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TWO-STEP LEACHING OF METAL VALUES FROM DISCARDED MOBILE PHONE PRINTED CIRCUIT BOARD USING BIOGENIC FERRIC ION WITH COPPER LEACHING KINETICS

Sugandha Aachhera[1], *Shatakshi Tiwari*[1], *Pradeep Verma*[1*], *Chandra Sekhar Gahan* [1, 2*]

Address(es):
[1] Department of Microbiology, School of Life Sciences, Central University of Rajasthan, Bandarsindri, Kishangarh, Ajmer-305817, Rajasthan, India.
[2] Department of Biotechnology, School of Studies of Interdisciplinary Education and Research, Guru Ghasidas Vishwavidyalaya (A Central University), Koni, Bilaspur, Chhattisgarh - 495009, India.

*Corresponding author: gahancsbiometal@gmail.com; pradeepverma@curaj.ac.in

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ABSTRACT

Discarded mobile phone printed circuit boards (mobile phone PCBs), a prominent component of electronic waste, represent a significant secondary resource for metal recovery. Mobile phone PCBs contain significant amounts of copper and other critical metals, making them a good option for bioleaching, which is a sustainable and environmentally friendly alternative to conventional metal extraction methods. However, slow reaction kinetics often hinder bioleaching, limiting its industrial-scale applicability. This study utilized mixed culture consortia of iron oxidizing microorganisms dominated by *Leptospirillum ferriphilum*, which were chemolithotrophic microbes thriving in highly acidic conditions and high ferric iron concentrations responsible for enhanced copper recovery through a two-step bioleaching process. All the bioleaching experiments were conducted at a controlled temperature of 35±5 °C and pH controlled to 1.5 with a Pulp Density (P.D.) of 10% while varying biogenic ferric iron concentrations from 9–18 g/L, which served as the main lixiviant for metal extraction. The results demonstrated that increasing biogenic ferric iron concentration significantly improved copper recovery and reduced leaching time. In the first leaching step, 50-83% copper extraction efficiencies were achieved at biogenic ferric iron concentrations of 9-18 g/L. During the second leaching step, copper extraction exceeded 99% within 5.5-3.5 days for the corresponding ferric concentrations. The elevated biogenic ferric iron levels facilitated this rapid and efficient copper extraction, accelerating the leaching kinetics. Post-leaching analyses, including XRF, SEM-EDS, and XRD, confirmed >95% copper recovery following solid-liquid separation. The study highlights the effectiveness of the two-step bioleaching process and underscores the potential of biogenic ferric iron in overcoming the limitations of traditional bioleaching methods. This research demonstrates a fast, simple, and sustainable bioleaching method for the efficient recovery of copper from mobile phone PCBs, offering a promising pathway for industrial applications in e-waste management and contributing to the circular economy.

Keywords: Two-step bioleaching; Biogenic ferric; Iron oxidizers; Mobile phone PCBs

INTRODUCTION

Electronic waste (e-waste) is escalating globally due to rapid technological advancements and the increasingly shorter lifespans of electronic devices. According to a report by Forti et al. (2020), global e-waste generation reached 53.6 million metric tons (Mt) in 2019 and is projected to rise to 74.7 Mt by 2030, posing significant challenges for effective e-waste management. Among the various components of e-waste, printed circuit boards (PCBs) play a critical role. PCBs, integral to almost all electronic devices, are predominantly manufactured in China, Taiwan, and South Korea **(Ilyas et al., 2022)** and contribute approximately 4% of the total e-waste volume **(Jadhao et al., 2020; Gu et al., 2019)**. The composition of PCBs typically includes 30% plastics for insulation, 30% non-conductive ceramic oxides for stability, and 40% metallic components for electrical conduction (Shah et al., 2014; Martelo et al., 2024). These boards are a rich source of valuable metals including copper (10–20 wt%) and nickel (1–3 wt%), with metal concentrations significantly exceeding those found in natural ores **(Zhao et al., 2020)**. However, PCBs also contain hazardous materials, including polyvinyl chloride and brominated flame retardants, which, if improperly managed, can lead to severe environmental contamination **(Dutta et al., 2023; Becci et al., 2020)**. Recycling waste PCBs offers a dual advantage: recovering valuable metals and mitigating ecological hazards. Proper recycling reintroduces these critical materials into the circular economy and reduces energy consumption and harmful gas emissions associated with conventional mining and processing **(Li et al., 2022)**. Furthermore, sustainable management of PCBs can address socio-environmental issues, particularly in developing nations, where the adverse impacts of improper e-waste recycling are most pronounced **(Kang et al., 2023)**. Biomining technology is used by the mining industry to extract copper, uranium and gold from low-grade ores as well as secondary mineral resources such as E-waste, spent batteries, spent catalysts from petroleum refineries etc. Microbial insight in the bioleaching process with respect to its metabolites and regulatory pathways provide a synergistic approach in bioleaching which is important for biomining microbial community to adapt into the changing environmental conditions for primary and secondary mineral resources for efficient and higher metal recovery **(Das et al., 2011)**. Another source of secondary metal resources like spent batteries from electronic vehicles which is highly increasing in last few years which is a promising source of Lithium recovery to support the demand of Lithium for the production of lithium ion battery considering the increasing demand of it, when the lithium mineral reserves are scanty and minimal today as Lithium is considered as white gold for electric vehicles (EVs) to meet the global demand of 3 million tons by 2030. Hence in the context of the circular economy, it is indispensable develop recycling technologies via biotechnology using diverse microorganisms. **(Priyadarsini & Das, 2025a)**. The research on Bioleaching of Lithium batteries supports a positive recycling opportunities on optimizing process with sustainability and cost-effective recycling of Lithium ion from spent batteries **(Priyadarsini & Das, 2025b)**.

Currently, several methods are being employed to extract valuable metals from PCBs. These methods encompass physical separation, high-heat treatment (pyrolysis), aqueous solution dissolution, and biological processes utilizing microbial activity **(Garole et al., 2020)**. Physical separation involves mechanical processing, primarily yielding metal concentrate while generating significant amounts of solid waste, while pyrolysis emits toxic compounds like dioxins and furans **(Kumar et al., 2018)**. Chemical dissolution using strong oxidants and acids produces substantial effluents, consuming large amounts of chemicals and energy, making it less sustainable **(Qiu et al., 2021; Heydarian et al., 2018)**. In contrast, biohydrometallurgy, particularly bioleaching, has emerged as a promising alternative for metal recovery. This microbial-mediated recycling process leverages the natural metabolic activities of microorganisms to extract metals from e-waste in an environmentally friendly and sustainable manner **(Faraji et al., 2018; Pourhossein & Mousavi, 2022)**. Acidophilic microbes, such as *Acidithiobacillus ferroxidans (A. ferroxidans)*, *Acidithiobacillus thiooxidans (A. thiooxidan)*, and *Leptospirillum* species, are widely studied for their ability to thrive in highly acidic environments **(Bajestani et al., 2014)**. These bacteria derive energy by oxidizing ferrous ions (Fe^{2+}) and reduced inorganic sulfur compounds (RISCs) into biogenic ferric (Fe^{3+}) and sulfuric acid (H_2SO_4) using carbon dioxide

(CO_2) as the primary carbon source. Optimal growth occurs at pH ~ 1.5 and temperature between 30°C and 45°C **(Fortin et al., 1996; Sajjad et al., 2019; Yu et al., 2020).**

Bioleaching has demonstrated its feasibility for recycling e-waste, including printed circuit boards (PCBs) and other metal-rich waste streams such as ashes and slags. For instance, **Hubau et al. (2020)** reported the recovery of 96% copper, 73% nickel, and 93% cobalt from PCBs using *Leptospirillum ferriphilum* and *Sulfolobus benefaciens* in a bioreactor. Similarly, **Kremser et al. (2021)** achieved 100% leaching efficiency for zinc, copper, and manganese from incineration residues such as ashes and slags, employing Fe^{2+} and reduced inorganic sulfur compound (RISC) oxidizing bacteria. Bioleaching has also been extensively explored for metal recovery from lithium-ion battery (LIB) waste. **Roy et al. (2021)** documented the recovery of 90% nickel, 82% cobalt, and 92% manganese from spent NMC-based LIBs using *A. ferrooxidans*. Additionally, cultures enriched from soil and mud samples in volcanic regions and tannery wastewater achieved 62.8% lithium dissolution from LIBs within 15 days **(Hartono et al., 2017).**

Most studies dealing with bioleaching have been performed in one step using different kinds of microorganisms. Iron oxidation and bio-mediated regeneration of leaching reagents co-occur within the same reactor in this approach. However, toxic compounds in e-waste have been reported to affect microbial activity negatively **(Isildar et al., 2016; Magoda et al., 2024).** To mitigate such toxicity and enhance metal recovery, a two-step bioleaching process has been proposed as a more practical alternative **(Heydarian et al., 2018).** The two-step bioleaching involves pre-culturing the microorganisms to their exponential phase in optimum growth conditions without metal waste, which is more efficient for mobilizing metals **(Benzal et al., 2020; Hubau et al., 2020).** Toxic components in polymetallic wastes like PCBs pose significant challenges to bioleaching, as metal ions, particularly copper, inhibit microbial growth, and non-metallic constituents further impede microbial activity (**Van Yken et al., 2021; Ahmadi et al., 2012; Zhu et al., 2011).** Considering these facts, the current investigation into discarded mobile phone PCBs bioleaching was performed using a two-step method. However, in this study, the two-step bioleaching was slightly adapted from the available literature and modified. In this modified approach, the second step of the experiment was divided into two sub-steps: the first leaching step and the second leaching step. In the second leaching step, the lixiviant was replaced with fresh media to diminish metal toxicity, enabling the utilization of higher Pulp Density (P.D.). This strategy could prove more effective on an industrial scale for processing highly diverse PCBs, which may influence microbial activity. The work primarily focuses on improved Cu recovery from PCBs at various biogenic ferric concentrations. The reason for the use of biogenic iron is because of the green ferric ion which is produced by biological or microbial oxidation of ferric iron, which is good for a green technology compared to chemical ferric ion because chemical iron upon oxidation will reduce to chemical ferrous iron which will never be oxidized back to ferric iron for continuation of ferric oxidation of minerals in the system, whereas in the biogenic ferric ion upon oxidation of minerals will reduce to ferrous iron and can further oxidized to biogenic ferric ion by the microbial ferrous oxidation to biogenic ferric iron which will provide a continuation of mineral oxidation until all the minerals are exhausted or oxidized completely naturally by the act of microorganisms, which is an advantage in itself especially in bioleaching. Further, the growth characteristic of mixed consortia, including a profile of pH, cell count, and redox potential, and the influence of bioleaching on PCB powder (both the initial feed and the residue after bioleaching) was investigated during the two-step bioleaching approach. Furthermore, a kinetic model was employed to identify the limiting step in metal leaching and determine the kinetic order of the copper leaching reaction.

