Enhanced bioleaching of copper from circuit boards of computer waste by *Acidithiobacillus ferrooxidans*

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**Abstract**

Computer circuit boards are a major electronic waste containing higher concentrations of copper, gold and silver. These metals may be recovered by bioleaching, an eco-friendly process to recover metals from natural ores. However, the application of the bioleaching to electronic waste is still in the infancy stage. Here, the bioleaching capability of *Acidithiobacillus ferrooxidans* to extract copper from printed circuit boards was investigated at laboratory scale using shake flasks. The effect of initial pH, amount and size of printed circuit boards, and volume of inoculum on copper dissolution rates were evaluated. Results show that the highest dissolution rate of 32.44% was achieved after 7 days of leaching at initial pH 2.0, 10 g/L of waste printed circuit board, 40% v/v of inoculum for 1 mm size of circuit board. The smallest size of 1 mm induces the higher dissolution rates, which is explained by higher surface area and thus better bacterial adhesion. Also, the copper dissolution rates increase with the inoculum volume. Overall, bioleaching of copper from waste printed circuit boards using *Acidithiobacillus ferrooxidans* is achievable.

**Keywords** Bioleaching · Electronic waste · Waste computer printed circuit boards · Copper · Iron-oxidizing bacterium

**Introduction**

Generation of electronic waste (E-waste) has become a primary concern of the world. The recent technological developments and innovation of highly advanced electrical and electronic equipment have escalated the amount of E-waste generation. In addition to this, the ever-shortening product lifespan of this waste urges toward modern and fashioned electronic goods. Reliable automated production contributes to the increased exploitation of E-waste (Herat and Agamuthu 2012). E-waste includes all types of electrical and electronic equipment and its components that have been discarded away by the holder as waste, without any intention of reusing or recycling them (STEP 2014). E-waste can be classified into six different categories such as temperature exchange equipment, screens and monitors, lamps, large equipment, small equipment, information technology (IT) and telecommunication equipment. Information technology and telecommunication equipment comprise of mobile phones, personal computers, printers and telephones (Baldé et al. 2015). Personal computers with a usual lifespan of 5–8 years constitute for the larger amount of wastes in this category (Gurumurthy and Annamalai 2019). Motherboards also referred as printed circuit boards are the integral component of personal computers, which carries out the overall operation of the system. Printed circuit boards are generally copper-clad laminate reinforced with epoxy resin consisting of non-metals and metallic materials including precious metals like gold and silver (Ghosh et al. 2015). Non-metal mainly consists of glass fibers, fillers, ceramics, insulators, capacitors, and resistors.

The metal content of computer printed circuit boards is 30.1% and copper accounts for around 14.6% of the total content, as it is the construction and connecting element of printed circuit boards (Jakub 2014). Printed circuit boards also comprise of notable hazardous elements including heavy metals, flame retardants that pose severe damage to the land and aquatic ecosystem upon conventional landfills and incineration (Debnath et al. 2016). Majority of the waste computer printed circuit boards generated from malfunctioning personal computers get disposed along with
the municipal waste in dump yards (Needhidasan et al. 2014). Such improper handling of waste printed circuit boards not only leads to the degradation of environmental systems, but also the inevitable resource of metal values present in them is diminished. Hence, recycling of waste printed circuit boards has become an inevitable option for proper handling and recovery of metal values present in them, so that waste printed circuit boards can be used as a boundless secondary source of metal concentrations, essentially copper.

