Article

Characteristics of Metals Leached from Waste Printed Circuit Boards Using Acidithiobacillus ferrooxidans

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Abstract: The aim of this study was to compare leaching characteristics of metals from printed circuit boards (PCBs), taken from waste electrical and electronic equipment in the presence and in the absence of the iron-oxidizing bacteria, Acidithiobacillus ferrooxidans. A. ferrooxidans not only increases the leached concentration of Cu from the PCBs, but also inhibits the components of the 0K medium and leached Cu from forming precipitates such as libethenite (Cu₂(PO₄)(OH)), thereby assisting Cu recovery from the PCBs. In addition, the leached concentration of Pb from PCBs decreased in the presence of A. ferrooxidans, due to Pb forming amorphous precipitates. It is expected that Pb is not highly toxic to A. ferrooxidans. Consequently, A. ferrooxidans can be used as a cost-effective and environmentally friendly way to leach out valuable metals from PCBs as low-grade urban ore.

Keywords: urban mining; bioleaching; waste electrical and electronic equipment; printed circuit boards; valuable metals recovery; Acidithiobacillus ferrooxidans

1. Introduction

Urban mining is the process whereby the widely distributed small quantities of metal resources available in industrial raw materials, in the form of products or waste, can be used as if from a natural mine [1]. In other words, it is the recycling of metals contained in products, as an industrial resource, through the collection, disassembly, crushing, grinding, separating, smelting, and refining of waste.

Due to the rapid development of electrical, electronic, information, and communications industries, a considerable number of new electrical and electronic products is manufactured and sold annually [2]. As the purchasing power of consumers has gradually increased and consumer preferences have changed, new product release cycles are becoming faster. As a result, the replacement cycle of electrical and electronic products has shortened, and consequently the amount of waste electrical and electronic equipment (WEEE) is rapidly increasing. The quantity of WEEE generated in 2014 worldwide was around 41,900,000 tons [3]. According to the data published by USEPA, the amount of electrical and electronic products, including televisions, printers, scanners, and fax machines discarded by enterprises and individual consumers in 2009 in the US was 2,370,000 tons [4]. Only 25% of the total discarded electrical and electronic products were recycled, and the rest were landfilled [4].

Electrical and electronic products perform their functions by conducting the appropriate electrical signals through a conductive pattern on an electrical insulator surface, which is referred to as a printed circuit board (PCB). PCBs are known to contain metals in a very wide range of concentrations. Ilyas et al. [5] reported that PCBs consist of 28–30% metal, of which 10–20% is copper, 1–5% lead, 1–3% nickel, and 0.3–0.4% trace metals such as silver, platinum, and gold [5]. Yang et al. [6] also reported that PCBs obtained from waste computer have 25.5% copper, 6.18% zinc, 6.32% aluminum, 3.17% iron, 2.29% lead, and 3.31% tin. Obviously, the quantity of metals varies widely depending on the source and type of PCBs, but the recyclability of PCBs is low due to the high content of impurities.
such as plastics and other insoluble compounds [5,6]. It is necessary to disassemble, crush, and separate PCBs to recover these valuable metals, a process which can be expensive and time-consuming. Evidently, there is a need for a more effective technique to recover valuable metals from low-grade urban ore such as PCBs, which could be used to relieve the worldwide shortage of metal resources.

To recover valuable metals from low-purity natural or urban ore, the ore is usually dissolved in an acidic solution; this method requires the use of large amounts of acid, resulting in a high cost and adverse effects on the ecosystem. If the element can be separated from the ore using the metabolism of bacteria, the process can be both cost-effective and environmentally friendly. Such a recovery method is called bioleaching. Bosecker [7] has demonstrated the commercial exploitation of bioleaching to recover valuable metals from low-grade ores with 0.5% (w/w) metallic content [7]. Typical metals recoverable through bioleaching include copper, uranium, and gold. In the 1970s, 200 tons of copper was produced daily from the 2.6 billion tons of dump through bioleaching at the Kennecott Copper Corporation located in Bingham, Utah, USA, which accounted for 25% of total copper production in the USA at that time [7]. For gold leaching, the bioleaching process, which is a low-cost and low-energy method, is preferred to the conventional cyanidation process [7].

