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Microbial Processing of Waste Shredded PCBs for Copper Extraction Cum Separation—Comparing the Efficacy of Bacterial and Fungal Leaching Kinetics and Yields

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Abstract: The recycling of electronic scrap is an important subject not only from an environmental aspect but also for recovering metal resources such as copper. In this work, the microbial extraction of copper and other metals (Cu, Ni, Co, Fe and Al) present in the depopulated and shredded printed circuit board (PCB) is elaborated. Bacterial strains of *A. ferrooxidans*, *A. thiooxidans* and a fungal strain, *A. niger* are used for copper extraction along with other metals from shredded PCBs. An optimum metal recovery of 93% Cu was obtained at 308 K, pH 2 using 8% pulp density in 10 days by a mixed culture of A. *ferrooxidans* and *A. thiooxidans*. Whereas using *A. niger*, a metal recovery of 66% Cu was reported using similar experimental conditions. The results show the higher potential ability of bacteria as compared to fungus to bioleach copper. Additionally, the kinetics and mechanism of copper bioleaching from this e-waste by the chemolithotrophs and heterotrophs were evaluated. The leach liquor obtained from the optimized leaching process was subjected to separation and purification of copper as >99% pure copper sulfate using Acorga M5640 by solvent extraction.

Keywords: shredded PCB; copper; microbial leaching; solvent extraction; copper product

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1. Introduction

In the modern-day, printed circuit boards (PCBs) are the key component for all electronic companies. The PCBs contain different kinds of metals and they possess potential toxic features that could generate deleterious effect on living organisms including human beings [1,2]. Thus, the production of PCBs and after utilization creates many problems for mankind. Most of the electronic products are subjected to landfilling after their consumption. Electronic waste not only has an environmental impact (possess toxic metals like Hg, Be, Cd, Cr (VI), As, Sb, and Bi) but it is also rich in various valuable (Ag, Au, and Pt) and useful metals (Cu, Al, Ni, Si, Zn, and Fe) along with it [3]. Recycling of PCBs is needed to extract the metal value, as well as preventing environmental concerns. E-wastes, especially PCB, are a significant resource of copper. The global e-waste generation rose from 33 million tons to 54 million tons from 2010 to 2020 [4]. With the rise of electric vehicles (EVs), the copper demand is expected to increase from 400 kt to 1800 kt in 2040. World demand for copper is projected as 765 Mt until 2044 at 1.9% annually. This will demand more mining. Without new copper projects, the supply gap will exceed 15 Mt by 2035. Around 50% of copper demand in the European Union (EU) is met by recycling [4]. India has emerged as the world's second-largest consumer of materials of which the import of copper is quite significantly high. The amount of electronic waste in India is growing at 7% every year. India generates nearly 7.17 Mt of hazardous waste annually, with waste electrical and electronic equipment (WEEE) contributing 10–15%, and currently, nearly

2.0 Mt of accumulated E-Wastes in India (fourth globally). India has 178 formal e-waste recycling/dismantling units recognized by Central Pollution Control Board (CPCB), using automated, semi-automated, or manual operations; these are located in different states. However, these units are not engaged in the extraction of metals and function as dismantlers for manual separation of components and selling to recyclers outside the country [5,6].

A variety of techniques are available for the processing of PCBs. Pyrometallurgical, hydrometallurgical, and bio-hydrometallurgical processes are the most common of those. Currently, microbial resources have been playing a major role in technological development to extract metals from WEEEs [7–10]. Microorganisms like bacteria as well as fungus are employed for recycling valuable metals from such wastes [11–15]. Wang et al. (2009) isolated the bacteria *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) and *Acidithiobacillus thiooxidans* (*A. thiooxidans*) from acidic mine drainage and adapted them in the presence of printed wire boards (PWBs) and then used them as bioleaching bacteria to solubilize metals from PWBs [16,17]. Chi et al. (2011) reported leaching of gold and copper from waste mobile phone printed circuit boards by using a cyanide-generating bacterium, *Chromobacterium violaceum* in YP (yeast extract and polypeptone with glycine) medium [18]. Additionally, fungi such as *Penicillium* sp. and *Aspergillus niger* are examples of some eukaryotic microorganisms used in bioleaching of industrial wastes for the recovery of metals [19]. Microbial leaching can be classified into two types (based on the contact of microbes with the ores/waste) as (i) direct (contact) leaching and (ii) indirect leaching. Bioleaching can be grouped into three types based on leaching mechanism: (i) redoxolysis, (ii) acidolysis, and (iii) complexolysis. Acidophilic bacterial strains (*Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*) could be involved in redoxolysis leaching and acidolysis [10,20]. Fungal strain *Aspergillus niger* (*A. niger*) could be involved in acidolysis and complexolysis [8,21].

Table 1 represents a comparison of previous works describing copper bioleaching from PCBs using lithoautotrophic and/or heterotrophic microorganisms. Based on the previous works, it was realized that most of the studies reported very high copper extraction by virtue of some common factors like low pulp density, role of additives, concentration of Cu in feed and treatment duration. The treatment of these voluminous e-waste resources (PCBs) can be environmentally and economically attractive, if the pulp ratio is high, the volume of feed raw material treated is high, it requires no additives (i.e., pyrite, Fe (II/III), elemental sulfur, oxidants), and the leaching duration is limited.

Table 1. Comparison of previous work on copper bioleaching from printed circuit boards (PCBs) using lithoautotrophic and/or heterotrophic microorganisms.

Type of PCB	Composition	Microbes Used	Additive	pH	Particle Size	Pulp Density	Temp (K)	Duration (h)	% Cu Extraction	Reference
PCB powder	759 ppm Cu	*At. ferrooxidans*	1 g/L citric acid	-	<1410 μm	1%	308 K	144 h	87%	[22]
Printed Wire Boards	3.38% Cu, 0.016% Ni	*At. ferrooxidans*, *At. thiooxidans and mixture*	-	2.5	0.5–1.0 mm	0.78%	308 K	216 h	99% (*At. ferrooxidans*) 74.9% (*At. thiooxidans*) 99.9% (mix)	[16]
Computer printed circuit boards	1.88% Cu, 0.25% Ni, 1.25% Al, 6.97% Fe	*At. ferrooxidans*	-	1.8–2.0	<2 mm	1.5%	303 K	720 h	56%	[23]
Scrap TV circuit boards	11.2% Cu, 0.02% Ni, 0.04% Co, 0.0043% Fe,	*At. ferrooxidans*, *L. ferrooxidans*, *At. thiooxidans*	8% pyrite	1.7	<250 μm	1%	308 K	115 h	89%	[24]

