Hindawi Publishing Corporation
Journal of Spectroscopy
Volume 2014, Article ID 269351, 8 pages
http://dx.doi.org/10.1155/2014/269351

Research Article

Characterization of Extreme Acidophile Bacteria (*Acidithiobacillus ferrooxidans*) Bioleaching Copper from Flexible PCB by ICP-AES

Weihua Gu,[1] Jianfeng Bai,[1] Jue Dai,[1] Chenglong Zhang,[1] Wenyi Yuan,[1] Jingwei Wang,[1] Pengcheng Wang,[2] and Xin Zhao[2]

[1] *Shanghai Cooperative Centre for WEEE Recycling, Shanghai Second Polytechnic University, Shanghai 201209, China*
[2] *China National Electric Apparatus Research Institute Co. Ltd., Guangzhou 510275, China*

Correspondence should be addressed to Jianfeng Bai; jfbai@sspu.edu.cn

Received 11 August 2014; Accepted 3 September 2014; Published 17 November 2014

Academic Editor: Wang Lin

In order to improve copper leaching efficiency from the flexible printed circuit board (PCB) by *Acidithiobacillus ferrooxidans*, it is necessary to quantitatively measure the bacteria bioleaching copper under extreme acidic condition from flexible PCB. The inductively coupled plasma-atomic emission spectroscopy (ICP-AES) is a very accurate way to analyze metals in solution; this paper investigated the optimal conditions for copper bioleaching by *Acidithiobacillus ferrooxidans* from flexible PCB through ICP-AES. The conditions included particle size of flexible PCB powder, quantity of flexible PCB powder, initial pH of culture medium, bacteria inoculation, bacteria activation time, and quantity of $FeSO_4 \cdot 7H_2O$. Prior to ICP-AES measurement, culture solution was digested by aqua regia. The experimental results demonstrated that flexible PCB contained one main metal (copper); this was associated with the structure of flexible PCB. The optimization conditions were in 50 mL medium, flexible PCB 10 g/L, particle size of flexible PCB 0.42~0.84 mm, culture medium initial pH 2.5, bacteria inoculation 5%, bacteria activation time 5 d, and quantity of $FeSO_4 \cdot 7H_2O$ 30 g/L. Under the optimization condition, the leaching rate of copper was 90.10%, which was 42.4% higher than the blank group. For the ICP-AES determination, it reached a conclusion that the best corresponding wavelength (nm) of copper will be 224.7 (nm).

1. Introduction

With the development of technology, printed circuit board (PCB) manufacturing technique has been widely improved. Traditional rigid PCB cannot be well applied in many areas because of its inflexibility, nonfolding, poor heat dissipation, and so forth. The flexible PCB, by using polyimide or polyester thin films as base material, with the advantages of high reliability and flexibility, enables the possibility of lighter, smaller, and thinner electronics (laptops, cell phones) [1]. However, if the flexible PCB were not recycled or treated appropriately, the heavy metals such as lead, copper, and nickel in flexible PCB would cause environmental pollution, which would be harmful to human [2–4]. In addition, according to its own characteristics, flexible PCB had a high metal content particularly the three metals gold, copper, and nickel. As metals resources were increasingly scarce today, waste flexible PCB were of the same importance as waste rigid PCB, which both were good "secondary resources," with high values, and recyclable.

Nowadays, physical and chemical methods were the main applications in extracting metals from rigid PCB [5–7]. Due to high consumption of energy and incapability in dealing with the depth of PCB, the physical method was not regarded as an economical way to extract metals from PCB [8]. The chemical method, whether acid or alkaline treatment, consumed large amounts of reagents and produced a lot of "three wastes," which would lead to the limitations in large-scale applications in industry [7]. In recent years, microbiological method has been of wide concern for scientists due to its low consumption of chemical reagents, energy, less environmental pollution, and so forth. The study found [9–11] that the metallurgical function microorganism contains *Acidithiobacillus*, *Aspergillus niger*, *Cyanide* bacteria, and so on, which could use their oxidation and acid soluble metabolites of chelate effect to extract zero-valent metals from discarded PCB. Particularly, *Acidithiobacillus ferrooxidans* could survive and play the role of bioleaching metals from PCB under extreme acidic condition.

The ICP-AES was a spectrum analysis method which used inductively coupled plasma moment as its excitation light source [12]. After years of development, the technology had been gradually matured. With its advantages such as rapid analysis, high accuracy, and multielement simultaneous analysis, ICP-AES had been widely applied to fields such as environment, food, medicine, and chemical industry, which need qualitative and quantitative analysis of metal cation [13].

The aim of the present work was the characterization of using *Acidithiobacillus ferrooxidans* bioleaching copper from flexible PCB by ICP-AES. Based on the results obtained by ICP-AES, series of optimizations were made to work out the best condition for bioleaching copper from the flexible PCB by *Acidithiobacillus ferrooxidans*; then the results could be the theoretical basis for its further industrial applications.

