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# Bioleaching and Kinetic Investigation of WPCBs by *A. Ferrooxidans*, *A. Thiooxidans* and their Mixtures

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## Abstract

Bioleaching was used to mobilize Cu, Zn and Ni from waste printed circuit boards (WPCBs) and eliminate hazardous metal species from these wastes. Pulp density (PD) and medium culture are two effective factors which have been optimized in this paper. The bacteria *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) and *Acidithiobacillus thiooxidans* (*A. thiooxidans*) and their mixture were grown and adapted in the presence of WPCBs and then used as bioleaching bacteria to solubilize metals from PCBs. The experimental results demonstrated that 15 g/L WPCB is the best solid concentration which can be tolerated by the bacteria. Comparing different inoculation ratios, Cu (86%), Zn (100%) and Ni (100%) were recovered after 25 days of bioleaching, which suggests that the rate of metal recovery is significantly influenced by PD. Kinetics of bioleaching reactions was investigated in this work and the shrinking core model (SCM) was used to describe the kinetics of the process of no pretreated WPCBs. A constrained multi-linear regression analysis using the least square technique was employed to determine the rate controlling mechanism in each operating condition. Based on the results, diffusion through solid product layer was the major controlling mechanism.

## Keywords

Bioleaching;  
Copper;  
Electronic scrap;  
Kinetics;  
Nickel;  
Zinc.

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## 1. Introduction

Electrical and electronic equipment (EEE) have been developed rapidly and their average life spans reduced due to changes in functions and designs. Personal computer (PC) board scraps are a type of waste electrical and electronic equipment (WEEE) that contain large amounts of precious metals such as gold, silver and palladium [1, 2]. Removing hazardous metal species by recycling of WEEE is an important subject, not only from the point of view of hazardous waste treatment but also from the recovery aspect of valuable materials. Some researchers have reviewed the differences in the e-waste amounts generated in each country [3]. WEEE is also the fastest growing waste category which emphasizes the need for their efficient recycling strategies [4]. Many countries have drafted legislation to improve the reuse, recycling and other forms of recovery of such wastes in order to reduce the disposal. However, conventional disposal methods of WEEE, such as landfill, incineration and hydrometallurgical recycling techniques, generate soil and water pollutants and harmful substances [5]. Thus, recycling of the WEEE is yet quite limited due to the heterogeneity of materials present in the product and the complexity of producing EEE [6].

Printed circuit boards (PCBs) are important parts of the electronic equipment and their composition is quite varied, containing polymers, ceramics and metals. The metal content of PCBs is around 28% (Cu: 10–20%, Pb: 1–5%, Ni: 1–3%) [7]. The quantity of metals, especially Cu, turns the electronic scrap into an interesting raw material from economic point of view. If waste printed circuit boards (WPCBs) are not recycled or treated appropriately (thus, dispersed in environment), their heavy metals such as Cu, Pb and Ni and the halogenated burn-resisting materials can cause serious environment problems [6].

Hydro- and pyro-metallurgical procedures are traditional metallurgical techniques which are commonly used to recover metals from electronic wastes [8]. However, pyro-metallurgical treatment raises concerns regarding formation of brominated and chlorinated di-benzo furans and dioxins in the burning process, thus, leading to serious atmospheric pollution [9, 10]. Hydrometallurgical processes are also associated with risks of environmental impacts owing to toxicity of re-

agents used and large amounts of by-products being generated [11]. Consequently, to meet environmental regulations, eco-friendly and energy-saving processes are urgently needed to recycle metals from WEEE.

Bioleaching is now emerging as a commercially exploitable technology applicable for metal extraction from low grade ores [12, 13]. This process is a promising technology which does not need large capital investment, labor need and energy consumption [14]. In the recent years, many investigations have been carried out on recovery of metals from WEEE [15, 16]. It was reported that some species of microorganisms show the potential for metal recovery [17–22]. Acidophilic bacteria are commonly used in the recovery of heavy metals from industrial wastes, among which are *A. ferrooxidans* and *A. thiooxidans* are currently used strains [23–25]. Undoubtedly, using microorganisms to leach metals from WEEE would be a promising alternative in comparison to the traditional methods.

Metal dissolution by the bacteria mainly depends on direct and indirect oxidation mechanisms. For *A. ferrooxidans* the main mechanism is indirect where metals are dissolved through the prior oxidation of ferrous ions [22, 26]. In the case of *A. thiooxidans*, conversion of metals into soluble state is mostly done by sulfuric acid production according to the following reaction [27]:



The hydrolytic reaction of  $Fe^{3+}$  may also be inhibited in acid condition [15, 22, 28].

