



Development of two-step process for enhanced biorecovery of Cu–Zn–Ni from computer printed circuit boards

Monal B. Shah, Devayani R. Tipre, Mamta S. Purohit, and Shailesh R. Dave*

Department of Microbiology and Biotechnology, School of Sciences, Gujarat University, Ahmedabad 380 009, Gujarat, India

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Metal pollution due to the huge electronic waste (E-waste) accumulation is widespread across the globe. Extraction of copper, zinc and nickel from computer printed circuit boards (c-PCB) with a two-step bleaching process using ferric sulphate generated by *Leptospirillum ferriphilum* dominated consortium and the factors influencing the process were investigated in the present study. The studied factors with 10 g/L pulp density showed that pH 2.0 was optimum which resulted in 87.50–97.80% Cu–Zn–Ni extraction. Pre-treatment of PCB powder with acidified distilled water and NaCl solution showed 3.80–7.98% increase in metal extraction corresponding to 94.08% Cu, 99.80% Zn and 97.99% Ni extraction. Particle size of 75 µm for Cu and Zn while 1680 µm for Ni showed 2-folds increase in metal extraction, giving 97.35–99.80% Cu–Zn–Ni extraction in 2–6 days of reaction time. Whereas; 2.76–3.12 folds increase in Cu and Zn extraction was observed with the addition of 0.1% chelating agents. When the studies were carried out with high pulp density, ferric iron concentration of 16.57 g/L was found to be optimum for metal extraction from 75 g/L c-PCB and c-PCB addition in multiple installments resulted in 8.81–26.35% increase in metal extraction compared to single addition. The studied factors can be implemented for the scale-up aimed at faster recovery of multimetals from E-waste and thereby providing a secondary source of metal in an eco-friendly manner.

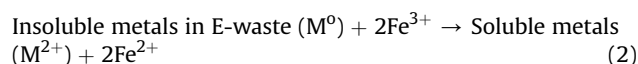
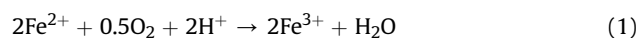
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[**Key words:** Chelating agent; Computer printed circuit board; High pulp density; *Leptospirillum ferriphilum*; Two-step bioleaching; Pre-treatment]

Electronic waste (E-waste) generation, its trans-boundary movement and disposal have created a major problem across the world (1). Almost every electrical and electronic equipments contain printed circuit board (PCB) as their basic component. The PCBs are the platform on which the microelectronic components such as semiconductor chips and capacitors are mounted and they help in providing electrical connections between the internal components (2,3). PCBs contain 28–32% metallic components like Cu, Zn, Pb, Ni, Al, Fe, Pd, Ag, Au, and 68% non-metallic components which mainly include plastics, glass and ceramics. The non-metallic portion of PCB viz. brominated flame retardants (BFR) and epoxy resins are the major cause of environmental pollution (4–6). Recycling of PCB is a daunting task due to the complexity of the materials and possible expulsion of toxic substances (7). Looking at the current scenario of E-waste generation, India is now emerging as one of the largest dumping grounds of E-waste for the developed countries of the world (8,9). In India, generation of E-wastes and their PCB has undergone a drastic increase. According to the United Nations Environment Programme (UNEP) report, India has generated 8,00,000 t of E-waste in the year 2012 and is expected to rise to 12,00,000 t or more by the year 2020 (10,11). Thus, proper management and safe disposal of E-wastes have become an urgent need.

Biohydrometallurgical process with relatively low capital costs, no gas/dust formation, operational selectivity and suitability for large scale applications makes it a treatment of choice for metal extraction from ores and concentrates (12–14). In the recent years, few researches have been carried out on the metal extraction from electronic scrap by applying one step bioleaching process with potential microbes like *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Chromobacterium violaceum*, and *Penicillium simplicissimum* (15–18). As the bacterial growth was adversely affected in the presence of high pulp density of E-waste, Brandl et al. (15) suggested the necessity to develop two-step process for enhanced metal extraction from E-waste.

In the two-step bioleaching process, Fe³⁺ iron is the oxidizing agent for metals present in the E-waste and the role of organisms is to regenerate Fe³⁺ from Fe²⁺ iron. This process is applied to reduce the toxic effects of E-wastes on micro-organisms (19). As in the first step, biomass is allowed to grow in absence of electronic scrap. When more than 90–95% Fe²⁺ has been oxidized by the acidophilic microbes, E-waste is added in the second step. The reaction mechanism of ferrous oxidation and solubilization of metals is expressed as follows:



* Corresponding author. Tel./fax: +91 079 26303225.
E-mail address: shaileshrdave@yahoo.co.in (S.R. Dave).

The Fe^{2+} iron formed due to reaction 2 is again oxidized to Fe^{3+} iron by the viable metal resistant consortium. The two-step process has following advantages which can be considered for metal extraction from E-wastes: (i) the Fe^{3+} iron can be continuously regenerated; (ii) higher waste concentration can be applied; (iii) metal yields can be increased (20). To the best of authors' knowledge, no reports are available on the factors influencing two-step bioleaching process for metal extraction from computer printed circuit boards (c-PCB).