	0.30% Al, 91 ppm Cr									
Crumbled fine PCB fines	4.1% Cu, 1.3% Ni, 72.4 ppm Cr	*Bacillus subtilis* PCM 2021 and *Bacillus cereus* PCM 2019	1% sulphur	-	<2.0 mm	0.5%	310 K	600 h	53–90%	[25]
Printed circuit board assemblies (PCBA)	NA	Mixed microbial consortium of *Acidiphilum spp.*, *Leptospirillum spp.*, *Thiobacillus ferroxidans*, *Thiobacillus caldus and Sulfobacillus*	-	2.38	12 cm × 6 cm	-	303 K	240 h	920 ppm	[26]
Computer flexible PCB	42.6% Cu	*At. ferrooxidans*	30 g/L $FeSO_4 \cdot 7H_2O$	2.5	0.42~0.84 mm	1%	301 K	120 h	90%	[27]
Computer printed circuit boards	3.92% Cu, 0.63% Ni, 1.98% Fe, 1.47% Al, 47 ppm Cr	*At. ferrooxidans*	8.4 g/L Fe (III)	3.0	<95 μm	2%	308 K	480 h	100%	[28]
Ground electronic wastes (copper-rich)	86.63% Cu, 0.25% Al, 0.063% Fe	*At. thiooxidans*	-	<1.0	40–104 μm	1%	308 K	12 h	90%	[29]
Waste computer motherboards	255 ppm Cu, 4.3 ppm Ni, 63.2 ppm Al, 31.7 ppm Fe	*At. ferrooxidans*	-	-	420 μm	1.5%	308 K	72 h	96.8%	[30]
Shredded, low-grade, waste PCBs	3.38% Cu, 0.41% Ni, 16.1% Fe, 0.04% Cr	*At. caldus BRGM3*, *L. ferriphilum BRGM1*, *Sb. benefaciens BRGM2*, *Fp. acidiphilum BRGM4*	5% pyrite	-	<2 mm	1%	308 K	96 h	100	[15]
Waste mixed PCBs	18–23% Cu, 0.09–1.15% Ni, 0.2–5.2% Fe, 1–3.6% Al, 0.2–1 ppm Cr	*At. ferrivorans* and *At. thiooxidans*	-	1.0–1.6	-	1%	296 K	168 h	98.14%	[31]
Mixed PCBs	24.8% Cu, 2.5% Al, 0.18% Fe	*At. ferrooxidans*	-	-	4–10 mm	5%	303 K	672 h	98%	[9]
Waste PCBs powder	63.37% Cu	*At. ferrooxidans*	12 g/L Fe(II)	-	420 μm	1.2%	303 K	168 h	96%	[32]
PCB powder	80.25% Cu, 0.26% Ni, 0.0059% Co, 56.28% Al, 2.10% Fe	*A. niger*	20 g/L citric acid + 3.18% H_2O_2	1.97	299.3 μm	1%	353 K	12 h	99%	[33]

Depopulated PCB	2.49% Cu, 0.03% Fe, 0.88% Al	*Purpureocillium lilacinum* and *Aspergillus niger*	-	-	150 µm–1 mm	8%	308 K	<528 h	56.1%	[34]
Waste computer motherboards	646 ppm, 11.79 ppm Ni, 1.69 ppm Fe	*At. ferrooxidans*	-	2.0	<1 mm	1%	303 K	168 h	32.44%	[35]
PCBs from end-of-life mobile phones	3.98% Cu, 1.15% Ni, 0.014% Co, 0.19% Fe, 0.13% Al	*At. ferrooxidans*	-	-	0.2–1.0 mm	3%	308 K	144 h	80% (3 cycles)	[36]

Therefore, we aim to investigate the microbial ability to extract copper and other metals from waste printed circuit boards by using a mixed culture of acidophilic bacterial strains (*A. ferrooxidans* and *A. thiooxidans*) and a fungal strain *A. niger*. The influence of various parameters like pH, pulp ratio and the temperature on bioleaching is explained which draws the details of the mechanism involved in metal solubilization. This study also aims to subdue the demerits of previous processes as reported in Table 1 and display a complete pathway vis-à-vis taking care of environmental and economic benefits.

2. Materials and Methods

2.1. Microorganisms

Consortium of bacterial strains *A. ferrooxidans* and *A. thiooxidans* were used in metal bioleaching experiments. Pre-isolated *A. ferrooxidans* was cultivated in 9K$^+$ medium [Composition (g/L): $(NH_4)_2SO_4$-3, KCl-0.1, K_2HPO_4-0.5, $MgSO_4 \cdot 7H_2O$-0.5, $CaNO_3 \cdot 4H_2O$-0.01, $FeSO_4 \cdot 7H_2O$-44.2, pH−1.6–1.8] and *A. thiooxidans* in 9K-medium (similar as 9K$^+$, except for replacement of ferrous sulfate with sulfur powder (20 g/L)). The fungus *A. niger* was cultivated in Czapek Dox broth media [Sucrose-30 g/L, Sodium Nitrate-3 g/L, Dipotassium Phosphate-1.0 g/L, Magnesium Sulfate-0.5 g/L, Potassium Chloride-0.5 g/L, Ferrous Sulfate-0.01 g/L].

2.2. Printed Circuit Board (PCB) Sample

The depopulated solder free PCB samples were shredded and subjected to physical separation. After crushing, the samples were sieved below 150 µm, digested adhering to USEPA 3050 procedure, and analyzed by ICP-OES (Thermo® ICP7000™, Thermo Scientific, Cambridge, UK) to contain 3.88% Cu, 0.169% Ni, 0.368% Co, 2.3% Fe, 1.92% Al, 0.021% W, and 0.006% Cr. The shredded printed circuit board samples that were used in this experiment for copper recovery are depicted in Figure 1 wherein thick venations embedded with high copper can be observed. The scanning electron microscope-energy dispersive (SEM-EDS) analysis using FEI® NovaNano SEM™ (FEI Company, Hillsboro, OR, USA) of the entire depopulated PCB of 0.25 cm × 0.25 cm size focused directly after carbon coating.

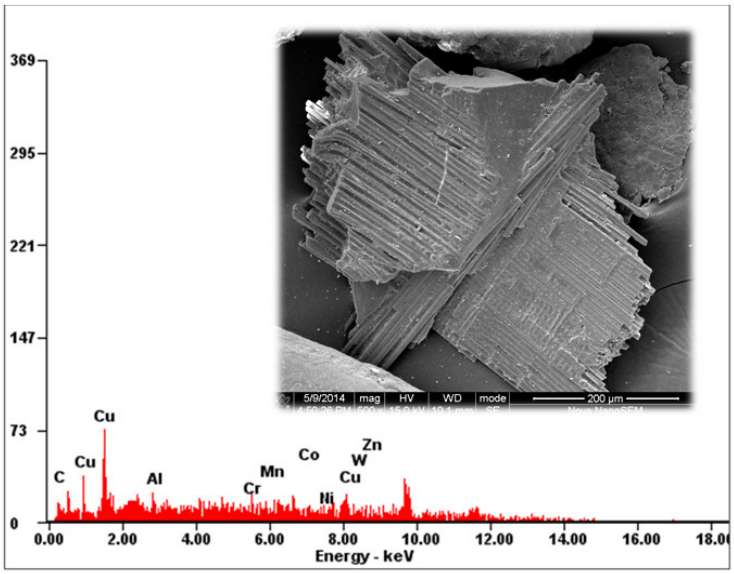

Figure 1. SEM-EDS of bulk sample analysis of shredded depopulated PCBs.