2. Materials and Methods

2.1. Source of Flexible PCB. The flexible PCB, which were used as main board of high-tech computers, were obtained from an electronics limited company in Yangzhou, Jiangsu Province, China. Since no components were assembled to the flexible PCB, they were crushed and fractionated by dry sieving directly in the laboratory. Pictures of flexible PCB before and after being crushed were shown in Figure 1.

The main metal concentration of flexible PCB was determined by ICP-AES rapidly screening at the following wavelengths (nm): copper (224.7, 324.7, and 327.3). And the particle sizes of sieve fraction in the crushed flexible PCB were 0.25~0.42 mm, 0.42~0.84 mm, and >0.84 mm, respectively. (Concentrations of metal in those three particle sizes were given in Table 1.)

2.2. Strains and Culture Medium. The isolates used in the experiments were recovered from the acidic mine drainage taken from Dexing Mine, Jiangxi Province, China. The isolates were identified as *Acidithiobacillus ferrooxidans* [14]. The acidophilic bacteria were grown in 9 k medium, with its components: $FeSO_4{\cdot}7H_2O$ 40 g, $(NH_4)_2SO_4$ 3.0 g, K_2HPO_4 0.5 g, $MgSO_4$ 0.5 g, KCL 0.1 g, $Ca(NO_3)_2$ 0.01 g, and distilled water 1000 mL, adjusted to pH value 2.5 by sulfuric acid.

2.3. Analytical Methods. During experiment, the culture solution pH was assayed by pH meter (Sartorius PB-10), and the copper in the flexible PCB was dissolved by aqua regia digestion (HCL and HNO_3, ratio 3 : 1) [14] and was analyzed by ICP-AES (A6300, Thermo, USA) [15]. To get accurate and reliable results, take the averaged measurement data and calculate the standard deviation and then plot it by origin. ICP-AES instrument parameter and the analysis of metal concentration parameter were given in Tables 2 and 3.

Table 1: Concentration of copper in three particle sizes in crushed flexible PCB.

Particle sizes (mm)	Copper
>0.84	33.4%
0.42~0.84	42.6%
0.25~0.42	39.7%

Table 2: ICP-AES instrument parameter.

Wavelength range	Radio frequency generator	Spectral band	Signal range
160–190 MHZ	40 MHZ	0.19 MHZ	6 orders of magnitude

2.4. Experiment Design. In order to get optimization conditions for copper bioleaching from the flexible PCB by *Acidithiobacillus ferrooxidans*, the dissolved metal was analyzed by ICP-AES, thus determining the optimal conditions with the results-based assay. In these experiments, on the basis of six factors, particle size of flexible PCB powder, quantity of flexible PCB powder, initial pH of culture medium, bacteria inoculation, bacteria activation time, and quantity of $FeSO_4{\cdot}7H_2O$, single factor experiment was designed. Table 4 listed the conditions of the experiment.

Experimental conditions setting: shaker (ZHWY-2112B) speed 125 r/min, temperature 28°C. Each experiment was repeated three times, and the average experimental data would be taken.

3. Results and Discussion

3.1. The Selection of Cu^{2+} Optimal Detection Wavelength. At different wavelengths, the different concentrations of standard samples intensity values were measured and linear equations were established between standard samples and intensity values in these experiments to determine the optimum detection wavelength. Different standard samples and intensity values were shown in Table 5.

According to Table 5, when the wavelength 224.7 nm was chosen, the linear equation was better than the other two, so the best detection wavelength was found (copper 224.7 nm).

3.2. Effect of Particle Size with Flexible PCB on Copper Leaching Efficiency. In experiment 1 condition, medium pH changed with time as shown in Figure 2(a). In this experiment, three different particle sizes were added, during 24 h; when the particle sizes of >0.84 mm and 0.42~0.84 mm PCB were added, the medium pH rose from initial 2.0 to near 3.5 rapidly, while in the corresponding time period the particle size of PCB was added 0.25~0.42 mm; granularity medium pH rose only to 2.5; 24–48 h, when particle size of PCB was added 0.25~0.42 mm, the medium pH increased to 3.3, while in the corresponding time period particle sizes of >0.84 mm and 0.42~0.84 mm PCB were added; the medium pH already rose above 3.5. After 48 h, the medium pH of these three different particle sizes decreased to some extent and tended to a smooth trend. Reasons for the increase of medium pH,