Environment-friendly and clean technologies for the treatment of E-waste have been investigated widely over the decade for efficient recycling and disposal. The existing conventional processes for the extraction of metal values from waste printed circuit boards are pyro-metallurgical and hydrometallurgical process. They are not considered environmentally friendly as they generate atmospheric pollution through the release of hazardous gases like dioxins, furans, and cause land degradation through the discharge of large volume of effluents (Ilyas et al. 2007). Electrochemical methods have also been applied for the extraction of metal fractions from waste printed circuit boards but found to be costly with high-energy consumption rates. Thus, exploration of a process, which is environment friendly, with less operating costs and less energy consumption, has been enthusiastic among researchers. Bio-hydrometallurgy is one such extensively encouraging technology used for extracting metal values from substandard ores and mineral deposits (Brierley 2016). Bio-hydrometallurgy includes bioleaching; bio-sorption and bioaccumulation employed for treating industrial wastes, tannery sludge, acid mine drainages, low-grade ores and mining wastes (Arshadi et al. 2015; Ganesapillai et al. 2017). The dissolution of metal ions from insoluble ores or wastes through biological oxidation and reduction processes is bioleaching. In general, the dissolution of metals from many mineral sources is carried out by several naturally prevailing microorganisms mostly archaea, bacteria, and fungi (Rawlings et al. 2003). Bioleaching has been effectively applied for the extraction of metals such as copper, iron, manganese, gold from sulfide ores like chalcopyrite, hematite, pyrites, and arsenopyrite (Pandey and Natrajan 2015). Bioleaching is at the level of maturity in the mineral processing of ores, whereas its application for the extraction of metals from waste printed circuit boards is at an infant stage. Thus, increased interest has shown toward microbial extraction of metals from waste printed circuit boards due to its capability to lower operational costs and energy requirements. Few studies have earlier been undertaken involving Acidithiobacillus species, Acidithiobacillus ferrooxidans (A. ferrooxidans) and Acidithiobacillus thio-oxidans for the metal extraction from E-waste (Lambert et al. 2015; Rodrigues et al. 2015; Yang et al. 2017). Some studies have also been investigated involving fungal strains like Aspergillus niger and Aspergillus flavus for the extraction of metals from E-waste (Chatterjee et al. 2019; Qu et al. 2015).

The present study involves A. ferrooxidans, which is of specific interest due to its remarkable broad metabolic capability. Acidithiobacillus ferrooxidans can oxidize Fe²⁺ (Eq. 1) in favorable environments that produce Fe³⁺ a powerful oxidant (Nemaí et al. 1998). Conditions favorable for oxidative leaching must be provided constantly for effective extraction of metals, as the metal elements present are in a natural form or as alloys in waste printed circuit boards. In situ generated ferric iron due to the oxidation of ferrous iron by bacteria in (Eq. 1) is a great oxidizing agent to solubilize copper from waste printed circuit boards (Eq. 2). Apparently, the initial concentration and the rate of ferric iron generation in the bioleaching environment controls the rate of copper solubilization from waste printed circuit boards (Janyasuthiwong et al. 2016).

\[
2\text{Fe}^{2+} + \frac{1}{2}\text{O}_2 + 2\text{H}^+ \rightarrow 2\text{Fe}^{3+} + \text{H}_2\text{O} \quad (1)
\]

\[
\text{Cu}^{2+} + 2\text{Fe}^{3+} \rightarrow \text{Cu}^{2+} + 2\text{Fe}^{2+} \quad (2)
\]

There are studies with less focus on increasing the efficiency of dissolution of metal values from waste printed circuit boards. Influencing factors, such as pH of the leaching medium, bacterial inoculum, waste printed circuit board size, and concentration, which have significant effects on the dissolution rate of copper from waste printed circuit boards have not been investigated deeply. This study emphasizes highly to assess the dissolution rate of copper from waste printed circuit boards by A. ferrooxidans and to determine the optimum conditions for efficient bioleaching process.

