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METAL SOLUBILIZATION FROM POWDERED PRINTED CIRCUIT BOARDS BY MICROBIAL CONSORTIUM FROM BAUXITE AND PYRITE ORES

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With the current rapid developments in technology, there is an increasing accumulation of outdated electronic equipment. The primary reason for this increase is the low rate of recycling due to the complex nature of such waste. Bioleaching offers a promising solution for this problem.

Study was conducted on the solubilization of heavy metals from electronic waste (e-waste). For this purpose, a microbial consortium from bauxite and pyrite ore samples was obtained using a simple “top down” approach. Essentially, printed circuit boards (PCB) were obtained and used as representative samples of e-waste. Various concentrations (1–5%) of PCB powder were subjected to bioleaching, and the effects on metal solubilization, changes in pH and concentration of ferrous iron produced were assessed. It was observed that a maximum of 96.93% Cu and 93.33% Zn was solubilized by microbial consortium from 10 g/l of PCB powder, whereas only 10.26% Ni was solubilized from 30 g/l of PCB powder. For lead, only 0.58% solubilization was achieved from 20 g/l of PCB powder. An analysis of the precipitate formed during bioleaching using scanning electron microscopy with energy dispersive x-ray analysis revealed the presence of Tin (59.96%), Cu (23.97%), Pb (9.30%) and Fe (5.92%).

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Rapid advancements in technology have led to an increasing amount of electronic waste (e-waste), as older electronic equipment, such as computers and mobile phones, are discarded or approach the end of their usefulness. Interestingly, e-waste has a low level of recycling because its complex composition makes the separation of each component difficult.

It is believed that biotechnology is one of the most promising technologies in metallurgical processing. For many years, bioleaching has been used for the solubilization of metals from ores [1]. Bioleaching is useful for treating ores with low concentrations of metals; it is also simple and cheap to operate. It has been successfully applied toward the leaching of metals from ores, though it has not yet been commercially applied toward the recovery of metals from printed circuit boards (PCB) [2]. Several authors [2–7] have recently published studies on the bioleaching of metals from electronic waste. Xin and colleagues [8] and Mishra with coworkers [9] have reported attempts to extract metals from waste batteries.

The aim of the study was to formulate microbial consortium for solubilization of metals from waste PCB and evaluate its efficacy.

MATERIALS AND METHODS

Growth of consortium. The bioleaching consortium was selected using the simple “top down” approach following Rawlings and Johnson in 2007 [10]. In the “top down” approach (“see-who-wins” approach), a mixture of microorganisms is used to inoculate the test material (in laboratory or pilot-scale operations), and it is assumed that a limited number of these acidophiles will emerge as a stable and effective bioleaching consortium.

In the present investigation, the microorganisms were derived from natural environments. Bauxite (Radhanagari, Kolhapur, India) and pyrite (Chitradurga, India) ore samples were used as a source of acidophiles. The acidophiles were cultivated in modified 9K medium having composition (g/l): part A – $(\text{NH}_4)_2\text{SO}_4$ – 3.0; KCl – 0.20; K_2HPO_4 – 0.050; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.050; part B – $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ – 45. Part A was sterilized by autoclaving at 121°C for 15 min at 15 lb pressure and part B was sterilized by filtration using 0.25 μm filter (Merck Millipore, USA). After sterilization equal amount of part B was added to part A, aseptically. The pH of the medium (2.4) was adjusted using 1.0 N H_2SO_4 . The enriched acidophiles obtained from both the ore samples were mixed and again cultivated in the same medium. The obtained consortium was used for the bioleaching studies. The complete bi-

Chemical analysis of metals (%) in waste PCB powder

Metal	Solvent use for extraction*	
	aqua regia	nitric acid
Copper	19.10	28.68
Iron	0.002	0.002
Zinc	0.81	0.46
Lead	2.81	5.14
Nickel	0.042	0.432
Aluminum	1.383	Nil
Silver	0.0044	Nil
Chromium	0.029	0.0033
Manganese	0.0007	0.00133
Cadmium	Nil	Nil
Silicon	0.1678	N.D.
Calcium	2.8405	9.77

* N.D. – not performed; Nil – absence of metal.

oleaching experiments were carried out in non-sterile conditions to obtain a stable and compatible consortium and mimic the conditions of the commercial bioleaching process.