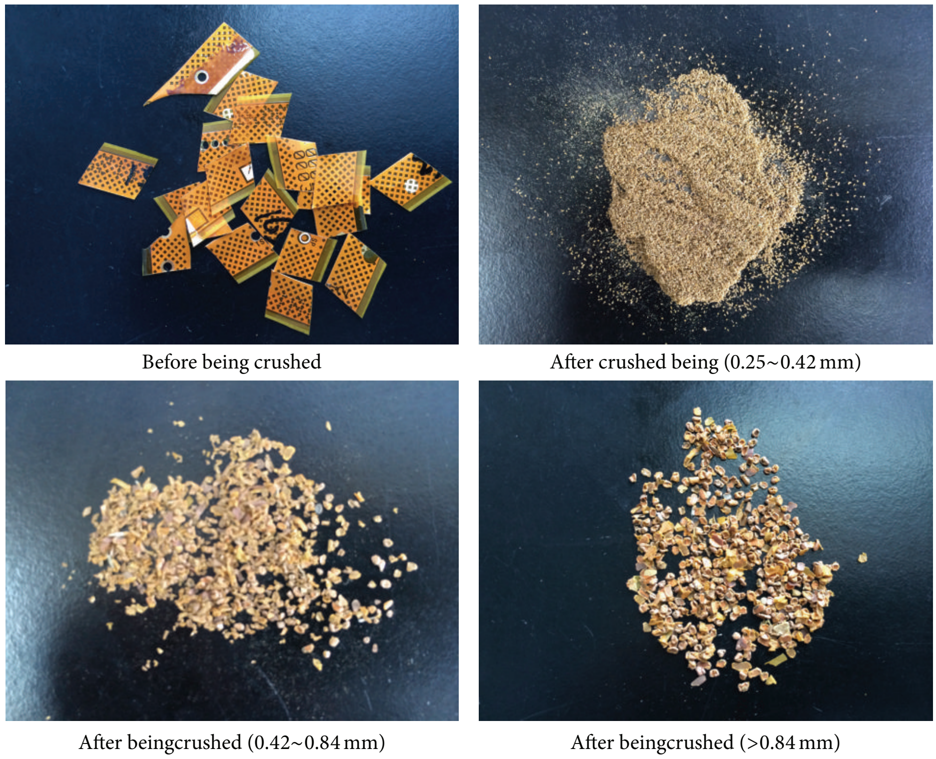

FIGURE 1: Flexible PCB before and after being crushed.

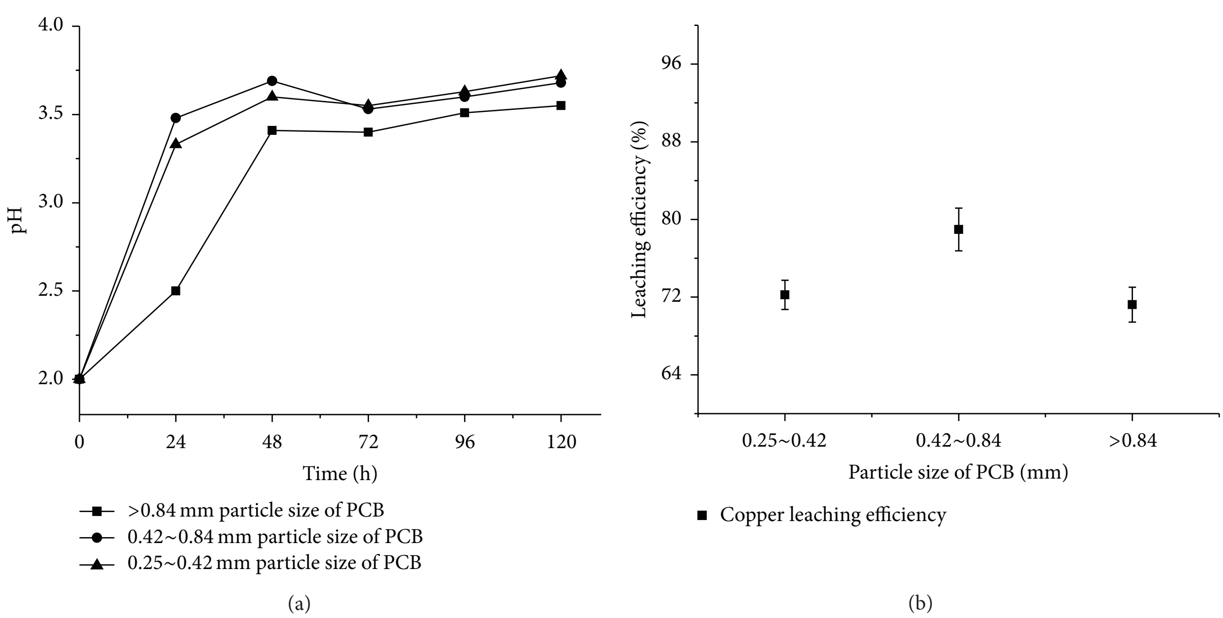

FIGURE 2: Different particle sizes of PCB and medium pH changed with time (a) and 5 days leaching and copper leaching efficiency (b).

TABLE 3: Analysis of metal concentration parameter.