Several parameters play role in bioleaching of WPCBs by *A. ferrooxidans* and *A. thiooxidans*. Zhu et al. [29] and Liang et al. [22] investigated the effect of initial pH, S and  $Fe^{2+}$  in different *A. ferrooxidans* to *A. thiooxidans* ratios on bioleaching of metals from WPCBs. At the optimum condition using only *A. ferrooxidans*, (initial pH of 2.00, initial  $Fe^{2+}$  concentration of 12 g/L, 60–80 mesh particle size, 12 g/L WPCB and 10% inoculation) Zhu et al. [29] reached 96.8% Cu, 88.2% Al and 91.6% Zn recoveries in 98 h. Similarly, Liang et al. [22] reported that at 28.8 g/L WPCBs, more than 90% of Cu was dissolved at optimum condition of initial inoculum ratio (*A. ferrooxidans* to *A. thiooxidans*) of 1:2, pH of 1.56,  $FeSO_4 \cdot 7H_2O$  concen-

tration of 16.88 g/L and S concentration of 5.44 g/L in 10 days. In bioleaching by these two strains, Bas et al. [8] employed pyrite ( $\text{FeS}_2$ ) instead of adding S and  $\text{Fe}^{2+}$  and managed to recover 84% Cu in 115 h (after heat treatment and removal of solder).

Using 80 days adapted *A. ferrooxidans* and plastic free PCBs (by soaking at salt water), Arshadi and Mousavi [30] leached entire Cu and Ni at initial pH of 3,  $\text{Fe}^{3+}$  concentration of 8.4 g/L, PD of 20 g/L and particle size of 95  $\mu\text{m}$ . In another report [31], using the same microorganism, they were able to recover 100% of Cu and Ni from mobile phone PCBs at the optimum condition (initial pH of 1,  $\text{Fe}^{3+}$  concentration of 4.18 g/L, PD of 8.5 g/L and particle size of 114.02  $\mu\text{m}$ ). Isildar et al. [32] leached 98.4% of Cu by a mixture of *A. ferrivorans* and *A. thiooxidans* at pH of 1.0–1.6 and 10 g/L WPCB in 7 days.

It can be concluded from above information that in the previous studies, the focus has been on optimization of bacterial bioleaching of metals from pretreated WPCBs (heating and soaking to remove solder and plastics) using *A. ferrooxidans* and *A. thiooxidans* and no kinetics investigation was performed. Therefore, the aim of this study was to investigate the effect of *A. ferrooxidans* and *A. thiooxidans* inoculation ratios on recovery of metals and understanding the kinetics of bioleaching of WPCBs. To minimize any exceeding preparation step, no treatment was performed on WPCBs. Furthermore, due to the influence of the inoculation ratios of these two bacteria on generation of sulfuric acid and  $\text{Fe}^{3+}$  ions in the medium as well as their different leaching behavior in presence of different metals, adapted *A. ferrooxidans* and *A. thiooxidans* were evaluated as potential microorganism for efficient leaching of Cu, Zn and Ni.

## 2. Experimental

### 2.1. Electronic scraps

Electronic scraps used in this study were collected from PCB scraps. No physical or mechanical separation process was used before transportation to the laboratory. The scraps were crushed and sieved to less than 100  $\mu\text{m}$  (the most suitable size, based on the previous researches [30, 31]). In order to determine its composition, the crushed scrap was digested in an aqua regia solu-

tion composed of nitric acid (Merck, 67% pure), hydrochloric acid (Merck, 37% pure) with the volume ratio of  $3\text{HCl}/1\text{HNO}_3$ . The solution after digestion was analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES, VISTA-PRO, VARIAN, Australia) and the results demonstrated that the main metallic part of WPCB contains 10935 (mg/kg) Cu, 384.96 (mg/kg) Zn, 190.77 (mg/kg) Ni, 963.14 (mg/kg) Pb, 696.07 (mg/kg) Fe and 1056.10 (mg/kg) Al.

### 2.2. Microorganisms and growth medium conditions

Mesophilic *A. ferrooxidans* and *A. thiooxidans* strains (PTCC 1646 and PTCC 1692) collected from the Microorganisms Collection Research Center (MCRC) at University of Tehran were chosen for bioleaching. The use of autotrophic *Thiobacilli* is advantageous because no organic carbon source is needed for its growth and also its ability for ferrous ion oxidation to ferric as a powerful oxidizing agent in a non-contact mechanism [34, 35]. *A. ferrooxidans* and *A. thiooxidans* strains as autotrophic bacteria were cultivated under sterile conditions in baffled 500 mL Erlenmeyer flasks and incubated at 30 °C on a rotary shaker at 180 rpm. The strains were grown in four media with different chemical compositions presented in Table 1.