The present work was carried out to develop a competent and speedy two-step bioleaching process for metal extraction from c-PCB. The inherent characteristic of ferrous iron oxidation and generation of ferric iron as biolixiviant by *Leptospirillum ferriphilum* dominated consortium was evaluated to enhance the extraction of metals from c-PCB. Further, the two-step process was improved by optimization of various factors influencing the extraction of copper (Cu), zinc (Zn) and nickel (Ni) from c-PCB E-waste. The optimized process was then applied for enhancing metal extraction from as high as 75 g/L of E-waste with the aid of the metal resistant consortium, which was responsible for the continuous faster regeneration of Fe^{3+} iron and increased metal extraction. These are the novel findings.

MATERIALS AND METHODS

Procurement and metal content analysis of waste computer printed circuit boards The waste c-PCB was purchased from an E-scrap market in Ahmedabad, India. For metal content analysis, 1 g powder of c-PCB E-waste was digested in 20 mL aqua-regia (3:2 – HNO_3 :HCl) at 150–180°C temperature till it was dry. It was followed by their further digestion in 10 mL concentrated H_2SO_4 for 15 min (21). The solution was allowed to cool down and filtered through Whatman No. 42 filter paper having 2.5 μm pore-size. The final volume of the solution was made up to 250 mL by double distilled water and total metal content was analyzed.

Culture conditions and two-step bioleaching process Multi-metal resistant iron oxidizer, *Leptospirillum ferriphilum* dominated consortium (22) was obtained from Microbiology Department, Gujarat University. The bioleaching experiments were conducted in 250 mL Erlenmeyer flasks containing modified SDB1 medium comprising of g/L: $(\text{NH}_4)_2\text{SO}_4$ 3.0; K_2HPO_4 0.5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5; KCl 0.1, pH 1.8 adjusted with 10% (v/v) H_2SO_4 , supplemented with 45.0 g/L of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. All the medium ingredients except ferrous sulphate were sterilized in an autoclave whereas; ferrous sulphate solution was filter sterilized using 0.22 μm nylon membrane filter (Pall Life Sciences, India). The sterilized medium was inoculated with 10% v/v of the activated consortium in its logarithmic growth phase having 2.5×10^7 cells/mL and agitated on thermostatic environmental shaker (Newtronics, India) rotating at 150 r/min at $32 \pm 2^\circ\text{C}$. The activated consortium refers to those cells that were oxidizing Fe^{2+} iron at the optimum rate. The iron oxidation rates of the selected consortium in presence and absence of 10 g/L c-PCB were noted down. Similarly for the two-step bioleaching experiments, when >95% of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was oxidized, 10 g/L of c-PCB was added to bio-generated Fe^{3+} iron containing medium and different experiments were performed to investigate the effect of system pH, pre-treatment, particle size and chelating agents whereas; effect of Fe^{3+} iron concentration and addition of pulp density in parts were studied at 75 g/L c-PCB concentration. Two-step bioleaching experiment before optimization of any of these factors was also carried out with 10 g/L of c-PCB having 149–500 μm particle size and pH of the bio-generated Fe^{3+} iron containing medium was adjusted to 1.8. Result of this experiment was used for calculating the improvement due to various optimizations. For all the studied parameters, results were recorded for change in pH, redox potential, acid consumed, Fe^{2+} oxidized and Cu–Zn–Ni solubilized.

Effect of system pH To check the influence of pH on leaching of c-PCB, 10 g/L of the PCB powder was added into the five different 250 mL Erlenmeyer flasks containing 100 mL of 0.8% Fe^{3+} iron generated by the consortium. The pH of leaching system was adjusted to 1.5, 1.8, 2.0, 2.5 and 3.0, respectively, with 10% (v/v) H_2SO_4 before addition of the c-PCB and was maintained throughout the experiments by addition of required H_2SO_4 .

Effect of pre-treatment In pre-treatment experiment, sample of c-PCB was prepared by suspending 5 g of c-PCB powder in 100 mL of saturated NaCl solution, pH 5.0 distilled water (D/W) and pH 2.0 distilled water (D/W) in separate 250 mL Erlenmeyer flasks. The pH of the distilled water used for pre-treatment was set with 10% (v/v) H_2SO_4 . The system was stirred for 30 min and allowed to stand till the heavier c-PCB particles settled at the bottom. The supernatant and the floating material were filtered and collected to determine the loss of metals during pre-treatment immediately, while the denser part which settled at the bottom was separated and dried to constant weight. The samples treated in this way were

labeled as NaCl solution, pH 5 D/W and pH 2 D/W pre-treatments. The c-PCB powder which was added to the bioleaching system without washing was referred to as No pre-treatment sample. The pre-treated and no pre-treated c-PCB samples were sterilized in an open petri-dish exposed to ultra-violet light up to 5 $\mu\text{Joule}/\text{mm}^2$ dose (260 nm) for 10 min at a 15 cm distance before their addition to the bioleaching system.

