

Research Article

Statistical Optimization of Bioleaching for Simultaneous Recovery of Cu, Sn, Pb, and Zn from Computer-Printed Circuit Boards

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Electronic garbage is one of the fastest-growing waste streams. Its disposal and appropriate management are a worldwide concern. Printed circuit boards (PCBs) are critical components in contemporary electronic gadgets that contain toxic elements. Bioprocessing of PCBs for metal recovery employing microbial methods has evolved as a green solution in metallurgical operations. *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* were used in this study to leach metals from powdered waste PCBs. The RSM is used for optimizing the leaching conditions. The optimal conditions obtained were a bacterial activation period of 28 days, a pulp density of 23 g/L, and a temperature of 31°C. A confirmatory experiment under these optimal circumstances yielded recovery rates of Cu^{2+} , Sn^{2+} , Pb^{2+} , and Zn^{2+} of 95.62%, 96.27%, 95.6%, and 98.25%, respectively.

1. Introduction

The rapid development of electrical and electronic equipment use and its fast obsolescence due to rapid technical innovation lead to the generation of massive volumes of electronic waste (e-waste) globally [1]. Rapid economic expansion and increased transboundary flows of secondary materials will necessitate 3R (reduce, reuse, and recycle) activities [2]. E-waste is becoming a global concern as a result of informal recycling and reuse activities, particularly in poor nations. Due to the presence of numerous harmful substances, electronic waste management is a major international environmental problem. Despite the fact that there are various restrictions concerning what to do with electronic trash, the majority of it is discarded or transported to developing nations without authorization.

Printed circuit boards (PCBs) are the most prevalent source of high-value critical metals in e-waste, and they are typically processed using conventional pyrometallurgical and/or hydrometallurgical processes [3]. The pyrometallurgical process consumes more energy, emits unpleasant and toxic gases, and creates massive volumes of secondary waste (slag). Hydrometallurgical recycling demands the use of a variety of toxic chemicals, some of which are carcinogenic. Furthermore, the hydrometallurgical process demands additional treatment operations. As a result, considerable effort has been expended in developing ecologically acceptable biotechnology for e-waste processing. In comparison to smelting and chemical processing, the use of microbial activity in metal recycling is rapidly expanding. Because bioleaching procedures are less expensive and use less harmful biogenic lixiviants, they may be used to recycle e-waste. Other potential benefits include the ability to modify how it operates, using less energy, and metal selectivity.

The most common source of high-value critical metals in e-waste is PCBs, which are normally treated using standard pyrometallurgical and/or hydrometallurgical procedures. Since these processes have a lot of limitations, as discussed, there has been a quest for alternative technologies that are effective, environmentally benign, and long-lasting. In terms of capital investment, labor effort, and energy consumption, bioleaching has the potential to be one of the most promising technologies. As a result, contemporary efforts have focused on improving biological e-waste processing. Bacteria and metals interact in bioleaching via reduction, oxidation, sorption, and sulfate precipitation. Metal recovery from low-grade ores has been accomplished using bacterial leaching techniques. Using less toxic and less expensive biogenic lixiviants provides additional benefits such as operational flexibility, lower energy use, and metal selectivity.

From literature studies, leaching by *Acidithiobacillus ferrooxidans* [4] and *Acidithiobacillus thiooxidans* [2, 5–13] was found to be extremely successful. A broad range of heterotrophic [14], chemolithotrophic [15], and hemophilic [16] bacteria and fungi [6, 17] have been tested for basic metal mobilization, including Cu, Zn, Fe, and Ni [2, 18, 19]. Sulfur-oxidizing bacteria are the most important microorganisms for heavy metal degradation [20, 21]. This is because iron chemolithotrophy oxidizes iron and sulfur chemolithotrophy oxidizes sulfur.

Table 1 summarizes the literature on heavy metal recovery by microorganisms.

In this study, experiments are carried out by generating perfect conditions for microorganism development. To establish the optimal conditions for recovering heavy metals such as copper, lead, tin, and zinc from dumped PCBs, the bacteria *A. ferrooxidans* and *A. thiooxidans* were used. The effect of process factors such as time, temperature, and pulp density on leaching was investigated. Furthermore, RSM was employed to find the optimal conditions.