2.3. Bioleaching Experiments

The leaching experiments were carried out in Erlenmeyer flasks in an incubator shaker. A total of 180 mL of slurry containing shredded PCB samples were inoculated with 10% (*v*/*v*) of an enriched consortium of both species. Conditions like pH (initial pH values 2, 4, 6, 8 for *A. niger* and 1.7, 2.0, 2.2 for a consortium of *A. ferrooxidans* and *A. thiooxidans*), pulp density (2–10%), temperature (298–313 K) were varied with constant shaking in the incubator (120 rpm) to evaluate parameters for leaching using 10% (*v*/*v*) *A. niger* and a consortium of *A. ferrooxidans* and *A. thiooxidans* in 2:1 ratio and incubated in a shaker at 120 rpm at 308 K for 10 days. A known amount of liquid sample was drawn in 2 days intervals for analysis of metals leached out. The pH of the solution in experimental flasks was maintained daily with 2N sulfuric acid. Redox potential and pH for each of the flasks were taken at a regular interval of 24 h. The samples were collected in intervals of 48 h and prepared for ICP-OES analysis (Thermo® ICAP7000™, Thermo Scientific, Cambridge, UK). On completion of the leaching experiment, the slurry was filtered on Whatman Filer Paper (No. 42); leach liquor and solid residue after drying were sent for ICP-OES analysis. The leaching experiments were carried out in triplicate sets and the results had a mean variation of ±2–3%. The recovery of metal in solution was estimated based on metal concentration in liquor at various time intervals against that in feed at a pre-determined pulp ratio. It is understood that without adding microorganism, some metals would be leached and thus this metal concentration was excluded from concentration at defined time interval.

9K media (Iron oxidizing medium-HIMEDIA™, Mumbai, India) was used for the cultivation of *A. ferrooxidans* and *A. thiooxidans*. Czapek Dox broth (HIMEDIA™) is used for the cultivation of *Aspergillus niger*. H_2SO_4 and NaOH used for maintaining the pH of the medium was of EMPARTA™ grade from Merck®.

2.4. Solvent Extraction Studies

The extractant used for copper was used an aldoxime extractant, Acorga™ M 5640 (a mixture of 5-nonyl-2-hydroxy-benzaldoxime with 2,4,4-trimethyl 1,3-pentanediol di-isobutyrate as fatty ester) supplied by CYTEC®, Stevensville, ON, Canada, and was diluted in kerosene for the desired concentration. All solvent extraction experiments were carried out by shaking an equal volume of an aqueous solution containing metal ion and the desired extractant of known concentration in a separating funnel for a predetermined time

interval. All the experiments were carried out at 30 °C. The pH of the aqueous solution was adjusted to the desired value by adding dilute H_2SO_4 or NaOH before equilibrium. Metal ion concentration in the aqueous phase was analyzed by Atomic Absorption Spectrophotometer (GBC 980™, GBC Scientific Equipment, Victoria, Australia). Metal contents of the organic phases were determined by mass balance. The effect of various parameters such as pH of aqueous feed, solvent concentration, and organic to aqueous (O/A) phase ratio was optimized. Stripping studies of metal ions from the loaded organic phase was carried out with dilute sulfuric acid.

3. Results and Discussion

3.1. Role of Microorganisms in Metal Solubilization

The PCBs scrap is generally alkaline, and hence their addition to medium leads to the immediate rise in the initial pH. Thus, the PCBs slurry pH was lowered to <1.5 before adding the microbial inoculum by using 2N sulfuric acid. *A. ferrooxidans* oxidizes ferrous to ferric form and *A. thiooxidans* has the inherent ability to oxidize the elemental sulfur (S°) to produce H_2SO_4 [20,37–40]. It was reported earlier that *A. niger* produces different kinds of organic acids like citric acid, oxalic acid, gluconic acid when the growth medium is enriched with sucrose as the source of energy [41–43]. These organic acids assist in the dissolution/complexation of metals from the printed circuit board scraps. Additionally, *A. niger* was adapted at low pH (2.0) which triggered leaching of copper at lower pH, as against standard conditions. The other factors which influenced the role of microorganisms in the extraction of copper from PCBs are discussed in further sections.

3.1.1. Influence of pH on Bioleaching

For the variation of pH using a consortium of *A. ferrooxidans* and *A. thiooxidans*, there was a steep change in redox potential, which principally governs galvanic interaction of metals and their leaching. At pH 1.7, the redox potential increased from 91 to 263 mV in 10 d giving a maximum of 78% Cu, 72% Ni, 68% Co, 82% Fe and 38% Al (Figure 2). At pH 2, the maximum recovery of metals was observed as 85% Cu, 66% Ni, 64% Co, 88% Fe and 37% Al by using a mixed culture of *A. ferrooxidans* and *A. thiooxidans* at 308 K, in 10 days with the rise in redox potential from 104 to 378 mV. At pH 2.2, the maximum metal dissolution was reported to be 70% Cu, 64% Ni, 60% Co, 76% Fe and 39% Al. Further, at pH 2.5, the metal extraction was further lowered to <60% Cu, Ni, Co, Fe, and <23% Al. The change in redox potential is depicted in Figure 3a.

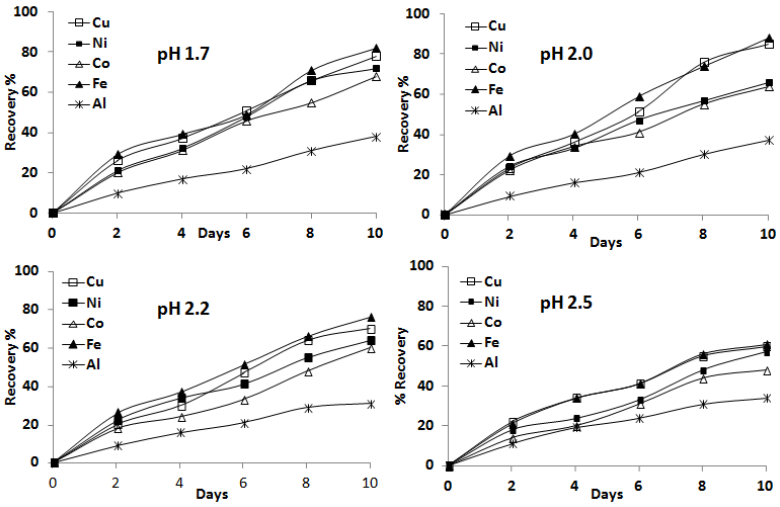

Figure 2. Metal recovery yields in various pH conditions during bacterial leaching of PCB at 2% PD, 308 K.

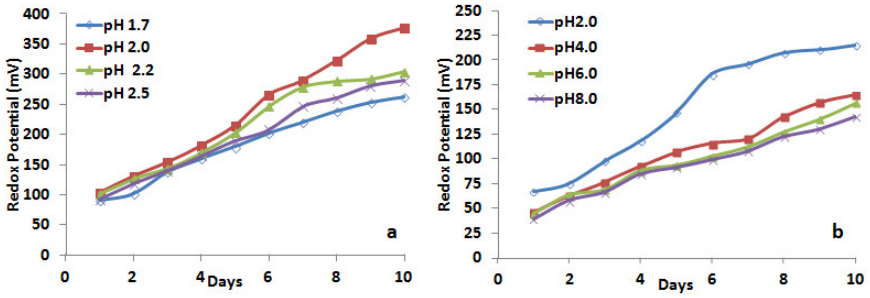

Figure 3. Variation in redox potential during leaching of PCB at different pH values, 308 K using <150 μm particles [(**a**) bacterial leaching; (**b**) fungal leaching].