MATERIAL AND METHODS

Sample collection, preparation, and characterization of Mobile phone PCBs

The present study used discarded mobile phone PCBs as an e-waste feed sample. The discarded mobile phones of various brands were collected from various local electronics shops in the Alwar district in Rajasthan, India. The phones were manually dismantled, which resulted in approximately 5.0 Kg of PCBs. The PCBs separated were crushed and ground by an impact crusher to an acceptable size of 250 microns (d80 = 250 μm) at the Mineral Processing department of CSIR-Institute of Minerals and Materials Technology (IMMT), Bhubaneswar, Odisha, India. The powder sample was thoroughly mixed and divided using the coning and quartering method to obtain sample homogeneity. The same sample was further analyzed for its metal content and their mineralogy.

Microbial culture and growth conditions

The microbial culture used in the experiments was obtained from the Hydrometallurgical Laboratory of Lulea University of Technology, Sweden and was a mixed culture of iron-oxidizing microorganisms dominated by *Leptospirillum ferriphilum* **(Gahan et al., 2009).** This mixed culture of iron oxidising microorganisms dominated by *Leptospirillum ferriphilum* was cultivated in 9K growth medium comprising of 3.0 g/L $(NH_4)_2SO_4$, 0.1 g/L KCl, 0.5 g/L K_2HPO_4, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.01 g/L $Ca(NO_3)_2{\cdot}4H_2O$ supplemented with 9 g Fe^{2+}/L (44.8 g/L $FeSO_4.7H_2O$) with 20% (v/v) inoculum in 80% (v/v) 9K Medium at a pH of 1.5 **(Silverman & Lundgren, 1959).** The pH of the solution was measured by a pH meter (EUTECH) with a plastic-body single junction gel-filled pH combination electrode and a temperature probe. Three-point pH meter calibration was carried out with pH 1.68, 4.01, and 7.0 buffer solutions to avoid any error in the pH readings due to low pH sensitivity. The slope value of the calibrated pH meter was always maintained between 100±5 for accuracy. The pH of the solution was controlled to 1.5±0.5 by the addition of 2M/5M H_2SO_4 regularly when required. The microbial growth was followed by measurement of Oxidation-Reduction Potential (ORP) or redox potential two to three times at different intervals daily, and ferrous iron was oxidized to ferric irons, resulting in an increasing trend in redox potential. The Redox potential was always measured immediately after adjusting the pH to 1.5 to ensure correct redox potential measurements. The ORP meter (EUTECH) with a platinum electrode with an Ag/AgCl reference electrode was used for redox potential measurement. The microbial population determined by Quantitative Polymerase Chain Reaction (qPCR) analysis from Bioclear B.V., Groningen, Netherlands, revealed the dominance of *Leptospirillum ferriphilum* (2.1 x 10^8 cells mL^{-1}) along with smaller amount of *Sulfobacillus sp.* (7.3 x 10^5 cells mL^{-1}), *Acidithiobacillus sp.* (4.9 x10^4 cells mL^{-1}), and *Acidithiobacillus thiooxidans* (2.6 x 10^4 cells mL^{-1}). The mixed culture of iron-oxidizing microorganisms dominated by *Leptospirillum ferriphilum* was repeatedly sub-cultured until minimum doubling time was attained; thereafter, this microbial culture was used as an inoculum for all the bioleaching experiments conducted in this study.

Batch Bioreactor Configuration

The microbial culture and bioleaching experiments were carried out in a 2000 mL borosilicate glass bioreactor with a working volume of 1000 mL mounted by a mechanical overhead stirrer (RIVOTEK) at 220 rpm connected with Polytetrafluoroethylene (PTFE) coated stainless steel straight four-blade turbine impeller for homogenous mixing of the mass and temperature solution. A dissolved oxygen level of 5 mg/L was maintained by blowing air at a flow rate of 3 L/min at the bottom of the reactor using an aquarium air pump to meet the CO2 and O2 requirements. Two baffles [14 cm (L) x 4 cm (B) x 1 cm (W)] made of acrylic material were mounted opposite to each other and perpendicular to the wall (inside) of the bioreactor to avoid vortex formation and achieve homogenous heat and mass transfer in the solution. A temperature of 35±5 °C was maintained in the bioreactor by placing a hot plate (IKA) beneath the borosilicate glass reactor, and the temperature was regulated as required to maintain a uniform temperature.

Bioleaching Experiment

Generation of biogenic ferric ion

The biogenic ferric iron (Fe^{3+}) was generated by the bio-oxidation of ferrous at determined concentrations of 9, 13.5, and 18 g/L by the iron-oxidizing microbial consortium dominated by *Leptospirillum ferriphilum* in basal salt medium (0K) as discussed above supplemented with varying concentrations of Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$), i.e., 44.82, 67.23, and 89.64 g/L^{-1} for 9K, 13.5K and 18K medium respectively, in the first step of experiment. A batch reactor was used for the culture activation to prepare biogenic Fe^{3+}. As discussed above, the bioreactor setup used for generating biogenic ferric ions is the same setup used for microbial culture growth. At the end of the culture activation, determined by the redox value >700mV, the respective concentration of biogenic ferric was achieved. This activated microbial culture, which has biogenic ferric, was used to bioleaching mobile phone PCBs.

The microbial cell population of inoculum for culture activation was 1.73 × 10^7 cells mL^{-1} as determined by microscopic cell count under Bright field compound microscope at a magnification of 1000x (10x eyepiece lens and 100x objective lens) using immersion oil on a Neubauer hemocytometer (0.1 mm depth and 0.0025 mm^2 area). The biogenic ferric ion generated from 9K, 13.5K, and 18K experiments had the ferric iron concentration of 9, 13.5, and 18 g/L respectively, at a pH value of 1.5. Each culture activation for 9K, 13.5K, and 18K biogenic ferric generation was repeated 4-5 times until minimum doubling time was achieved. The shortest time required for the complete oxidation of ferrous ions in 9K, 13.5K, and 18K experiments was 21 h, 24 h, and 26 h, respectively. The biogenic ferric ion generated from the 9K, 13.5K, and 18K experiments was used as a lixiviant for the bioleaching of mobile phone PCBs.

Two-step bioleaching of mobile phone PCBs

The bioleaching experiment for mobile phone PCBs was conducted in two-step mode in a 2000 mL batch bioreactor with a working volume of 800 mL. In the first leaching step of the experiment, 80 g of pulverized mobile phone PCBs (particle size < 250 μm) was introduced into 800 mL of biogenic ferric iron solution, achieving a P.D. of 10% (w/v). Deionized water was added daily to the bioreactor to compensate for water loss due to evaporation and maintain a constant volume of 800 mL. The pH of the bioleaching solution was carefully controlled at 1.5 by adding 2M or 5M H_2SO_4 as required. The total cumulative acid additions made during each experiment (9K, 13.5K, and 18K) were used to calculate and determine the amount of kg concentrated (conc.) H_2SO_4 per ton of mobile phone PCBs. The redox potential was measured twice or thrice daily to follow the ferric consumption trend and oxidation-reduction reaction during bioleaching. The microbial cell count of planktonic viable cells in the bioleaching pulp was carried out daily by withdrawing samples from the bioreactor. Metal concentration in the bioleaching pulp for Cu, Ni, and Zn was analyzed by Atomic Absorption Spectroscopy (AAS) (Thermos Scientific iCE 3000 Series) for the determination of their leaching kinetics and follow the leaching trend in the bioreactor. AAS measured total iron concentration in the bioleaching solution, and the ferrous iron concentration was determined by titrimetric method using cerium sulfate as titrant and 1, 10-phenanthroline as an indicator **(Kolthoff & Sandel, 1963).** The experiments were carried out until a stable metal concentration (Cu, Ni, Zn) and redox potential was achieved. Thereafter, the stirring of the reactor was stopped, and the solid particles in the slurry were allowed to settle down for 1 h; then 640 mL (80% of working volume) of bioleach liquor was removed from the bioreactor and replaced with 640 mL of 9K, 13.5K, and 18K growth medium in respective experiments for the second step.