Materials and methods

Source of waste printed circuit boards

Waste computer motherboards that do not function anymore were chosen as a source of waste printed circuit boards. For the bioleaching studies, waste printed circuit boards were purchased from local scrap dealers in Vellore, Tamil Nadu, India. Waste printed circuit boards were reduced to smaller pieces using stainless blades, after manually dismantling the major electronic components such as capacitors, connectors, resistors, sensors, and diodes. Waste printed circuit board scraps were then hammered and crushed into smaller fractions, sieved with sieves of different mesh sizes to obtain different sizes of a scrap of 1 mm, 2 mm, > 2 mm, and > 10 mm.
Analysis of waste printed circuit boards

The metal analysis of waste printed circuit board samples was performed with acid digestion techniques. About 1 g of waste printed circuit board sample was dissolved in 100 mL of aqua regia (3HNO₃ + 1HCl) and refluxed in a round bottom flask for 1 h. Once the solution gets cooled down, the final volume was made up to 100 mL. All the experiments were conducted in duplicates. Dissolved copper and other metal ion concentrations like zinc, nickel, lead, and iron in the digested solutions were determined through flame atomic absorption spectroscopy (AAS) (PerkinElmer Pinnacle 500).

Preparation of waste printed circuit board sample for bioleaching studies

Crushed waste printed circuit board samples after sorting out ferrous and non-ferrous metals were used to carry out bioleaching experiments. Waste printed circuit boards were washed by adding 10 g of electronic scrap in 100 mL of sodium chloride solution prepared initially. The mixed feed was continuously stirred for 10 min and allowed to stand for the settling down of heavier particles. All the particles floating were decanted off, and the denser parts that settled down at the bottom were collected, wiped and dried to constant weight. Such treated waste printed circuit board samples were called “washed samples,” and 10 g/L of these washed waste printed circuit board samples was used for every set of bioleaching experiments. Autoclaving at 121 °C 15 psi for 20 min for these waste printed circuit board samples was performed for sterilization prior to bioleaching experiments.

Bacterial strain and culture conditions

Iron-oxidizing bacterium *Acidithiobacillus ferrooxidans* (ATCC 23270) was purchased from National chemical laboratory, Council of Scientific and Industrial Research (CSIR), India. The bacterial strain was activated in 9 K medium proposed by Silverman which is composed of: (NH₄)₂SO₄ 3.0 g/L, K₂HPO₄ 0.5 g/L, MgSO₄·7H₂O 0.5 g/L, KC1 0.1 g/L, Ca (NO₃)₂ 0.01 g/L, and 45 g/L of FeSO₄·7H₂O, which is the only energy source for the iron-oxidizing bacterium (Silverman and Lundgren 1959). The pH of the 9 K medium was altered to 2.0, initially with 1 N H₂SO₄, and sterilization was carried out by autoclaving at 121 °C and 15 psi for 20 min. Ferrous sulfate solution was filter-sterilized with nitrocellulose membrane syringe filter (0.45 μm) and added separately to the sterilized medium prior to inoculation. The inoculated medium in Erlenmeyer flasks was kept for incubation at 30 °C for 5–7 days in a shaking incubator at 160 rpm. After the incubation period, grown bacterial cultures were preserved by adding glycerol (20%) and stored at −80 °C for further bioleaching experiments.

The metal adapted cultures were obtained through repeated sub-culturing of the bacterium in the presence of Cu²⁺ ion solution. 100 mL of 9 K media was prepared in 250 mL Erlenmeyer flasks and sterilized through autoclaving. Cu²⁺ stock solutions of concentrations 10 mM, 20 mM, 30 mM, 40 mM, and 50 mM were prepared from copper sulfate (CuSO₄·5H₂O) and deionized water. Metal solutions were filter sterilized by passing through nitrocellulose membrane syringe filter (0.45 μm), and the desired volume of metal concentrations was added to the above-prepared 9 K medium. About 1 mL of inoculum containing 3 × 10⁹ cells/mL was added to the flask and kept for incubation at 30 °C in a rotating shaker-incubator at 160 rpm for 5–7 days. This procedure was repeated with increasing Cu²⁺ ion concentrations, and successive transfers were made. Finally, such adapted cultures were harvested and stored in −80 °C as glycerol stocks. The adapted culture was sub-cultured in prior to bioleaching tests and had been inoculated into medium containing waste printed circuit board sample.