2. Materials and Methods

2.1. Raw PCB Collection and Sample Preparation. The PCBs were retrieved from an e-waste disposal site. To remove dust particles, the sample was first cleaned with an air blower. Mechanical tools (a saw metal cutter, a sheet metal cutter, a metal lathe cutting tool, cutting pliers, and a material separation tool kit) were employed to separate other parts such as capacitors, resistors, integrated circuits, diodes, and transistors. The crushed sample was ground into powder using a pulverizer with a disk diameter of 175 mm and a 3-phase motor running at 1400 rpm on a 225–445 volt supply. The weight fraction of the bottom products obtained (sieves from 52 B.S.S. to pan) is insufficient for the expected

recovery since the decrease in size increases the rate of metal ion recovery [30–32]. As a consequence, the crushed PCB powder is further processed in a ball mill with a 500 g ball weight, a mill diameter of 200 mm, and a 0.25-HP, 3-phase motor operating at 60–120 rpm.

Cultivation. The 2.2. Microorganism's microbe's A. ferrooxidans and A. thiooxidans were procured from the National Chemical Laboratory in Pune, India. The A. ferrooxidans were grown in a 9 K medium containing the following components: Ca (NO₃)₂: 0.01 g/L, (NH₄)₂SO₄: 3.0 g/L, KCl: 0.1 g/L, K₂HPO₄: 0.5 g/L, MgSO₄·7H₂O: 0.5 g/L, and FeSO₄.7H₂O: 45 g/L. A. thiooxidans were also cultivated on a 9 K medium in the same way; the only difference is that instead of ferrous sulfate, sulfur powder at a concentration of 20 g/L is employed. Using 1 N sulfuric acid, the pH of the growth medium was changed to the desired level of 2.5. Following that, the strain inoculation flasks were shaken in an incubator for 48 hrs at 30°C with an agitation speed of 170 rpm. The bacterial cells were collected by filtration after growth, and the medium was centrifuged at 10,000 rpm for 20 min to eliminate any leftover bacteria. The bacterial count was obtained as 1×10^9 cells/mL. The cell pellets were collected and kept in deionized water at 4°C for further investigation.

2.3. Bioleaching Experimentation. A. ferrooxidans and A. thiooxidans bacteria were used in bioleaching experiments. The PCB powder samples are added to the nutrient broth solutions, and the influence of various leaching parameters is studied. At the beginning of the bioleaching experiment, the stock cultures of A. ferrooxidans and A. thiooxidans are injected into the PCB sample in a conical flask. The parameters studied were PCB size fraction (0.25 mm-3 mm), temperature (20–30°C), pulp density (5–25 g/L), and time intervals (7 days, 14 days, 21 days, and 28 days). The metal leaching rate was analyzed using the following equation:

Removal efficiency(%) =
$$\frac{(C_0 - C_e)}{C_0} \times 100$$
, (1)

where C_o is the initial concentration of metal ions from sample PCBs. C_e is the concentration of metal ions after bioleaching. The PCB powdered samples are added into the nutrient broth solutions, and the various leaching parameters are studied. The bioleaching is carried out under specified parameters and constants. The composition of metals is analyzed by the energy-dispersive X-ray spectroscopy (EDXs).

2.4. Modelling and Statistical Analysis for Retrieving of Cu, Sn, Zn, and Pb by RSM. The RSM is used to examine the influence of numerous independent factors on the response. RSM integrates statistical and arithmetic techniques for the design, parameter analysis, and process optimization of experiments. The Box–Behnken design (BBD) is used in this investigation. This design employs three coded levels: low

used (microbes/species)CuSnZnPbCrAcidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans 95.6 92 90 $$ $$ Aspergillus niger 65 65 $$ $$ $$ $$ $$ $$ Aspergillus niger $$ <th>Leaching media</th> <th></th> <th></th> <th>Heavy metal</th> <th>s recovery (%)</th> <th></th> <th></th> <th></th> <th>J - U</th>	Leaching media			Heavy metal	s recovery (%)				J - U
Acidithiobacillus ferrooxidans and Acidithiobacillus thriooxidans 95.6 92 90 $ -$ Aspergillus niger 65 65 65 $ -$ <	used (microbes/species)	Cu	Sn	Zn	Pb	Cr	AI	Ż	Kelerences
Aspergillus niger6565Penicillium simplicissimum9491-Acidithiobacillus ferrooxidansAcidithiobacillus ferrooxidans74.9Acidithiobacillus thermosulfidooxidans99.9Sulfobacillus thermosulfidooxidans99.9Sulfobacillus thermosulfidooxidans89-83Sulfobacillus thermosulfidooxidans86-80Sulfobacillus thermosulfidooxidans86-80Sulfobacillus thermosulfidooxidans8680	Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans	95.6	92	06	I	I		I	[22]
Penicillium simplicissimum $ 94$ 91 $-$ Acidithiobacillus ferrooxidans 99 $ -$ <td< td=""><td>Aspergillus niger</td><td>65</td><td>65</td><td> </td><td> </td><td>I</td><td></td><td>I</td><td>[22]</td></td<>	Aspergillus niger	65	65			I		I	[22]
Acidithiobacillus ferrooxidans99 $ -$ Acidithiobacillus thiooxidans74.9 $ -$ Acidithiobacillus thermosulfidooxidans99.9 $ -$ Sulfobacillus thermosulfidooxidans99.9 $ -$ Sulfobacillus thermosulfidooxidans89 $-$ 83 $ -$ Sulfobacillus thermosulfidooxidans86 $-$ 80 $-$ 80 $ -$ Mixed fungal cultures (<i>Purpureocillium lilacinum</i> and Aspergillus niger)56.1 \pm 0.69%8.1 \pm 0.34%20.5 \pm 0.78%1:Streptomyces albidoftavus TN1068% 68% 82%46%1:	Penicillium simplicissimum	I	I	94	91			93	[22]
Acidithiobacillus thiooxidans 74.9 $ -$	Acidithiobacillus ferrooxidans	66	I						[23]
Acidithiobacillus ferrooxidans and Acidithiobacillus theoxidans99.9 $ -$	Acidithiobacillus thiooxidans	74.9	I						[23]
Sulfobacillus thermosulfdooxidans 89 83 -	Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans	6.66	I	I	I	I		I	[23]
Sulfobacillus thermosulfidooxidans Thermoplasma acidophilum Mixed fungal cultures (<i>Purpureocillium lilacinum</i> and Aspergillus niger) 56.1 ± 0.69% 8.1 ± 0.34% 49.5 ± 0.38% 20.5 ± 0.78% 1: Streptomyces albidoflavus TN10 Acidithiohacillus ferroxidans	Sulfobacillus thermosulfidooxidans	89	Ι	83				81	[24, 25]
Inermoplasma actaopnium Mixed fungal cultures (<i>Purpureocillium lilacinum</i> and Aspergillus niger) 56.1±0.69% 8.1±0.34% 49.5±0.38% 20.5±0.78% Streptomyces albidoflavus TN10 Acidithiohacillus ferroxidans	Sulfobacillus thermosulfidooxidans	86	I	80	I	I		74	[24, 25]
Streptomyces albidoflavus TN10 68% 82% 46%	inermopiasma aciaopnium Mixed fungal cultures (Purpureocillium lilacinum and Aspergillus niger)	$56.1 \pm 0.69\%$	$8.1\pm0.34\%$	$49.5 \pm 0.38\%$	$20.5 \pm 0.78\%$		$15.7 \pm 0.87\%$		[26]
Acidithinhacillus ferroaxidans	Streptomyces albidoflavus TN10	68%		82%	46%		66%	81%	[27]
Acidiphilium acidophilum 94.5 74.5 74.5	Acidithiobacillus ferrooxidans Acidiphilium acidaphilum	96		94.5	74.5			75	[28]
Penicillium simplicissimum 94 100 99.8	Penicillium simplicissimum	94		100		99.8		100	[29]

TABLE 1: Recovery data of metals with different microbes.

