



Bioleaching of metals from printed wire boards by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* and their mixture

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ARTICLE INFO

Article history:

Received 5 February 2009

Received in revised form 25 July 2009

Accepted 27 July 2009

Available online 3 August 2009

Keywords:

Bioleaching

Pure culture of *Acidithiobacillus ferrooxidans*

The mixed culture of *Acidithiobacillus*

ferrooxidans and *Acidithiobacillus*

thiooxidans

Printed wire boards

Copper

Other minor metals

ABSTRACT

Bioleaching processes were used to mobilize metals from printed wire boards (PWBs). The bacteria *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) and *Acidithiobacillus thiooxidans* (*A. thiooxidans*) isolated from an acidic mine drainage were grown and acclimated in presence of PWBs and then used as bioleaching bacteria to solubilize metals from PWBs. The experimental results demonstrate that all the percentages of copper, lead, zinc solubilized into the leaching solution from actual PWBs basically increased with decrease of sieve fraction of sample and decrease of PWBs concentration. The concentration of PWBs should be controlled under the range from 7.8 to 19.5 g l⁻¹. Under 7.8 g l⁻¹ of the concentration of PWBs, the percentages of copper solubilized are 99.0%, 74.9%, 99.9% at 0.5–1.0 mm of sieve fraction at 9 d of leaching time by the pure culture of *A. ferrooxidans*, the pure culture of *A. thiooxidans*, and mixed culture of *A. ferrooxidans* and *A. thiooxidans*, respectively, while the percentages of copper, lead and zinc solubilized are all more than 88.9% at <0.35 mm of the sieve fractions of sample at 5 d of leaching time by the above three kinds of cultures. Variation of pH and redox potential of leaching solution with time implied that Fe³⁺ oxidized from Fe²⁺ in the culture medium in presence of *A. ferrooxidans* caused the mobilization of metals. It is concluded that *A. ferrooxidans* and *A. thiooxidans* were able to grow in the presence of PWBs and the pure culture of *A. ferrooxidans*, and the mixed culture of *A. ferrooxidans* and *A. thiooxidans* can not only efficiently bioleach the main metal copper but also bioleach other minor metals such as lead, zinc as well.

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1. Introduction

Recycling of Waste Electrical and Electronic Equipment (WEEE) is an important subject not only from the point of waste treatment but also from the recovery aspect of valuable materials. Some authors have reviewed the data on generation of waste electronic equipments in different parts of the world [1]. It is also affirmed that WEEE are the fastest growing waste category; this finding emphasizes the need for efficient WEEE recycling strategies [2]. The US Environmental Protection Agency has identified seven major benefits when scrap iron and steel are used instead of virgin materials. Using recycled materials in place of virgin materials results in significant energy savings. Many countries have drafted legislation to improve the reuse, recycling and other forms of recovery of such wastes so as to reduce disposal. However, in the US and EU, the treatments of an enormous amount of WEEE are simply relying on incineration or landfill. Up to now, the recycling of WEEE is still quite limited due to the heterogeneity of materials present on the

product and the complexity in producing electrical and electronic equipment.

Printed wire boards (PWBs) are important parts of the electronic equipments and their composition is quite varied, containing polymers, ceramics and metals. The metal content is around 28% (copper: 10–20%, lead: 1–5%, nickel: 1–3%). The content of the most important materials remaining is: plastics 19%, bromine 4%, glass and ceramics 49% [3]. In addition, precious metals like silver, platinum and gold are also present in the electronic scrap to a total of 0.3–0.4%. Besides these inorganic elements, the following important organic compounds are found in PWBs: isocyanates and phosgene from polyurethanes, acrylic and phenolic resins, epoxides and phenols [4]. The quantity of metals, especially copper, turns the electronic scrap into an interesting raw material according to the economic point of view. If PWBs are not recycled or treated appropriately and therefore they are made to be dispersed to environment, the heavy metals such as copper, lead and nickel and the halogenated burn-resisting materials in them can cause serious environment problems. Especially, the lead presence in their composition stimulates studies for recycling according to the environmental point of view.

The existing processes of recycling PWBs use pyrometallurgical [4–7] or hydrometallurgical methods [8–12], which generate

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Table 1
Concentration of metals in crushed scraps of actual PWBs^a.