In this study, experiments on leaching metals from PCBs were performed using iron-oxidizing bacteria to develop a technology to effectively recover valuable metals from poorly recyclable WEEE PCBs. Acidithiobacillus ferrooxidans is an iron and sulfur-oxidizing bacteria, mainly used for the industrial recovery of copper by bioleaching or biomining [8]. A. ferrooxidans uses Fe$^{2+}$ as an electron donor and carbon dioxide as a carbon source, producing Fe$^{3+}$ under acidic conditions [8]. The produced Fe$^{3+}$ acts as a strong oxidizing agent, leaching metals in the ore [8]. Therefore, the ability of A. ferrooxidans to oxidize iron in PCBs, along with the resulting leaching behavior of each element, was evaluated. Since the purpose of this study is to recover valuable metals from PCBs, the addition of metals through culture medium was excluded. Recently, Yang et al. [6] observed that the mixture of 4.5K medium (i.e., the same as 9K medium but containing half as much FeSO$_4 \cdot 7$H$_2$O) and acid with A. ferrooxidans effectively recovered copper (96.8%) from PCBs in waste computers. Hubau et al. [9] used double-stage continuous bioreactors with an acidophilic consortium comprising Leptospirillum ferriphilum and Sulfobacillus benefaciens to leach out metals from PCBs, and achieved high dissolution yields including 96% copper and 73% nickel when iron only was added from the comminuted PCBs. To our knowledge, few studies have used PCBs themselves as a sole iron source with A. ferrooxidans, one of the most widely used bacteria for bioleaching experiments. In brief, the goals were to confirm whether A. ferrooxidans can be grown using only iron contained in PCBs, and whether valuable metals can be leached from PCBs through the metabolic pathways of A. ferrooxidans.

2. Materials and Methods

2.1. Microorganisms and Cultivation

A. ferrooxidans (KCTC4516) was obtained from the Korean Collection for Type Cultures (KCTC). One liter of 9K medium [10], composed of 3 g of (NH$_4$)$_2$SO$_4$, 0.1 g of KCl, 0.5 g of K$_2$HPO$_4$, 0.5 g of MgSO$_4 \cdot 7$H$_2$O, 0.0144 g of Ca(NO$_3$)$_2 \cdot 4$H$_2$O, 1 mL of 10 N H$_2$SO$_4$, and 44.22 g of FeSO$_4 \cdot 7$H$_2$O, was added to a 1 L glass bottle. A. ferrooxidans was inoculated and cultured under oxic conditions in a shaking incubator (30 °C, 180 rpm). Five milliliters of the sample was collected once a day for a week. The oxidation-reduction potential (ORP) of the collected samples was measured using the Orion five-star portable meter (Thermo Fisher Scientific, Waltham, MA, USA) connected with the ORP probe (9678BNWP). The Fe$^{2+}$ concentration of the collected samples was measured with the Ferrozine method described by Viollier et al. [11]. The growth rate of A. ferrooxidans was studied by counting through the microscope (Axio imager A1, Carl Zeiss, Oberkochen, Germany) using the Petroff-Hauser method.
2.2. Leaching Experiments