Argon plasma channel	Assist the argon gas flow	Spray the argon gas flow	Peristaltic pump power rating	High pressure photomultiplier tube	Integral time
12 L/MIN	0.6 L/MIN	0.6 L/MIN	1.0 L/MIN	600 MV	150 MS

Table 4: Conditions of the experiment.

Experimental conditions	Particle size of flexible PCB (mm)	Quantity of flexible PCB (g/L)	Initial pH of culture medium	Bacteria inoculation n (%)	Bacteria activation time (d)	Quantity of $FeSO_4 \cdot 7H_2O$ (g/L)
1	0.84, 0.42, 0.25	20	2.0	10	4	40
2	0.42	10, 20, 30	2.0	10	4	40
3	0.42	20	1.5, 2.0, 2.5	10	4	40
4	0.42	20	2.0	5, 10	5	40
5	0.42	20	2.0	10	4, 5, 6	40
6	0.42	20	2.0	10	6	10, 20, 30, 40

Table 5: Standard concentrations and intensity values under different wavelengths.

Measurement wavelength (nm)	224.7		324.7		327.3	
Standard concentration (ppm)	Intensity values	Linear equation/R^2	Intensity values	Linear equation/R^2	Intensity values	Linear equation/R^2
0	227.9		763.7		934.6	
0.5	279.4	$Y = 0.0089x + 0.0194$ $R^2 = 0.9999$	994.8	$Y = 0.0020x - 0.0007$ $R^2 = 0.9996$	1066	$Y = 0.0033x + 0.0134$ $R^2 = 0.9995$
1	338.6		1273		1240	
2	451.1		1788		1546	
4	676.7		2754		2136	

on the one hand, were the oxidation of Fe^{2+} to Fe^{3+} under the effect of bacteria, with acid consumption during the process; on the other hand, PCB itself was composed of some alkaline substances. Because of the larger size of the PCB, its alkaline substances could not release immediately; when different sizes of PCB were added, the medium pH rose inconsistently. Only if the reaction proceeded to some extent, would the alkaline be released. After 48 h, the pH appeared to decline, illustrated Fe^{3+} reaction had occurred, copper was a portion of the reaction, and hydrolysis was the other portion of the reaction. After 72 h, the pH slowly rose; the possible reason would be that Fe^{3+} had been turned back to Fe^{2+} and the Fe^{2+} was oxidated to Fe^{3+} under the action of bacteria.

As shown in Figure 2(b), after 5 days' bioleaching, when the particle size was 0.42~0.84 mm, the copper leaching efficiency had reached 78.97%, which was the highest of the three particle sizes. The possible reason was that, with the particle size becoming smaller, the surface contact areas between bacteria and PCB became bigger, so the copper leaching efficiency was increased; but when PCB particle size was too small, the oxygen in the solution would be reduced and the growth of bacteria would be hindered, so leaching efficiency was reduced.

3.3. Effect of Flexible PCB Quantity on Copper Leaching Efficiency. Figure 3(a) shows that when 1.0 and 1.5 g PCB powders were added, the change trend of pH was consistent; when 0.5 g PCB powder was added, culture medium pH rose to the peak within 72 h and then leveled off and dropped. The more PCB powders the higher its culture medium pH. Possible reason was that the PCB itself has alkaline substances. The more PCB powder was added, the more alkaline substances got into the culture medium, which resulted in the solution pH getting higher.

With the quantity increasing of PCB powders added, 5 days later, the leaching rate of copper in the PCB was reducing as shown in Figure 3(b); the best PCB powder quantity was 10 g/L. The reasons for this phenomenon might be that, without the domestication, bacteria in the experiments could not tolerate high doses of PCB powder, leading to PCB powder quantity inversely proportional to the copper leaching rate.

3.4. Effect of Initial Culture Medium pH on Copper Leaching Efficiency. Whether initial pH of experimental group was 1.5, 2.0, or 2.5, as the experiment proceeded, the culture medium pH rose to the peak during 48 h and then leveled off, as shown in Figure 4(a). In conditions of initial pH being 1.5, 2.0, or 2.5, 5 days later, leaching rate difference was not significant, as shown in Figure 4(b), which showed that culture medium initial pH was not the main factor to affect the copper leaching efficiency. As the optimum growth pH of *Acidithiobacillus ferrooxidans* is 2.0~3.5, pH 2.5 was chosen as the optimum leaching pH in this experiment.