(-1), middle (0), and high (1), with regularly spaced gaps between them. Equation (2) was used to calculate the uncoded actual levels, Equation (3) was used to calculate the connection between the actual and coded values, and equation (4) was used to calculate the total number of experiments (*N*) in a Box-Behnken design (4). As a result, 17 trials were required for a three-variable (n=3) and three-replicate $(c_p=3)$ centre point. Based on the experimental data collected in equation (5), the second-order mathematical models were developed as follows [8, 33]:

$$X_{\text{Centre}} = \left(\frac{X_{\text{centre}} - X_{\text{low}}}{2}\right),\tag{2}$$

$$X_{\text{Code d}} = \left(\frac{X_{\text{actual}} - X_{\text{centre}}}{X_{\text{centre}} - X_{\min}}\right),\tag{3}$$

$$N = n^2 + n + c_p, \tag{4}$$

$$\gamma = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3, \tag{5}$$

where X_{actual} , X_{centre} , and X_{min} are the corresponding actual values, the actual value in the canter, and the minimum (low) actual value, respectively, and X_{Coded} is the coded value. The number of parameters (variables) and replicates in the central point is *n* and c_p , respectively. γ is the predicted response, β is the model constant, β_1 , β_2 , β_3 , and β_4 are the linear coefficients, β_{12} and β_{13} are the interaction coefficients, β_{23} , β_{11} , β_{22} , and β_{33} are the quadratic coefficients, and X_1 , X_2 , X_3 , and X_4 are the symbols for the independent variables. In this present study, the effect of the parameters (i.e., pulp density, time of bioleaching, and temperature) on the bioleaching was studied.

Table 2 lists the different parameters (variables) and their associated values used in the bioleaching experiments. The experimental data were statistically analyzed using Design Expert 13 software. Three-dimensional response surface graphs are shown. The fit model and optimal conditions for independent variables were estimated using the ANOVA technique.

3. Results and Discussion

3.1. Metal Content of PCBs. The collected PCBs were processed in the manner specified in Section 2.1. Figure 1 displays the sequence of processes. Using energydispersive X-ray spectroscopy (EDXs), the final collected samples (size range of 4 mm-0.05 mm) revealed that the principal metals contained in the sample were copper (13.15 wt%), tin (4.24%), lead (2.78%), zinc (1.16%), and other metals (2.55%) (Table 3). The total metallic content was found to be 23.88%. The samples evaluated in other studies had an average metal level of 27% [30]. The typical PCBs comprise 30% organic fraction, 40% inorganic fractions, and 30% metallic fraction. The vast range of board types employed, the varying characterization methods used by the various researchers, and the change in PCB composition through time can all explain this difference.

3.2. Parameter Optimization Studies. Bioleaching is used to remove the metallic part of PCBs. The effect of process factors such as the particle size, time, pulp density, and temperature was investigated.

3.2.1. Effect of Pulp Density on the Recovery Rate. Pulp density is a key factor in the bioleaching process. For PCBs with a diameter of 0.25 mm, samples with varying pulp densities (5, 10, 15, 20, and 25 g/L) are prepared. Other factors, such as temperature and time, are held constant at 200°C and 21 days. After inoculating the bacteria, the mixture is placed on a magnetic stirrer, and the flasks are incubated. The leaching efficiency progressively improves with an increase in the pulp density, but it is essentially steady at pulp densities over 12 g/L. The toxic metal removal was computed using (1), and the results are Cu^{2+} of 92.80%, Sn²⁺ of 95.83%, Zn²⁺ of 87.61%, and Pb²⁺ of 88.88%. Poor bioleaching at higher pulp densities might be owing to the toxic effects of WPCB metallic and nonmetallic components on bacteria or to oxygen mass transfer restrictions, which are a barrier to the process's practical industrial applicability. The precipitate appears to form near the flacks' bottom. With a prolonged incubation period, the precipitate is continually generated, and the heavy metal lixiviating effectiveness is somewhat lowered (Figure 2). The microorganism's A. ferrooxidans and A. thiooxidans do not exist in the sample due to the increase in pulp density. This might be because the precipitate formed diminishes with increasing metal ion pulp density in the solution [34, 35].

3.2.2. Effect of Temperature on the Recovery Rate. Temperature is an important operational parameter in microorganism activation. Leaching tests for PCB metallic waste will be conducted up to 30° C [36, 37]. Previous research has shown that *A. ferrooxidans* and *A. thiooxidans* work well at the optimal temperature of 28–35°C [13, 14]. As a result, the current study was also conducted at tempera-

TABLE 2: Levels of different process variables in coded and uncoded forms for biological leaching studies.