**Table 1.** Chemical composition of different culture media

| Media Composition (g)                     | 9k    | 119   | 123  | MIX   |
|---|-------|-------|------|-------|
| $(\text{NH}_4)_2\text{SO}_4$              | 3.00  | -     | 2.00 | 4.00  |
| KCl                                       | 0.10  | -     | 0.10 | 0.10  |
| $\text{K}_2\text{HPO}_4$                  | 0.50  | -     | 0.10 | 0.50  |
| $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ | 0.50  | -     | 0.25 | 0.50  |
| $\text{Ca}(\text{NO}_3)_2$                | 0.01  | -     | -    | 0.01  |
| $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ | 44.22 | -     | -    | 22.10 |
| S   | -     | 10.00 | 5.00 | 5.00  |
| $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ | -     | 0.14  | -    | 0.13  |
| $\text{KH}_2\text{PO}_4$                  | -     | 3.00  | -    | -     |
| $\text{NH}_4\text{Cl}$                    | -     | 0.10  | -    | -     |
| $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ | -     | -     | 0.10 | -     |

It is worth noting that 123 and 119 solutions contained *A. thiooxidans*, 9k had *A. ferrooxidans* bacteria and the MIX media (50:50) included both types simultaneously. One liter of each medium was prepared for experiments and its pH was adjusted to 2.5 by adding sulfuric acid. Growth of

bacteria was monitored by measuring pH, redox potential and cell counts by means of Neubauer counting chamber.

### 2.3. Adaptation and bioleaching

In order to reduce toxic effect of scraps composition on the microorganisms, a prolonged adaptation process was performed. Bacteria adaptation was done by serial bioleaching stages until the PD reached 20 g powder per 1 L medium. Adaptation was initiated by adding 0.1 g of powder to 100 mL of culture medium and after 15 days, the adapted bacteria was inoculated into a fresh medium with a higher scrap concentration. The adaptation period was chosen by controlling the pH and Eh of the medium. Serial passage was continued till a PD of 20 g/L was reached. Bacterial resistance was monitored at different PDs by measuring pH, Eh, cell counts and measuring recovery of metals. In higher PDs, the bacterial count did not reach to the acceptable value and decreased to near zero. Thus, a PD of 15 g/L and the adapted bacteria were chosen for bioleaching experiments.

Bacterial leaching was carried out in two steps. In the first stage, the bacteria were added to 100 mL of culture media without scrap and kept at 30°C in an incubator shaker for 8 days with rotation speed of 180 rpm. Eh and pH of media were measured during these 8 days. In the second stage, the scrap was added at a specific solid to liquid ratio and every 2 days a sample was taken from the solution by pipet, filtered and sent for metal analysis by ICP.

## 3. Results and Discussion

### 3.1. Metal solubilization by bacteria

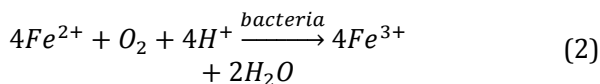
Biological activity consumes sulfur and produces sulfuric acid which consequently leads to a significant pH reduction in the media after a while. Considering the indirect leaching mechanism of microorganisms, the required energy is supplied by the oxidation of  $\text{Fe}^{2+}$  into  $\text{Fe}^{3+}$ . Knowing the fact that the change in the oxidation-reduction potential is proportional with  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  ions ratio [35], it can be concluded that in the period between days 12 and 16, the increase in the ferric ion concentration leads to growth of Eh.

Bacterial growth, measured as pH decrease of culture of different bacterial systems on different PDs, is shown in Fig. 1. It can be seen that addition of scrap has led to increase of initial pH in the first days of biological leaching. This is the result of toxicity and alkalinity properties of WPCBs which results in decrease in the number of bacteria. Afterwards, due to the biological activity, the acid is produced which leads to decrease of pH of the media. It means that after the lag phase resulted from the presence of WPCBs in the growth medium, the bacteria started their growth phase, produced acid and metabolites and adapted to the media. Therefore, the pH of the medium began to reduce after the lag phase.