3.5. Effect of Different Bacteria Inoculation on Copper Leaching Efficiency. Figure 5(b) shows the results that when the inoculation of *Acidithiobacillus ferrooxidans* amount was at 5%, 5 days later, the copper leaching rate was 99.79%. In 0–96 h period, though different in inoculation amount, the culture medium pH changing trend was consistent over time. After 96 h, in the group with 10% inoculation amount, medium pH

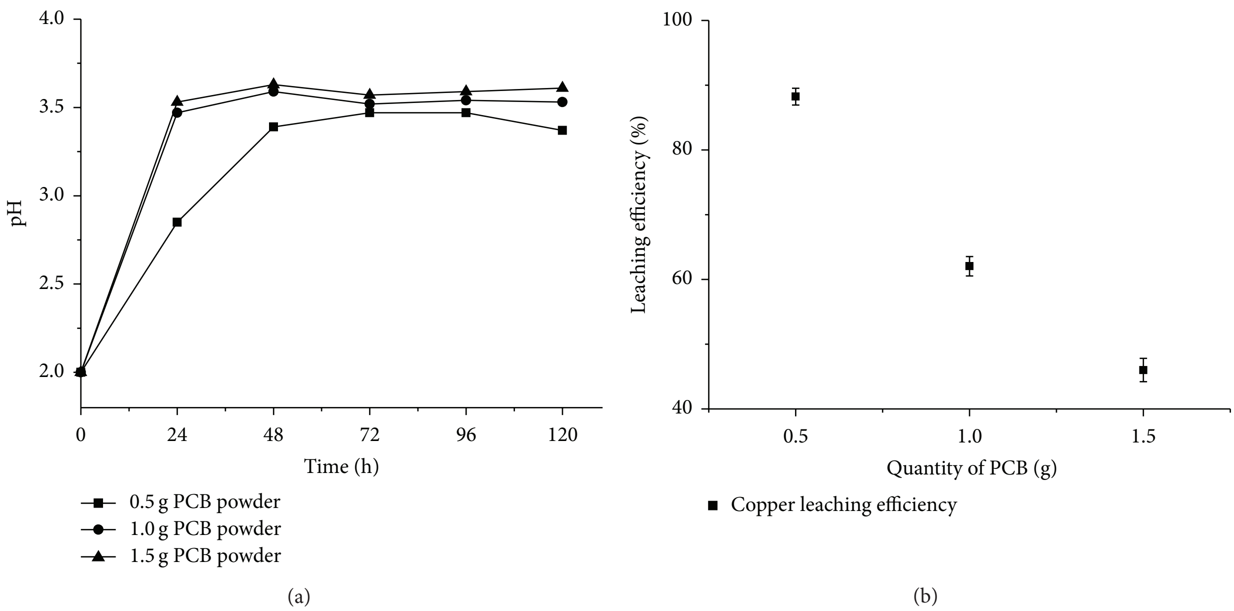

Figure 3: Different quantity of PCB and medium pH changed with time (a) and 5 days leaching and copper leaching efficiency (b).

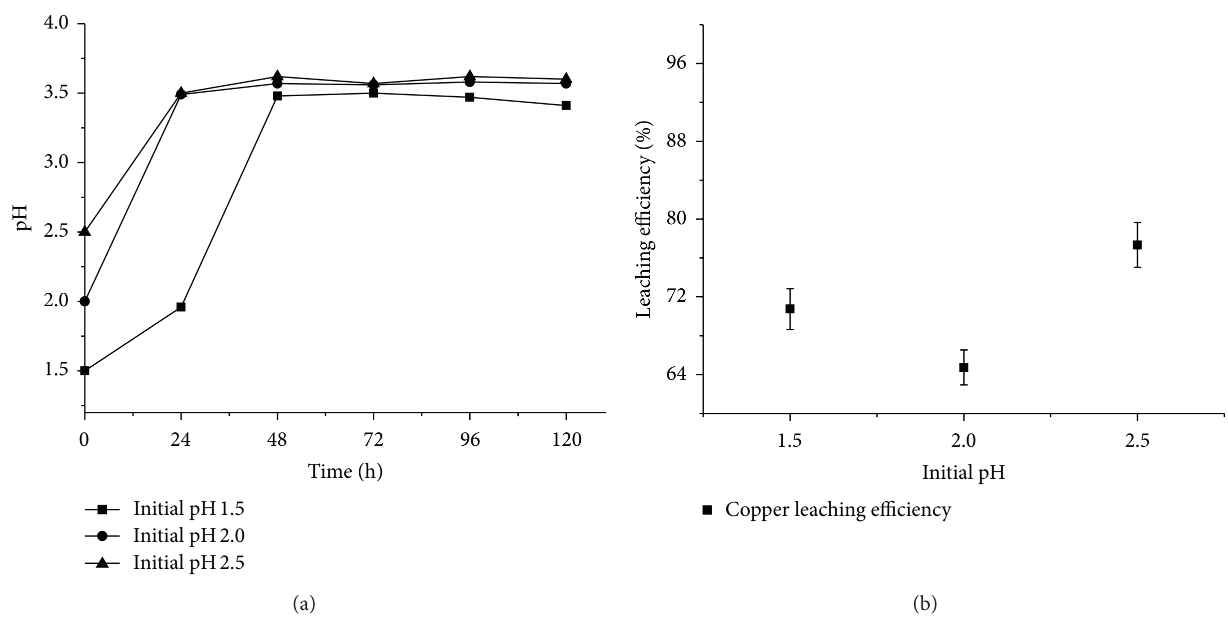

Figure 4: Different initial pH and medium pH changed with time (a) and 5 days leaching and copper leaching efficiency (b).