The individual organisms from the consortium were not purified. However, the presence of iron oxidizers and acidophilic heterotrophs was confirmed using solid Fe-TSB non-overlay medium [11]. The Fe-TSB non-overlay medium was prepared in 3 parts. Part A was prepared as follows: 0.36 g ammonium sulfate, 0.005 g di-potassium hydrogen phosphate, 0.14 g magnesium sulfate, and 0.070 g tryptone soya broth (TSB) was added to 140 ml of double distilled water in a 250-ml Erlenmeyer flask. The pH was adjusted to 2.3. For part B, 2.0 g of agarose was dissolved in 70 ml of double distilled water in a 150 ml conical flask and maintained at a neutral pH. Parts A and B were autoclaved at 121°C and 15 lbs of pressure for 15 min. For part C, 6.9 g of ferrous sulfate heptahydrate was added to 10 ml of double distilled water, pH 2.15–2.3, and the concentration of the final solution was 2.5 M. The solution was filter-sterilized using a 0.25- μ m filter (Merck Millipore, USA). A volume of 1.5 ml of part C was added to part A. Part B was added to the mixture of A and C, and the plates were poured. All reagents used were of analytical grade.

Preparation of the waste PCB sample. Printed circuit board assemblies (PCBA) were collected from a scrap market. The PCBA consisted of different electronic components, such as random access memory (RAM) chips, peripheral component interconnect (PCI) slot, condensers, transistors, heat sink, etc., attached to PCB. The PCB consisted of metals required for the functioning of electronic equipment and non-metallic support.

The attached plastic parts, including RAM, PCI slots, and chip slots were removed from the PCBA. The PCB were shredded using a file (a file is a metal and woodworking tool used to cut fine particles of material from a workpiece). The obtained powder was composed of metallic and nonmetallic components of varied composition (mainly epoxy or phenolic resin).

The powder was sieved using a –14/+20 mesh. It was not pretreated prior to the bioleaching studies.

Chemical analysis of the waste PCB powder. A chemical analysis of the waste PCB powder was conducted to determine the heavy metal content in it. The content was determined using 2 different methods:

I) Approximately 1.0 g of homogenized powder was added to 100 ml of aqua regia, heated at 100°C for 1 h, cooled and subsequently filtered through Whatman filter paper no. 1 (UK). The volume was increased to 100 ml with double distilled water. The prepared sample was used for the detection of heavy metals with an atomic absorption spectrophotometer (Perkin Elmer A Analyst 300, USA).

II) A few drops of HNO₃ were added to 1.0 g of homogenized powder in a 100-ml volumetric flask, and the volume was made to 100 ml with double distilled water. The sample was filtered with Whatman filter paper no. 1 prior to heavy metal analysis. The heavy metals were detected using method of atomic absorption spectroscopy.

Determining the metal solubilization ability and tolerance of the consortium. The metal solubilizing ability was determined using pure copper and solder. This metal adaptation increased the efficiency of the consortium in solubilizing the metals from PCB powder.

Solubilization of elemental copper. To determine the solubilization of elemental copper, we used 0.1–0.8% copper turnings of <0.5 mm in length and <0.05 mm in width. Copper turnings were added to a 500-ml Erlenmeyer flask containing 200 ml of modified 9K medium and a 15% inoculum of consortium. The flask was incubated at 30°C on a rotary shaker at 140 rpm for 10 days.

Solubilization of lead from solder. The solder was purchased from the market and shredded into pieces of 1–2 cm in length. Approximately 0.1% of these pieces were treated in the same way as copper chips.