The second step, bioleaching, was started immediately after replenishing 9K, 13.5K, and 18K growth medium to the bioreactor. As per the experiment, the objective of replacing the bioleach liquor with a fresh nutrient medium was to avoid eventual metal toxicity caused to the microbes and to avail fresh nutrients for their luxuriant growth, resulting in an improved leaching yield. The pH was maintained at 1.5 by the addition of 2M/5M H_2SO_4. Other parameters include redox potential, viable planktonic cell count, Fe^{2+} concentration, Fe (Total) concentration, Cu, Ni, and Zn concentrations, and amount of conc. H_2SO_4 required per ton of mobile phone PCBs was measured and determined by similar methods adopted in the first step of bioleaching. Finally, the second bioleaching was harvested only after stable redox potential and stable metal (Cu, Ni, Zn) concentration was observed in the bioleaching pulp. Solid/Liquid (S/L) separation of the bioleaching slurry/pulp was carried out upon harvesting of an experiment by filtration using a 1 L borosilicate filtering Erlenmeyer flask with Buchner porcelain funnel of 110 mm diameter connected to a water jet aspirator vacuum pump (Cole -Parmer). At the end of the filtration, the bioleach residue cake was thoroughly washed with 100 ml of acidified distilled water (by adding 5M H_2SO_4) with a pH of 1.5. The purpose of washing the filter cake with pH 1.5 acidified water was to avoid precipitation of residual metal ions or ferrous or ferric iron in the filter cake. Further, the bioleach residue was dried at 60 °C for 48 h until no change in dry weight was observed. Finally, the dry bioleach residue was ground using porcelain mortar and pestle and subjected to elemental analysis by XRF and mineralogy analysis XRD and FESEM. The weight of the feed sample, residue, and leach liquor is summarized in Table 1.

Table 1 Summary of Biogenic ferric ion bioleaching experiment

Leaching parameters	**Experiments**		
	9K	**13.5K**	**18K**
Weight of feed (Mobile phone PCBs), g	80	80	80
Weight of bioleach residue wt., g	53.92	56.09	54.25
Volume of bioleach Liquor, mL	0.74	0.78	0.74
Weight Loss after bioleaching (%)	32.6	29.9	32.2
Acid Consumption, kg Conc. H_2SO_4 per Ton Mobile phone PCBs	336.6	334.5	382.7

The leaching yield percentage of the two-step bioleaching experiment was calculated by the formula given in Eq.1 after the completion of the leaching experiment.

$$\text{Leaching Yield}(\%) = \left[\frac{(\mathit{Metal\ content\ in\ the\ feed} - \mathit{Metal\ content\ in\ the\ bioleach\ residue})}{\mathit{Metal\ content\ in\ the\ feed}}\right] \times 100 \qquad (1)$$

RESULTS AND DISCUSSIONS

Material Composition and Characterization

The elemental analysis of pulverized mobile phone PCBs sample was performed by an X-ray fluorescence (XRF) analyzer (Bruker), which resulted in a significant content of 26.3% (Cu), 21.7% (Si), and 14.7% (Ca) and a less significant amount of Br, Al, Fe, and Ba together with other elements such as Sn, Ni, P, and Zn etc. as shown in Table 1

Table 2 Elemental composition of mobile phone PCBs (wt%)

Mg	Al	Si	P	Cl	K	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn	Br	Sr	Ag	Sn	Sb	Ba	Ta	Au	Pb
0.4	6.1	21.7	1.2	0.2	0.1	14.7	0.8	0.3	0.1	5.3	1.4	26.3	1.1	9.6	0.1	0.4	3.1	0.3	4.3	0.3	0.1	0.7

The mineralogy of the pulverized mobile phone PCBs and bioleached residues was carried out by powder X-ray diffraction (XRD) using a PAN-analytical X-ray diffractometer equipped with a continuous scanning device Cu Kα radiation of 40 kV and 30 mA with a sample rotation of 30 rpm. Diffraction patterns were measured from 5° to 75° with a resolution of 0.02 angle/second, whereas the crystalline phases were identified using the instrument's Joint Committee for Powder Diffraction Standards (JCPDS) file. The dominating mineralogical phases identified in the powder mobile phone PCBs were Cu^0, $(Cu_{0.3}Fe_{0.7})$, (FeNi), Zn^0, and SiO_2 (Figure 1) **(Marra et al., 2018; Erust et al., 2020).**

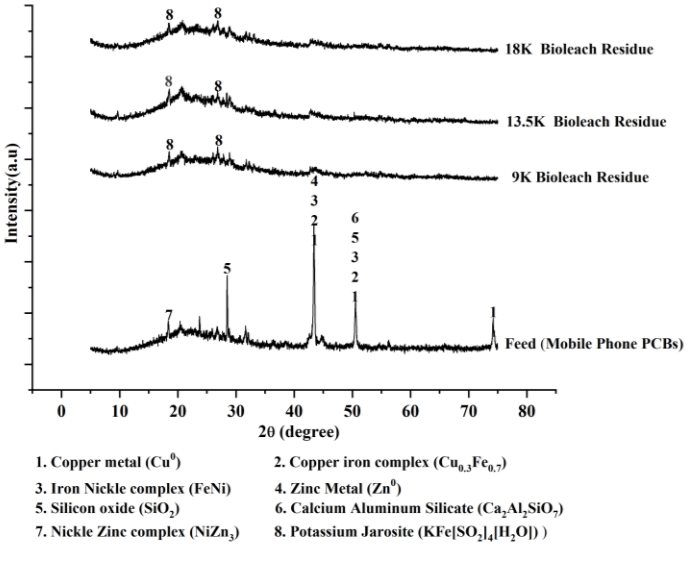


Figure 1 X-Ray diffractogram of Pulverized mobile phone PCBs and bioleach residue of 9K, 13.5K, and 18K experiments.

However, the XRD study outcomes matched the feed sample's XRF analysis (Table 2), showing Cu presence. The XRD pattern of the feed sample peak reveals that two distinct peaks at 44° and 51° are related to Cu, which was similar to the observation made in waste PCBs powder samples of mobile phones in other studies (Trivedi & Hait, 2021). Field Emission Scanning Electron Microscopy (FESEM) of the powder of mobile phone PCBs was carried out by FEI- Nova Nano SEM 450 model Scanning Electron Microscope (Figure 2).

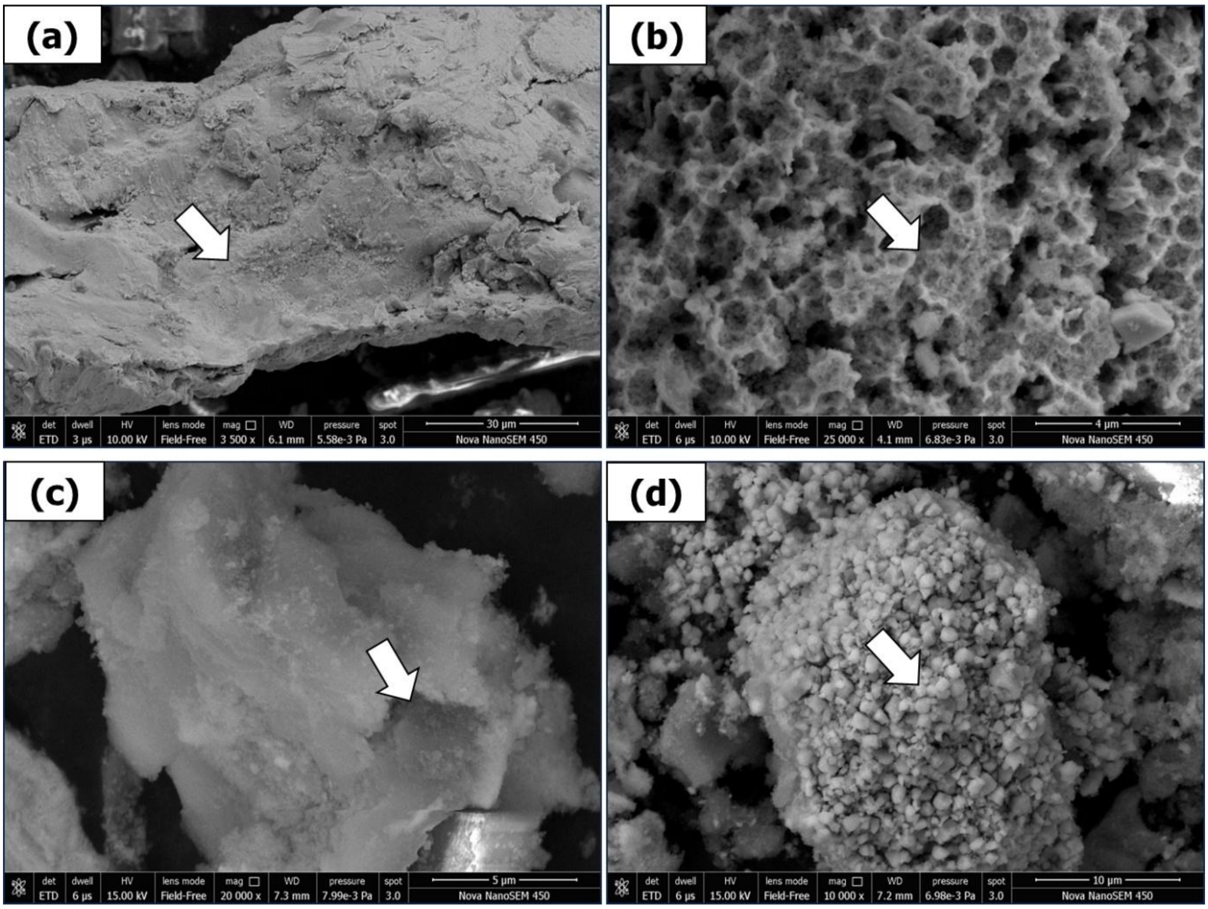


Figure 2 FESEM micrograph of **(a)** feed material of mobile phone PCBs and Bioleach residue from the experiment **(b)** 9K **(c)** 13.5K **(d)**18K

Production of Biogenic Ferric Ion

The biogenic ferric iron solution for 9K, 13.5K, and 18K bioleaching experiments were generated in 9K, 13.5K, and 18K nutrient growth medium, respectively. The oxidation of Fe^{2+} to biogenic Fe^{3+} was facilitated by a microbial consortium dominated by *Leptospirillum ferriphilum*. This microbial-mediated oxidation of ferrous iron is a proton-consuming process (Eq. 2), leading to an increase in the pH of the growth medium. To maintain the pH at 1.5, 5 M H_2SO_4 was added incrementally, with cumulative additions of 6 mL, 8 mL, and 10 mL required for the 9K, 13.5K, and 18K growth media, respectively.