Fig. 2 demonstrates variation of Eh versus time in different media and PDs. This figure shows that the organisms grow well at PDs of less than 15 g/L. Figs. 1 and 2 show that presence of scrap in higher PDs has led to an increase in the pH range of medium growth and also decreased the oxidation potential of bacterial systems. It can be seen in Fig. 2 that in the first 3 days of bioleaching, Eh increases slowly and after diminishing the toxicity of the media, when the bacteria have become more adopted to the growth in the culture medium, Eh increases faster. The figures also demonstrate that the highest pH reduction and Eh increase occur between days 15 and 18 (when the highest biological activity occurs). Also, it can be seen that the medium with greater amount of Fe (from either Fe exists in WPCB or in different medium) has higher Eh value however, the differences are sometimes small (because of low content of Fe ions).

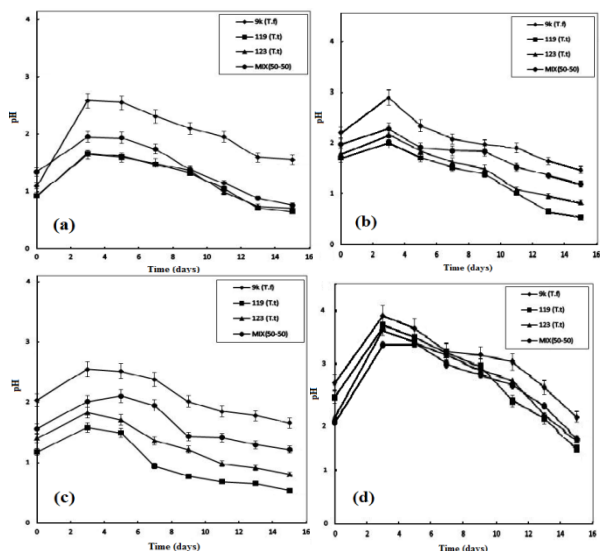
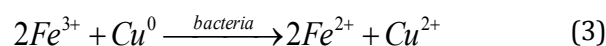
Trends of Eh and pH versus leaching time given in Figs. 1 and 2 show an increase in pH and also a decrease in Eh during the first days of bioleaching, following by decrease in pH and increase trend in Eh. This trend suggests existence of two stages in the process in the presence of  $\text{Fe}^{3+}$ . In the first stage (the first 3 days of bioleaching), leaching of Cu occurs due to the attack of WPCBs by  $\text{Fe}^{3+}$  ions which eventually transforms into  $\text{Fe}^{2+}$  leading to the increase of concentration of this ion in the solution. In the second stage (after the 3<sup>rd</sup> day),  $\text{Fe}^{2+}$  ions are consumed by the bacteria as an energy source and produce  $\text{Fe}^{3+}$  and these two stages continue simultaneously. It should be noted that, based on the ICP analysis, small quantity of Fe existed in WPCBs which can be influential on the aforementioned cycle. Oxida-

tion of  $Fe^{2+}$  into  $Fe^{3+}$  can be expressed as below [28]:



It can be concluded from Fig. 1 that the activity of *A. ferrooxidans* bacteria in transforming sulfur to acid sulfuric has a slower rate (reflected in lower pH drop of 9k media) in comparison with other *A. thiooxidans* media. The dominant mechanism of leaching of Cu for this type of microorganism is indirect oxidation of Cu through the prior oxidation of ferrous ions by *A. ferrooxidans*. In addition, sharper drop of pH in both 119 and 123 medium suggests higher rate of sulfuric acid production from sulfur in the presence of 1:1 *A. ferrooxidans* and *A. thiooxidans* than 9k media.

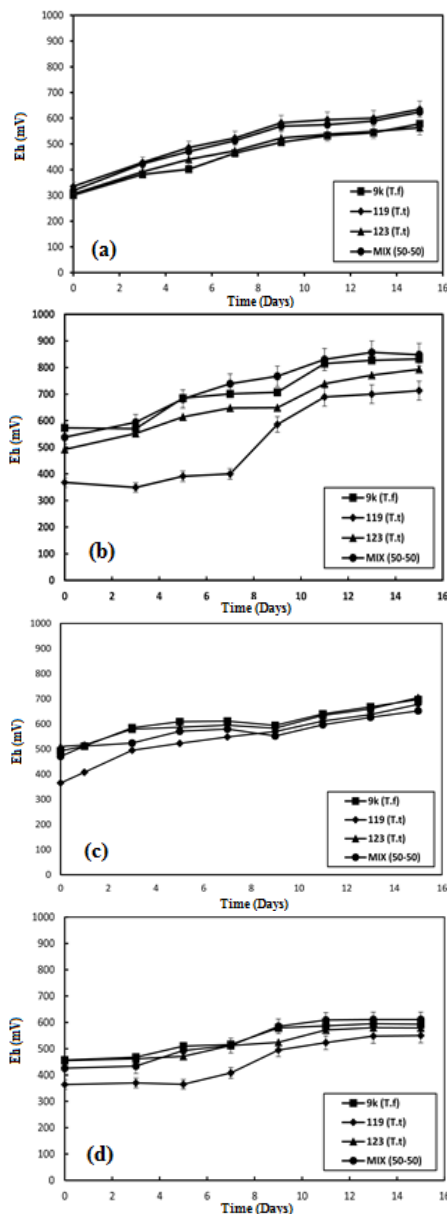
Cu can be dissolved as in the presence of acid and  $Fe^{3+}$  [28]:



**Figure 1.** pH versus time in the leaching process with different media for PDs of (a) 5, (b) 10, (c) 15 (d) 20 g/L.

While  $Fe^{2+}$  ion oxidation by *A. ferrooxidans* bacteria is faster than its acid production, *A. thiooxidans* bacteria shows a balance between producing ferric ions and acid [22, 26]. An acidic environment is conducive to the growth of bacteria and helps converting metals into soluble state by means of sulfuric acid produced by *A. thiooxidans* and  $Fe^{3+}$  ions produced by *A. ferrooxidans*. There-

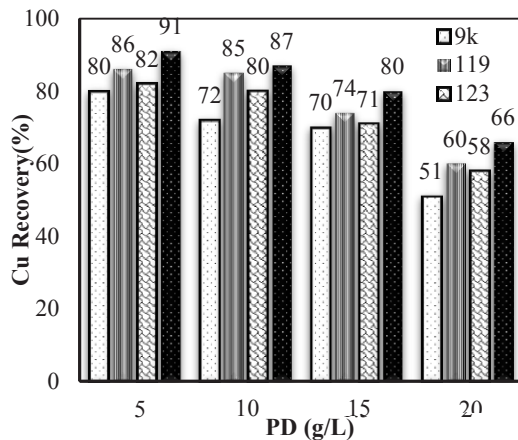
fore, a mixed culture of both bacteria seems to be a better culture for dissolving Cu.



**Figure 2.** Eh values versus time of leaching process at different media for PDs of (a) 5, (b) 10, (c) 15 (d) 20 g/L.

Fig. 3 shows the Cu recovery in a two-step bi-leaching process at different PDs in different bacterial systems. It can be seen in this figure that Cu recovery decreases with increasing the amount of scrap in the culture medium after 15 days of bioleaching because of the toxicity and alkalinity of the scrap. At a higher concentration of scrap (20 g/L), recovery of Cu decreases sharp-

ly, due to bacterial inability to adapt. Referring to Figs. 1 and 2, higher pH and lower Eh values are expected at higher PDs where lower biological activity as well as acid and ferric ions production are seen. This can be attributed to higher concentration of metal species presented in the WPCBs composition, leaching of them can be harmful for bacterial activity and also the alkaline nature of scrap which results in decrease in bacterial growth. Therefore, it can be concluded from Fig. 3 that higher Cu recovery can be obtained at lower PDs which can be confirmed by the results shown in Figs. 1 and 2. Therefore, highest recovery of Cu (91%) can be attained in the lowest S/L ratio of 0.5 and in the MIX medium containing both bacteria.



**Figure 3.** Cu recovery at different PDs in different bacterial systems.

Simultaneous presence of *A. thiooxidans* and *A. ferrooxidans* bacteria in the MIX medium results in a higher Cu recovery compared to other media since production of sulfuric acid and oxidation of  $Fe^{2+}$  ions at the same time increase the leaching efficiency. In order to investigate the effect of each bacterium in the MIX medium, three different bioleaching tests with different amount of *A. thiooxidans* and *A. ferrooxidans* in the culture medium (70:30, 30:70 and 50:50; counted under optical microscope using Neubauer chamber) were conducted. Fig. 4 shows the recovery of Cu, Zn and Ni in the MIX medium with different amount of *A. thiooxidans* and *A. ferrooxidans* inoculation. Fig. 4-a shows that recovery of Cu is 86% after 25 days of bioleaching in the system with equal amounts of each bacteria inoculation. The

results also show that the percentages of Cu solubilized in the culture medium with different amount of each bacteria inoculation are all less than 70%. As mentioned before, bioleaching of Cu depends on presence of both oxidizing ( $Fe^{3+}$ ) and acidic leaching medium ( $H_2SO_4$ ) simultaneously provided by the bacteria (regarding to reactions 1 and 2).