Fraction (mm)	Cu (g kg ⁻¹)	Cr (g kg ⁻¹)	Ni (g kg ⁻¹)	Pb (g kg ⁻¹)	Zn (g kg ⁻¹)
<0.35	90 ± 6	0.093 ± 0.013	0.119 ± 0.019	2.05 ± 0.17	2.15 ± 0.04
0.35–0.50	586 ± 24	0.046 ± 0.001	0.155 ± 0.011	2.53 ± 0.14	2.00 ± 0.17
0.50–0.75	338 ± 31	0.041 ± 0.000	0.163 ± 0.001	3.26 ± 0.01	2.47 ± 0.06
0.75–1.00	371 ± 11	0.043 ± 0.003	0.198 ± 0.002	4.41 ± 0.09	2.64 ± 0.11
1.00–3.00	308 ± 30	0.037 ± 0.001	0.151 ± 0.021	2.74 ± 0.81	1.69 ± 0.04
>3.00	294 ± 15	0.032 ± 0.000	0.151 ± 0.010	1.79 ± 0.11	1.97 ± 0.14

^a Values are the means of three experiments.

atmospheric pollution through the release of dioxins and furans [13] or high volumes of effluents. Mechanical processing [14,15] has been used as an alternative for recycling PWBs to concentrate the metals in one fraction and the polymers and ceramics in another. The metal concentrate fraction can be sent to electrochemistry processes [16–18] in order to separate the metals among themselves. However, such processes are not thought to be economical due to high consumption of energy and are not regarded as economical way to extract valuable components from waste PWBs [19,20].

Biological processes have to be integrated into future industrial processes and “should be on every industrial agenda”. Process-integrated biotechnology is characterized by the application of biocatalysts (e.g., microorganisms, enzymes) in an industrial process and the substitution of existing processes. Regarding sustainability, biological processes can contribute to a large extent to future technologies, including waste treatment.

Sulfuric acid is the main inorganic acid found in leaching environments [21,22]. It is formed by sulfur-oxidizing microorganisms such as *Acidithiobacillus* species. A series of organic acids are formed by bacterial (as well as fungal) metabolism resulting in organic acidolysis, complex and chelate formation [23,24]. These microbial activities can be applied in the industry for the recovery of metals from solid materials [25]. Use of microorganisms for the recovery of metals from waste electronic equipment could be an economical alternative to such processes as pyrometallurgical processes or hydrometallurgical processes, or mechanical processing processes, since using biological techniques for recycling PWBs, the recovery efficiency could be increased whereas thermal or physico-chemical methods alone are less successful, as shown in copper and gold mining where low-grade ores are biologically treated to obtain metal values [26]. Though, this process has been successfully applied for the leaching of metals from ores [27], data pertaining to its application for the extraction of electronic waste material is still scanty. Recently, a few studies have been undertaken for the extraction of metals from electronic scrap [28–32].

The present studies were undertaken to evaluate the potential of the pure culture of *Acidithiobacillus ferrooxidans*, the pure culture of *Acidithiobacillus thiooxidans*, and/or the mixed culture of *A. ferrooxidans* and *A. thiooxidans* to solubilize metals such as copper, lead and zinc from PWBs to figure out strategies for enhanced metals recovery and to answer some basic questions about the relationship among pH and redox potential of the culture media and percentages of metal solubilization during the bioleaching process.

2. Materials and methods

2.1. Source of electronic scraps

The scraps of actual PWBs, which are used as main board of personal computers, were obtained from Shanghai Weimei Corporation of Science and Technology, China. These scraps were crushed and then were fractionated by dry sieving in the laboratory. Metal concentrations of PWBs were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Analytical Instruments, Thermo, A6300, USA) rapid screening at the following

wavelengths (nm): Cu (324.7), Pb (216.9), Zn (206.2), Cr (284.3), Ni (221.6). The percentages of the ranges of <0.35, 0.35–0.50, 0.50–0.75, 0.75–1.00, 1.00–3.00, >3.00 mm of sieve fraction in the crushed samples are 8.9%, 4.7%, 5.7%, 5.1%, 28.6%, 47.0%, respectively; the concentrations of metals in all fractions are given in Table 1.

