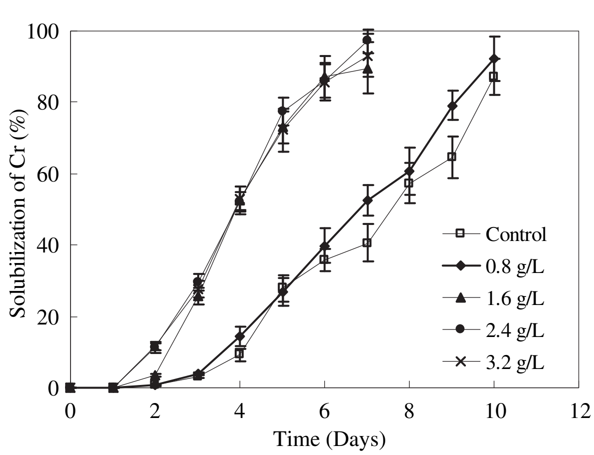

Fig. 6 – Change of Cr solubilization during tannery sludge (with 2.34% of total Cr present) bioleaching process with/without the supplementation of KH_2PO_4 at 0.8, 1.6, 2.4 and 3.2 g/L.

the three treatments supplemented with more than 1.6 g/L of KH_2PO_4 were not significant. Therefore, inorganic phosphate supplementation is an effective and feasible method to stimulate the growth of *Acidithiobacillus* species and further enhance Cr removal efficiency during tannery sludge bioleaching process, and the optimum dosage of KH_2PO_4 was 1.6 g/L for tannery sludge with 5.1% of total solids.

4. Conclusion

Inorganic mineral nutrients were investigated to reveal the potential limiting nutrients for tannery sludge bioleaching process driven by *Acidithiobacillus* species. It was found that inorganic phosphate (Pi) was the most probable restricting nutrient for the growth of *Acidithiobacillus* species due to its low concentration throughout the whole bioleaching process. Further experiments revealed that the deficiency of inorganic phosphate could seriously influence the growth of *A. thiooxidans* and lower its oxidization capacity for S^0, and the limiting concentration of Pi for the growth of *A. thiooxidans* was 6 mg/L. Sorption test indicated that the low concentration of inorganic phosphate in sludge matrix was resulted from the extremely strong sorping/binding capacity of tannery sludge for phosphate. The supplementation of more than 1.6 g/L of KH_2PO_4 into tannery sludge bioleaching system could effectively stimulate the growth of *Acidithiobacillus* species and further enhance the removal rate of sludge-borne Cr. Therefore, inorganic phosphate supplementation is an effective and feasible method to accelerate tannery sludge bioleaching process, and the optimum dosage of KH_2PO_4 was 1.6 g/L for tannery sludge with 5.1% of total solids.

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REFERENCES

Alvarez, S., Jezez, C.A., 2004. Copper ions stimulate polyphosphate degradation and phosphate efflux in *Acidithiobacillus ferrooxidans*. Appl. Environ. Microbiol. 70 (9), 5177–5182.
APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. Amer. Public Health Assoc., Washington, D.C.
Bartlett, R.J., 1991. Chromium cycling in soils and water: links, gaps, and methods. Environ. Health Persp. 92, 17–24.
Choi, H., Choi, C., Lee, S., 2009. Analyses of phosphorus in sewage by fraction method. J. Hazard. Mater. 167 (1–3), 345–350.
Chuan, M.C., Liu, J.C., 1996. Release behavior of chromium from tannery sludge. Water Res. 30 (4), 932–938.
Couillard, D., Mercier, G., 1993. Removal of metals and fate of N and P in the bacterial leaching of aerobically digested sewage sludge. Water Res. 27 (7), 1227–1235.