PCBs were obtained from a waste computer and crushed to pieces <1 cm². The PCB fragments were washed in deionized (DI) water and oven-dried. Five grams of PCB fragments were placed in a 50 mL conical tube, along with 50 mL DI water, 0K medium (i.e., the same as 9K medium but without FeSO₄·7H₂O), and 0K medium inoculated with 1 mL of A. ferrooxidans concentrate (i.e., grown in 9K medium for 7 d, and estimated to have a concentration of A. ferrooxidans of about 7 × 10⁷ cells/mL, as presented in Figure 1a. Hence, the final biomass concentration contacted with PCBs, calculated at about 1.4 × 10⁶ cells/mL, was added. All tests were repeated three times. During the reaction at 30 °C in the shaking incubator, the concentrations of the metals dissolved were evaluated every day for seven days. After collecting the sample, the solution containing PCBs (DI water, 0K medium, 0K medium + A. ferrooxidans) was filtered using a decompression pump and 0.2 µm acetate nitrate membrane filter (Whatman™, Cytiva, Marlborough, MA, USA). The concentration of metals (i.e., Fe, Cu, Ni, and Pb) in the filtered solution was measured with an inductively coupled plasma-optical emission spectrometer (ICP-OES, ICAP 7400 DUO, Thermo Fisher Scientific). The detection limits for Fe, Cu, Ni, and Pb were 0.007 mg/L, 0.005 mg/L, 0.0134 mg/L, and 0.065 mg/L, respectively. The precipitates remaining on the filter were characterized with an X-ray diffraction spectrometer (XRD) (D8 ADVANCE with DAVINCI, Bruker, Billerica, MA, USA), and a high-resolution scanning electron microscope (HR-SEM)(SU8220, Hitachi, Tokyo, Japan) with an energy-dispersive X-ray spectrometer (EDS). The XRD was performed under the conditions of 40 kV and 40 mA, and the results were obtained under a 2theta range of 5° to 90°, a step of 0.02, and a scan speed of 0.5 s/step. The HR-SEM-EDS analysis was carried out on the Pt-coated samples under 5 kV voltage.

3. Results and Discussion

3.1. Growth of A. ferrooxidans and Changes in ORP and Fe²⁺ Concentrations

A. ferrooxidans was inoculated into the 9K medium [10] with 9000 mg/L of Fe²⁺, and the consumption of Fe²⁺ by its oxidation (Figure 1b) with the growth of A. ferrooxidans (Figure 1a) was studied, along with the corresponding increase in oxidation-reduction potential (Figure 1c).

As shown in Figure 1a, at the exponential phase (days 5–10), bacteria rapidly grew and vigorously oxidized Fe²⁺ to Fe³⁺ (Fe²⁺ + 1/4O₂ + H₂O → Fe³⁺ + 1/2H₂O) in the 9K medium. Between days 5 to 10, a considerable amount of Fe²⁺ in the solution disappeared (Figure 1b), and the ORP of the solution reached 800 mV, matching the phenomenon at which it reaches the maximum point (Figure 1c). As A. ferrooxidans used in this study grew well in the iron-containing medium, oxidized the iron, and increased the ORP, the
study also investigated how well this oxidizing bacteria grows in iron present in PCBs, and whether it can additionally dissolve valuable metals.

3.2. Characteristics of Metals Leached from PCBs

Figure 2 shows the concentration of metals leached from the PCBs with different reaction times. Cu was hardly leached in the PCBs mixed with DI water (Figure 2a). When 0K medium was added, approximately 41 mg/L and 413 mg/L were leached on day 1 and day 7 of the reaction, respectively, showing a tenfold increase in the leached amount with increasing reaction time. Meanwhile, when the solution of 0K medium and \textit{A. ferrooxidans} was injected, only 18 mg/L of Cu dissolved on day 1 while 1047 mg/L of Cu was dissolved on day 7. This suggests that using \textit{A. ferrooxidans} could accelerate the recovery of Cu from the PCB. Yang et al. [12] confirmed that \( \text{Fe}^{3+} \) formed by \textit{A. ferrooxidans} oxidizes the elemental Cu contained in PCBs to \( \text{Cu}^{2+} \) through the following reaction (\( \text{Cu}_0 + 2\text{Fe}^{3+} \rightarrow 2\text{Fe}^{2+} + \text{Cu}^{2+} \)). This reaction has \(-82.90 \text{ kJ/mol}\) of Gibbs free energy, indicating that it can take place spontaneously. However, in their study [12], \( \text{Fe}^{3+} \) was additionally injected through an external stock culture, whereas in this study, Fe, which is contained in the PCBs, was used without additional injection of Fe. Becci et al. [13] conducted a kinetic experiment on leaching Cu from PCBs using \( \text{Fe}^{3+} \) as an oxidant, and reported that the Cu recoverable from PCBs was highly dependent on \( \text{Fe}^{3+} \) concentration. Overall, the presence of \textit{A. ferrooxidans} can produce \( \text{Fe}^{3+} \) from PCBs through its metabolic pathway, and \( \text{Fe}^{3+} \) plays an important role in oxidizing elemental Cu into \( \text{Cu}^{2+} \).

