INFLUENCE OF USED BACTERIAL CULTURE ON ZINC AND ALUMINIUM BIOLEACHING FROM PRINTED CIRCUIT BOARDS

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Abstract: Bioleaching processes were used to solubilize metals (Cu, Ni, Zn and Al) from printed circuit boards (PCBs). In this study, a PCBs-adapted pure culture of *Acidithiobacillus ferrooxidans,* pure culture of *Acidithiobacillus thiooxidans* and PCBs-adapted mixed culture of *A. ferrooxidans* and *A. thiooxidans* were used for recovery of the metals. The study showed that the mixed bacterial culture has the greatest potential to dissolve metals. The maximum metal bioleaching efficiencies were found to be 100, 92, 89 and 20% of Cu, Ni, Zn and Al, respectively. The mixed culture revealed higher bacterial stability. The main factor responsible for high metal recovery was the ability of the mixed culture to maintain the low pH during the whole process. The pure culture of *A. thiooxidans* had no significant effect on metal bioleaching from PCBs.

Keywords: bioleaching, electronic waste, zinc, aluminium, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*

1. Introduction

Waste from printed circuit boards (PCBs) belongs to the typical waste of electric and electronic equipments and their recycling has attracted a great attention not only from the perspective of waste treatment but also of valuable metal recovery. To date, PCBs waste recycling was still limited due to heterogeneity and complexity in their components. The content of most components present in PCBs is: major metals 28 % - 30 %, plastics 19 %, bromine 4 %, glass and ceramics 49 %. The content of metals can be expressed as follows: copper: 10-20 %, lead: 1-5 %, nickel: 1-3 % and precious metals like silver, platinum and gold are also present in the electronic scrap to a total of 0.3-0.4 % (ILYAS *et al.*, 2013; LIANG *et al*., 2013).

The quantity of metals turns the electronic scrap into an interesting raw material according to the economic point of view. If PCBs are not recycled or treated appropriately and consequently they are made to be dispersed to environment, the heavy metals such as copper, lead and nickel and halogenated burn – resisting materials being a part of them can lead to serious environment problems (WANG *et al*., 2009).

The use of microorganisms for metal recovery from waste electronic equipment could be an economical alternative to such processes as pyrometallurgy, hydrometallurgy or mechanical processes, since using biological techniques for

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recycling PCBs, 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 (WANG *et al*., 2009; XIANG *et al*., 2010). *Acidithiobacillus ferrooxidans* is a major participant in consortia of microorganisms used for industrial recovery of copper (bioleaching or biomining) (VALDÉS *et al*., 2008; BÁLINTOVÁ and LUPTÁKOVÁ, 2012). Initially, an acid environment was established by *Acidithiobacillus thiooxidans* through producing H_2SO_4 to dissolve PCBs fine powder (Eq. 1). Then, *A. ferrooxidans* oxidized Fe^{2+} into Fe^{3+} (Eq. 2) which can oxidize metals into corresponding ionic forms (Eq. 3). Afterwards, Fe^{2+} will be oxidized into Fe^{3+} to begin the next cycle reaction. *A. ferrooxidans* and *A. thiooxidans* can grow and utilize ferrous iron or elemental sulphur and thus produce ferric ions or sulphuric acid that are important as leaching agents of metals (ZHAO *et al*., 2008; LEE and PANDEY, 2011; LIANG *et al*., 2010; LIANG, *et al.*, 2013).

$$S^0 + 1{,}5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+ \tag{1}$$

$$2Fe^{2+} + 0{,}5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \tag{2}$$

$$M\ (Zn, Al) + Fe_2(SO_4)_3 \rightarrow MSO_4 + 2FeSO_4 \tag{3}$$

High amounts of different metals present in PCBs have a toxic effect on bacteria; however, as it was reported by BRANDL *et al*. (2001) the bacterial strains of *A. ferrooxidans* and *A. thiooxidans* can to a certain extent tolerate high metal concentrations. It is known, that metals bioleaching from PCBs closely relate to bacterial growth.

The aim of this study was to evaluate the influence of the pure cultures of the acidophilic bacteria and their mixture on copper, nickel, zinc and aluminium recovery from PCBs. Additionally, the contribution of particular bacterial strains to bioleaching mechanisms was examined.

2. Materials and methods

The isolates used in the experiments were recovered from the acid mine drainage water in Smolník, Slovakia. The pure culture of *A. ferrooxidans* and *A. thiooxidans* and their mixture, as well, were cultured and acclimated in presence of PCBs for two weeks and consequently used as the bioleaching bacteria for zinc and aluminium recovery from PCBs waste. The pure culture of *A. ferrooxidans* and *A. thiooxidans* and mixed culture of *A. ferrooxidans* and *A. thiooxidans* are designed as main influential factors in examination of percentage of zinc and aluminium solubilisation from the PCBs. The chemical composition of nutrient media needed for bacterial growths, which were also used as the bioleaching media, are shown in Table 1. The pH values of the bioleaching media were adjusted to 1.5 with 10 M H_2SO_4. The unwashed scraps of actual PCBs, which were used in our experiments, were crushed to particle size of 1 - 1 000 mm. Analysis of the electronic scrap before bioleaching revealed the presence of Zn (1.2 %) and Al (1.7 %). Metal ion concentration in the leach liquor was measured by an atomic absorption spectrophotometer (Perkin Elmer 3100).