Bioleaching of waste printed circuit board

Bioleaching experiments for the dissolution of copper from waste printed circuit boards were performed in 250-mL Erlenmeyer flasks added with 100 mL of 9 K medium and 10% v/v of freshly activated adapted bacterial inoculum. The pH of the medium was set to have pH 2.0 adjusted with sulfuric acid, which provides the optimum condition for the bacterial growth. The flasks were subjected to sterilization by autoclaving at 121 °C and 15 psi pressure for 20 min prior to bioleaching. Then, about 10 g/L of sterilized washed waste printed circuit board was added to each flask aseptically. All the flasks were monitored for constant pH of 2.0 and were supplied with 2.0 M H₂SO₄ for maintaining stable pH of 2.0 throughout the leaching period. The iron oxidation capability of the bacterium is higher at pH 2.0, resulting in higher copper dissolution rates (Yang et al. 2009). After compensation of initial acid requirements, the bioleaching flasks were inoculated with 1.0 mL inoculum (3 × 10⁹ cells/mL) of the adapted culture of *A. ferrooxidans* aseptically. Flask without adding the bacterial inoculum ran in parallel as a control. All the flasks are checked for their weights and kept for incubation in a shaking incubator at 30 °C temperature at a shaking speed of 160 rpm for 5–7 days. With an intent to investigate the effect of initial pH, initial concentration of waste printed circuit board samples and different waste printed circuit board sizes, a sequence of flasks was arranged with initial pH at 1.6, 1.8, 2.0, 2.2 and 2.4. Maintaining constant pH, dissolution experiments were carried out with different initial concentrations of waste printed circuit board likely 10 g/L, 20 g/L, 30 g/L and 40 g/L, and different sieve fractions of 1 mm, 2 mm, >2 mm, and >10 mm. The optimum conditions were maintained throughout the
bioleaching period. The leaching flasks were weighed initially and kept for incubation in a shaking incubator at 30 °C, 160 rpm. All the experiments were carried out in duplicates, and the average data were stated.

**Analytical determination of samples**

The bioleaching flasks were balanced prior to sampling, and any loss because of evaporation during incubation period was recompensed by adding the respective volume of sterilized deionized water. To monitor the pH of the flasks daily, a considerable volume of medium is collected aseptically from the flasks and measured for the pH levels. The solid particles were removed from the samples by filtering through the Whatman No. 1 filter paper, and bacterial cells were removed through centrifugation at 10,000 rpm for 10 min. Cell pellets were removed, and the supernatant was analyzed for Cu²⁺ ion concentrations. Hydrochloric acid was added to the samples to a final concentration of 6 M HCl to remove any jarosites formed (Zhu et al. 2011). Copper ion concentrations in the leached liquor were analyzed through AAS analysis.

**Results and discussion**

**Elemental composition of waste printed circuit boards**

Waste printed circuit board metal composition after acid digestion is shown in Table 1. The concentration of metals in the acid digested liquor reflects the actual metal content of the waste printed circuit boards. The concentration of metals analyzed after the acid digestion was used for the assessment of the level of metal concentrations recovered during the bioleaching process as the maximum level of dissolution of metals from the waste printed circuit board samples, which can be 100% recovery.