![Figure 2](image-url)

**Figure 2.** Concentrations of (a) Cu, (b) Fe, (c) Ni, and (d) Pb leached from printed circuit boards (PCBs) for 7 d in the absence (○ deionized (DI) water, ▽ 0K medium) and presence of \textit{A. ferrooxidans} (□ 0K + \textit{A. ferrooxidans}). Error bars indicate the standard deviations (\( n = 3 \)).
The concentration of Fe in the filtrate of PCBs mixed with DI water was 13 mg/L and 9 mg/L on day 1 and day 7 of the reaction, respectively, showing no significant increase with the reaction time (Figure 2b). In contrast, for 0K medium, the Fe concentration almost doubled from 62 mg/L to 117 mg/L over the course of the reaction, which can be explained by Fe dissolution occurring due to the low pH of the 0K medium. This is because the 0K medium is titrated to be pH 2 to create a suitable condition for the growth of *A. ferrooxidans*. When injecting the 0K medium and *A. ferrooxidans* mixed solution, an increase of 76% ± 8% Fe concentration was detected over the total reaction time compared with 0K medium used alone in the reaction. This may be due to the oxidation of Fe depending on the presence of *A. ferrooxidans*, and the produced Fe$^{3+}$ precipitates as iron oxides such as Fe(OH)$_3$ and jarosite (KFe$_3$(SO$_4$)(OH)$_6$) in the aqueous phase [14], disappearing from the solution.

Ni did not dissolve in the PCBs mixed with DI water (Figure 2c). Contrarily, Ni leaching increased up to tenfold over the reaction time in the PCBs mixed with 0K medium. A very similar Ni leaching behavior was found in PCBs mixed with 0K medium and *A. ferrooxidans*. In other words, the leaching of Ni from the PCBs was significantly affected by the characteristics of 0K medium (i.e., sulfuric acid was titrated to bring the pH to 2) and the reaction time.

The concentration of Pb in the filtrate tended to increase over time until the fourth day, then decreased from day 5 when *A. ferrooxidans* was injected (Figure 2d). When only the 0K medium was added, the Pb concentration in the filtrate increased over time, suggesting that the Pb leached by the 0K medium from the PCBs precipitates due to the effect of *A. ferrooxidans*. If Pb is continuously leached from the PCBs, the activity of *A. ferrooxidans* may be inhibited due to Pb toxicity, but this study confirmed the recovery process of valuable metals from PCBs using *A. ferrooxidans* and 0K medium can be a useful method, because the leached Pb precipitates out of the solution.

### 3.3. Precipitate Analysis

The results of XRD analysis on the precipitates retrieved from the filtration of each leachate are shown in Figure 3. The crystalline mineral libethenite (Cu$_2$(PO$_4$)(OH)) was observed in the sample when 0K medium was injected without *A. ferrooxidans*. This Cu precipitate is likely to have been formed after Cu leached from the PCBs reacts with anions in the 0K medium. In contrast, no libethenite was found in the sample with *A. ferrooxidans*, and the XRD pattern of this sample was similar to that of DI water (Figure 3a,c). These results show that inoculation with *A. ferrooxidans* increases the ORP and promotes additional leaching of Cu, but it also inhibits the precipitation of Cu leached due to the characteristics of the 0K medium. Therefore, Cu is expected to be effectively recoverable by injecting the mixed solution composed of *A. ferrooxidans* and 0K medium into the PCBs.