2.2. Leaching experiment

The isolates used in the experiments was recovered from the acidic mine drainage taken from Dexing Mine, Jiangxi Province, China. The isolates were identified as *A. ferrooxidans* and *A. thiooxidans*. *A. ferrooxidans*, *A. thiooxidans* and the mixture of *A. ferrooxidans* and *A. thiooxidans* were cultured in the media salts with three energy sources, which were Fe²⁺, S and a mixture of Fe²⁺ and S, adjusted to pH value 2.5 by sulfuric acid. The chemical composition of the three media containing the individual energy source is shown in Table 2. 9K medium, S medium and mixed medium are the culture media for *A. ferrooxidans*, *A. thiooxidans* and the mixture of *A. ferrooxidans* and *A. thiooxidans*, respectively. Erlenmeyer flasks of 500 ml were used, containing 200 ml culture medium and 10% of inoculum. The flasks were shaken at 150 rpm on an orbital shaker at 28 °C. Before the bioleaching period of the experiment, these bacteria had been sub-cultured in their own medium containing 1% PWBs samples at 28 °C for 3 d so that they could adapt to the electronic scrap circumstance faster.

Two sieve fractions (1.0–3.0, 0.50–1.0 mm), three kinds of bacterium culture media (the pure culture of *A. ferrooxidans*, the pure culture of *A. thiooxidans*, and the mixed culture of *A. ferrooxidans* and *A. thiooxidans*) and two conditions of inoculation treatments (culture medium non-inoculated and culture medium inoculated) are designed as three main influential factors in examination of effect of leaching time on the percentage of copper solubilization from the PWBs samples. These experiments lasted for 9 d at most. By the end of cultivation, the samples of leaching solution were used for determining the concentration of metal ions, pH value and redox potential.

Leaching experiments were carried out in 150 ml Erlenmeyer flasks, which were shaken in an air-conditional shaker. 50 ml of culture media depicted in Table 2 was added with 0.40 g of PWBs samples at pH 2.5. Solutions and substrates were sterilized by autoclaving at 115 °C and 1.05 kg cm⁻² for 20 min before inoculation. Each flask was inoculated with 1 ml inoculum of *A. ferrooxidans*

Table 2
Chemical composition of three kinds of cultures (g l⁻¹).

Composition	9K medium	Mixed medium	S medium
(NH ₄) ₂ SO ₄	3.0	4.0	0.4
KCl	0.1	0.1	–
K ₂ HPO ₄	0.5	0.5	3.0
MgSO ₄ ·7H ₂ O	0.5	0.5	0.5
Ca(NO ₃) ₂	0.01	0.01	–
FeSO ₄ ·7H ₂ O	44.7	22.1	0.01
CaCl ₂ ·2H ₂ O	–	0.13	0.25
Sulfur powder	–	5	10.0

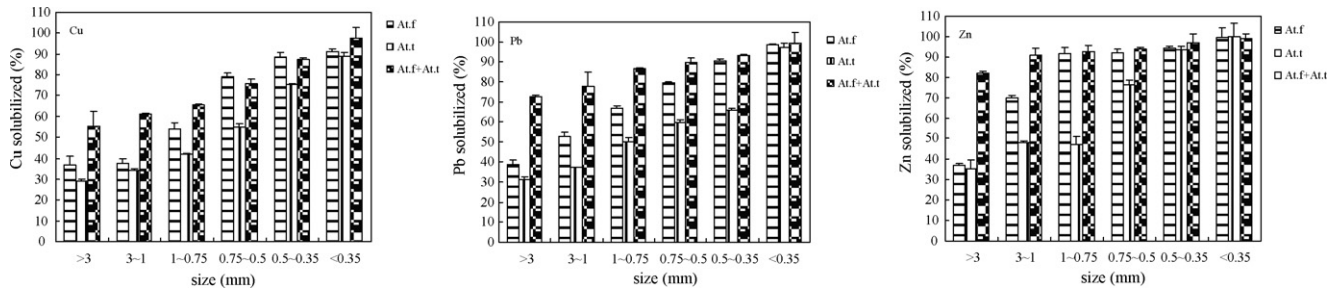


Fig. 1. Percentage of copper, lead and zinc solubilized in leaching solution with size of PWBs at 5 d of leaching time by pure culture of *A. ferrooxidans* (At.f), pure culture of *A. thiooxidans* (At.t), and mixed culture of *A. ferrooxidans* and *A. thiooxidans* (At.f+At.t). The data are the average of three replicates (\pm S.D.).