Effect of particle size of c-PCB powder In order to determine optimal particle size, bioleaching tests were carried out with various particle sizes. The c-PCB used in the study contained more than 80% of 3360, 1680, 250, 149, 74 and 53 μm particle size, which ranged between 2830–4000, 1410–2000, 149–500, 125–177, 63–88 and 44–63, respectively. The desired particles were obtained by sieving the c-PCB powder passed through the first British Standard Sieve Series (BSS) mentioned and retained by the second BSS mesh number 6 (5–7), 12 (10–14), 60 (35–100), 100 (80–120), 200 (170–230) and 270 (230–325), respectively (23). From the obtained c-PCB with different particle sizes, 10 g/L c-PCB powder was added to the flasks containing 0.8% bio-generated Fe^{3+} iron having an initial pH adjusted to 2. The concentration of metals extracted was analyzed periodically.

Effect of chelating agents Ethylene diamine tetraacetic acid (EDTA), ethylene diamine disuccinic acid (EDDS), diethylene triamine penta acetate (DTPA), nitrile triacetic acid (NTA), citric acid and oxalic acid were individually tested for their effect on Cu–Zn–Ni extraction from c-PCB. Ten grams per liter of c-PCB powder and 0.1% of the chelating agents were added to the 100 mL of medium containing 0.8% of bio-generated Fe^{3+} iron. One set was kept as no chelant system. Initial pH of all the test flasks was adjusted to 2.0 with 10% (v/v) H_2SO_4 . Thereafter, the leaching system was mixed well for 10 min at room temperature. The c-PCB was allowed to settle down and appropriate amount of samples from all the flasks were withdrawn to determine the 0 h reading. All the flasks were then incubated on a rotary shaker and the aliquots were withdrawn at definite time intervals till maximum metal ions were brought into the solution.

Effect of bio-generated Fe^{3+} iron concentration for metal extraction from 75 g/L pulp density To determine the optimum concentration of Fe^{3+} iron for 75 g/L pulp density, 10 mL of the actively growing consortium was inoculated into 90 mL of modified SDB1 medium with initial concentrations of 65, 85, 105 and 125 g/L of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, which correspond to 13.07, 17.10, 21.12 and 25.15 g/L Fe^{2+} iron, respectively, in different flasks. All the flasks were incubated for 2–5 days till almost complete Fe^{2+} iron was oxidized. After removal of the precipitate by filtration from different inoculated test flasks, the solutions were analyzed to determine the concentrations of soluble Fe^{3+} iron generated by the consortium. Seventy five grams per liter of c-PCB was added to the different concentrations of bio-generated Fe^{3+} iron and aliquots were withdrawn at definite time interval to determine the amount of Cu–Zn–Ni extracted.

Effect of c-PCB addition in parts In order to study the effect of mode of c-PCB addition on metal extraction from 75 g/L of c-PCB, 10 mL of the actively growing consortium was inoculated into 90 mL of modified SDB1 medium containing 85 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The concentration of bio-generated Fe^{3+} iron and c-PCB added were kept constant for all the experiments, however the c-PCB addition amounts and time of addition were varied to understand the effect of addition mode. The c-PCB concentration of 75 g/L was added at 0 h in set 1; 25, 25 and 25 g/L c-PCB was added at 0, 24 and 48 h, respectively, in set 2; 37.5 and 37.5 g/L c-PCB was added at 0 and 48 h, respectively, in set 3 whereas; 37.5 and 37.5 g/L c-PCB was added at 0 h and 24 h, respectively, in set 4. The comparison was carried out to check the effect of c-PCB added in parts compared to c-PCB added in one lot on Cu–Zn–Ni extraction efficiency.

Analytical methods In all the experiments, quantitative analysis of Cu, Zn, Ni and total iron was carried out by atomic absorption spectrophotometer (AAS) (Elico SL-243, Elico, India). The Fe^{2+} iron was estimated by titrating with 0.1 N $\text{K}_2\text{Cr}_2\text{O}_7$ using diphenylamine as an indicator in acidic condition (21). The pH was measured using microprocessor based digital pH meter (Systronics 362, Systronics, India). Redox potential was measured using pH ionics Pt combination redox electrode with the reference calomel electrode. Ferric iron concentration was calculated from the difference of total iron and Fe^{2+} iron estimated by AAS and titrimetric method, respectively. The amount of acid 10% (v/v) H_2SO_4 consumed during the entire experiment was also recorded. All the experiments were conducted in triplicates to ensure the reliability of the process and the data were analyzed for standard deviation and a significance at 95% confidence limit was considered.

RESULTS AND DISCUSSION

Metal content analysis of c-PCB The total metallic content of c-PCB was 593.46 mg/g of c-PCB. The major metal component detected by AAS was copper followed by lead, iron, aluminium, zinc, arsenic, nickel and chromium, with the concentration of 300.00, 136.50, 60.12, 45.93, 37.00, 7.82, 3.84 and 1.61 ± 0.80 to ± 1.70 mg/g, respectively. Precious metals like palladium, silver and gold were also detected from c-PCB with the concentration of 0.27, 0.23 and 0.14 ± 0.30 to ± 0.50 mg/g, respectively. Under the

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