With *A. niger*, the redox potential at pH 2 increased up to 215 mV from the initial (67 mV) and the value remained positive throughout the experiment at a constant pH 2 and 308 K thus promoting a maximum recovery of 60% Cu, 66% Ni, 64% Co, 72% Fe and 37% Al in 10 d (Figure 4). At pH 4, the recovery of metals was found to be 58% Cu, 64% Ni, 60% Co, 64% Fe and 39% Al, and redox potential was 185 mV on the 10th day. At pH 6, dissolution of metal was reported to be 55% Cu, 62% Ni, 60% Co, 64% Fe and 48% Al. At pH 8, the recovery of metals was found to be 54% Cu, 60% Ni, 58% Co, 60% Fe and 39% Al. The change in redox potential is depicted in Figure 3b.

3.1.2. Influence of Pulp Density on Bioleaching

Solid-liquid ratio (% *w*/*v*) was varied by using the mixed culture of *A. ferrooxidans* and *A. thiooxidans* at optimum pH (2.0). At 4% pulp ratio, 88% Cu, 68% Ni, 66% Co, 89% Fe and 38% Al were recovered in solution with redox potential rising from 152 to 198 mV in 10d (Figure 5). Maximum recovery of metal was observed at 8% pulp density (PD) which recorded 93% Cu, 70% Ni, 69% Co, 94% Fe and 41% Al dissolution at pH 2, 308 K. There appears a clear correlation of the formation of biogenic iron [39] which influences the dissolution of other metals with the concurrent increase in redox potential (Figure 6a).

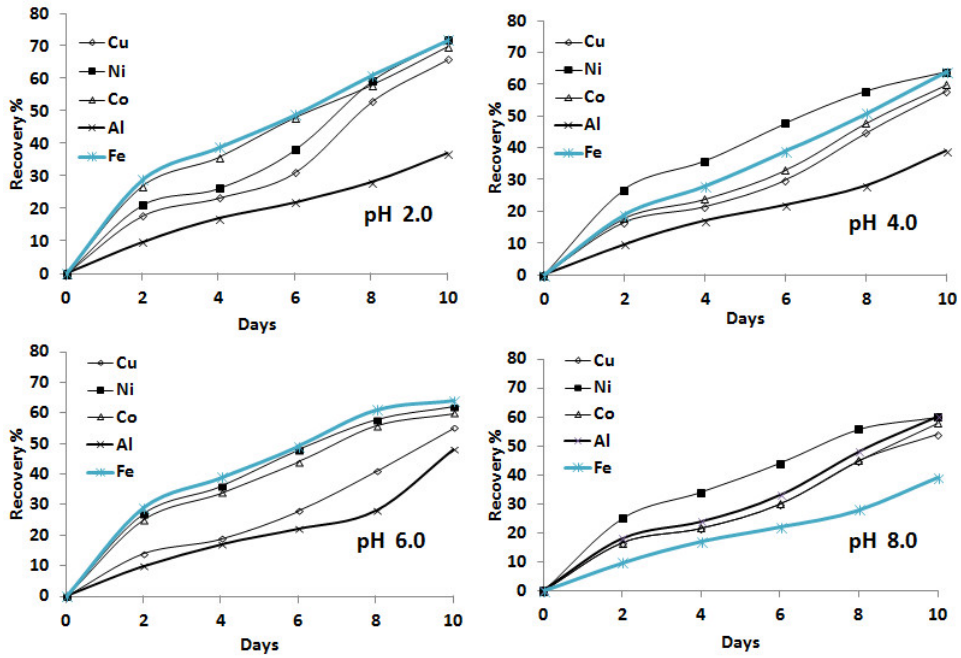

Figure 4. Metal recovery yields in various pH conditions during fungal leaching of PCB at 2% PD, 308 K.

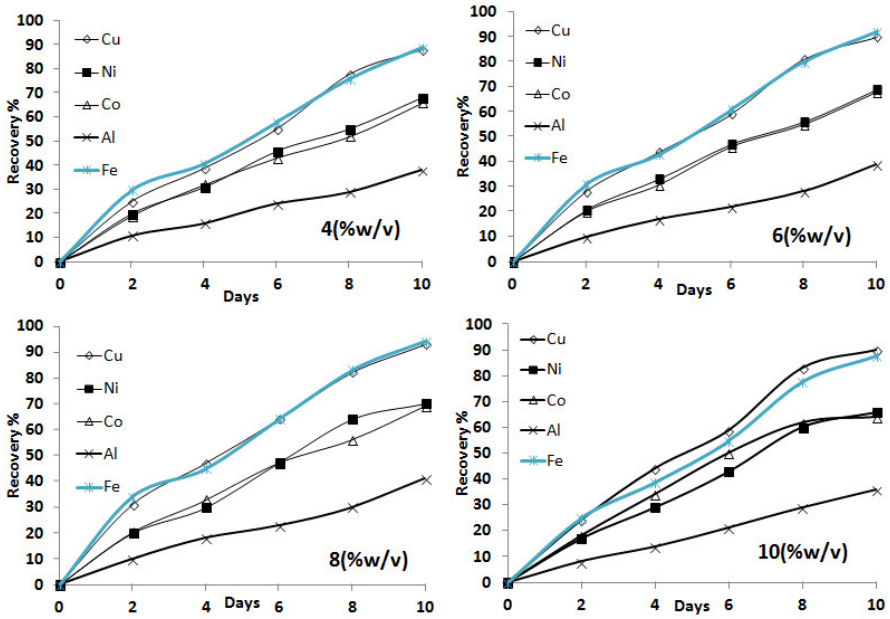

Figure 5. Metal recovery yields at various pulp densities during bacterial leaching of PCB at 308 K, pH 2.0.

Experiments were performed using *A. niger* in CZB media at different pulp densities (4%, 6%, 8%, and 10% *w*/*v*) to optimize the solid-liquid ratio. At 4% pulp density, redox potential was observed to be positive (65 mV) at the very beginning of the experiment and after 10 days, it reached 182 mV, and the metal recovery was recorded 60% Cu, 66% Ni, 64%Co, 72% Fe and 37% Al (Figure 7). At 6% pulp density, redox potential was observed initially 110 mV which raised to 228 mV in 10 days. The metal recovery after 10 days of leaching at 6% pulp density was 62% Cu, 66% Ni, 65% Co, 72% Fe and 39% Al. Maximum recovery of metals was observed at 8% pulp density with 66% Cu, 72% Ni, 70% Co, 78% Fe and 43% Al at pH 2, 308 K. Initially, the redox potential was observed at 118 mV which is positively increased and reached 261 mV after 10 days. At 10% PD, 58% Cu, 56% Ni, 54% Co, 62% Fe and 31% Al recovery was reported. Figure 6b elucidates the changes in redox potentials during fungal leaching.