started to rise again; on the contrary, in the group with 5% inoculation amount, culture medium pH began to decline as shown in Figure 5(a). The possible reason was that, with the increase in amount of bacteria inoculation, bacteria were increased dramatically in the medium, which made the Fe^{2+} turn into Fe^{3+} again and caused the culture medium pH second time to rise. Five days later, the leaching rate of the group with 5% inoculation amount was higher than that with 10%.

3.6. Effect of Bacteria Activation Time on Copper Leaching Efficiency. Seen from Figure 6(a), during 96 h, were different bacteria working with different activation time; the culture medium pH changing trend was consistent over time, after 96 h, for the group of bacteria activation time being 5 d, whose culture medium pH began to rise for the second time, which was consistent with the pH change of inoculation capacity being 10% in Figure 5(a). As seen from Figure 6(b), when the bacteria activation time was 5 d, the leaching rate of

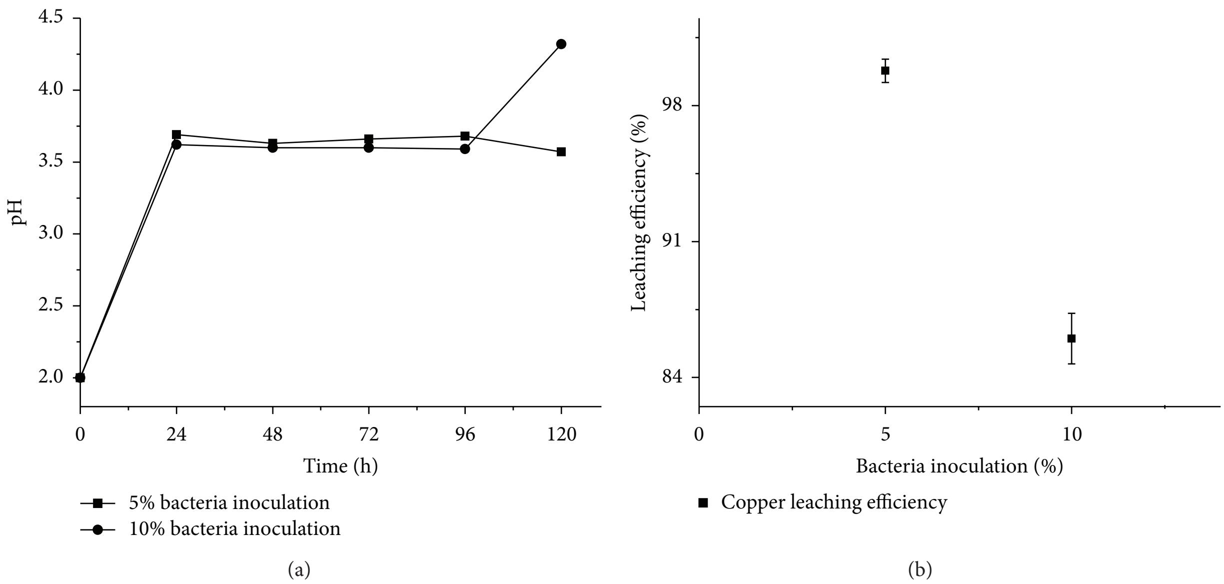

Figure 5: Different bacteria inoculation and medium pH changed with time (a) and 5 days leaching and copper leaching efficiency (b).