Variables	Nama of the process	Range and levels				
	variable	Low	Centre	High		
	variable	-1	0	1		
Α	Time (hr)	14	21	28		
В	Temperature (T) (°C)	24	28	32		
С	Pulp density (g/L)	15	20	25		

tures ranging from 20 to 35° C. As the temperature climbs over 20°C, the rate of dissociation slows. It slows the rate of recovery. The recovery rates are Cu²⁺ of 97.19%, Sn²⁺ of 96.23%, Zn²⁺ of 93.67%, and Pb²⁺ of 96.9% (Figure 3). The leaching efficiency exceeds 90%.

3.2.3. Effect of Time on the Recovery Rate. The rate of leaching grows with time until a certain point is reached and then declines [4]. The time period during which the measured leaching rate is at its peak is referred to as the "effective leaching rate" [30]. The samples used in this study were grown for 7 days, 14 days, 21 days, and 28 days, respectively. Figure 4 depicts the effect of inoculation on the long-term leaching of metal ions from PCB samples, such as Zn^{2+} , Sn^{2+} , Cu^{2+} , and Pb²⁺. The highest leaching efficiency was 96.49% for Cu²⁺, 96.31% for Sn²⁺, 97.64% for Zn²⁺, and 98.6% for Pb²⁺ when the leaching procedure was extended from 14 to 21 days. The leaching procedure was extended for 28 days, but no significant improvement in metal removal was observed after 21 days. As a result, the best time interval between treatments is 21 days [31, 38].

3.2.4. Effect of Size on the Recovery Rate. The size of the PCB has a significant impact on leaching efficiency. When the size fraction is adjusted, the contact duration between the bioleaching contact material (species) and test samples is reduced. The smaller the particle size, the higher the recovery rate. The current investigation addresses the leaching of metals with different particle sizes, i.e., 4 mm, 2.3 mm, 0.6 mm, and 0.05 mm. Figure 5 depicts the variation in the particle size and the corresponding % metal recovery. The

rate of recovery is highest for a 0.05 mm-sized PCB metallic sample. Since the particles are so small, the bacteria can easily leach the metals, resulting in a process efficiency of up to 95.34% for Cu^{2+} , 67.30% for Sn^{2+} , 85.80% for Pb^{2+} , and 96.84% for Zn^{2+} . As a result, 0.05-mm PCB samples are considered to be the best possible size for the experiment, in line with earlier research [38, 39].

3.3. Modelling and Statistical Analysis for Retrieving of Cu, Sn, Zn, and Pb. The BBD-RSM method was used to investigate the interaction effects of parameters on the removal of Cu, Sn, Zn, and Pb from PCB samples using bioleaching techniques. Table 4 shows the coded and uncoded levels of independent factors from 17 experiments that correspond to BBD along with their responses.

3.3.1. Analysis of Variance (ANOVA). An analysis of variance corresponding to the experimental results was presented in Tables 5 and 6. The low probability (<0.05) with greater *F*-values implied that the model was accurate. Also, the acceptable and reasonable value of the lack of fit indicates the suitability of the method for good presentation of experimental data. As presented in Table 7, the model presents the high R^2 value for the metals as follows: 0.9917 for Cu, 0.9319 for Sn, 0.9713 for Zn, and 0.9659 for Pb indicate that there was a good agreement between the experimental and predicted results. Also, the predicted R^2 values were in reasonable agreement with the adjusted R^2 values. The high values for the model's adequate precision (the signal to noise ratio) indicate that this model can be used to navigate the design space.

3.3.2. Development of the Model Equation. A model equation is a representative equation that mathematically relates the response to factors.