Bioleaching of metals from PCB. PCB powder (10, 20, 30, 40 and 50 g/l) was added to separate 500-ml Erlenmeyer flasks containing 200 ml of non-sterile modified 9K medium previously adjusted to pH 2.4 and a 15% microbial consortium inoculum. The flasks were subsequently incubated at 30°C on a rotary shaker at 140 rpm for 10 days. Samples were collected every 48 h for 10 days to determine the pH, and the ferrous iron and soluble metal content. The pH was measured using a digital pH meter (Elico LI 127, India). The ferrous iron content was measured using phenyl anthranilic acid [12], and the soluble metal content (Cu, Pb, Zn and Ni) was detected using method of atomic absorption spectroscopy. After 240 h, the precipitate formed during bioleaching was collected, dried and

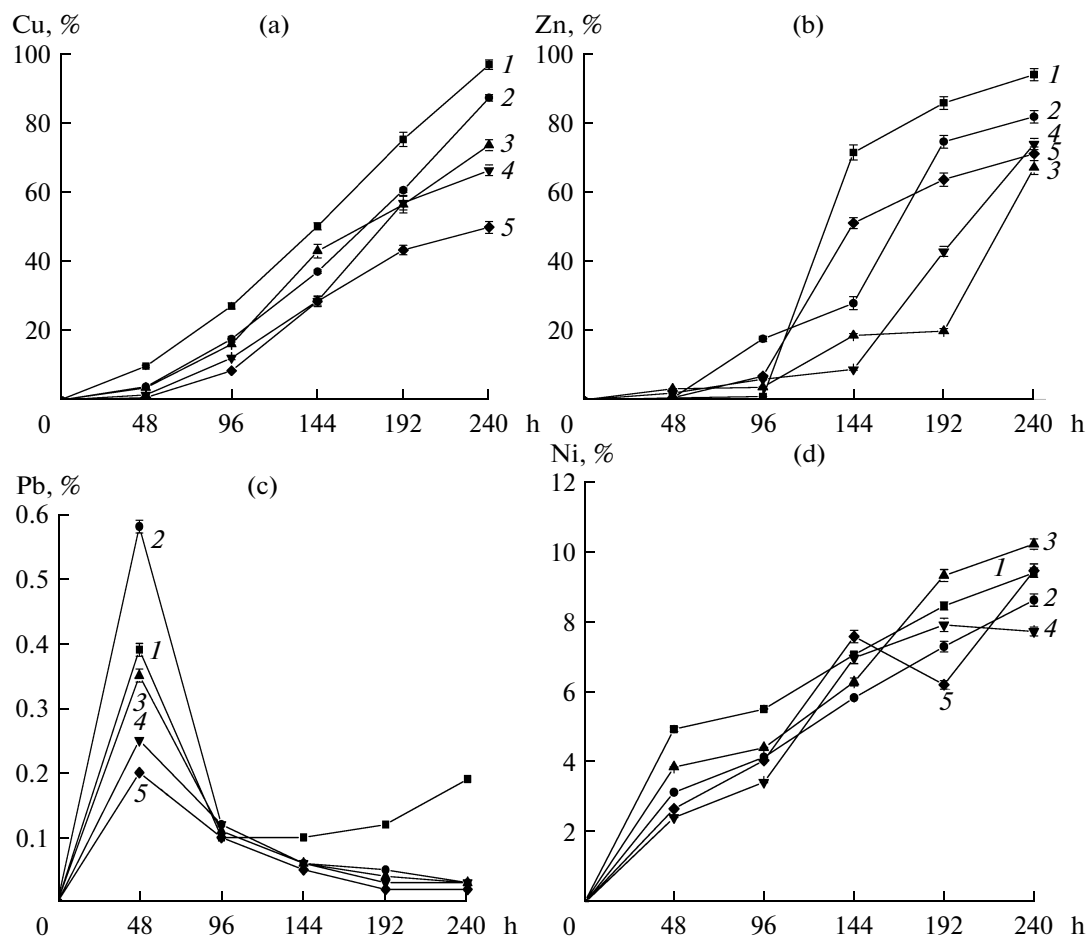


Fig. 1. Copper (a), zinc (b), lead (c) and nickel (d) mobilization under different concentrations of PCB powder (g/l: 1 – 10; 2 – 20; 3 – 30; 4 – 40; 5 – 50) relative to the time of incubation with microbial consortium. The data present the average of 3 replicates.

mounted on carbon-coated copper grids for scanning electron micrograph and energy dispersive X-ray analysis (SEM-EDAX). The micrographs were obtained using a JEOL (6360) JED-2300 analysis station (Japan) operating at 200 keV.