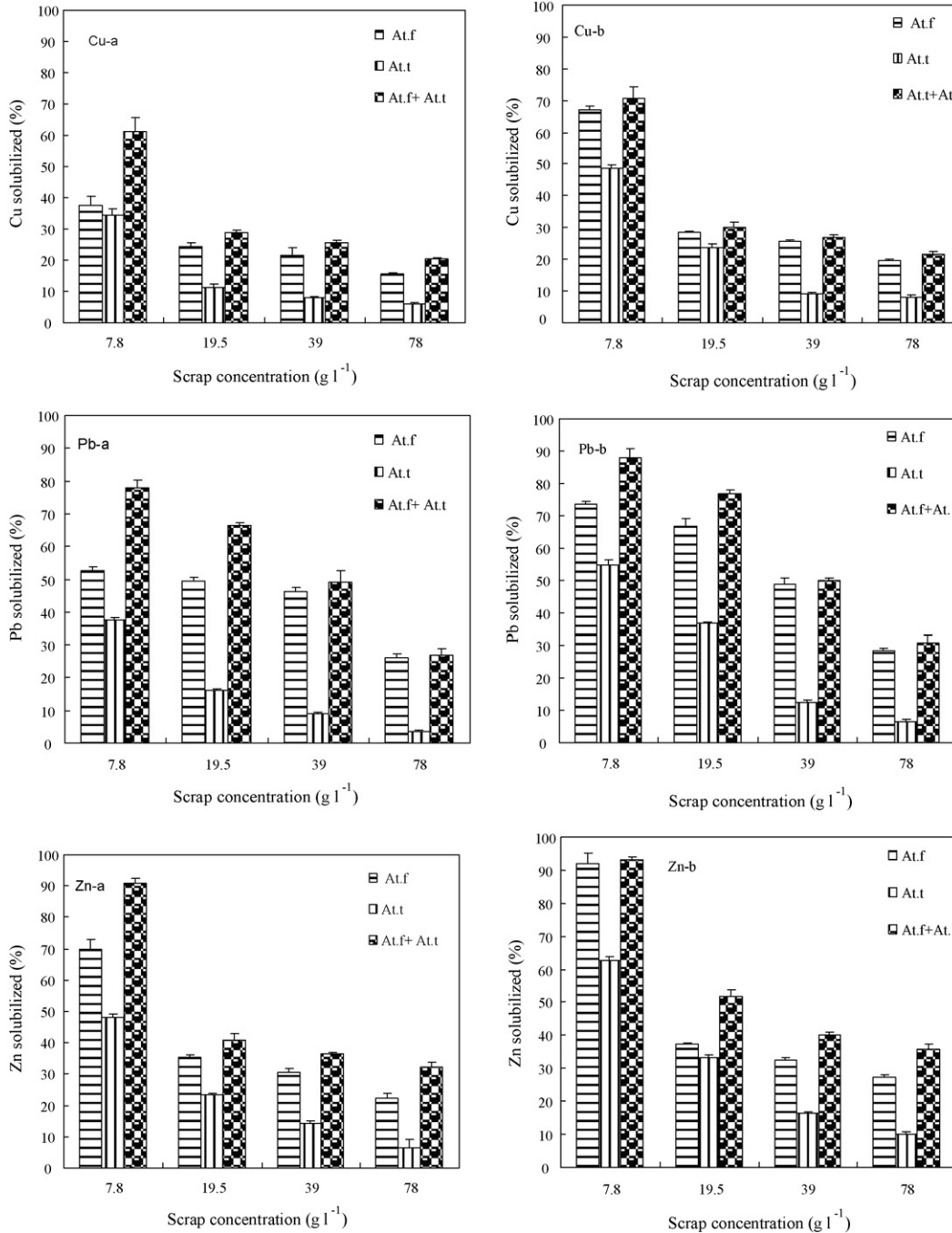


Fig. 2. Percentage of copper, lead and zinc solubilized in leaching solution with scrap concentration of PWBs at (a) 1.0–3.0 and (b) 0.5–1.0 mm of sieve fraction. The data are the average of three replicates (\pm S.D.).