Fig. 4-b shows the effect of MIX bacteria system with different inoculation ratio on the bioleaching recovery of Zn. This figure shows that after 17 days of bioleaching, 100% of Zn was recovered in the bacteria system with inoculation ratio (At:Af) of 70:30. This can be related to the Zn leaching process which highly depends on the presence of acidic solution produced by *A. thiooxidans* while in the other two systems, Zn recovery is less than the system with higher amount of *A. thiooxidans*. A higher ratio of *A. thiooxidans* in the MIX bacteria system with inoculation ratio (At:Af) of 70:30 results in a higher amount of sulfuric acid which dissolves a higher amount of Zn as follows:

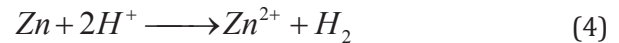


Fig. 4-c presents bioleaching of Ni in the MIX bacteria system with different inoculation ratio of *A. thiooxidans* and *A. ferrooxidans*. It is shown in Fig. 4-c that Ni recovery becomes complete after 12 days of bioleaching in the presence of both *A. thiooxidans* and *A. ferrooxidans* with equal ratio. In the first step, *A. thiooxidans* convert  $S^0$  into sulfuric acid and decrease pH of the medium. Therefore, oxidation of  $Fe^{2+}$  to  $Fe^{3+}$  ions by *A. ferrooxidans* becomes possible. Presence of  $Fe^{3+}$  in the bacterial system results in oxidation of  $Ni^0$  and transferring it into solution as  $Ni^{2+}$  ions according to the following reaction:



### 3.2. Bioleaching Kinetics

It was shown in Fig. 4 that the recovery of metals increases during the bioleaching period with different rates which means that there may be different kinetics mechanisms for controlling the bioleaching process. Shrinking core model (SCM) can be used to study the kinetics of bioleaching process since it was observed that the size of scrap particles remains approximately constant during bioleaching. The constant particle size

during the process may be attributed to the fact that the metallic components of the WPCB were mostly covered with resins and jarosite is usually formed during bioleaching of WPCBs using bacteria, they can act as a barrier similar to the ash product [28, 36]. Therefore, any leaching mechanism which is governed by diffusion through ash layer is suspected to be in relation with this covering resin and the probable jarosite phase. There are three possible controlling mechanisms in the SCM which are given below [24]:

Liquid film diffusion control:

$$\frac{t}{\tau_F} = X \tag{6}$$

where

$$\tau_F = \frac{\rho_B R_0}{3bk_l C} \tag{7}$$

Solid product layer diffusion control:

$$\frac{t}{\tau_P} = 1 - 3(1 - X)^{\frac{2}{3}} + 2(1 - X) \tag{8}$$

where

$$\tau_P = \frac{\rho_B R_0^2}{6bD_0 C} \tag{9}$$

Chemical reaction control:

$$\frac{t}{\tau_R} = 1 - (1 - X)^{\frac{1}{3}} \tag{10}$$

where

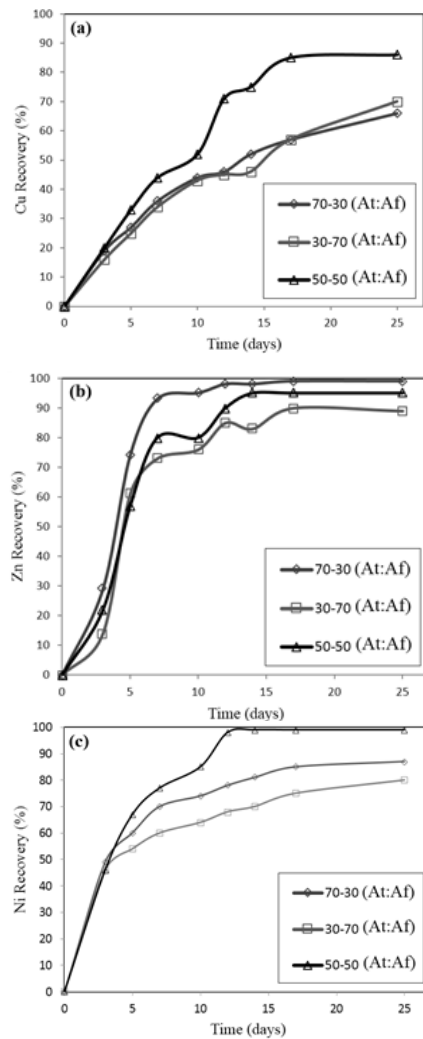
$$\tau_R = \frac{\rho_B R_0}{bk_s C} \tag{11}$$

where,  $\tau$  is the time for complete conversion of the reactant particle to product,  $t$  is the time of recovery and  $X$  is the metal recovery. Where  $\rho_s$  is density ( $\text{kg/m}^3$ ),  $R_0$  is initial radius of particle (m),  $k_l$  is mass transfer coefficient of the liquid film ( $\text{m}^3 \text{ liquid}/(\text{m}^2 \text{ surface.s})$ ),  $C_{ab}$  is concentration of sulfuric acid ( $\text{kg/m}^3$ ),  $D_e$  is effective diffusion coefficient in porous structures ( $\text{m}^3 \text{ liquid} / (\text{m}^2 \text{ surface.s})$ ),  $K_s$  is reaction rate constant at the particle surface ( $\text{s}^{-1}$ ).