RESULTS AND DISCUSSION

Growth of microbial consortium. The objective of this study was to evaluate the solubilization of metals from PCB; therefore, the specificity of microorganisms that carried out this process was not important. However, the most efficient organisms for bioleaching were typically the most desirable. Because the process was conducted under non-sterile conditions, there was a continuous selection of more efficient microorganisms for the bioleaching system, which included the selection for genes present in the horizontal gene pool (e.g., genes for metal resistance) that might improve the efficiency of the resident microorganisms [10, 13, 14]. Furthermore, in many leaching experiments conducted in laboratories, minerals or e-waste were inoculated with a pure or defined mixture of cultures, and it was uncertain whether these organisms would be

competitive in the non-sterile conditions of the commercial bioleaching process [1].

The presence of iron oxidizers and acidophilic heterotrophs in microbial consortium obtained using a simple “top down” approach was confirmed using solid Fe-TSB non-overlay medium, which yielded reddish brown-colored colonies of iron oxidizers and off-white colored colonies of acidophilic heterotrophs.

Chemical analysis of the PCB. Chemical analysis of the PCB was performed to determine its heavy metal content. It was conducted using 2 different dissolving agents: aqua regia and nitric acid. The metals in the solution were detected by atomic absorption spectroscopy. The results obtained are given in table. When aqua regia was used as a dissolving agent, the major metals detected were copper (19.1%), lead (2.81%), aluminum (1.383%) and zinc (0.81%). When nitric acid was used as a solvent, the main metals revealed were copper (28.68%), lead (5.14%) and zinc (0.46%).

Aqua regia was a good solvent for the most metals, which were less soluble in nitric acid. However, copper and lead were more soluble in nitric acid than in aqua regia.

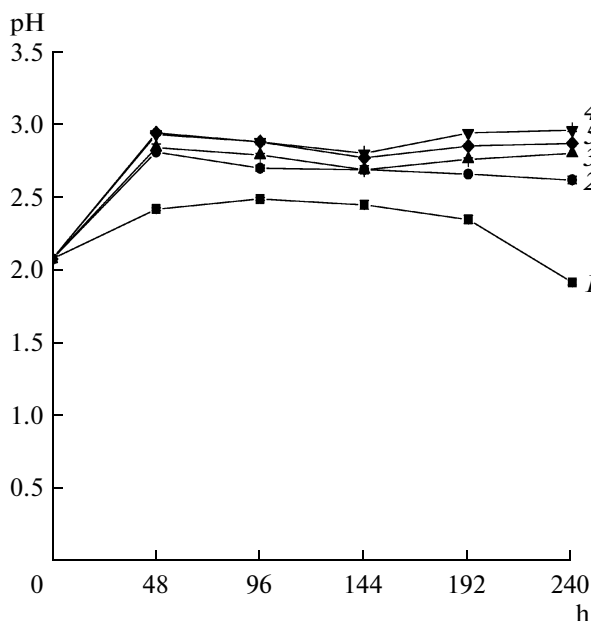


Fig. 2. Changes in pH under different concentrations of PCB powder (1 – 10; 2 – 20; 3 – 30; 4 – 40; 5 – 50 g/l) relative to the time of incubation with microbial consortium. The data present the average of 3 replicates.

Detection of metal solubilization ability and metal tolerance of microbial consortium.

In the pure metal solubilization experiments, it was observed that 0.8% (w/v) copper was completely solubilized (100% solubilization) by microbial consortium within 7 days. This result confirmed the copper bioleaching activity of the consortium and its tolerance to 0.8% (w/v) copper. In contrast, even 0.1% solder (w/v) did not get completely solubilized, and only 0.004% solubilization of lead was achieved in 10 days. These results demonstrated the high copper solubilization and poor lead solubilization ability of the microbial consortium used.

Bioleaching of PCB. The purpose of using a consortium of iron oxidizers and acidophilic heterotrophs was to provide natural conditions for the oxidation of iron. Acidophilic heterotrophs do not directly participate in iron oxidation but contribute to the stability of the mixed mineral-oxidizing population by consuming organic excretion products produced by mineral oxidizers [15].