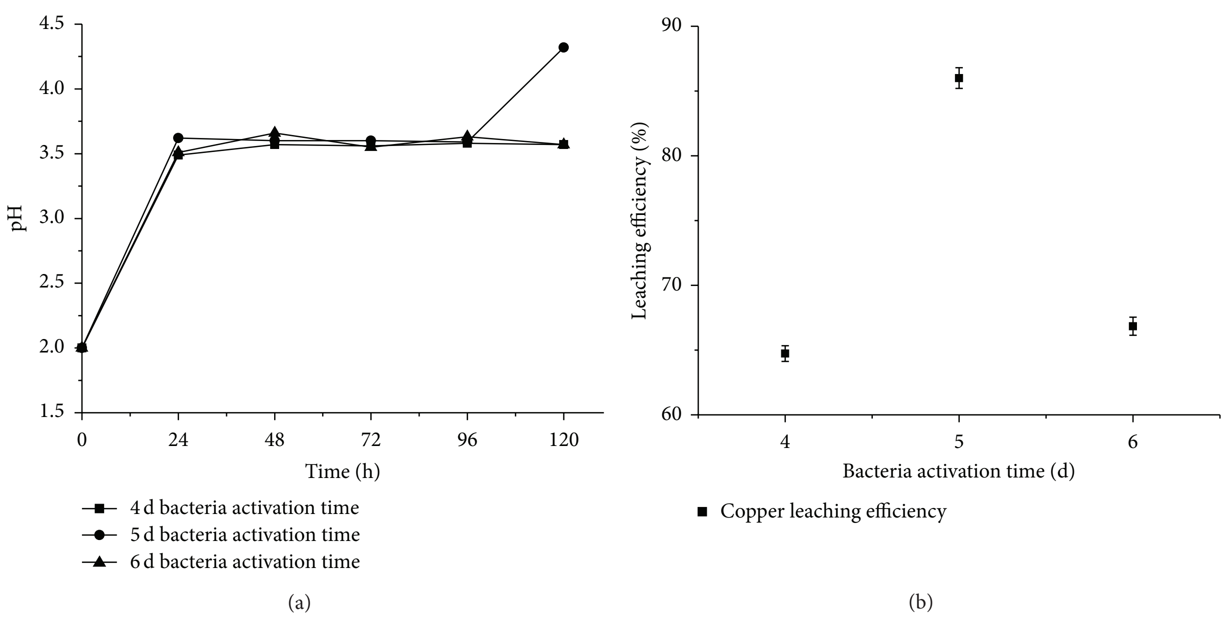

Figure 6: Different activation time and medium pH changed with time (a) and 5 days leaching and copper leaching efficiency (b).

copper was the highest, reaching 86%. Possible reason was that bacteria were just in the logarithmic phase after 5 d's bacterium activation, with the highest activity.

3.7. Effect of $FeSO_4 \cdot 7H_2O$ Quantity on Copper Leaching Efficiency. As shown in Figure 7(a), the greater amounts of Fe^{2+} was, the lower culture medium pH would be, which was generally believed that Fe^{2+} become Fe^{3+} was the process of acid consumption, and the results of the bacteria and air cooxidation. The reasons for these results might be that the *Acidithiobacillus ferrooxidans* had certain tolerance for Fe^{2+}. High levels of Fe^{2+} had a certain extent inhibiting bacteria from oxidation, which resulted in culture medium pH generally low. As Figure 7(b) shows, as the quantity of $FeSO_4 \cdot 7H_2O$ was 30 g/L, the leaching rate of copper was the highest.

4. Conclusion

(1) ICP-AES is a very accurate way to analyze metals in solution. The concentration of copper in the solution was determined by ICP-AES after bioleaching in this paper. The best wavelength (nm) was copper (224.7), which gave full play to the advantages of ICP-AES as

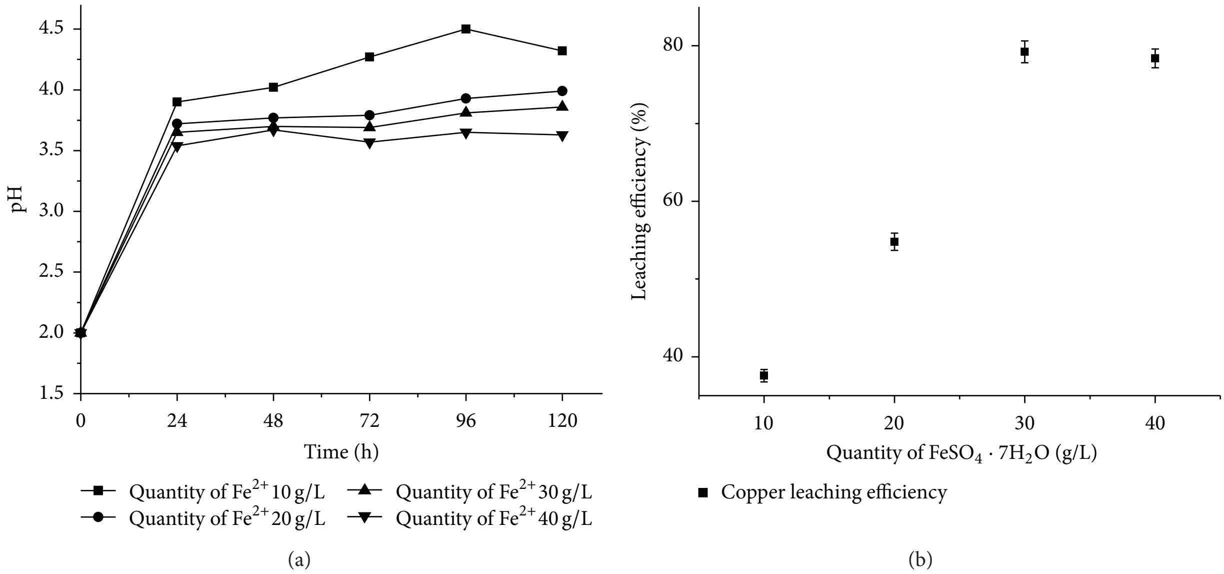

FIGURE 7: Different $FeSO_4 \cdot 7H_2O$ quantity and medium pH changed with time (a) and 5 days leaching and copper leaching efficiency (b).

simple in operation process and multielement analysis at the same time and accurate in experimental results.