**Effect of initial pH on copper dissolution**

The pH of the medium is a critical parameter in the leaching process generally controlled by bacterial growth (Jensen and Webb 1995). The effect of pH on Cu dissolution was studied by varying the pH while keeping the initial concentration of waste printed circuit board and volume of bacterial inoculum as constant. Experiments were carried out with a variation of pH ranging 1.6, 1.8, 2.0, 2.2 and 2.4 over a constant weight of 10 g/L of waste printed circuit board sample and constant volume of 10% v/v of *A. ferrooxidans* inoculum for 7 days. From Fig. 1a, it can be observed that the highest percentage of 25.58% dissolution of Cu²⁺ has been achieved at pH 2.2 followed by percentage dissolutions of 20.66%, 20.23%, 19.66%, and 17.9% for pH 2.0, 1.8, 2.4 and 1.6, respectively. It can be also observed that copper dissolution rate was lower at initial pH 1.6, mainly caused by the inhibitory effect of lower pH values on the metabolism of the bacterium, and apparently even higher pH values could have the inhibitory effect on the growth of bacteria, which results in low copper dissolution efficiencies. These results have shown that the optimal pH for maximum dissolution of Cu²⁺ is pH 2.2, which proves that efficiency of *A. ferrooxidans* over the dissolution of metals is pH-dependent, and relative changes in the pH will affect the efficiency of metal recovery. Chemical leaching could be witnessed in the control test ran parallel with initial pH 2.0 without adding any bacterial inoculum into the medium.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Element</th>
<th>Composition (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Iron</td>
<td>1.659</td>
</tr>
<tr>
<td>2</td>
<td>Lead</td>
<td>2.72</td>
</tr>
<tr>
<td>3</td>
<td>Nickel</td>
<td>11.79</td>
</tr>
<tr>
<td>4</td>
<td>Copper</td>
<td>646</td>
</tr>
<tr>
<td>5</td>
<td>Zinc</td>
<td>313.30</td>
</tr>
</tbody>
</table>

**Effect of different waste printed circuit board size fractions on copper dissolution**

Experiments with four different sieve fractions of 1 mm, 2 mm, > 2 mm and > 10 mm crushed waste printed circuit boards were conducted at initial pH 2.0. In this study, the highest copper recovery of about 32.44% had been observed with waste printed circuit board sieve fraction of 1 mm size followed by 2 mm, > 2 mm and > 10 mm of sizes with 25.56%, 10.60%, and 4.42%, respectively, as shown in Fig. 1b after 7 days of bioleaching. The higher dissolution rate of copper from 1 mm size fraction waste printed circuit boards may be due to the greater attachments of bacteria over the surface of the waste printed circuit board. This indicated that the surface area of the sample increases with the decrease in sample size and more surface area is favorable for higher adhesion rate of *A. ferrooxidans* onto these metal surfaces. Therefore, the ratio of Cu solubilized by *A. ferrooxidans* increases with the decrease in the size fraction of the samples. Hence, the smaller sieve fractions of 1 mm were used for further bioleaching experimental studies.

**Effect of concentration of waste of printed circuit board on copper dissolution**

Bioleaching flasks with actively adapted *A. ferrooxidans* cultures were fed with different dosage levels of crushed waste printed circuit boards. During this investigation, the
maximum level of copper dissolution was obtained at 10 g/L, of about 24.47%. The leaching rates at 20 g/L and 30 g/L dosages were almost similar after 7 days. A small decrease in the dissolution rate was observed at 40 g/L with the maximum dissolution of about 13%. After 7 days of leaching, the percentage of dissolution of copper was 25%, 16%, 13% and 11% at 10 g/L, 20 g/L, 30 g/L and 40 g/L, respectively, as depicted in Fig. 2a. These results illustrate that dissolution of copper decreases with the increase in waste printed circuit board sample dosage. It was evident that higher dosages of

![Graph](image)

**Fig. 2** Dissolution of copper from waste printed circuit boards. (a) Note the effect of varying waste printed circuit board dosages (10 g/L, 20 g/L, 30 g/L and 40 g/L of 1 mm waste printed circuit board) on Cu²⁺ dissolution by A. ferrooxidans (pH 2.0, 10% v/v inoculum, 30 °C). (b) Note the effect of varying inoculum volume of A. ferrooxidans (10% v/v, 20% v/v, 30% v/v and 40% v/v) on Cu²⁺ dissolution at (pH 2.0, 10 g/L waste printed circuit board (1 mm), 30 °C)
waste printed circuit board resulted in low copper dissolution partially due to restraint in air distribution and oxygen supply (Silva et al. 2015). Another important reason could be the toxicity of metal ions when waste printed circuit board dosage reaches the threshold level of the active bacteria in the solution. It was also observed that the growth of bacteria was critically reduced, which may be due to the poisoning of non-metallic portions and plastics present along with the waste printed circuit board sample. In a similar study, the presence of lead, plastics and other non-metals was found to be responsible for the inhibition of bacterial growth (Uoeoka et al. 2016).