and/or *A. thiooxidans* containing 1.0×10^7 cells ml^{-1} and incubated at 28°C in a rotary shaker at 150 rpm. The progress of bioleaching was monitored by measuring pH and redox potential with a pH-meter (Sartorius PB-10, Germany), and measuring the concentrations of copper ions Cu^{2+} and other minor metal ions such as Pb^{2+} , Zn^{2+} (ICP-AES) into the leaching solutions transferred from the samples of PWBs. Every three flasks were taken out every day from the air-conditional shaker and kept in a refrigerator at 4°C until at the end of the leaching time when experiments for all samples were completed. Then, each 2 ml of leaching solution was withdrawn from these flasks for determining concentrations of the soluble metal ions such as Cu^{2+} , Pb^{2+} , Zn^{2+} and the left leaching solutions in these three flasks were used for measuring pH value and redox potential. The experiment followed completely randomized block design with three times.

The concentration of total biomass was determined by counting in a Neubauer chamber with optical microscope following its classical method. Viable biomass determination was made by plate counting [33]. For chemical analysis of the concentrations of the metals of the PWBs samples or the concentrations of the copper ion Cu^{2+} of the leaching solution, 0.15 g of the PWBs samples or 2 ml of leaching solution was dissolved in 10 ml of aqua regia by refluxing in a round bottom flask for 3 h. The solution was vaporized to be nearly dry and then washed to scaled cuvette up to 20 ml of volume using distilled water. This 20 ml solution was used to determine the concentrations of dissolved metal ions by ICP-AES and the data were analyzed for calculating the concentrations of various metals in the PWBs samples or in the leaching solutions.

3. Results and discussion

Percentages of copper, lead and zinc solubilized in leaching solution with size of PWBs by the pure culture of *A. ferrooxidans*, the pure culture of *A. thiooxidans*, and the mixed culture of *A. ferrooxidans* and *A. thiooxidans* at 5 d of leaching time under 7.8 g l^{-1} of the concentration of PWBs are shown in Fig. 1. The experimental results also showed that the percentages of copper, lead and zinc solubilized in the culture medium non-inoculated under the same other experimental conditions as in Fig. 1 are all less than 2.0%. It was indicated that the pure culture of *A. ferrooxidans* and/or the mixed culture of *A. thiooxidans* and *A. thiooxidans* can not only efficiently leach the main metal copper but also leach other minor metals such as lead and zinc as well, and the pure culture of *A. thiooxidans* also has considerable solubilizing ability for these metals. Moreover, the percentage of copper, lead and zinc solubilized into the leaching solution in the three kinds of culture basically increased with decrease of sieve fraction of sample, which can be explained for the reason that the ratio area (area per volume of sam-

ple) increase with decrease of sample size and the more ratio area is favorable for the attachment of *A. ferrooxidans* and/or *A. thiooxidans* onto the metals such as copper, zinc, lead and therefore percentages of these metals solubilized by *A. ferrooxidans* and/or *A. thiooxidans* increase with the decrease of sieve fractions of sample. It can be seen that the percentages of copper, lead and zinc solubilized are all more than 88.9% for these three kinds of bacteria cultures when the sieve fractions of sample are all less than 0.35 mm, and that within all the sieve fractions, these three kinds of bacteria cultures have the solubilizing capabilities for copper, lead and zinc by the following order: the mixed culture of *A. ferrooxidans* and *A. thiooxidans* > the pure culture of *A. ferrooxidans* > the pure culture of *A. thiooxidans*. This explains that *A. ferrooxidans* and *A. thiooxidans* have both considerable leaching function for copper, lead and zinc and they have positive cooperative function for leaching the metals from PWBs when they coexist in the leaching culture. The sieve fractions of 1.0–3.0 and 0.5–1.0 mm were chosen for further experimental research.