In order to determine the overall rate of the leaching processes when none of the above mechanisms is the rate controlling step, all these three steps can be taken into account for describ-

ing the overall kinetics of leaching by the SCM [37]:

$$t = \tau_F X + \tau_P \left[ 1 - 3(1 - X)^{\frac{2}{3}} + 2(1 - X) \right] + \tau_R \left[ 1 - (1 - X)^{\frac{1}{3}} \right] \tag{12}$$



**Figure 4.** Effect of *A. thiooxidans* and *A. ferrooxidans* inoculation ratio on recovery of (a) Cu, (b) Zn, (c) Ni during 25 days of bioleaching at PD of 15 g/L.

Share of each of the above mentioned steps in the kinetics of leaching can be revealed by fitting the experimental data to Eq. (12) and evaluating the constants of this equation. For this purpose, the method proposed by Nazemi et al. [38] was used in this study. Employing this multi-linear regression analysis based on the least squares technique not only eliminates the need for extra experiments and calculations but also will lead to

determine the share of each controlling mechanism, if more than one. Furthermore, difficulty in the recognition of the rate controlling step in a case of close correlation coefficients will be removed when testing different controlling mechanisms [38-40]. Table 2 presents the SCM parameters (dissolution times) calculated by fitting the experimental data shown in Fig. 4 to Eq. (12) by minimizing  $\phi$  in the equation bellow ( $\tau_F$ ,  $\tau_P$  and  $\tau_R > 0$ ).

$$\phi = \sum_i \left[ \tau_F X + \tau_P [1 - 3(1 - X)^{\frac{2}{3}} + 2(1 - X)] + \tau_R \left[ 1 - (1 - X)^{\frac{1}{2}} \right] - t_i \right]^2 \quad (13)$$

Regarding to Table 2 the fastest bioleaching of Cu is at the inoculation ratio (At:Af) 50:50. Also, in the bacteria systems with inoculation ratio (At:Af) 70:30 and 50:50, diffusion through the product layer and chemical reaction are both effective mechanisms for Cu bioleaching. In the system with inoculation ratio (At:Af) 70:30 the amount of *A. ferrooxidans* is lesser than in other systems which can be result in a decrease in the amount of  $Fe^{3+}$  ions. Since oxidation of elemental Cu to ion needs the presence of  $Fe^{3+}$  as an oxidant, diffusion of  $Fe^{3+}$  ions through the product layer to reach the surface of unreacted Cu will be the slowest step and can control the leaching rate. On the other hand, in the system with inoculation ratio (At:Af) 30:70 the amount of  $Fe^{3+}$  ions are sufficient for the biooxidation reaction in the favorable direction. However, the rate of surface oxidation is bigger than the rate of  $Fe^{3+}$  ions diffusion through the product layer and so the diffusion steps would be the most limiting step. In the system with inoculation ratio (At:Af) 50:50,  $Fe^{3+}$  ions produced by *A. ferrooxidans* can diffuse through the liquid media and the product layer as the presence of sulfuric acid produced by *A. thiooxidans* can facilitated the transfer of ferric ions. However, the chemical reaction rate is lower than the rate of diffusion and so is the limiting step.

Referring to Zn data in Table 2, its bioleaching becomes faster when At:Af ratio increases. As it was mentioned in reaction (4), dissolution of Zn needs the presence of acidic medium which can be provided by higher proportion of *A. thiooxidans* by producing sulfuric acid. Higher At:Af ratio results in higher production of  $H^+$  compared to other two bacterial ratios and thus higher Zn leaching. It can be seen in Table 2 that in inoculation ratio (At:Af) 30:70, diffusion through the

product layer is the controlling mechanism as the chemical reaction will consume the produced sulfuric acid rapidly. However, the amount of acid produced is not as much as in the other two systems and the diffusion of this acid through the product layer is limiting step. On the other hand, in the bacterial systems with inoculation ratios (At:Af) 50:50 and 70:30, dissolution by chemical reaction on the surface of un-reacted core and diffusion through solid product layer are both effective mechanisms in the Zn bioleaching rate. In fact, the sulfuric acid produced in the presence of *A. thiooxidans* diffuses through the solid product layer to reach the unreacted core and converts  $Zn^0$  to  $Zn^{2+}$ . Then,  $Zn^{2+}$  ions diffuse through the product layer to reach the liquid. Of course, Table 2 suggests that at inoculation ratios (At:Af) 50:50 and 70:30, diffusion through the solid product layer is faster than the chemical reaction and this is more evident at the inoculation ratio (At:Af) 50:50.