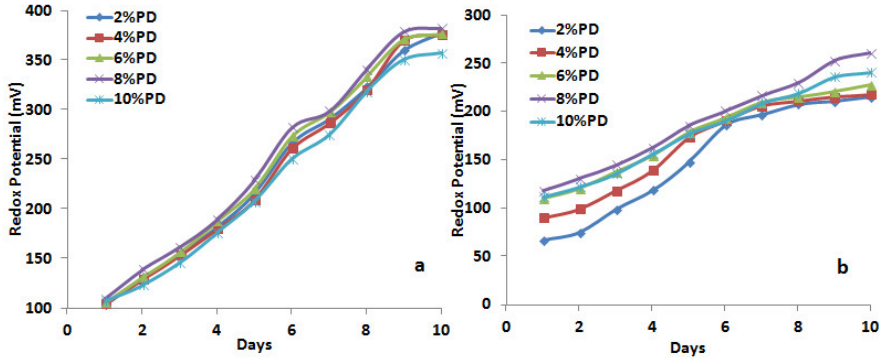

Figure 6. Variation of redox potential during bio-leaching of PCB at the different solid-liquid ratios, 308 K and pH 2.0 [(**a**) bacterial leaching; (**b**) fungal leaching].

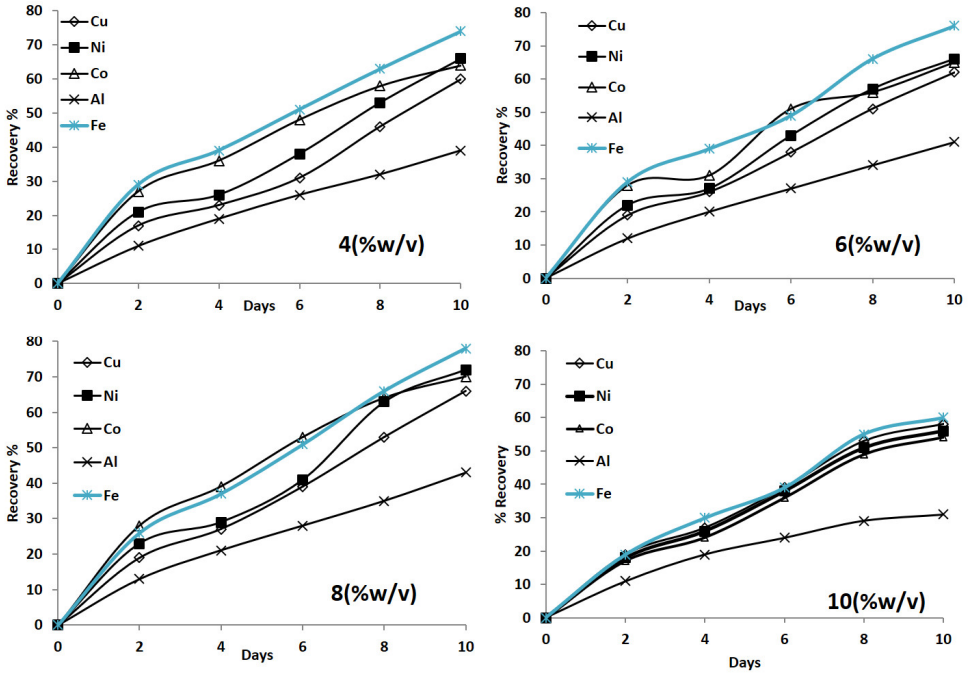

Figure 7. Metal recovery yields at various pulp densities during fungal leaching of PCB at 308 K, pH 2.0.

3.1.3. Influence of Temperature on Bioleaching

For the variation of temperature, a consortium of *A. ferrooxidans* and *A. thiooxidans* was added in 100 mL of 9k medium in the temperature range 298–313 K. At 298 K, 57% Cu, 60% Ni, 58% Co, 70% Fe and 30% Al was extracted (Figure 8a) with rise in redox potential from 153 to 263 mV in 10d. At 303 K, 70% Cu, 64% Ni, 60% Co, 76% Fe and 39% Al were recovered with a rise in redox potential from 189 to 287 mV (Figure 8b). At 308 K, 93% Cu, 70% Ni, 69% Co, 94% Fe and 41% Al extraction were reported after 10 d leaching (Figure 8c). At 313 K, 88% Cu, 68% Ni, 66% Co, 89% Fe and 38% of Al were reported with 8% pulp density (Figure 8d). The changes in redox potentials during bacterial leaching at various temperatures are illustrated in Figure 9a.

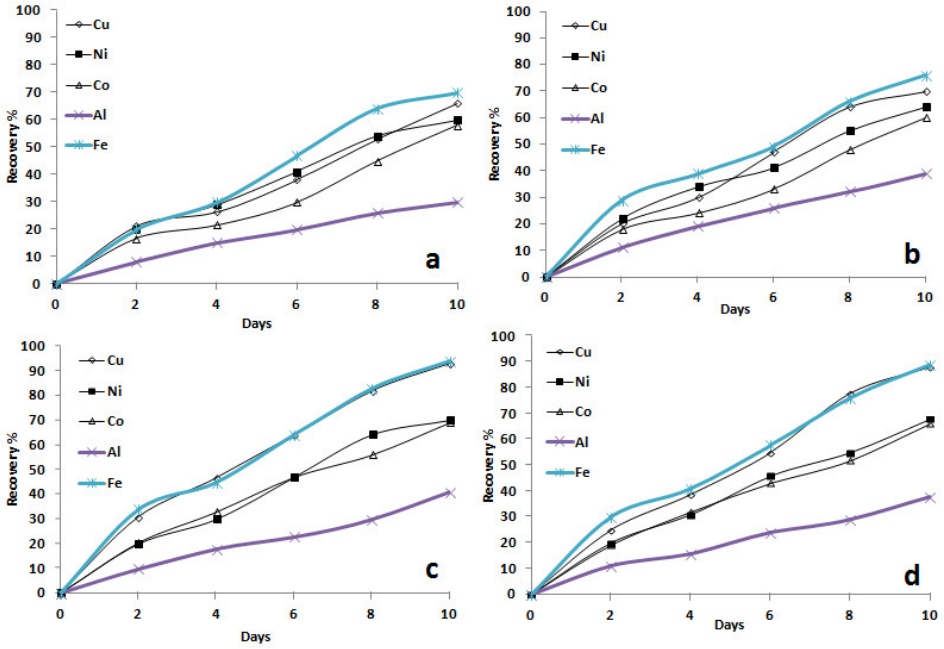

Figure 8. Metal recovery yields at various temperatures during bacterial leaching of PCB using 8% pulp density at pH 2.0 in 10 d [(**a**)-298 K, (**b**)-303 K, (**c**)-308 K, (**d**)-313 K].

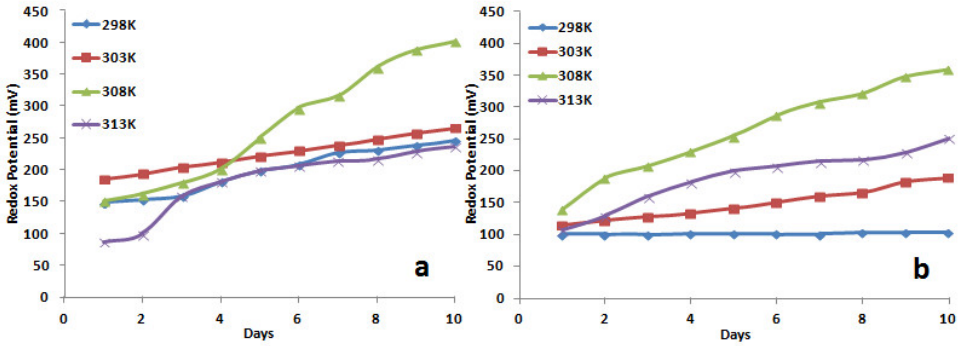

Figure 9. Variation of redox potential during bio-leaching of PCB at different temperatures using 8% pulp density at pH 2.0 in 10 d [(**a**) bacterial leaching; (**b**) fungal leaching].