Percentage of copper, lead and zinc solubilized in leaching solution with scrap concentration of PWBs at (a) 1.0–3.0 and (b) 0.5–1.0 mm of sieve fraction by the pure culture of *A. ferrooxidans*, the pure culture of *A. thiooxidans*, and the mixed culture of *A. ferrooxidans* and *A. thiooxidans* after 5 d of leaching time are shown in Fig. 2. The experimental results also showed that the percentages of copper, lead and zinc solubilized in the culture medium non-inoculated under the same other experimental conditions as in Fig. 2 are all less than 2.0%. It is shown that percentages of copper, lead and zinc solubilized decrease with increase of concentration of PWBs in the both cases of 1.0–3.0 and 0.5–1.0 mm of sieve fraction. Especially, when the concentration of PWBs decreases from 7.8 to 19.5 g l^{-1} , percentages of copper, lead and zinc solubilized decreased considerably for most of the experimental cases, which illustrates that the concentration of PWBs should be controlled under a reasonable range although *A. ferrooxidans* was reported to be able to tolerate high metal concentrations [34,35].

Percentage of copper solubilized in leaching solution with leaching time by the pure culture of *A. ferrooxidans*, the pure culture of *A. thiooxidans*, and the mixed culture of *A. ferrooxidans* and *A. thiooxidans* at (a) 1.0–3.0 and (b) 0.5–1.0 mm of sieve fraction under 7.8 g l^{-1} of the concentration of PWBs are shown in Fig. 3. The experimental results also showed that the percentages of copper, lead and zinc solubilized in the culture medium non-inoculated under the same other experimental conditions as in Fig. 3 are all less than 1.30%. It can be seen that at 9 d of leaching time, percentages of copper solubilized are 89.0%, 74.7% and 94.0% at 1.0–3.0 mm of sieve fraction, and percentage of copper solubilized are 99.0%, 74.9%, 99.9% at 0.5–1.0 mm of sieve fraction, for the pure culture of *A. ferrooxidans*, the pure culture of *A. thiooxidans*,

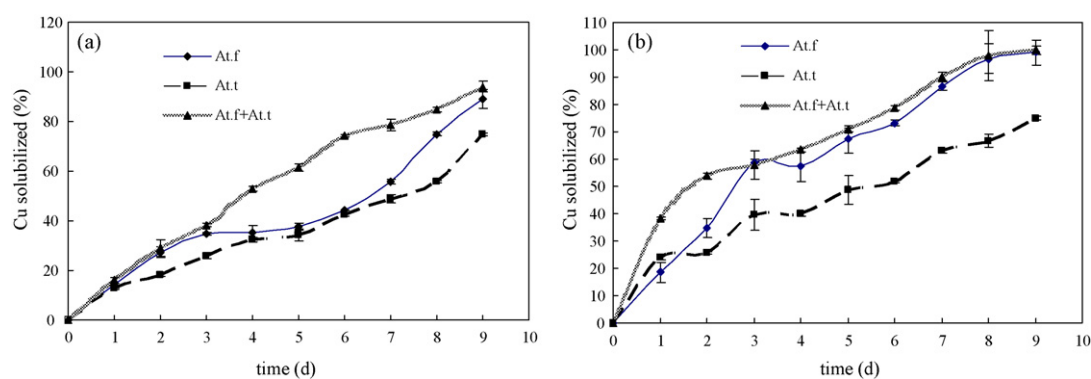


Fig. 3. Percentage of copper solubilized in leaching solution with leaching time at (a) 1.0–3.0 mm and (b) 0.5–1.0 mm of sieve fraction. The data are the average of three replicates (\pm S.D.).