The regression equations (6)-(9) for the statistical analysis data plots for the metals Cu, Sn, Zn, and Pb from PCBs are as follows:

The final equations in terms of coded factors are as follows:

$$Cu = +94.78 + 0.79 \times A + 0.74 \times B + 1.12 \times C - 0.48 \times A \times B + 0.71 \times A \times C - 0.29 \times B \times C + 1.7 \times A^{2} - 3.03 \times B^{2} - 3.25 \times C^{2},$$
(6)

$$Sn = +91.59 + 1.45 \times A + 0.34 \times B + 0.73 \times C - 0.5 \times A \times B + 2.45 \times A \times C + 2.95 \times B \times C,$$
(7)

$$Zn = +94.7 - 0.45 \times A + 1.2 \times B - 0.54 \times C + 1.2 \times A \times B + 0.78 \times A \times C + 2.12 \times B \times C - 0.6 \times A^{2} - 0.6 \times B^{2} - 1.49 \times C^{2},$$
(8)

 $Pb = +96.60 + 0.96 \times A + 0.40 \times B - 0.34 \times C - 0.67 \times A \times B + 0.71 \times A \times C + 2.49 \times B \times C + 0.92 \times A^{2} - 1.32 \times B^{2} - 1.76 \times C^{2}.$ (9)



FIGURE 1: Schematic diagrams of primary raw PCBs into stepwise size reduction under the various mechanical operations and metal composition analysis performed by SEM with EDAX (reproduced from Murugesan et al., [31] under https://creativecommons.org/licenses/by/4.0).



TABLE 3: Metal composition results in a sample.

FIGURE 2: Heavy metals' $(Zn^{2+}, Sn^{2+}, Cu^{2+}, and Pb^{2+})$ leaching efficiency under different pulp densities of PCBs.

FIGURE 3: Heavy metals' $(Zn^{2+}, Sn^{2+}, Cu^{2+}, and Pb^{2+})$ leaching efficiency under different temperatures.



FIGURE 4: Heavy metals' $(Zn^{2+}, Sn^{2+}, Cu^{2+}, and Pb^{2+})$ leaching efficiency under different time periods of contact of the PCB sample and leaching media.



FIGURE 5: Heavy metals' (Zn²⁺, Sn²⁺, Cu²⁺, and Pb²⁺) leaching efficiency under different particle sizes of the PCB sample.

Figure 6 shows that the predicted value of the responses from the model was in agreement with observed values over the selected range of independent variables with reasonable higher values of the coefficient of determination (R^2).

3.3.3. 3D Response Plots of Interaction Effects. Figures 7–11 show 3D response surface plots of metal removal vs. leaching time, temperature, and pulp density interactions. 3D surface plots can aid in the determination of response values [40]. Each contour plot represents various combinations of two test parameters, with the other value set to zero. The contour plot's shape reveals whether or not the variables' reciprocal interactions are significant. The interactions between related variables in a circular contour plot are low but significant in an elliptical contour plot. The effects and combinations of the variables' leaching time, temperature, and pulp density on Cu removal are depicted in Figures 7(a)-7(c). The figures show a significant interaction of time with temperature and pulp density (Figures 7(a) and 7(b)) but no interaction between pulp density and temperature (Figure 7(c)). Cu bioleaching performed best at high temperatures (28° C) and for an extended period of time (28 days). Cu leaching is increased when pulp density rises (Figure 7(b)).

The effects and combinations of the parameters on Sn removal are depicted in Figures 8(a)-8(c). The variables had a significant influence on tin bioleaching. Increasing cyanide production in response to increased pulp density will reduce bacterial growth. As shown in Figure 8(b), Sn removal was high at low pulp density. The effects and combinations of parameters on Zn removal are depicted in Figures 9(a)-9(c).

Pb

96.60

93.76

90.29

97.42

96.34

96.07

91.76

96.60

96.60

95.84

96.60

96.87

95.94

94.17

96.30

97.08

96.60

94.39

94.70

95.45

94.06

93.95

93.95

92.41

94.70

91.86

91.59

92.88

93.47

89.30

90.97

96.23

91.59

		TAB	LE 4: The	RSM-BBD e	xperimental	design: expe	erimental an	d predicted	values.	
							Leaching of	heavy metal	s	
Run No.	Α	В	С		Experi	mental			Pred	icted
				Cu	Sn	Zn	Pb	Cu	Sn	Zn
1	0	0	0	94.6	91.24	94.2	96.2	94.78	91.59	94.70
2	-1	0	1	92.88	88.76	91.88	93.4	92.85	88.41	91.75
3	0	-1	1	89.24	89.46	88.76	90.24	89.17	89.03	88.74
4	1	-1	0	94.16	93.88	90.24	97.46	93.98	93.20	90.65
5	1	0	-1	92.16	88.68	91.8	96.7	92.19	89.85	91.93
6	0	1	1	90.23	95.7	94.86	96.46	90.08	95.61	95.40
7	0	1	-1	88.34	88.68	92.2	91.81	88.41	88.24	92.22
8	0	0	0	94.8	91.6	94.66	96.4	94.78	91.59	94.70
9	0	0	0	94.88	91.86	94.88	97.2	94.78	91.59	94.70