Figure 4 represents the results of the SEM-EDS analysis of precipitates retrieved from the filtration of each leachate. When only DI water was injected into the PCBs, the surface of the filter used for the filtration was measured (Figure 4a). Meanwhile, the amount of Cu precipitate was similar to the XRD analysis results from the sample where only 0K medium was added (Figure 4b,e). When 0K medium and *A. ferrooxidans* were injected, the precipitates had a smaller particle shape were compared with when only 0K medium was injected (Figure 4c). Based on the EDS analysis results, Pb precipitates were also observed (Figure 4f).
Figure 3. XRD patterns of residue on 0.2-µm acetate nitrate membrane filter after filtering the leachate of (a) printed circuit boards (PCBs) and deionized (DI) water, (b) PCBs and 0K medium, and (c) PCBs and 0K inoculated with *Acidithiobacillus ferrooxidans*.

As mentioned in Section 3.2, the formation of iron oxide precipitates (e.g., Fe(OH)_3, KFe_3(SO_4)(OH)_6) is expected depending on the injection of *A. ferrooxidans*, and the amorphous iron oxide contained in the precipitate was detected by the SEM-EDS analysis (Figure 4e,f). Moreover, the higher amount of Fe (i.e., the peak height) in Figure 4f compared with that in Figure 4e suggests that more iron oxide precipitates were formed in the case of *A. ferrooxidans* injection because *A. ferrooxidans* oxidized Fe^{2+} to Fe^{3+}. This is consistent with the study of Priya and Hait [14], which reported that the hydrolysis of accumulated Fe^{3+} in low pH and high Eh conditions can lead to the formation of reddish-brown precipitates known as jarosite (KFe_3(SO_4)(OH)_6). Although the peak of Zr was shown in Figure 4e due to proximity between Zr-Lα (2.042 keV) and Pt-M (2.048 keV), it is not actually contained in the precipitate (Pt is measured due to the surface coating of the sample during the pretreatment of SEM-EDS analysis).
Figure 4. Scanning electron microscope (SEM) images and energy-dispersive X-ray spectrometer (EDS) results of residue on 0.2-µm acetate nitrate membrane filter after filtering the leachate of (a,b) printed circuit boards (PCBs) and deionized (DI) water, (c,d) PCBs and 0K medium, and (e,f) PCBs and 0K inoculated with Acidithiobacillus ferrooxidans.

4. Conclusions

In this study, the applicability of using bacteria as a technique to recover valuable metal from low-grade urban ore was investigated. *A. ferrooxidans* oxidized iron to obtain the energy required for its growth, and during this process it increased the ORP of the solution. As a result, it was found that using iron in the PCBs collected from the waste computer could recover valuable metals in the PCBs, especially Cu. *A. ferrooxidans* not only increases the leached amount of Cu from the PCBs but also inhibits the components in the 0K medium and the leached Cu from forming precipitates, thereby assisting the recovery of Cu. The concentration of Pb in the leachate was low, as it formed an amorphous precipitate under the presence of *A. ferrooxidans*. It was confirmed that the bioleaching technique can be effectively applied as a cost-effective means of recovering valuable metals from low-grade urban ores such as PCBs. Bioleaching is expected to diversify the raw material sources of metal and decrease the landfill throughput of PCBs. Future studies should focus on the optimization of bioreactor operation conditions, including biomass, supernatant, or precipitate separation to achieve significant economic and ecological benefits through applying this bioleaching processes.

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