Table 1. Chemical composition of nutrient and bioleaching media, as well ($g.l^{-1}$).

Composition	Waksman and Joffe medium (*A. thiooxidans*)	9K medium (*A. ferrooxidans*)	Mixed medium (*A. ferrooxidans* and *A. thiooxidans*)
KCl	-	0.1	0.1
$(NH_4)_2SO_4$	0.2	3.0	2.0
K_2HPO_4	3.0	0.5	0.25
$MgSO_4.7H_2O$	0.5	0.5	0.25
$Ca(NO_3)_2.4H_2O$	-	0.014	-
$CaCl_2.6H_2O$	0.25	-	-
$FeSO_4.7H_2O$	trace amount	44.2	44.2
H_2SO_4	1-5 ml	1-5 ml	1-5 ml
Sulphur powder	10	-	5

Bioleaching experiments were carried out in 250 ml Erlenmeyer flasks containing 190 ml of the nutrient media, depicted in Table 1, to which 2 g PCBs were added. Each flask was inoculated with 10 ml PCBs-adapted pure bacterial culture of *A. ferrooxidans,* PCBs-adapted pure bacterial culture of *A. thiooxidans* or mixture of *A. ferrooxidans* and *A. thiooxidans*.

3. Results and discussion

The overall Cu, Ni, Zn and Al bioleaching efficiencies using the pure culture of *A. nferrooxidans*, *A. thiooxidans,* and the mixed culture of *A. ferrooxidans* and *A. thiooxidans* are shown in Fig. 1.

As it can be seen the pure culture of *A. ferrooxidans* was the most effective in Zn and Cu dissolution, when 100 % and 85 %, respectively, were dissolved. The percentages of Ni and Al dissolved were 35 % and 33 %, respectively.

The effectiveness of the pure culture of *A. thiooxidans* was the lowest. During the whole bioleaching process only 1 %, 3 % and 17 % of Cu, Zn and Al were leached out. However, the bioleaching efficiency of Ni was 50 % that was higher than by the pure culture of *A. ferrooxidans.* Contrary to the results obtained by the pure bacterial cultures, shown in Fig. 1, it is obvious that mixed bacterial culture was the most effective. The percentages of Cu, Ni, Zn and Al bioleaching after 28 days were 100 %, 92 %, 89 % and 20%, respectively.

Compared to the results obtained during the bioleaching by the pure culture of *A. ferrooxidans* Zn and Al bioleaching efficiency was slightly lower. Positive influence of the mixed bacterial culture on metal dissolution was observed by several authors. ILYAS *et al.* (2007) observed maximum bioleachability of metals from PCBs using mixed consortium of metal – adapted culture of acidophilic heterotrophs (*Sulfobacillus thermosulfidooxidans*) and acidophilic autotrophs (*A. ferrooxidans*). In their study 81, 79 % and 83 % of Ni, Al and Zn, respectively, were leached out. One of the main advantages of using heterotrophs in above mentioned mixed culture was the crossfeeding. The heterotrophs use extracellular metabolites from autotrophs as a carbon source enhancing the stability of the mixed bacterial culture (FOURNIER *et al.*, 1998).

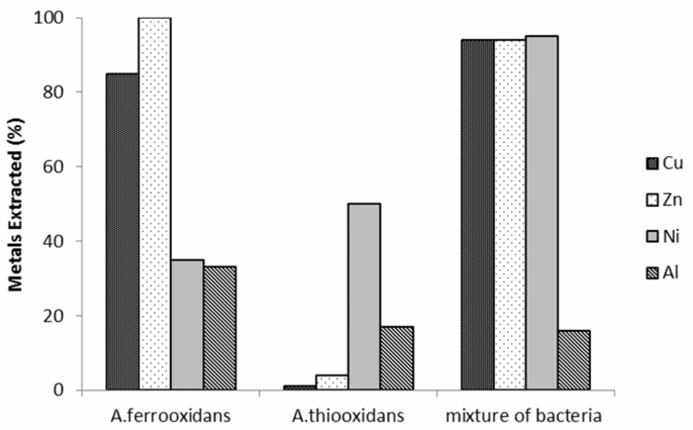

Fig. 1. Percentages of copper, nickel, zinc and aluminium solubilized from PCBs by the pure culture of *A. ferrooxidans, A. thiooxidans* and mixed culture of *A. ferrooxidans* and *A. thiooxidans*.

The presence of *A. thiooxidans* in mixed culture obviously enhanced the bioleaching process. Although the main leaching bacteria (especially in the case of Cu and Zn recovery) were probably *A. ferrooxidans* the positive effect of *A. thiooxidans* was in the maintaining of the low pH values in media. Many authors agreed that acid environment and strong oxidants are needed for dissolution of metals present in PCBs (LIANG *et al.*, 2010). Thus, the maximal metal recovery could be achieved if these two strains were cultured together to generate H_2SO_4 and Fe^{3+} simultaneously. In the presence of *A. ferrooxidans* ferrous ions are oxidized into ferric ions (Eq. 2) which as the strong oxidation agents consequently oxidized metals from M^0 into $M^{2+\,(3+)}$ form (Eq. 3). The oxidation of Fe^{2+} into Fe^{3+} and metals dissolution resulted in a rapid increase of pH from the initial pH = 1.5 up to value of 2.2 observed in the first ten days of the bioleaching processes (Fig. 2). On the following days the pH remained stable until the end of the experimental period.