The percent metal mobilization was determined from the higher values obtained in the chemical analysis of the corresponding metals, irrespective of the solvent used for dissolving the PCB powder.

We observed that the solubilization of Cu by microbial consortium after 240 h of incubation was 96.93, 87.34, 73.68, 66.42 and 49.93% at 10, 20, 30, 40 and 50 g/l of PCB powder in the medium, respectively (Fig. 1a). The dissolution of Zn after the same time reached 93.33, 81.23, 66.66, 73.45, 73.45 and 70.61% at 10, 20, 30, 40 and 50 g/l of PCB powder in the broth, respectively (Fig. 1b). The maximum

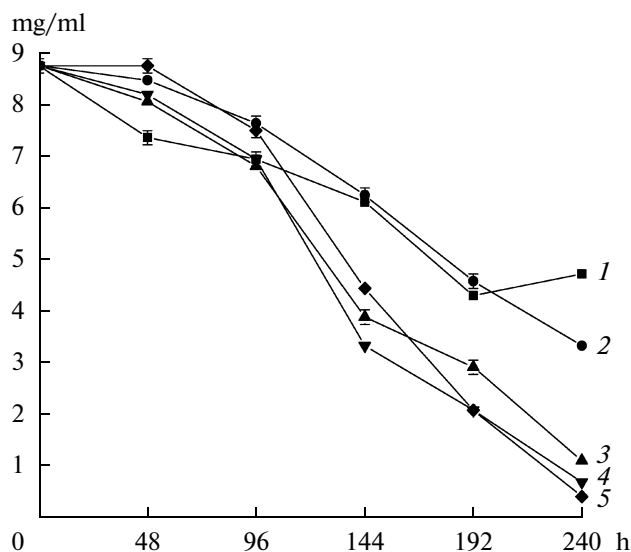


Fig. 3. Fe^{2+} amount under different concentrations of PCB powder (1 – 10; 2 – 20; 3 – 30; 4 – 40; 5 – 50 g/l) relative to the time of incubation with microbial consortium. The data present the average of 3 replicates.

solubilization of Pb during bioleaching after 48 h of incubation was 0.39, 0.58, 0.35, 0.25 and 0.20% at 10, 20, 30, 40 and 50 g/l of PCB powder in the broth, respectively (Fig. 1c). After 240 h the concentration of Pb in bioleachate amounted 0.19, 0.03, 0.03, 0.03 and 0.02% at 10, 20, 30, 40 and 50 g/l of PCB powder, respectively. The solubilization of Ni by microbial consortium after 240 h was 9.44, 8.66, 10.26, 7.76 and 9.50% at 10, 20, 30, 40 and 50 g/l of PCB powder in the medium, respectively (Fig. 1d).

During the bioleaching of the PCB powder, the solubilization of copper was achieved at a faster rate compared with the other metals (Fig. 1). The maximum copper solubilization (96.93%) and zinc (93.33%) was obtained at 10 g/l of PCB powder (Figs. 1a and 1b). The solubilization rate of these metals increased with time. In case of lead mobilization (Fig. 1c), the maximum mobilization (0.58%) was achieved when 20 g/l of PCB powder was used. Interestingly, the lead content slowly decreased with increasing incubation time, reaching 0.03%. However, when 10 g/l of PCB powder was used, the soluble lead content decreased during bioleaching from 2.038 mg/l (0.40%) to 0.520 mg/l (0.10%), and subsequently increased, reaching 0.998 mg/l (0.19%). One possible reason for the later increase would be the decreasing pH. Compared with the leaching of copper and zinc, the lead solubilization rate was low. The nickel solubilization was less compared with Cu and Zn. The highest dissolution achieved was 10.26% (Fig. 1d). Moreover, even at 50 g/l of PCB powder, the solubilization was 9.5%.

Brandl with coworkers [3] observed that at scrap concentrations of 5 and 10 g/l, *Thiobacilli* were able to leach more than 90% of the available Al, Cu, Ni and

Zn, but at higher scrap concentrations, metal solubilization was reduced, especially for Al and Cu.