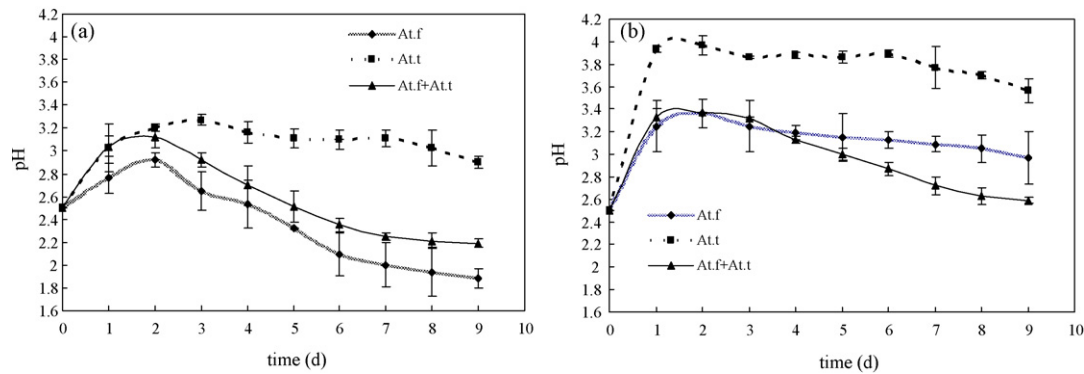


Fig. 4. Variation in pH of leaching solution with leaching time by pure culture of *A. ferrooxidans* (At.f), pure culture of *A. thiooxidans* (At.t) and mixed culture of *A. ferrooxidans* and *A. thiooxidans* (At.f+At.t) at (a) 1.0–3.0 mm and (b) 0.5–1.0 mm of sieve fraction. The data are the average of three replicates (\pm S.D.).

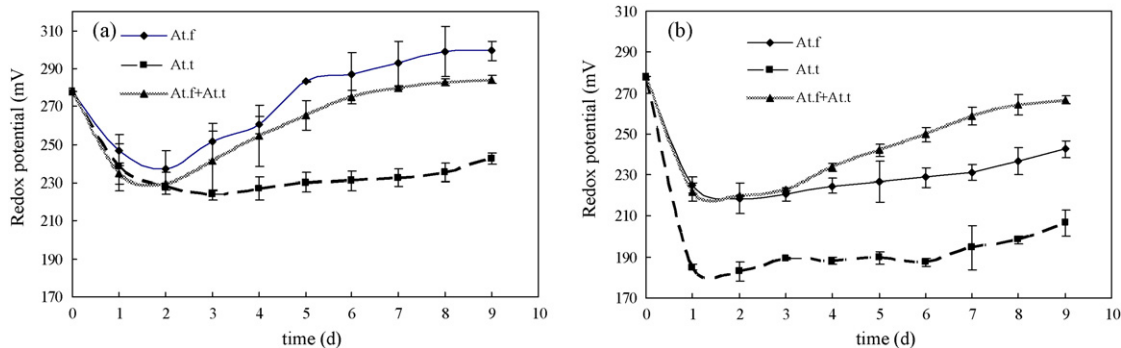


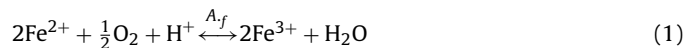
Fig. 5. Variation in redox potential of leaching solution with leaching time by pure culture of *A. ferrooxidans* (At.f), pure culture of *A. thiooxidans* (At.t) and mixed culture of *A. ferrooxidans* and *A. thiooxidans* (At.f+At.t) at (a) 1.0–3.0 mm and (b) 0.5–1.0 mm of sieve fraction. The data are the average of three replicates (\pm S.D.).

and the mixed culture of *A. ferrooxidans* and *A. thiooxidans*, respectively.

Variations in pH and variations in redox potential of leaching solution with leaching time by the pure culture of *A. ferrooxidans*, the pure culture of *A. thiooxidans* and the mixed culture of *A. ferrooxidans* and *A. thiooxidans* at (a) 1.0–3.0 mm and (b) 0.5–1.0 mm of sieve fraction are shown in Figs. 4 and 5, respectively. The experimental results also showed that the values of pH and redox potentials in the culture medium non-inoculated under the same other experimental conditions as in Figs. 4 and 5 are around 2.60 ± 0.10 and 270 ± 10 mV within 9 d of leaching time, respectively. In these two figures, the curves of the evolutions of the pH values and/or the redox potential with leaching time could be divided into two periods. In the first period, the curves of pH values ascended and the curves of redox potential dropped with increase of leaching time. In the second period, the curves of pH values dropped and the curves of redox potential ascended with increase of leaching time. For instance, at 1.0–3.0 mm of sieve fraction, the initial pH of 2.5 increased to 2.92, 3.20, 3.12 at 2 d of leaching time and then declined to 1.88, 2.90, 2.19 at 9 d of leaching time and the initial redox potential of 278 mV decreased to 237, 228, 230 mV at 2 d of leaching time and then increased to 229, 243, 284 mV at 9 d of leaching time by the pure culture of *A. ferrooxidans*, the pure culture of *A. thiooxidans* and the mixed culture of *A. ferrooxidans* and *A. thiooxidans*, respectively. At 0.5–1.0 mm of sieve fraction, the trends of variation of pH and redox potential with leaching time are similar to those at 1.0–3.0 mm of sieve fraction.