**Table 2.** SCM parameters obtained by fitting experimental data to Eq. (12) for Cu, Zn and Ni bioleaching

| Metal | $\tau_F$ | $\tau_P$ | $\tau_R$ | R <sup>2</sup> | Culture medium (At:Af) |
|-------|----------|----------|----------|----------------|------------------------|
| Cu    | 0        | 81.92    | 21.44    | 0.9977         | 70:30                  |
|       | 0        | 8.94     | 34.08    | 0.9608         | 50:50                  |
|       | 15.31    | 56.44    | 0        | 0.9946         | 30:70                  |
| Zn    | 0        | 3.39     | 12.50    | 0.9553         | 70:30                  |
|       | 0        | 0.92     | 20.78    | 0.9777         | 50:50                  |
|       | 0        | 33.80    | 0        | 0.9118         | 30:70                  |
| Ni    | 0        | 38.96    | 0        | 0.9532         | 70:30                  |
|       | 5.07     | 9.71     | 0        | 0.9897         | 50:50                  |
|       | 0        | 55.70    | 0        | 0.9541         | 30:70                  |

The parameters calculated for Ni in Table 2 demonstrate that bioleaching of Ni at (At:Af) 50:50 is the fastest. The leaching of Ni depends on its oxidation by means of  $Fe^{3+}$  as the oxidant agents; however, this reaction is not the controlling step in any of the bacterial systems which means that this reaction occurs considerably faster than diffusion of  $Fe^{3+}$  ions through the solid product layer. At the inoculation ratio (At:Af) 50:50 the mass transfer through the liquid film is also effective in the dissolution rate of Ni which suggests that in this condition diffusion through the product layer has become such fast that it is comparable with the mass transfer through the liquid film. In the system with inoculation ration (At:Af) 70:30 the amount of ferric ions produced



by the *A. ferrooxidans* is lower and diffusion of these ions can decrease the rate of Ni dissolution. In the present work, the important role of product layer in limiting the leaching progression can be explained by the persistence of resin cover on the metallic component of the non-pretreated WPCBs.

#### 4. Conclusion

In this study, for removing the hazardous metal species, *A. thiooxidans* and *A. ferrooxidans* and their mixed culture were used to investigate the Cu, Zn and Ni recovery from non-pretreated WPCBs. In order to reduce the toxic effect of scraps composition on the microorganisms, a prolonged adaptation process was performed and a PD of 15 g/L was considered in the bioleaching experiments. Metals recovery was higher in the MIX medium than the other media and different inoculation ratios were used to investigate the effect of each bacterium. Extraction of Cu was 86 % after 25 days of bioleaching in the MIX medium and Ni and Zn were completely recovered during this period. Employing a multi-linear regression analysis based on the least squares technique, the kinetics of bioleaching was investigated to determine the mechanism of the process. In this study, diffusion through the solid layer was found to be the major controlling mechanism. It was suggested that stability of the covering resins around the metallic components in WPCBs is the main reason for this trend.

#### Nomenclature

|                |   |
|----------------|---|
| b              | stoichiometric coefficient  |
| C              | acid concentration (kg/m <sup>3</sup> )   |
| D <sub>0</sub> | diffusion coefficient of sulfuric acid (m <sup>2</sup> /s)                                      |
| k <sub>l</sub> | mass transfer coefficient of the liquid film (m <sup>3</sup> liquid/(m <sup>2</sup> surface.s)) |
| k <sub>s</sub> | reaction rate constant (s <sup>-1</sup> )   |
| R <sup>2</sup> | coefficient of regression   |
| R <sub>0</sub> | initial radius of particle (m)  |
| X              | conversion  |

#### Greek letters

|                |   |
|----------------|---|
| ρ <sub>B</sub> | density of solid (kg/m <sup>3</sup> )                                   |
| τ <sub>F</sub> | time of complete dissolution in liquid film control mechanism (s)       |
| τ <sub>P</sub> | time of complete dissolution in product diffusion control mechanism (s) |
| τ <sub>R</sub> | time of complete dissolution in chemical reaction control mechanism (s) |

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