$$4Fe^{2+} + 4H^{+} + O_2 \xrightarrow{\text{Bacteria}} 4Fe^{3+} + 2H_2O \qquad (2)$$

The iron oxidation profile was monitored and followed by the trend of the Redox potential of the growth medium from the start of the experiment till completion. The increasing trend in ferric iron concentration in the growth medium correlated with the rising ferric iron concentration throughout the experiment. However, the ferrous iron concentration determined the growth rates in accordance with redox potential, and the rate of ferrous iron oxidation for 9K, 13.5K, and 18K were found to be 0.333 g Fe^{2+} L^{-1} h^{-1}, 0.466 g Fe^{2+} L^{-1} h^{-1}, and 0.563 g Fe^{2+} L^{-1} h^{-1} respectively. The shortest time to complete the oxidation of ferrous iron into biogenic ferric iron for 9K, 13.5K, and 18K growth medium were 27 h, 29 h, and 32 h, respectively. The ferrous iron concentration in the growth medium was found to be zero at the end of the microbial growth as determined by the titrimetric method, which validated the complete oxidation of ferrous iron in the growth medium. Two different types of microbial cells, namely planktonic cells (freely moving in the solution) and sessile cells (attached to the wall, stirrer, and baffles in the reactor), are responsible for the oxidation of ferrous iron into ferric iron. The viable planktonic cell count determined by microscopic observations resulted in 9 x10^7 cells mL^{-1} at the beginning of the microbial growth and increased to 4.5 x 10^8, 5.5 x 10^8, and 4.5 x 10^8 cells mL-1 for 9K, 13.5K, and 18K growth medium, respectively. The cell counts slightly decreased for the 18K medium, but interestingly, the cell elongation was observed compared to cells grown in 9K and 13.5K growth medium. The possible reason could be due to high substrate concentration and in feed resulting in high ionic strength as calculated from the electrical conductivity data measured from both the feed of 9K, 13.5K, and 18K growth medium and biogenic ferric solution. Studies conducted by Arias et al 2024 suggest that filamentation of *L. ferriphilum* in response to sulfate could be a strategy which enables bacteria to survive in challenging environmental conditions by temporarily halting cell division and elongating their shape. This adaptation would allow them to better handle osmotic imbalances until conditions become more favorable for optimal growth and cell division **(Ariaska et al., 2024; Nepal and Kumar, 2020).** The electrical conductivity measured for the 9K, 13.5K, and 18K growth medium were 47740, 59520, 71300 µS/cm, and the biogenic ferric solution after oxidation was 16740, 21080, 25420 µS/cm. The ionic strength calculated from the electrical conductivity for the 9K, 13.5K, and 18K growth medium and the biogenic ferric solution was 0.77, 0.96, 1.15 mol L-1, and 0.27, 0.34, 0.41 mol L^{-1}, respectively. Interestingly, a drastic drop of 65% in the ionic strength was observed in the biogenic ferric iron compared to their respective 9K, 13.5K, and 18K growth medium. This drop in ionic strength could be due to stable ferric sulfate complexes forming upon bio-oxidation of ferrous sulfate when the solution was oxidized **(Sundkvist et al., 2008).**

Investigation of the Two-Step Bioleaching of Mobile Phone PCBs

Bioprocess Parameters

pH dynamics and acid consumption

The initial and final pH profile in Figure 3 illustrates a continuous increase in pH based on daily measurements attributed to the consumption of acid or ferric ions during the leaching of metal ions from the metal oxides/metals present in mobile phone PCBs. In all three experiments with 9K, 13.5K, and 18K, the pH was maintained at 1.5 using 5M H_2SO_4, creating a favorable environment for microbial activity and preventing iron precipitation.

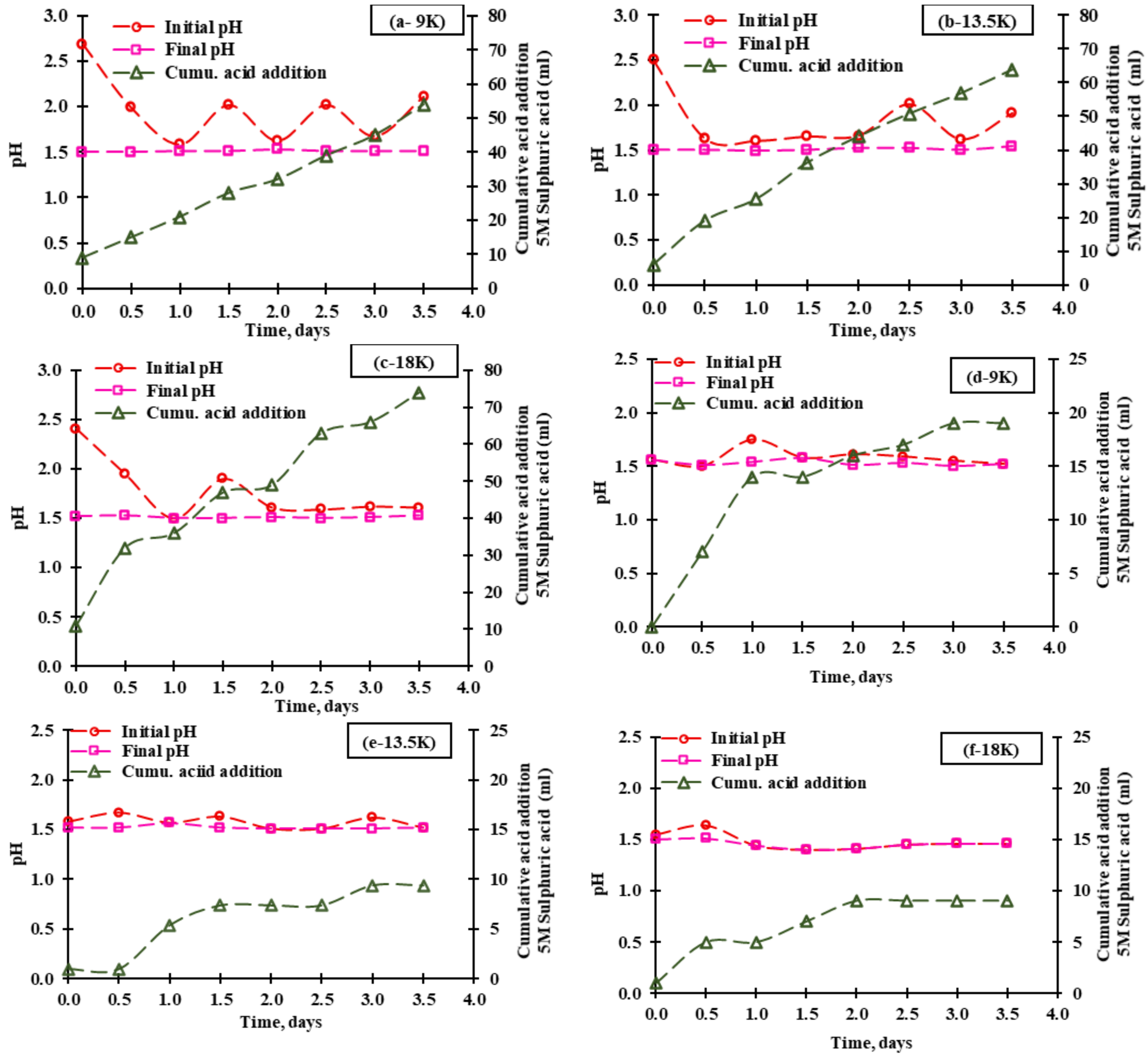


Figure 3 Initial and final pH profile along with cumulative addition of 5M H_2SO_4 using biogenic Fe^{3+} in the first-step bioleaching **(a)** 9K **(b)** 13.5K **(c)**18K growth medium; Second-step bioleaching **(d)** 9K **(e)** 13.5K **(f)** 18K growth medium.

Initially, adding feed material to the biogenic ferric solution in the first bioleaching step caused a notable rise in pH (Figure 3a-c), reaching approximately 2.5 ± 0.2 due to acid neutralization during the dissolution of metals such as copper, nickel, and zinc (Eq. 3) (**(Dave et al., 2018),** and acid-neutralizing gangue minerals (Yang et al., 2009; Arshadi & Mousavi, 2014). Furthermore, the bacterial-mediated oxidation of ferrous iron (Eq. 2) also consumed acid.

$$M^{o} + \frac{n}{2}O_2 + nH^{+} \rightarrow M^{n+} + \frac{n}{2}H_2O \qquad (3)$$

Following this, a slight decrease in pH (without acid addition) was observed, likely attributed to a reduction in the rate of acid-consuming reactions **(Rastegar et al., 2016).** In the second leaching step, there was only a slight increase on the first day in all the experiments, and no significant changes were observed in the pH afterward (figure 3d-f).