Based on the fact that the pH remained stable since day 10 up to the end of the bioleaching processes, it might be assumed that there was a balance between the simultaneous consumption of protons (Eq. 2) and release of protons during the, bacterial sulphur oxidation (Eq. 1), hydrolysis of Fe^{3+} ions (Eq. 4, 5, 6) and jarosite formation (Eq. 7).

$$Fe^{3+} + H_2O \leftrightarrow FeOH^{2+} + H^+ \tag{4}$$

$$Fe^{3+} + 2H_2O \leftrightarrow Fe(OH)_2^- + H^+ \tag{5}$$

$$Fe^{3+} + 2H_2O \leftrightarrow Fe(OH)_2^- + H^+ \tag{6}$$

$$3Fe^{3+} + 6H_2O + M^+ + 2HSO_4 \rightarrow MFe_3(SO_4)_2(OH)_6 \; + 8H^+ \tag{7}$$

As it was reported by DAOUD and KARAMANEV (2006) the presence of jarosite has negative effect on the metal dissolution due to the kinetic barrier formation which inhibits the diffusion of reactants and products through the precipitation zone.

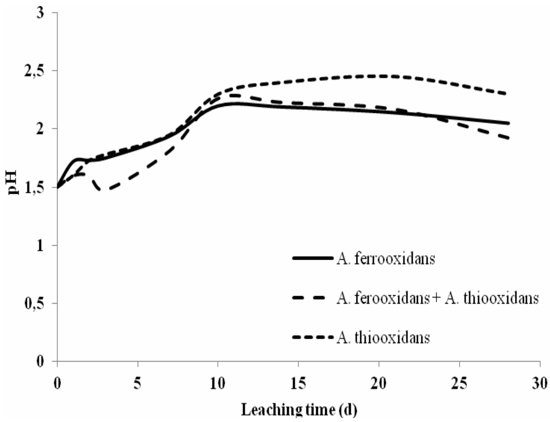

Fig. 2. Changes in pH during bioleaching studies.

The presence of jarosite was confirmed by the X-ray diffraction analysis in the studied system (Fig. 3).The maintenance of low pH values by the mixed bacterial culture delayed the jarosite formation significantly resulting in the higher recovery of metals in comparison with the pure *A. ferrooxidans* culture utilization.

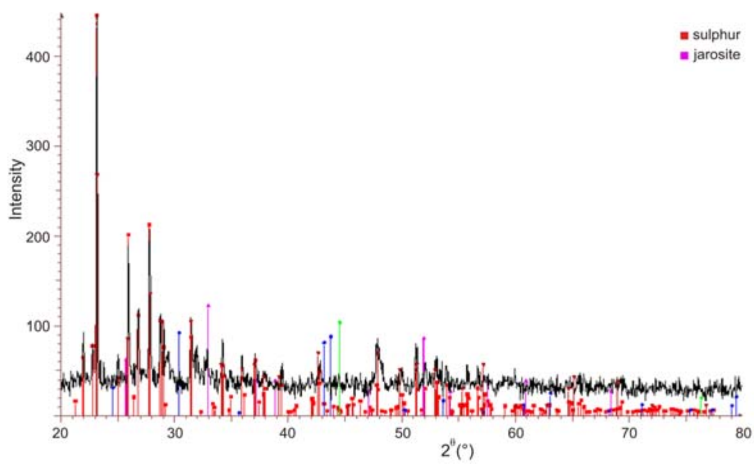

Fig. 3. X – ray diffraction of PCBs residuum after bioleaching process.

The maintenance of low pH values by the mixed bacterial culture delayed the jarosite formation significantly resulting in the higher recovery of metals in comparison with the pure *A. ferrooxidans* culture utilization.

4. Conclusion

The main purpose of this work was to investigate the influence of the three kinds of acidophilic bacterial cultures: the pure culture of *A. ferrooxidans,* of *A. thiooxidans* and mixed culture of *A. ferrooxidans* and *A. thiooxidans* on copper, nickel, zinc and aluminium solubilisation from PCBs. The results from this study demonstrate that mixed culture of *A. ferrooxidans* and *A. thiooxidans* had the highest metals bioleaching capacity. The mixed consortium was able to dissolve 100 %, 92 %, 89 % and 20 % of Cu, Ni, Zn and Al, respectively. The main advantage of the mixed bacterial culture was the ability of the bacteria to maintain the low pH values resulting in higher metal bioleaching efficiencies. The experimental results also showed that the pure culture of *A. ferrooxidans* was the most effective in Zn (100 %) and Cu (85 %) dissolution. The pure culture of *A. thiooxidans* was not efficient.

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