According to Ilyas and colleagues [6], approximately 64, 80, 86 and 74% of Al, Zn, Cu and Ni, respectively, were leached out (in 300 days) using a mixed consortium of metal-adapted cultures of *Sulfobacillus thermosulfidoxidans* and the acidophilic heterotrophs.

During the bioleaching studies, the pH of the medium was continuously monitored as a change in pH affects the leaching efficiency [2]. The changes in pH during the metal solubilization by microbial consortium in this study are shown in Fig. 2. We observed that the pH of the medium increased initially due to the alkalinity of the PCB powder that corresponded to previous results [3] and subsequently decreased.

Fig. 3 shows the pattern of Fe^{2+} change during bioleaching. The results obtained demonstrated that when 10 g/l of PCB powder was used, the rate of Fe^{2+} oxidation (biological reaction) was minimal, but the rate of oxidation of copper and zinc by Fe^{3+} (chemical reaction) was maximal (Figs. 1a, 1b, 3). In contrast, when 50 g/l of PCB powder was used, the rate of Fe^{2+} oxidation was maximal, while the rate of oxidation of copper and zinc by Fe^{3+} was minimal. The rate of Fe^{2+} oxidation increased with increasing PCB powder concentration. Hence, it is rational to conclude that the PCB powder concentration stimulated Fe^{2+} oxidation (biological reaction) but adversely affected the rate of oxidation of copper and zinc by Fe^{3+} (chemical reaction).

Figs. 4a, 4b show the slope of the curves obtained in Figs. 1a, 1b, respectively. The largest positive slope obtained for Cu was at 192 h of incubation with microbial consortium when 40 g/l of PCB powder was used. In the case of Zn, the largest slope was observed at 144 h using 10 g/l of PCB powder. Because there was a continuous selection for microorganisms during bioleaching, the largest positive slope might suggest the point where the optimum consortium composition and pH for the bioleaching of PCB powder could be obtained. These results suggest that pH 2.94 and 2.45 could be the optimum pH for the solubilization of Cu and Zn, respectively (Fig. 2).

For the overall bioleaching efficiency, we observed that the microbial consortium used could solubilize up to 7620 mg/l of copper, 286 mg/l of zinc, 6 mg/l of lead and 21 mg/l of nickel. This result indicates not only the high metal tolerance of the consortium but also its high metal solubilization ability.

The mechanism for the dissolution of metals from PCB powder is similar to that of the metal sulfide [4]. Under acidic conditions, iron oxidizers use ferrous iron as an energy source and convert it to ferric iron. The last oxidizes zero valent metals present in the PCB powder and converts them to soluble forms (Equations 1 and 2) [2].

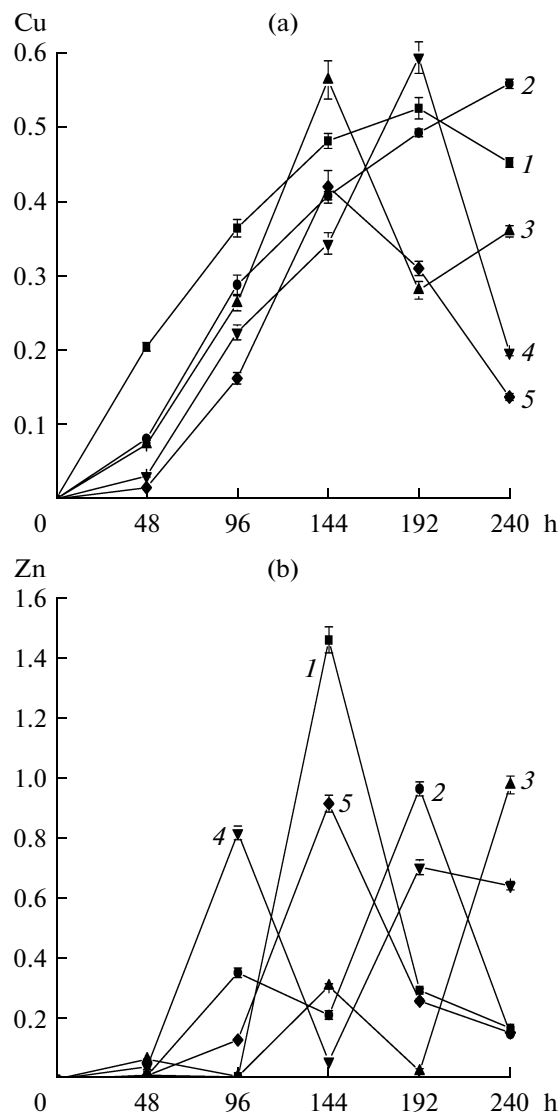
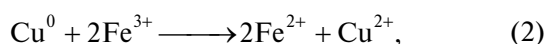
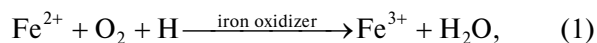