The cumulative acid addition varied between the experiments, with the highest addition observed in the 9K experiment during the second step and the 18K experiment during the first step. The final consumption of concentrated H_2SO_4 showed significant variations across experiments with different ferric ion (Fe^{3+}) concentrations, increasing proportionally with higher biogenic Fe^{3+} levels (Brandl et al., 2001). Notably, the 18K bioleaching experiment, which had the highest ferric iron concentration (18 g/L), exhibited the highest total H_2SO_4 consumption, reaching 382.71 kg per ton of discarded mobile phone PCBs, as summarized in **Table 1.**

Redox and iron profile

In a bioleaching system, the ratio of ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron, known as the redox value, is a crucial indicator of microbial activity **(Ballor et al., 2006; Sapieszko et al., 1977).** The redox potential of the biogenic ferric solution started to decrease or drop from 700 mV to nearly 100 mV due to the consumption of ferric ions for oxidation of the metal complexes of the mobile phone PCBs **(Ilyas et al., 2013; Akbari & Ahmadi, 2019).** This process reduced Fe^{3+} to Fe^{2+}, leading to a redox potential drop as predicted by the Nernst equation where oxidation-reduction potential or redox potential decreases with the decrease oxidized species (Fe^{3+}) and an increase in reduced species (Fe^{2+}) as stated in (Eq. 4).

$$E = E^{0} + \frac{RT}{nF}\ln\frac{[Fe^{3+}]}{[Fe^{2+}]} \qquad (4)$$

The oxidation-reduction reaction depended on the variable concentration of ferric ions ([Fe^{3+}]). At the same time, other parameters like the standard electrode potential (E_o), gas constant (R), temperature (T), Avogadro's number (n), and Faraday's constant (F) remained constant. The iron profile confirmed ferric reduction, with ferric concentrations of ~1, 2, and 3 g/L in 9K, 13.5K, and 18K, respectively, due to its reduction into ferrous ions during metal oxidation. After 2.5 days, the pH stabilized below 1.65, while redox potential increased to 355 and 382 mV in 13.5K and 18K, respectively. In contrast, 9K showed lower redox potential (~144 mV) due to microbial inhibition caused by metal toxicity, reducing viable cell counts **(García-Balboa et al., 2022)**. In the second bioleaching step, 13.5K and 18K displayed stable redox trends, while 9K exhibited a distinctive peak exhibited in Figure 4.

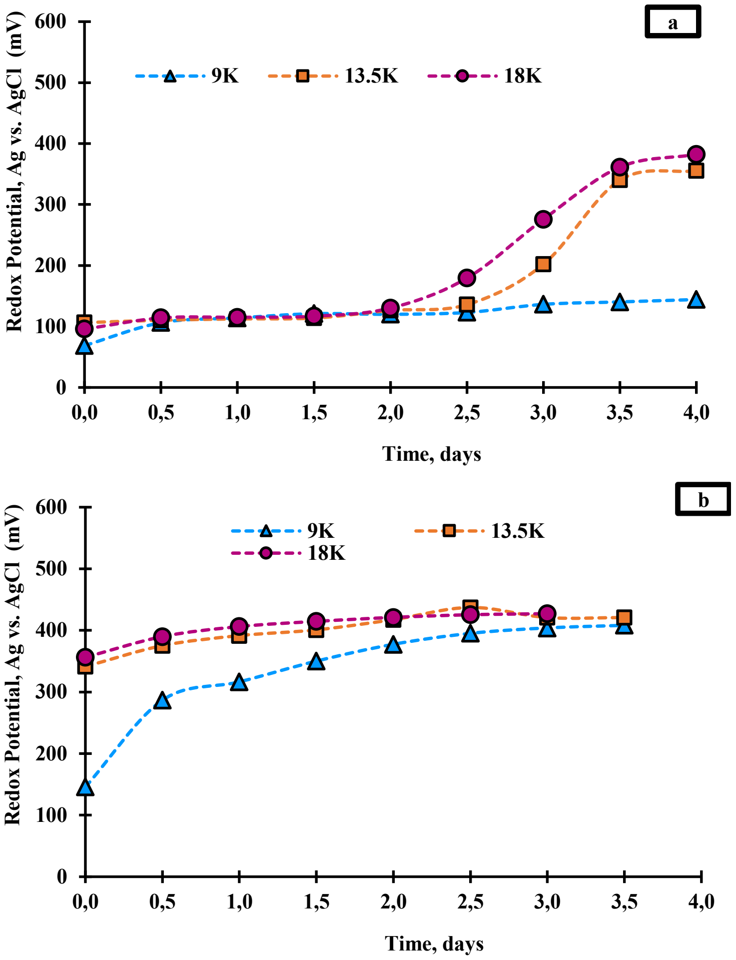
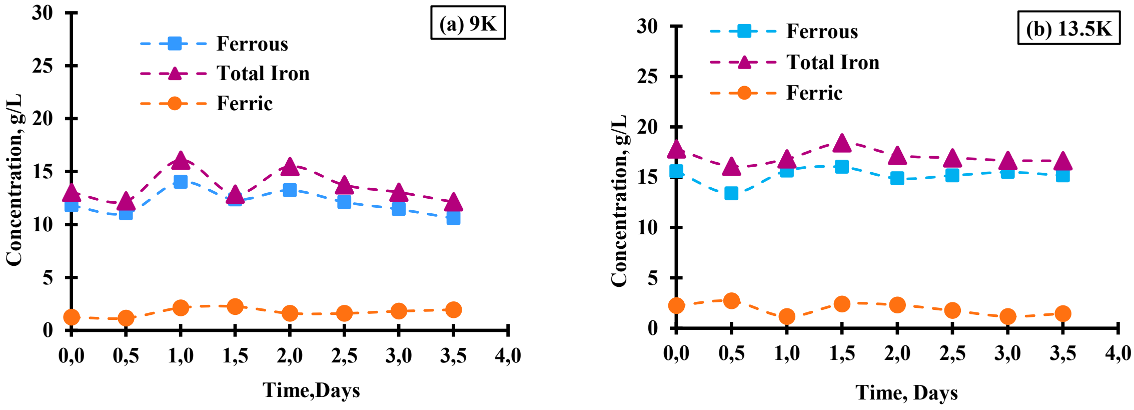


Figure 4 Redox potential trend during biogenic ferric ion bioleaching in 9K, 13.5K, and 18K experiment **(a)** First-step bioleaching; **(b)** Second-step bioleaching.

The maximum redox potential in all experiments (~400 mV) suggested incomplete ferrous oxidation, likely caused by microbial activity limitations, iron oxidation constraints, or restricted ferrous availability. The lixiviant in these experiments contained 9, 13.5, and 18 g/L of ferric ions, which were reduced to ferrous ions during mineral oxidation, resulting in Fe^{2+} concentrations of 11.78, 15.56, and 21.04 g/L on the first day in first bioleaching step (Figure 5a-c). Fe^{2+} trend in the 9K and 13.5K showed a slight increase from day 1 to day 2 but later decreased or stabilized, whereas, in 18K, the Fe^{2+} concentration rose from 21 g/L on day 1 to 23 g/L on day 2 before stabilizing at approximately 18–19 g/L. The higher dissolution of the feed material in 18K explains the trend of Fe^{2+} concentration, which decreased in the order 18K > 13.5K > 9K, as shown in Figures 5a-c.



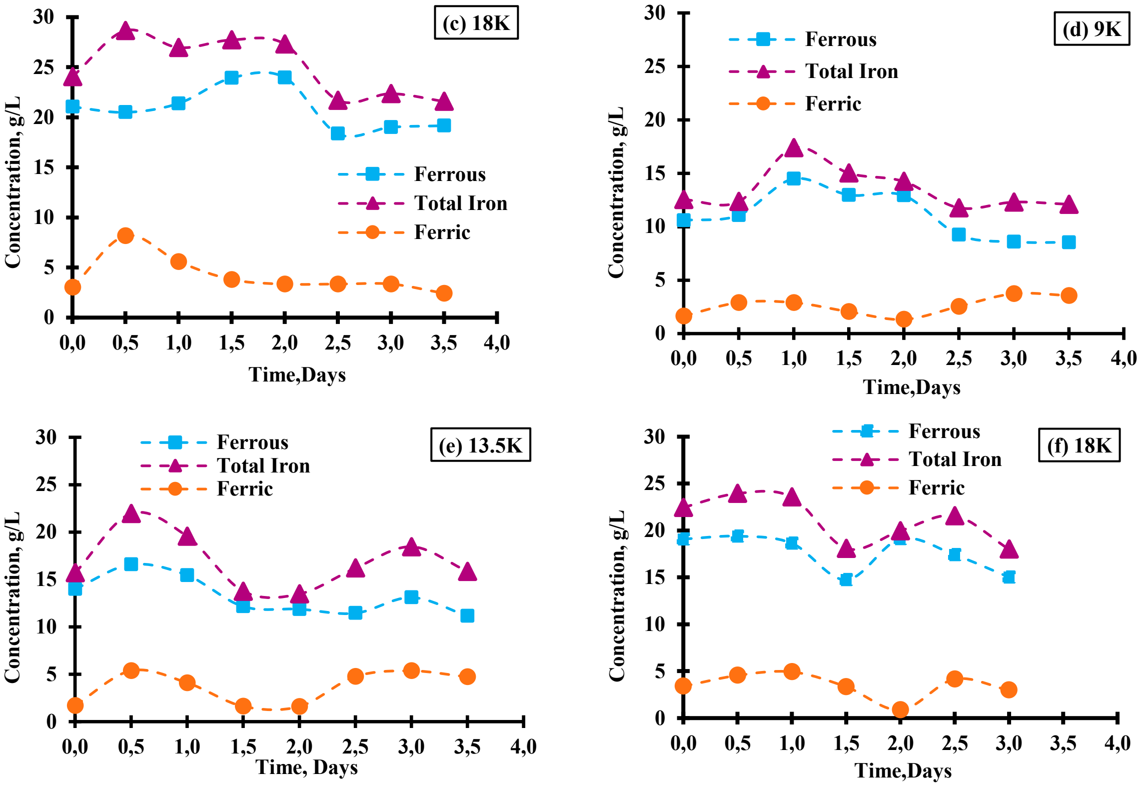


Figure 5 Trend of Fe^{2+} ion, Fe^{3+} ion, and Fe (total) during first-step biogenic ferric leaching in experiment **(a)** 9K **(b)** 13.5K **(c)** 18K; Second-step biogenic ferric leaching in experiment **(d)** 9K **(e)** 13.5K **(f)** 18K.