For the bioleaching mechanism of metals such as copper, lead and zinc by *A. ferrooxidans*, it could be speculated that PWBs have been reported to be alkaline in nature [28], resulting in increase of pH of the culture media. In above experiments, the first period in evolutions of pH and redox potential with leaching time was due

to the alkalinity of PWBs and the oxidation of Fe^{2+} into Fe^{3+} in the culture media as well, resulting in increase of pH and decrease of redox potential in the culture media. The oxidation reaction of Fe^{2+} into Fe^{3+} in the culture media can be expressed as Eq. (1) [36].

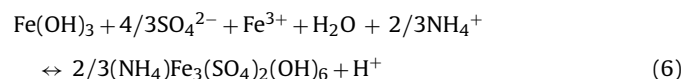


Then, Fe^{3+} , produced in the first period acted as oxidant, which oxidizes metals copper Cu^0 , lead Pb^0 , zinc Zn^0 into their corresponding metal ions Cu^{2+} , Pb^{2+} , Zn^{2+} , is transferred into Fe^{2+} as shown in Eqs. (2)–(4).



The population of bacteria also increases with the oxidation of Fe^{2+} to Fe^{3+} and the subsequent accumulation of Fe^{3+} . Curves of pH and redox potential with leaching time show that the whole process can be divided into two continuous periods when Fe^{3+} exists in the leaching systems. First, the dissolution of metals such as copper is due to an attack of PWBs by Fe^{3+} , as a result of attack, Fe^{3+} is reduced to Fe^{2+} and causes the increase of Fe^{2+} concentration in leaching solution. Then, bacteria use Fe^{2+} as an energy source, which leads to a rapid increase in bacterial population and the regeneration of Fe^{3+} upon Fe^{2+} oxidation. Therefore, a cycle between Fe^{3+} and Fe^{2+} is built and copper in PWBs is continuously leached. As leaching time increased from 2 to 9 d of leaching time when the first period ended and the second period began, hydrolysis reactions of Fe^{3+} occurred, producing H^+ as shown in Eqs. (5) and (6) [37] and then resulting in decrease of pH and increase of redox potential in the leaching solution. Therefore, in order to prevent Eqs. (5) and (6) to

happen excessively and consequently to seriously affect the process of Eq. (1), controlling the leaching solution under strongly acidic environment is very necessary and important so that the metals such as copper, lead and zinc could be mobilized from the electronic scraps smoothly.



4. Conclusions

The results from this work demonstrate that *A. ferrooxidans* and *A. thiooxidans* were able to grow in the presence of PWBs and the metals such as copper, lead and zinc can be recovered from PWBs by the pure culture of *A. ferrooxidans* and the mixed culture of *A. ferrooxidans* and *A. thiooxidans*, and the pure culture of *A. thiooxidans* also has considerable solubilizing ability for these metals. Various percentages of metals can be leached depending on the sieve fractions of experimental samples, PWBs concentration within 9 d of leaching time. Copper as the main metal of PWBs, almost complete solubilization was achieved. For other minor metals such as lead and zinc present in PWBs, considerably high percentages of metal solubilization were also achieved. These three kinds of bacteria cultures have the solubilizing capabilities for copper, lead and zinc by the following order: the mixed culture of *A. ferrooxidans* and *A. thiooxidans* > the pure culture of *A. ferrooxidans* > the pure culture of *A. thiooxidans*. The results might have the potential for industrial applications regarding the treatment of metal-containing solids such as PWBs of WEEE since *A. ferrooxidans* and *A. thiooxidans* are widely used bacteria in nature and from 5 to 9 d of bioleaching time when most of metals are solubilized into leaching solution is acceptable in industrial applications.

Acknowledgements

The financial supports for this work from the projects of “Innovation Program of Scientific Research of Shanghai Municipal Education Commission” under grant number “06RZ003” and “09YZ448”, the project of “Excellent Young Teacher Fund of Shanghai Municipal Education Commission” under grant number “egd-07008”, the project of “Chinese National Plan of High Technological Research and Development (863 Plan)” under grant number “2006AA06Z366” are gratefully appreciated.

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