91.86

91.46

93.89

94.24

88.8

90.8

95.4

90.66

94

94.88

95.6

94.6

93.8

94.36

92.8

94.88

95.82

96.01

96.46

95.55

94.58

96.27

97.1

97.2

92.03

94.78

94.51

86.35

91.45

93.88

95.85

94.78

TABLE 5: The ANOVA table for the model to predict percent of leaching of copper and tin.

	Model	l to predi	ict percent of	f leaching of	copper	Mod	el to pre	dict percent	of leaching	of tin
Source	Sum of squares	df	Mean square	<i>F</i> value	<i>p</i> value Prob > F	Sum of squares	df	Mean square	<i>F</i> value	<i>p</i> value Prob > F
Model	118.14	9	13.13	93.35	< 0.0001	82.01	6	13.67	22.81	< 0.0001
A-time	4.96	1	4.96	35.28	0.0006	16.91	1	16.91	28.21	0.0003
B-temp	4.37	1	4.37	31.05	0.0008	0.9045	1	0.9045	1.51	0.2474
C-pulp den	10.06	1	10.06	71.53	< 0.0001	4.29	1	4.29	7.16	0.0232
AB	0.9025	1	0.9025	6.42	0.0390	0.9900	1	0.9900	1.65	0.2277
AC	2.02	1	2.02	14.34	0.0068	24.11	1	24.11	40.23	< 0.0001
BC	0.3306	1	0.3306	2.35	0.1690	34.81	1	34.81	58.09	< 0.0001
A^2	12.16	1	12.16	86.46	< 0.0001					
B^2	38.61	1	38.61	274.59	< 0.0001					
C^2	44.56	1	44.56	316.92	< 0.0001					
Residual	0.9843	7	0.1406			5.99	10	0.5993		
Lack of fit	0.2656	3	0.0885	0.4927	0.7063	5.17	6	0.8618	4.19	0.0933
Pure error	0.7187	4	0.1797			0.8219	4	0.2055		
Cor total	119.12	16				88.00	16			

The graphs demonstrate a strong link between time and temperature (Figure 9(a)), as well as between pulp density and temperature (Figure 9(c)). The interaction between pulp density and time is not significant, as seen in Figure 9(b). Figures 10(a)-10(c) depicts how each of the parameters influences Pb removal and how they interact.

3.3.4. Optimization of Parameters. In numerical optimization, the desirability function helps people understand the multiresponse parameters better when the parameters are being optimized. The current study's objective function is to maximize metal recovery percent. Figure 11 depicts the desirability profile for heavy metal removal percentage vs.

factors. The desirability scale ranges from 0.0 to 1.0, corresponding to the transition from an unpleasant to a much desired state [41-44]. At a time interval of 28 days, a temperature of 31°C, a pulp density of 23 g/L, and optimal removal of Cu²⁺ of 95.62%, Sn²⁺ of 96.27%, Zn²⁺ of 95.6%, and Pb^{2+} of 98.25% were reached with a desirability of 1. The experimental tests were carried out at the optimal parameters specified by the statistical technique, and the findings produced were consistent with the RSM predicted values. Figure 12 provides the SEM-EDAX analysis of the PCB sample after leaching under optimal conditions.