In the case of fungal leaching, the recoveries of metals were 57% Cu, 60% Ni, 58% Co, 66% Fe, and 30% Al at 298 K (Figure 10a). At 303 K, 59% Cu, 65% Ni, 62% Co, 68% Fe and 38% Al were recovered (Figure 10b). At 308 K, metal extractions were 66% Cu, 72% Ni, 70% Co, 78% Fe and 43% Al (Figure 10c). At 313 K, the recoveries of metals were shows 44% Cu, 45% Ni, 40% Fe and 39% Al (Figure 10d). The maximum redox potential rose from 140 mV to 352 mV in 10 days at 308 K with 8% pulp density (Figure 9b).

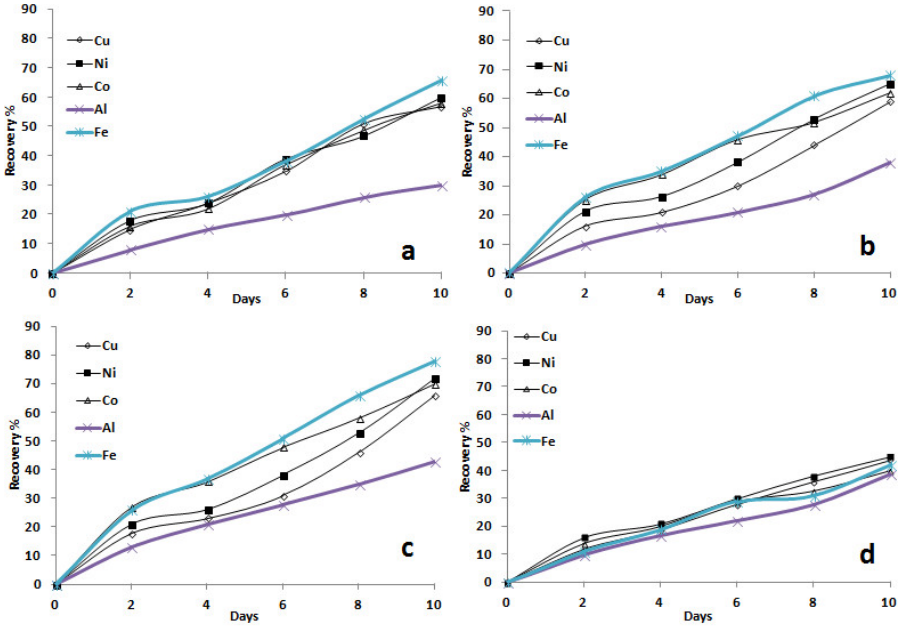

Figure 10. Metal recovery yields at various temperatures during fungal leaching of PCB, pH 2.0 at 8% pulp density [(**a**)-298 K, (**b**)-303 K, (**c**)-308 K, (**d**)-313 K].

The optimal copper bioleaching performances reported in Table 2 is compared with the optimized parameters reported in previous studies (Table 1). One can clearly see that the bacterial leaching (93% yield of copper extraction) performs better than the fungal leaching (66% yield of copper extraction). The better performance of the bacterial leaching can be explained by the role of bacteria in maintaining the cyclic regeneration of Fe(III) ions and by their capacity to generate sulfuric acid that can compensate the acid consumption of electronic scrap and promote the growth of acidophiles by stabilizing pH of leaching medium. Fe (III) ions can further chemically oxidize metallic copper into its soluble form (Cu^{2+}) and reduce to Fe (II) [8]. In contrast, the fungal leaching only relies on the acidity generated by the organic acids produced which contribute to acidolysis copper leaching and on the complexolysis mechanism relying the organic acids capacity to form metallic complex with the metals from the PCBs material to be bioleached [44,45]. However, these combined processes are less efficient than the bacterial leaching as previously reported by Brandl et al. [7].

Table 2. Optimal Cu bioleaching performance obtained in the present study using desktop shredded PCBs.

Composition of PCBs	Microbes Used	Additive	pH	Particle Size	Pulp Density	Temp (K)	Duration (h)	% Cu Extraction
3.88% Cu, 0.169% Ni, 0.368% Co, 2.3% Fe, 1.92% Al, 0.021% W, 0.006% Cr.	*At. ferrooxidans*, *At. thiooxidans*	-	2.0	<150 µm	8%	308 K	240 h	93%
	A. niger	-	2.0	<150 µm	8%	308 K	240 h	66%

3.1.4. Kinetics and Mechanism of Bacterial Leaching

Owing to the higher dissolution rates using bacteria, kinetics, and mechanism for leaching of PCBs by bacteria were compared with fungus. The rate for PCBs bioleaching was tested against shrinking core models through diffusion control, chemical control, and mixed control. The validity of the experimental data into the integral rate was tested and

kinetic analysis results for the dissolution process are found to be consistent with a chemically controlled reaction and the integral rate expression showed a good fit to the rate Equation (1).

$$1 - (1 - x)^{1/3} = k_c \cdot t \quad (1)$$

The apparent rate constants (k_c) can be evaluated by plotting $1 - (1 - x)^{1/3}$ versus t as shown in Figure S1, where in "x" represents the fraction leached in time "t". The dissolution process did not align with the diffusion-controlled model, which was evident from the lower correlation coefficient (Figure S2). An Arrhenius plot for the leaching of copper by bacteria was obtained by plotting the values of slopes of the straight lines (apparent rate constant) versus ln (1/*T*) as shown in Figure 11. The value of activation energy by the leaching of copper by bacteria (19.17 kJ/mol) depicts the phenomena being controlled by biochemical reaction. The higher activation energy of 55.36 kJ/mol exhibits the absence of a diffusion-based shrinking core model in Cu extraction.

The mechanism of bio-chemical leaching of copper was further investigated by observing the surface morphology through SEM-EDX (Figure 12) under optimal conditions of 8% PD and pH 2.0 at 308 K. The SEM image of the leach residue exhibits extensive corrosion that would have led to the direct attachment of bacteria on PCB surface leading to copper dissolution and significant lowering of copper (wt%) in the bulk analysis. The bacterial consortia of *A. ferrooxidans* and *A. thiooxidans* can oxidize the ferrous sulfate and elemental sulfur [20], respectively, thus producing sulfuric acid (H_2SO_4) which is a strong acid in respect to organic acids like citric acid, oxalic acid produced by the fungus *A. niger* [42]. Thus, the results show the higher efficacy of bacterial consortia in the dissolution of metals in comparison to fungus.