A significant decrease in the Fe^{3+} ion trend in the case of the 18K experiment was observed compared to the decreasing trend in the 9K and 13.5K experiments. In the second leaching step, no significant change was observed in the Fe^{2+}, Fe^{3+}, and total iron trend except a decrease in Fe^{3+} concentration on 2nd day of all the experiments 9K, 13.5K, and 18K (figure 5d-f). The possible reason for the decrease in Fe^{3+} ion concentration could be due to the precipitation of Fe^{3+} ion as jarosite (Eq.5), where jarosite formers such as potassium ion, ammonium ion, hydronium ion, sodium ions are plentily available in the growth medium are responsible for the jarosite formation in ferric rich solutions.

$$3Fe^{3+} + X^{+} + 2SO_4^{2-} + 6H_2O \rightarrow XFe_3(SO_4)_2(OH)_6 + 6H^{+}$$

[$X^+ = K^+, Na^+, H_3O^+, NH_4^+$ ….] (5)

The mineralogical studies of bioleach residue by X-ray diffraction confirm the presence of jarosite and is in occurrence with the Fe^{3+} ion precipitation as jarosite (Figure 1). The total iron concentration was also in coherence with the Fe^{2+} ion as well as Fe^{3+} ion concentration trend, stating that whatever decline in Fe^{3+} ion from the solution was not reduced to Fe^{2+} ion but rather precipitation in the form of jarosite or any other ferric hydroxide.

Microbial dynamics in bioleaching experiment

The histogram in Figure 6 illustrates the dynamics of planktonic microbial populations in bioleaching experiments conducted with varying concentrations of biogenic ferric (9K, 13.5K, 18K). The addition of feed sample into a bioleaching reactor initially results in an immediate decrease in bacterial populations, possibly due to stress of metals exceeding the tolerance of bacterial membranes (Figure 6a) **(Dopson et al., 2003),** coupled with limited gas diffusion at higher pulp densities **(Lee et al., 2015).** The low cell counts reflect microbial environmental adaptation, potentially involving gradual attachment to metal-containing minerals. Following adaptation, these microorganisms oxidize ferrous iron to ferric, maintaining high ferric concentrations and facilitating metal dissolution **(Arshadi et al., 2015; Nagar et al., 2019).**

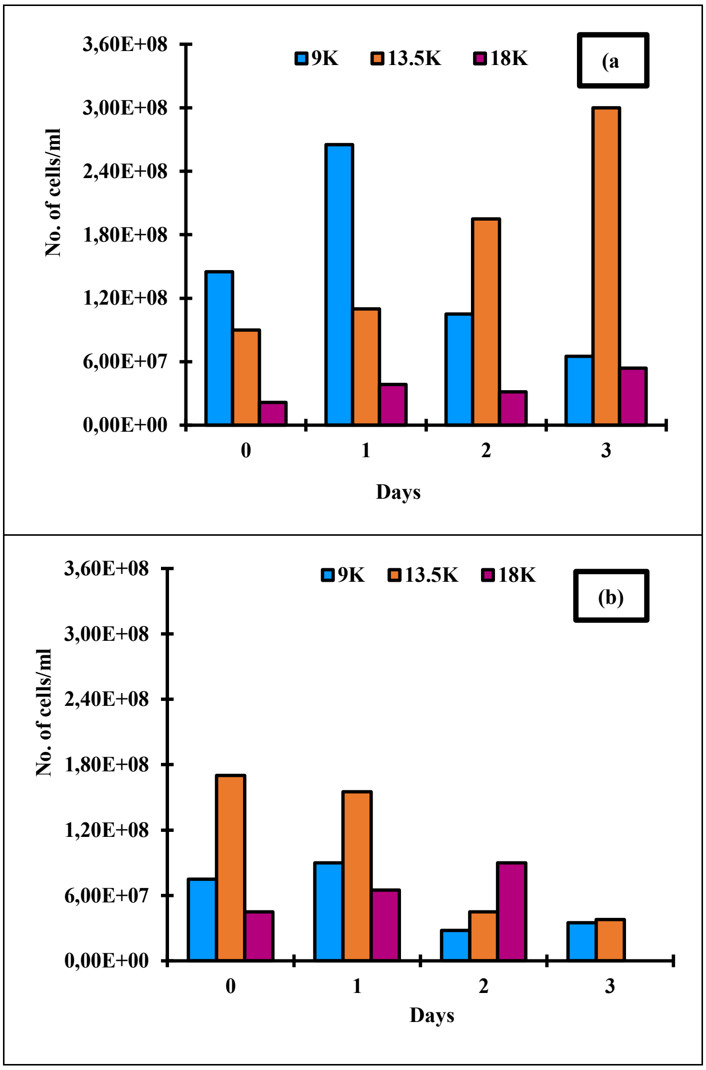


Figure 6 Viable planktonic cell count profile during biogenic ferric ion **(a)** First-

step leaching; **(b)** Second-step leaching

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The high feed concentration possibly created a harsh environment for microbes, leading to a significant decrease in bacterial population and, consequently, a reduction in biogenic ferric production **(Haghshenas et al., 2009).** This cell number trend was similar in first and second-step bioleaching (Figures 6a & b).

Characterization of feed and bioleached residues of bioleaching experiments

However, the XRD diffractogram of bioleached residues shows that the peak of Cu completely disappeared due to the high extraction efficiency of Cu achieved under optimal conditions during biogenic ferric leaching. Moreover, after bioleaching, the presence of excess ferric iron in the solution is more prone to precipitation as jarosite with monovalent cations (Figure 1) **(Watling, 2006).**

Figure 2 shows the variation in e-waste surface morphology before and after bioleaching. The initial sample photomicrograph (Figure 2a) indicates the presence of a heterogeneous mixture of particles with different sizes and textures. Before bioleaching, the powder surface was seen to be almost smooth. The impact of the bioleaching process on the surface of the particles is presented in Figure 2b-d for experiments 9K, 13.5K, and 18K, respectively. The photomicrograph obtained after bioleaching showed considerable changes in shape, size, and morphological structures with reduced particle size and increased surface porosity, also reported by **Maluleke et al. (2024).** The oxidation-reduction process of iron and microbial metabolites causes the acidic environment to erode the powdered e-waste **(Rasoulnia et al., 2016).** Metal solubilization results from a corrosive chemical reaction on the particle surface **(Horeh et al., 2016).**

Trend of copper extraction from mobile phone PCBs

Figure 7(a) demonstrates that the two-step bioleaching process significantly enhances Cu dissolution from e-waste.

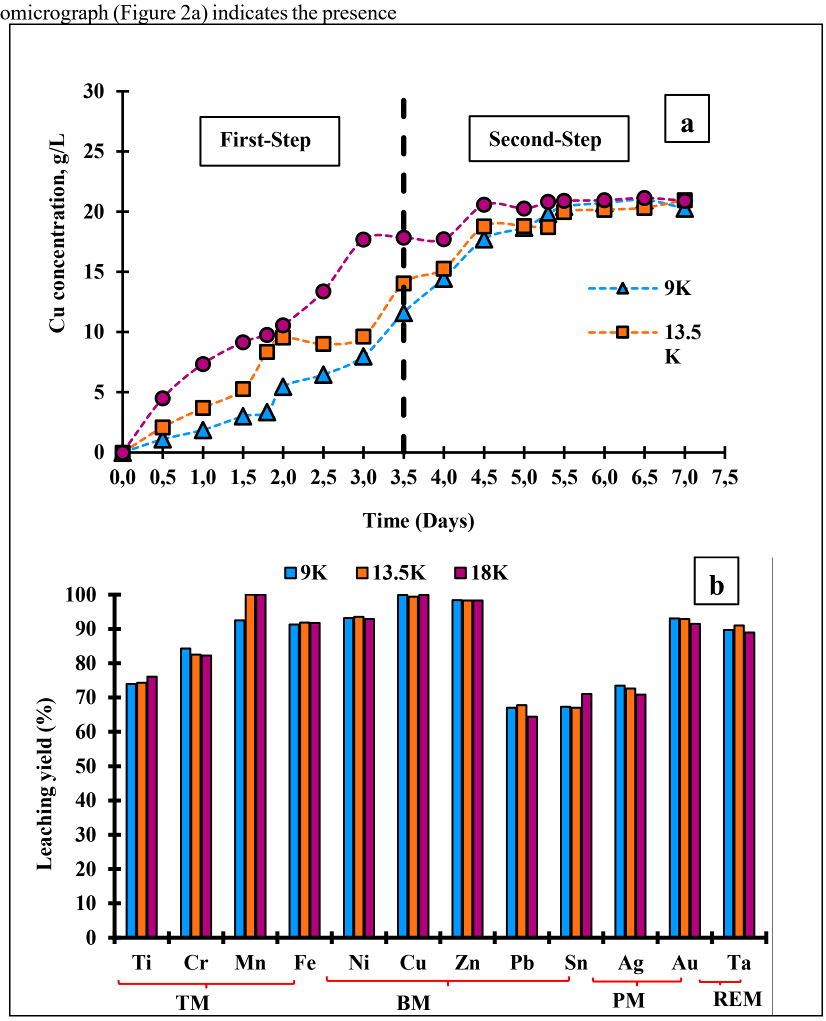

Figure 7 Metal leaching profile in first and second step biogenic Fe^{3+} ion leaching **(a)** Cu leaching in g/L vs. time; **(b)** Metal leaching yield (%) of metals of mobile phone PCBs sample (TM)- Transition Metals; (BM)- Base Metals; (PM)- Precious Metals; (REM)- Rare earth Metals

At a P.D. of 10% (100 g/L feed content), Cu extraction in the first leaching step was observed at concentrations of 10, 13, and 17 g/L in media containing biogenic ferric ions produced in 9K, 13.5K, and 18K, respectively. In the second leaching step, Cu recovery increased beyond 20 g/L, with the required timeframes reduced to 5.5, 4, and 3.5 days for 9K, 13.5K, and 18K media, respectively. The results suggest that higher biogenic Fe^{3+} concentrations significantly accelerate the Cu leaching process from mobile phone PCBs. However, 9 g/L biogenic Fe^{3+} achieved only 50% Cu leaching in the first step, likely due to metal toxicity effects. Introducing fresh medium in the second step led to a substantial increase in leaching efficiency, as depicted in Figure 7(a). During the initial phase, biogenic ferric ions (Fe^{3+}) and protons (H^+) attacked the metal lattice, initiating the following reaction:

$$Cu^0(s) + Fe^{3+} \rightarrow Cu^{2+} + Fe^{2+} \qquad (6)$$

This reaction facilitates the rapid dissolution of Cu^{2+} into the solution, while ferrous ions (Fe^{2+}) are subsequently oxidized back into ferric ions (Fe^{3+}) by functional bacteria. This process is accompanied by the generation of H_2SO_4, indicating that biological enzymatic activity accelerates the reaction rate. The resulting cyclical mechanism effectively enhances Cu recovery, as **Vargas et al. (2020)** described.