The bioleaching process addresses the issues of high energy consumption, significant environmental contamination, and complex operation, and as a consequence, it is

10

11

12

13

14

15

16

17

0

0

1

-1

-1

1

0

0

 $^{-1}$

0

0

-1

0

0

1

0

-1

0

1

0

 $^{-1}$

-1

1

0

92.28

95.4

94.6

86.2

91.36

93.7

95.6

94.24

	Mode	el to prec	lict percent o	of leaching of	of zinc	Mod	Model to predict percent of leaching of lead				
Source	Sum of squares	df	Mean square	F value	<i>p</i> value Prob > F	Sum of squares	df	Mean square	F value	p value Prob > F	
Model	55.19	9	6.13	26.32	0.0001	61.97	9	6.89	22.06	0.0002	
A-time	1.62	1	1.62	6.95	0.0336	7.32	1	7.32	23.44	0.0019	
B-temp	11.57	1	11.57	49.66	0.0002	1.26	1	1.26	4.02	0.0848	
C-pulp den	2.31	1	2.31	9.92	0.0162	0.8978	1	0.8978	2.88	0.1337	
AB	5.76	1	5.76	24.73	0.0016	1.81	1	1.81	5.80	0.0469	
AC	2.43	1	2.43	10.45	0.0144	1.99	1	1.99	6.37	0.0396	
BC	18.06	1	18.06	77.55	< 0.0001	24.80	1	24.80	79.46	< 0.0001	
A^2	1.48	1	1.48	6.35	0.0399	3.53	1	3.53	11.30	0.0121	
B^2	1.55	1	1.55	6.67	0.0363	7.39	1	7.39	23.67	0.0018	
C^2	9.32	1	9.32	40.00	0.0004	13.08	1	13.08	41.89	0.0003	
Residual	1.63	7	0.2329			2.18	7	0.3121			
Lack of fit	1.28	3	0.4272	4.90	0.0794	0.9167	3	0.3056	0.9639	0.4916	
Pure error	0.3488	4	0.0872			1.27	4	0.3170			
Cor total	56.82	16				64.15	16				

TABLE 6: The ANOVA table for the model to predict percent of leaching of zinc and lead.

TABLE 7: Quality of the quadratic model for the leaching of heavy metals.

Parameters	Cu	Sn	Zn	Pb
Standard deviation (SD)	0.375	0.7741	0.4826	0.5587
Mean	92.63	91.59	93.44	95.58
Coefficient of variation (CV %)	0.4048	0.8452	0.5165	0.5845
R -squared (R^2)	0.9917	0.9319	0.9713	0.9659
Adj R -squared (R^2)	0.9811	0.891	0.9344	0.9222
Pred R -squared (R^2)	0.9549	0.6989	0.7295	0.7405
Adequate precision (AP)	33.0102	16.0795	18.1342	16.643



FIGURE 6: Continued.



FIGURE 6: The comparison plot between the experimental data and predicted data for the metals.



FIGURE 7: 3D interaction plots between the pulp density, temperature, and time for Cu removal.



FIGURE 8: 3D interaction plots between the pulp density, temperature, and time for Sn removal.

regarded as a potential strategy for metal recovery. In most situations, the known and recognized mode of PCB bioleaching is the indirect contact mechanism. The metal dissolution from PCBs may be divided into two phases. In the first phase, bacteria oxidize ferrous ions to produce ferric ions. After releasing the metal from the PCBs that it was linked to, the ferric ions are transformed to ferrous ions in the second phase of the process. Ferrous ions improve the leaching process by acting as an oxidizing agent.



FIGURE 9: 3D interaction plots between the pulp density, temperature, and time for Zn removal.



FIGURE 10: 3D interaction plots between the pulp density, temperature, and time for Pb removal.



FIGURE 11: The desirability plot for the metal recovery rate with optimal conditions.



FIGURE 12: (a) The spectrum SEM image of metal isolation. (b) EDX result of metals present after bioleaching.

4. Conclusions

The fundamental advantage of bioleaching is that it produces no hazardous waste into the environment, resulting in safer waste disposal and paving the way for the development of a sustainable toxic metal recovery technology. Metal recovery by bioleaching is an effective method for handling the toxic metals present in PCBs. In this study, *A. ferrooxidans* and *A. thiooxidans* are used to leach metals from powdered waste PCBs. Using the RSM, the optimum removal of Cu²⁺ of 95.62%, Sn²⁺ of 96.27%, Zn²⁺ of 95.6%, and Pb²⁺ of 98.25% was reached with a desirability of 1 at a time interval of 28 days, a temperature of 31°C, and a pulp density of 23 g/L. The challenge that must be addressed is the scalability of bioleaching. Extensive research is required to develop suitable microorganisms for commercial activities.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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