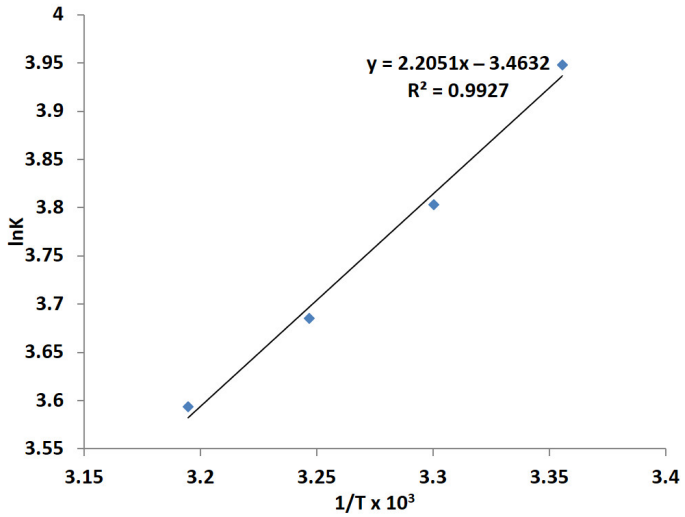

Figure 11. Arrhenius plot for Cu during bacterial leaching of PCB, pH 2.0 at 8% pulp density in the temperature range 298–313 K.

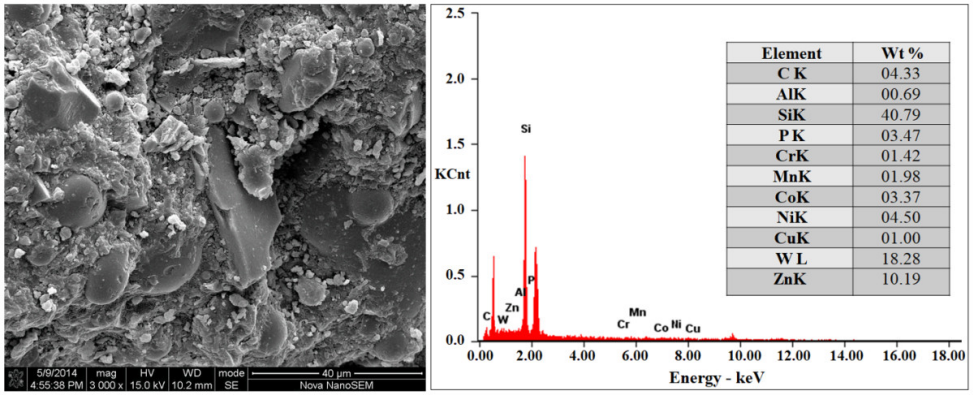

Figure 12. SEM-EDAX analysis of bacterially leached depopulated shredded PCBs at 8% PD, pH 2.0, and 308 K.

3.2. Solvent Extraction of Pregnant Leach Liquor (PLS)

The pregnant leach liquor (PLS) generated from bacterial leaching was used for the recovery of copper after PCBs bioleaching. The composition of the bacterial PLS under optimized conditions was 2.79 g/L Cu, 0.093 g/L Ni, 0.20 g/L Co, 1.72 g/L Fe, 0.63 g/L Al. The liquor was used for the separation of copper and other metals as described in Wang et al., 2009 b [46].

To study the effect of extractant concentration on extraction of copper, the concentration of Acorga M 5640 was varied in the range 5–30% (*v*/*v*) in kerosene. The experimental results show that the extraction of copper increases with an increase in extractant concentration (Figure 13a). As one can see, copper extraction increases from 76.4% to 95.2% at the equilibrium pH of 1.4 by increasing solvent concentration from 5–30% in single contact within 5 min. It is also observed that about 93.5% copper was extracted with 10% Acorga M 5640. The effect of phase ratio on the extraction and separation of copper against iron from the bacterial PLS containing 2.79 g/L Cu and 1.72 g/L Fe were determined using 10% (*v*/*v*) Acorga M5640 as the extractant. Figure 13b shows that the phase ratio significantly affected the extraction of copper and iron. The copper extraction percentage increased from 65% to 93.5% as the phase ratio (O/A) was varied from 1/5 to 1/1, the co-extraction percentage of iron decreased from 22% to 4%. This result can be explained by the crowding effect of copper onto iron extraction. However, when copper extraction approaches saturation, the co-extraction percentage of iron increases as the phase ratio is continuously increased. Therefore, the optimum separation efficiency of copper and iron can be obtained at a phase ratio of 1/1. During the separation of copper using 10% (*v*/*v*) Acorga M5640, other metals viz., Ni, Al, Co were not extracted and did not affect the extraction of copper [46,47]. Raffinate rich in Co, Ni, Al can be subjected to further stages of separation.

Stripping was carried out to recover copper from the loaded Acorga M5640 using sulfuric acid at various acid concentrations and contact times as shown in Figure 13c,d. Figure 13c depicts the effect of changing the concentration of sulfuric acid from 1 to 20% on stripping of copper. The stripping of copper was significantly stimulated from 27% to 92% as sulfuric acid concentration increased from 1 to 10%, beyond which it decreased, confirming 10% acid concentration to be most suitable. Figure 13d displays the influence of contact time (1–30 min) on the stripping efficiency of copper at 10% acid concentration. It was observed that a contact time of 20 min is sufficient to strip 68% copper in the first stage and the second stage (not shown) of 10 min with the same acid concentration offloads the complete copper in solution.

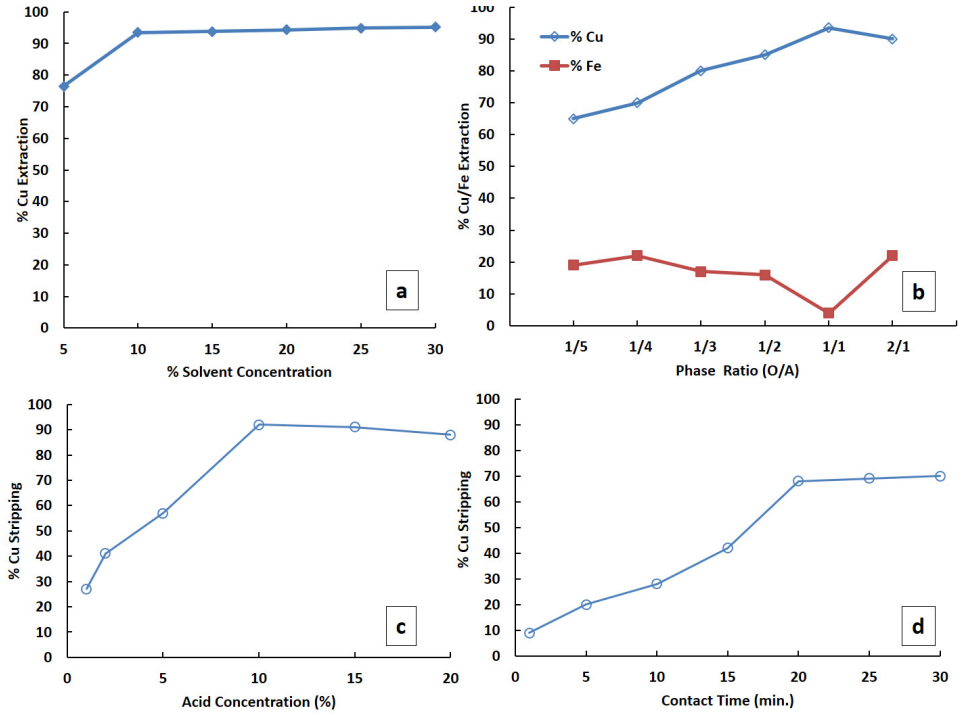

Figure 13. Variation of solvent extraction parameters for separation and stripping of copper from the bacterial PLS using Acorga M5640 at 30 °C [(**a**) variation of solvent concentration (O/A:1); (**b**) variation in phase ratio (10% Acorga); (**c**) concentration of stripping reagent; (**d**) contact time of stripping].