Erdem, M., 2006. Chromium recovery from chrome shaving generated in tanning process. J. Hazard. Mater. 129 (1–3), 143–146.
Esmaeili, A., Mesdaghi, A., Vazirinejad, R., 2005. Chromium (III) removal and recovery from tannery wastewater by precipitation process. Am. J. Appl. Sci. 2 (10), 1471–1473.
Fang, D., Zhou, L.X., 2007. Enhanced Cr bioleaching efficiency from tannery sludge with coinoculation of *Acidithiobacillus thiooxidans* TS6 and *Brettanomyces* B65 in an air-lift reactor. Chemosphere 69 (2), 303–310.
Fang, D., Zhou, L.X., 2006. Effect of sludge dissolved organic matter on oxidation of ferrous iron and sulfur by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. Water Air Soil Pollut. 171 (1–4), 81–94.
Hugues, P., Joulian, C., Spolaore, P., Michel, C., Garrido, F., Morin, D., 2008. Continuous bioleaching of a pyrite concentrate in stirred reactors: population dynamics and exopolysaccharide production vs. bioleaching performance. Hydrometallurgy 94 (1–4), 34–41.
Liao, Y., Zhou, L., Bai, S., Liang, J., Wang, S., 2009. Occurrence of biogenic schwertmannite in sludge bioleaching environments and its adverse effect on solubilization of sludge-borne metals. Appl. Geochem. 24 (9), 1739–1746.
Luedecke, C., Hermanowicz, S.W., Jenkins, D., 1989. Precipitation of ferric phosphate in activated sludge: a chemical model and its verification. Water Sci. Technol. 21 (4–5), 325–337.
Maurer, M., Boller, M., 1999. Modelling of phosphorus precipitation in wastewater treatment plants with enhanced biological phosphorus removal. Water Sci. Technol. 39 (1), 147–163.
Niemelä, S.I., Riekkola-Vanhanen, M., Sivelä, C., Viguera, F., Tuovinen, O.H., 1994. Nutrient effect on the biological leaching of a black-schist ore. Appl. Environ. Microbiol. 60 (4), 1287–1291.
Rawlings, D.E., 2002. Heavy metal mining using microbes. Annu. Rev. Microbiol. 56, 65–91.
Rawlings, D.E., 2005. Characteristics and adaptability or iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. Microb. Cell Factories 4, 1–13.
Rohwerder, T., Gehrke, T., Kinzler, K., Sand, W., 2003. Bioleaching review part A: progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. Appl. Microbiol. Biotechnol. 63 (3), 239–248.
Sand, W., Gehrke, T., Jozsa, P.-G., Schippers, A., 2001. (Bio) chemistry of bacterial leaching - direct vs. indirect bioleaching. Hydrometallurgy 59 (2–3), 159–175.
Seeger, M., Jerez, C.A., 1993a. Response of *Thiobacillus ferrooxidans* to phosphate limitation. FEMS Microbiol. Rev. 11 (1–3), 37–41.
Seeger, M., Jerez, C.A., 1993b. Phosphate-starvation induced changes in *Thiobacillus ferrooxidans*. FEMS Microbiol. Lett. 108 (1), 35–41.
Silverman, M.P., Lundgren, D.G., 1959. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields. J. Bacterial 77, 642–647.
Smolders, A.J.P., Lamers, L.P.M., Moonen, M., Zwaga, K., Roelofs, J. G.M., 2001. Controlling phosphate release from phosphate-enriched sediments by adding various iron compounds. Biogeochemistry 54 (2), 219–228.
Suzuki, I., Takeuchi, T.L., Yuthasastrakosol, T.D., Oh, J.K., 1990. Ferrous iron and sulfur oxidation and ferric iron reduction activities of *Thiobacillus ferrooxidans* are affected by growth on ferrous iron, sulfur, or a sulfide ore. Appl. Environ. Microbiol. 56 (6), 1620–1626.
Suzuki, I., Lee, D., Mackay, B., Harahuac, L., Oh, J., 1999. Effect of various Ions, pH, and osmotic pressure on oxidation of elemental sulfur by *Thiobacillus thiooxidans*. Appl. Environ. Microbiol. 65 (11), 5163–5168.
Takeuchi, T.L., Suzuki, I., 1994. Effect of pH on sulfite oxidation by *Thiobacillus thiooxidans* cells with sulfurous acid or sulfur dioxide as a possible substrate. J. Bacteriol. 176 (3), 913–916.
Tuovinen, O.H., Niemela, S.I., Gyllenberg, H.G., 1971. Effect of mineral nutrients and organic substances on the development of *Thiobacillus ferrooxidans*. Biotechnol. Bioeng. 13 (4), 517–527.
Varela, P., Levicán, G., Rivera, F., Jerez, C.A., 1998. An immunological strategy to monitor in situ the phosphate starvation state in *Thiobacillus ferrooxidans*. Appl. Environ. Microbiol. 64 (12), 4990–4993.
Vera, M., Pagliai, F., Guiliani, N., Jerez, C.A., 2008. The chemolithoautotroph *Acidithiobacillus ferrooxidans* can survive under phosphate limiting conditions by the expression of a C-P lyase operon that allows it to grow on phosphonates. Appl. Environ. Microbiol. 74 (6), 1829–1835.
Wang, Y.S., Pan, Z.Y., Lang, J.M., Xu, J.M., Zheng, Y.G., 2007. Bioleaching of chromium from tannery sludge by indigenous Acidithiobacillus thiooxidans. J. Hazard. Mater 147 (1-2), 319–324.
Wang, S., Zheng, G., Zhou, L., 2010. Heterotrophic microorganism *Rhodotorula mucilaginosa* R30 improves tannery sludge bioleaching through elevating dissolved CO_2 and extracellular polymeric substances levels in bioleach solution as well as scavenging toxic DOM to *Acidithiobacillus* species. Water Res. 44 (18), 5423–5431.
Zheng, G., Zhou, L., Wang, S., 2009. An acid-tolerant heterotrophic microorganism role in improving tannery sludge bioleaching conducted in successive multibatch reaction system. Environ. Sci. Technol. 43 (11), 4151–4156.
Zhou, L.X., Fang, D., Wang, S.M., Wong, J.W.C., Wang, D.Z., 2005. Bioleaching of Cr from tannery sludge: the effects of initial acid addition and recycling of acidified bioleached sludge. Environ. Technol. 26 (3), 277–284.
Zhou, S.G., Zhou, L.X., Wang, S.M., Fang, D., 2006. Removal of Cr from tannery sludge by bioleaching method. J. Environ. Sci. 18 (5), 885–890.