Analysis of Leaching yield of different metals in mobile phone PCBs

Bioleaching efficiency is intrinsically linked to the interaction between metal species and the powdered surface of e-waste. Copper (Cu), which predominantly exists in its elemental form, has its extraction rate significantly influenced by bacterial catalytic oxidation **(Deng et al., 2012).** Within the bioleaching system, bacteria directly oxidize Cu to release Cu^{2+}. At the same time, Fe^{3+} acts as an oxidizing agent to further facilitate the dissolution of other metals in the e-waste, including transition metals (TM), base metals (BM), precious metals (PM), and rare earth metals (REM). The interplay between the morphological and electrochemical characteristics of metals in mobile phone PCBs enhances metal recovery efficiency. The total leaching yield percentage (LY%) for TM, BM, PM, and REM at the end of the second leaching step was determined for 9K, 13.5K, and 18K conditions, as depicted in Figure 7(b). The leaching yield was calculated using Eq. 1, which quantifies metal extraction efficiency by comparing the metal concentrations in the feedstock and bioleached samples.

The leaching yields achieved in the present study for Cu, Ni, and Zn at the highest biogenic ferric-iron concentration (18 g/L) and pH 1.5 were consistent with results from other studies utilizing a two-step bioleaching approach. While some studies have achieved higher leaching yields of >98% copper, these often involve lower pulp densities and variations in PCB metal content (14.6- 25.1% for Cu), particle sizes, and types, which can affect leaching efficiency **(Bas et al., 2013; Işıldar et al., 2019; Hubau et al., 2020).** Previous studies have explored various methods to address the negative impact of e-waste from PCBs on bacterial activity during one-step bioleaching processes demonstrated in Table 3.

Table 3 Previous research on metal extraction yields from PCBs using one-step and two-step methods.

Metal sources and initial metal content (%)	**Bioleaching methods**	**Bacteria**	**Operation parameters**					**Metal removal efficiency (%)**	**References**
			pH	**T°C**	**Time**	**P.D (%).**	**Working volume (L)**		
Mobile phone PCBs Cu - 24.8	One-step	*A. ferrooxidans*	2.0	30	28 days	1	0.1	Cu- 94.8	**Chen et al., 2015**
Mobile phone PCBs Cu - 28.2	One-step	*A. ferrooxidans; L. ferroxidase; A. thiooxidans*	1.8	30	192h	10	1	Cu-97.3 Zn-66.8 Ni-79.3	**Erust et al., 2020**
Mobile phone PCBs Cu -14.58	Two-step continuous	*L. ferriphilum; Sulfobacillus benefaciens*	1.1	36	48h	1	2	Cu-96 Zn-85 Ni-73	**Hubau et al., 2020**
Mobile phone PCBs Cu - 58.7	Indirect non-contact	*A. ferrooxidans*	0.6-1.2	25	72h	1	0.1	Cu-86.17 Zn- 100 Ni- 100	**Van Yken et al., 2020**
Mobile phone PCBs Cu – 38.9	Indirect non-contact	*A. thiooxidans*	--	30	72h	1.6	--	Cu-98 Ni-82	**Kadivar et al., 2021**
PCBs (not specified)	Indirect non-contact	*Moderate thermophilic bacteria*	1.2	55	216h	10	0.1	Cu – 93.4	**Wu et al., 2018**
Mobile phone PCBs Cu - 17.6-23.01	Two-step	*A. ferrivorans; A. thiooxidans*	2.25	35	168h	1	0.1	Cu-96 Ni- 73 Zn- 85	**Işıldar et al., 2016**
Mobile phone PCBs Cu – 39.0	Two-step	*Acidithiobacillus ferrooxidans*	1.75	30	48h	0.75	0.35	Cu-100	**Benzal et al., 2020**
PCBs (not specified) Cu – 35.0	Two-step	Mixed Culture	1.8	37	120h	1	0.5	Cu- 68.2	**Magoda et al., 2024**
Mobile phone PCBs Cu-26.1	Two-step	*Leptospirillum* dominated culture	1.5	35	84h	10	1	Cu-99 Zn- 99 Ni- 70	This study

These strategies include PCBs pretreatment **(Zhu et al., 2011),** bacterial strain adaptation **(Arshadi & Mousavi, 2015),** and the implementation of a two-step approach **(Bryan et al., 2015).** Pretreatment methods can enhance leaching yields, including mechanical activation through milling **(Gu et al., 2019)** and washing or pre-leaching processes **(Xia et al.,2017).** Additionally, the concentration of biogenic ferric iron and the required leaching time play crucial roles in the leaching process **(Pourhossein & Mousavi, 2019).** The two-step process, involving pre-culturing bacteria under optimized conditions before introducing PCBs, has been shown to enhance leaching efficiency and reduce leaching time **(Shah et al., 2015; Hubau et al., 2020).** However, adding discarded mobile phone PCBs powder inevitably influences bacterial oxidation activity, decreasing leaching efficiency. To overcome this challenge, a novel approach is proposed in this investigation, involving periodic lixiviant removal and replenishment with fresh medium containing an energy source for microorganisms. To overcome this challenge, a novel approach proposed in this investigation of periodic lixiviant removal and replenishment with fresh medium containing an energy source for microorganisms successfully leached many metals present in the e-waste powder.

Bioleaching kinetics study of discarded mobile phone PCBs

A kinetics study is crucial for comprehending the nature and mechanism involved in bioleaching. Two kinetic models have been extensively studied for the heterogeneous reaction of dissolution of Cu leaching: diffusion-controlled and chemically controlled models. The models were utilized assuming that the ore is a homogeneous solid phase with a spherical shape and that the reaction proceeds at a constant temperature. These models consider the following steps which control the leaching process: first is diffusion of the lixiviant via the liquid film, second is diffusion of lixiviants over the solid film shell, third is between lixiviant and solid, a chemical reaction takes place, fourth is product (resultant species) diffusion through the solid and finally the last fifth step is a product diffusion through the liquid boundary film of the solution. The bioleaching process occurred under high mixing conditions; therefore, the initial and end steps were not considered rate-determining **(Levenspiel, 1998).** Hence, the rate-determining step can be identified as the third step **(Chen et al., 2015; Nagar et al., 2019).**

A chemical reaction is regulated in diffusion-controlled leaching (shrinking core model) by creating a porous layer of product on the particle surface to be leached. Compared to the diffusion of particles, the reaction happens at the solid surface more quickly. Therefore, using Eq.7, the diffusion and penetration of the product layer are estimated for the rate-determining phase.

$$1-\frac{2}{3}.\alpha-(1-\alpha)^{\frac{2}{3}}=\frac{2.M.D.C}{\beta.\rho.r_0^2}.t \qquad (7)$$

Here, α = Metals fraction leached, M = Molecular weight of solid, D = Diffusion constant, C = Leach solution concentration, t = Time, r_o = Original particle radius, ρ = Density, β = Stoichiometric coefficient. Likewise, the diffusion constant (D) can be determined by graphing the right-hand side of Eq.7 against time (t) (Figure 8a), with the assumption that M, C, ρ, and β remain constant.