The stripped solution was crystallized in an evaporator at low temperature to obtain blue crystals of 99.9% pure copper sulfate having dendritic morphology (Figure 14a–c), which can be electro-wined to copper metal. Acorga M5640 has excellent reuse performance and can be recycled more than 10 times, which demonstrates their industrial application value in the extraction of copper from shredded PCBs leach solution [47].

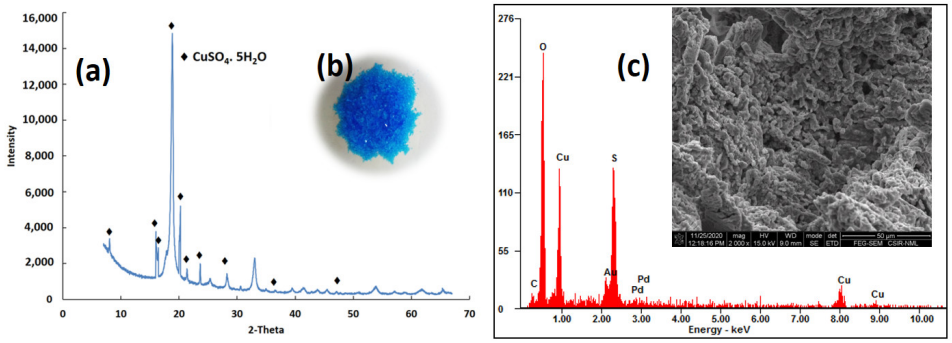

Figure 14. Characterization of the stripped and crystallized product of copper sulfate (**a**) XRD diffractogram; (**b**) 99.9% pure copper sulfate crystals; (**c**) SEM of Cu product.

Based on the optimized results of bacterial leaching followed by solvent extraction and separation of copper, a process flowsheet of shredded PCBs bio-recycling to extract Cu is shown in Figure 15. The raffinate after the first stage of solvent extraction (SX-1) contains Co, Ni, Al and traces of Fe. Fe and Al can be removed by oxidative precipitation using hydrogen peroxide at pH 1.5, followed by the separation of Co and Ni using D2EHPA and Cyanex 272 in 2nd stage of solvent extraction [48]. This synergistic mixture

would load cobalt, and Ni is obtained in raffinate. These metals can be converted to respective salts.

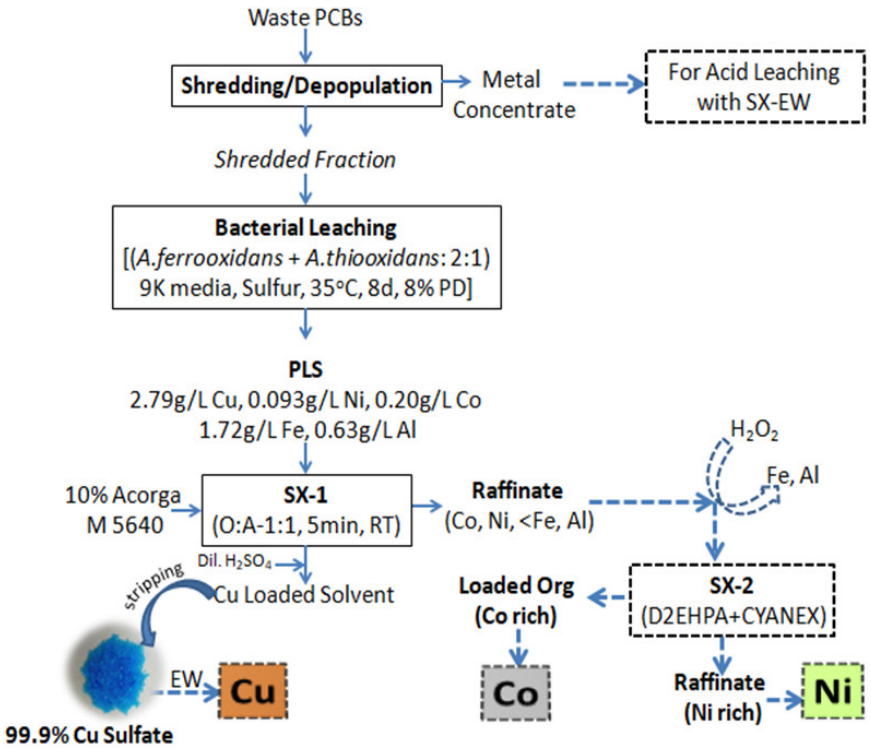

Figure 15. Process flowsheet for bio-recycling of depopulated shredded PCBs to extract copper.

The process described in Figure 15 depicts a clear advantage of using hybrid methods to recover copper and other metals. It is well in tandem with the assessment done on the environmental impact for base metal recovery from PCBs [49] making bioleaching the most sustainable method for metal recovery from PCBs. In addition to its environmental benefits, bioleaching requires less investment than conventional metal recovery methods [50].

4. Conclusions

The ability of acidophilic chemo-lithoautotroph bacteria, *A. ferrooxidans*, and *A. thiooxidans* were tested for the extraction of metals from waste shredded printed circuit boards. Both the bacteria *A. ferrooxidans* and *A. thiooxidans* were grown at pH 2 and experiments were carried out using these non-adapted microorganisms. 93% Cu, 70% Ni, 69% Co, 94% Fe and 41% Al were extracted at the optimum conditions of 308 K, pH 2, 8% pulp density from <150 µm fractionated and shredded PCB samples using the consortium (2:1) of bacteria, *A. ferrooxidans* and *A. thiooxidans*. It was noted that at optimum pH, pulp density, and temperature, the higher value of redox potential corresponds to the higher oxidation rate by microorganisms, better generation of Fe (III) and acid, thereby promoting metal dissolution. 66% Cu, 72% Ni, 70% Co, 78% Fe and 43% Al were recovered at the optimum condition of 308 K, pH 2, 8% pulp density from <150 µm fractionated and shredded PCB samples using *A. niger*. The bacterial leach liquor was processed by solvent extraction using 10% Acorga M 5640 to obtain 99.9% pure copper sulfate.

Supplementary Materials: The following are available online at www.mdpi.com/2075-4701/11/2/317/s1, Figure S1: Chemical controlled model for leaching of metals at various temperatures during bacterial and fungal leaching of PCB, pH 2.0 at 8% pulp density in the temperature range 298-313K [a-Bacterial Cu, b- Fungal Cu, c-Bacterial Ni, d- Fungal Ni, e-Bacterial Co, f- Fungal

Co]. Figure S2: Diffusion controlled model for leaching of metals at various temperatures during bacterial and fungal leaching of PCB, pH 2.0 at 8% pulp density in the temperature range 298-313K [a-Bacterial Cu, b- Fungal Cu, c-Bacterial Ni, d- Fungal Ni, e-Bacterial Co, f- Fungal Co].

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