**Effect of inoculum volume on copper dissolution**

In copper dissolution process, the volume of inoculum is also a critical factor which can directly influence the rate and degree of bacterial leaching, as the number of active bacteria in the medium rises with a rise in the volume of the bacterial inoculum (Yang et al. 2014). Figure 2b depicts that there was a significant increase predominantly in the initial frequency of copper dissolution with an increase in the volume of inoculum from 10 to 40% v/v. After 7 days of bioleaching the 23%, 25%, 28% and 31% of copper had been leached out from 10 g/L waste printed circuit board dosage at pH 2.0 for the inoculum volumes 10% v/v, 20% v/v, 30% v/v and 40% v/v, respectively. This rate of dissolution of copper can be related to the immediate conversion of ferrous (Fe^{2+}) into ferric ions (Fe^{3+}) due to the increased bacterial population. The increased availability of Fe^{2+} that was consumed over by the inoculum might be responsible for the improvement in the rate of copper dissolution. It also can be distinguished that the Fe^{3+} generation rate tends to increase with the increase in inoculum volume, eventually controlled by the transport of oxygen and carbon dioxide into the bioleaching medium resulting in higher dissolution rates.

**Conclusion**

*Acidithiobacillus ferrooxidans* (ATCC 23270) showed a potential performance in copper dissolution from waste printed circuit boards. The pre-adaption of the bacterial strain to copper metal concentration had a significant effect on the rate of copper dissolution from waste printed circuit boards. The dissolution of copper from waste printed circuit boards was accomplished indirectly through the oxidation of ferrous (Fe^{2+}) into ferric ions (Fe^{3+}). The results also indicated the influence of critical factors like initial pH, size of waste printed circuit boards, waste printed circuit board dosage and inoculum volume on copper dissolution rates. The initial pH around 2.25 is suggested for greater dissolution of copper from waste printed circuit boards. It is evident from the results that the rate of copper dissolution increases with the decrease in size fraction of the samples, which can be considered during the preparation of waste printed circuit board samples for bioleaching. The highest dissolution rate of 32.44% was achieved after 7 days of leaching at initial pH 2.0, 10 g/L waste printed circuit board dosage, 40% v/v of inoculum for 1 mm size of waste printed circuit boards. The volume of inoculum had a direct influence on the bioleaching of copper from waste printed circuit boards as the dissolution rate increases with increase in inoculum volume. These results validate the theoretical basis for the dissolution of copper from waste printed circuit boards. Techniques like electro-winning and stripping processes can be applied for the recovery of copper from leached solutions. From this investigation, we suggest that preprocessing of waste printed circuit boards into smaller sizes and removal of non-metallic parts and plastics completely are some of the key factors to achieve complete dissolution of copper by bioleaching.

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**References**


Jensen AB, Webb C (1995) Ferrous sulphate oxidation using Thioacido-
org/10.116032.959(095)85003.1
.org/. Accessed 29 Jun 2019
Yang T, Xu Z, Wen J, Yang L (2009) Factors influencing bioleaching copper from waste printed circuit boards by Acidithiobacillus fer-
Yang Y, Chen S, Li S et al (2014) Bioleaching waste printed cir-
cc.2014.01.008
Zhu N, Xiang Y, Zhang T et al (2011) Bioleaching of metal concen-
trates of waste printed circuit boards by mixed culture of acido-
org/10.1016/j.jhazmat.2011.05.062

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