(2) The results from this work demonstrated that the best leaching conditions were particle size of flexible PCB 0.42~0.84 mm, quantity of flexible PCB 10 g/L, initial culture medium pH 2.5, bacteria inoculation 5%, bacteria activation time 5 d, and quantity of $FeSO_4 \cdot H_2O$ 30 g/L.

(3) Under the best condition, the copper leaching rate can reach 90.10% after 5 days of incubation.

Disclosure

Weihua Gu is joint first author.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Weihua Gu and Jianfeng Bai contributed equally to this work.

Acknowledgments

The financial support for this work from the Project of "National High Technology Research and Development Program (2013AA040207)," "National Natural Science Foundation of China (21307080)," "Innovation Program of Scientific Research of Shanghai Municipal Education Commission (12ZZ194)," "Strategic Emerging Industry in Guangdong Province (2012A032300017)," and the key subject of Shanghai Polytechnic University (XXKYS1404) is gratefully appreciated.

References

[1] S. X. Wang, W. He, and M. Zhang, *The Principle and Process of Printed Circuit*, University of Electronic Science and Technology, 2010.

[2] J. Cui and L. Zhang, "Metallurgical recovery of metals from electronic waste: a review," *Journal of Hazardous Materials*, vol. 158, no. 2-3, pp. 228–256, 2008.

[3] R. Kahhat and E. Williams, "Product or waste? Importation and end-of-life processing of computers in Peru," *Environmental Science & Technology*, vol. 43, no. 15, pp. 6010–6016, 2009.

[4] O. Tsydenova and M. Bengtsson, "Chemical hazards associated with treatment of waste electrical and electronic equipment," *Waste Management*, vol. 31, no. 1, pp. 45–58, 2011.

[5] M. J. Noakes, "Using mineral processing technologies for the treatment of hazardous substances," *Enabling Materials Resource Sustainability*, no. 33, pp. 27–36, 1999.

[6] H. M. Veit, C. D. C. Pereira, and A. M. Bernardes, "Using mechanical processing in recycling printed wiring boards," *JOM*, vol. 54, no. 6, pp. 45–47, 2002.

[7] A. Tuncuk, V. Stazi, A. Akcil, E. Y. Yazici, and H. Deveci, "Aqueous metal recovery techniques from e-scrap: hydrometallurgy in recycling," *Minerals Engineering*, vol. 25, no. 1, pp. 28–37, 2012.

[8] K. Bosecker, "Bioleaching: metal solubilization by microorganisms," *FEMS Microbiology Reviews*, vol. 20, no. 3-4, pp. 591–604, 1997.

[9] H. Brandl, S. Lehmann, M. A. Faramarzi, and D. Martinelli, "Biomobilization of silver, gold, and platinum from solid waste materials by HCN-forming microorganisms," *Hydrometallurgy*, vol. 94, no. 1–4, pp. 14–17, 2008.

[10] R. C. Ivănuş, "Bioleaching of metals from electronic scrap by pure and mixed culture of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*," *Metalurgia International*, vol. 15, no. 4, pp. 62–70, 2010.

[11] D. Mishra, D.-J. Kim, D. E. Ralph, J.-G. Ahn, and Y.-H. Rhee, "Bioleaching of metals from spent lithium ion secondary batteries using *Acidithiobacillus ferrooxidans*," *Waste Management*, vol. 28, no. 2, pp. 333–338, 2008.

[12] A.-Y. Cheng, J.-Q. Yu, C.-L. Gao, L.-S. Zhang, and X.-H. He, "Study on trace elements of lake sediments by ICP-AES and XRF core scanning," *Spectroscopy and Spectral Analysis*, vol. 33, no. 7, pp. 1949–1952, 2013.

[13] Y. N. Zhao, Y. Han, and J. F. Wu, "Application research progresses of ICP-AES analysis," *Guangdong Trace Elements Science*, vol. 17, pp. 18–24, 2010.

[14] J. Wang, J. Bai, J. Xu, and B. Liang, "Bioleaching of metals from printed wire boards by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* and their mixture," *Journal of Hazardous Materials*, vol. 172, no. 2-3, pp. 1100–1105, 2009.

[15] Y. Q. Cui, *Study on analyses and callback of the metals in waste computer with ICP [Ph.D. thesis]*, Qingdao University, 2005.