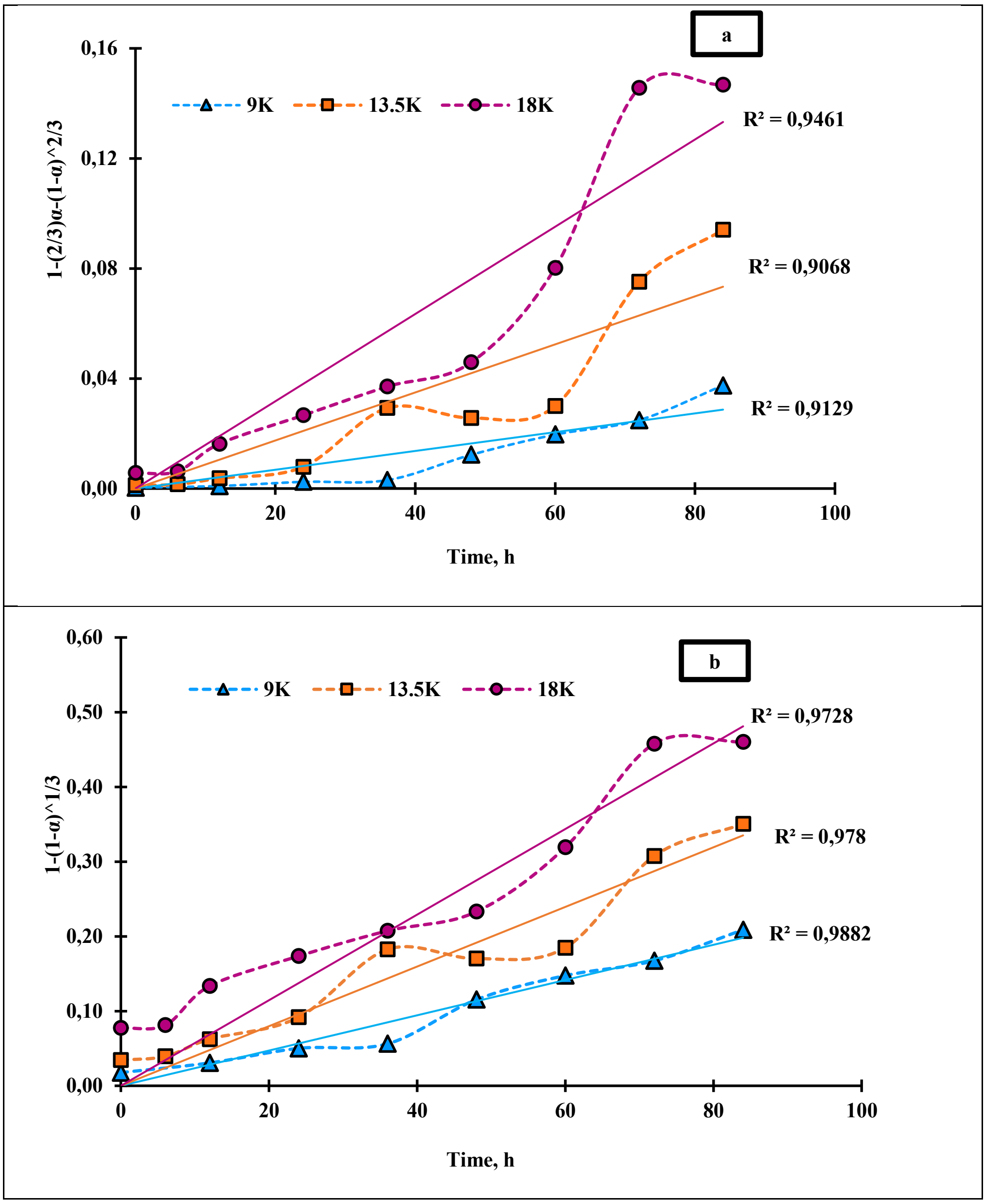

Figure 8 Leaching kinetics of copper during first-step bioleaching experiments **(a)** Diffusion controlled model; **(b)** Chemically controlled model.

In chemically controlled leaching (shrinking particle model), the chemical reaction is slower than the diffusion between a solid substance and the reactant. At the solid surface, the reactant concentration equals the solution's total amount. Chemically controlled leaching is expressed as Eq. 8.

$$1-(1-\alpha)^{\frac{1}{3}} = \frac{k.C}{r_o.\rho}.t \qquad (8)$$

Where α = Metal fraction leached, k = Rate kinetics constant, r_o = solid particle original radius, C = Leach solution concentration, ρ = Density, t = Time. Under the assumption that C, r, and r_o remain constant, the rate constant (k) can be determined by graphing the right-hand side of Eq.8 against time (t) (Figure 8b).

The leaching process of discarded mobile phone PCBs consistently adheres to a chemically controlled kinetic model across all concentrations of biogenic ferric iron. Chemically controlled kinetics (shrinking particle model) demonstrates a better fit with co-regression values of R^2 = 0.96, 0.94, and 0.97 in the 9K, 13.5K, and 18K experiments. In other words, the model suggests that chemical reactions on the surface of a solid limit the leaching rate, and no layer forms around the particles that would slow down the process.

Study of Rate of Reaction

The study of kinetic behavior or models is crucial for designing and scaling up the leaching process, as they characterize the rate of copper dissolution by biogenic ferric solutions as the lixiviant. Various reaction kinetic models have been explored to determine their appropriateness in describing the leaching process rate. Among the models considered are the first-order and second-order equations.

First-order kinetic model: - can be represented in linear form as follows:

$$\ln C = \ln C_0 - Kt \qquad (9)$$

where, C and C_0 = Leached element concentration at time t (hrs) and t = 0 respectively, and K= first-order rate constant (time^{-1} or per minute). The value of K can be determined from plots of lnC against t (as shown in Figure 9a) for copper.

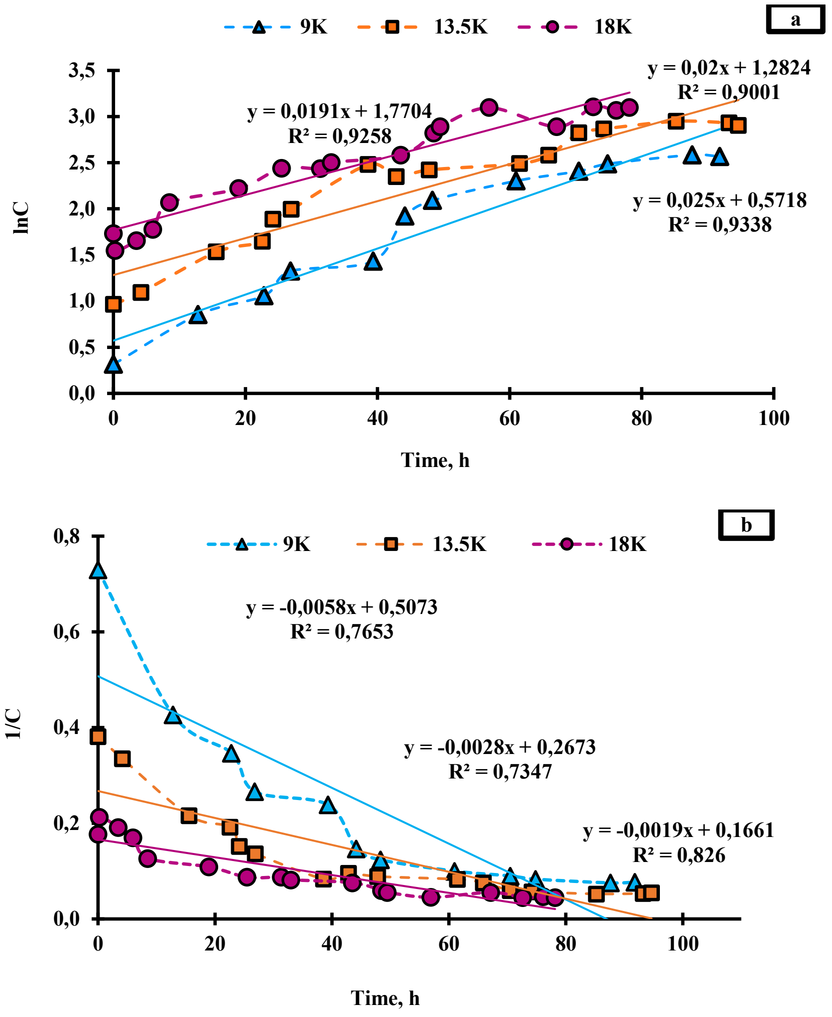

Figure 9 Rate of reaction of copper during first-step bioleaching experiments **(a)** First Order; **(b)** Second order reaction.

Second-order kinetic model: - can be expressed in linear form as follows:

$$\frac{1}{C} = C_0 + Kt \qquad (10)$$

Where C and C0 = dissolved metal concentration at time t (hrs) and t = 0, respectively, and K rate constant of second-order dissolution (L (mol min)$^{-1}$). The second-order kinetics rate constant, K, is determined from the slope of the plots (as depicted in Figure 9b). Eq. 10 illustrates the second-order reaction, which is graphed as a plot between the reciprocal of concentration (1/C) and time (t).

The correlation coefficients for the first-order kinetics in the 9K, 13.5K, and 18K experiments are 0.93, 0.90, and 0.92, respectively, with corresponding slope (k) values of 0.025, 0.020, and 0.019 in the first step bioleaching. Observation indicates that the first-order reaction kinetics were appropriate for copper leaching in the investigated leaching process **(Pradhan et al., 2011).** However, it was noted that the second step, bioleaching, did not exhibit the best fit in any of the reaction order plots.

CONCLUSION

The study investigates the impact of increasing biogenic ferric iron concentration on Cu leaching yield and kinetics in a two-step bioleaching process. Iron oxidizers were employed under optimal conditions of 10% P.D., temperature of 35 ± 5°C and pH 1.5 ± 0.5 with ferric iron concentrations of 9, 13.5, and 18 g/L for both steps of Bioleaching. The elemental analysis showed that Cu is 26 g/100 g of pulverized e-waste of discarded mobile phone PCBs. Thus, Cu leaching yield and kinetics were studied across all experiments. In the first step (9K, 13.5K, and 18K), recovery of Cu was 50%, 70%, and 83%, respectively. After the second step, Cu recovery exceeded 99% within 5.5, 4, and 3.5 days, respectively, in all the experiments. Increasing ferric concentration significantly reduces Cu leaching time from discarded mobile phone PCBs. Kinetic models, diffusion-controlled (shrinking core) and chemically controlled (shrinking particle), were applied, with the latter exhibiting better fit (R^2 = 0.96, 0.94, and 0.97 for 9K, 13.5K, and 18K, respectively), indicating surface chemical reaction as the rate-limiting step. Additionally, the bioleaching process follows first-order kinetics, directly linked to Fe^{3+} concentration acting as the lixiviant. The leaching process resulted in the extraction of other metals in the feed sample, with yields exceeding 50%. However, strontium (Sr) exhibited a leaching yield of only 22%. These findings introduce a novel metal solubilizing two-step bioleaching technique and suggest the potential for developing a three-phase recycle reactor system, offering more efficient and sustainable industrial e-waste management practice for Cu recovery from discarded mobile phone PCBs. The future studies can be scaled up by calculations of cost effectiveness and environmental issues and the potential results obtained can be used for future development of metal recovery from E-waste using Biogenic green technology of Bioleaching operations.

Declaration of Competing Interest:
The authors state that they do not have any competing financial interests or personal relationships that could have influenced the work reported in this paper.

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ORCIDs

Sugandha Aachhera	https://orcid.org/0000-0002-3093-7254
Shatakshi Tiwari	https://orcid.org/0000-0002-7498-5370
Pradeep Verma	https://orcid.org/0000-0003-2266-9437
Chandra Sekhar Gahan	https://orcid.org/0000-0003-3848-2802

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