Fig. 4. Rate of change in copper (a) and zinc (b) mobilization under different concentrations of PCB powder (1 – 10; 2 – 20; 3 – 30; 4 – 40; 5 – 50 g/l), relative to the time of incubation with microbial consortium. The data present the average of 3 replicates.

$$G_r^{\circ} = -82.90 \text{ kJ/mol.}$$

A reddish brown-colored precipitate was formed during the bioleaching process (Fig. 5). After its analyzing using SEM-EDX, the presence of Sn, Cu and Pb was confirmed. The precipitate obtained in the bioleaching experiment was composed of Tin (59.96%), Cu (23.97%), Pb (9.30%) and Fe (5.92%). According to the previous reports, Pb and Sn were not detected in the leachate, rather these metals were speculated to be precipitated as PbSO_4 and SnO , respectively [3]. The presence of Pb and Sn was also confirmed by Ilyas with coworkers [6] through a mineralogical analysis of the precipitate, which also contained $0.65 \pm 0.08\%$ aluminum, $1.88 \pm 0.05\%$ copper, $2.5 \pm 0.07\%$ iron, $27.0 \pm 0.08\%$ lead, $0.22 \pm 0.005\%$

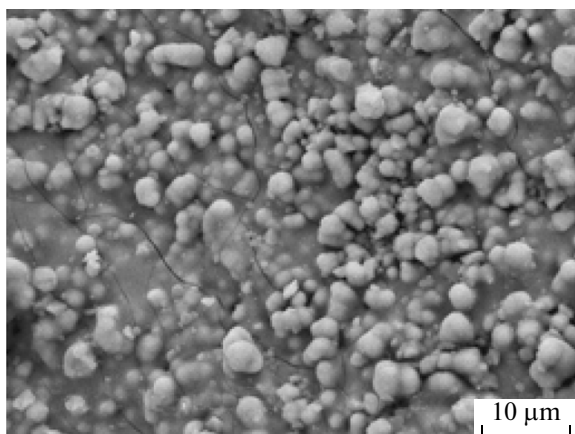
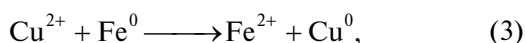


Fig. 5. Scanning electron micrograph of the precipitate formed during the bioleaching of the PCB powder.

nickel, $74 \pm 0.07\%$ tin and $0.10 \pm 0.01\%$ zinc. Choi and colleagues [4] observed the presence of 63% copper in the precipitate, though the copper content was minimized to approximately 18% by the addition of 1.0% citric acid.

The copper precipitation could occur due to a cementation reaction in which the presence of Fe, Zn or Al led to copper precipitation [16] as shown in Equation 1 [17]. The process of cementation generally recovers soluble copper from pregnant liquor following the addition of scrap iron after the bioleaching process is complete. In the present investigation, the precipitation of copper during bioleaching could be attributed to the presence of Fe, Zn or Al in the PCB powder, which could have led to the precipitation of copper, assuming that the rate of the solubilization of copper was faster than that of Fe, Zn or Al. Due to the faster solubilization of copper, dissolved copper would be cemented on Fe, Zn or Al. The occurrence of 'early cementation' also makes it difficult to distinguish whether any PCB powder is left after the completion of bioleaching experiment.



$$G_f^0 = -144.6 \text{ kJ/mol.}$$

From the present study, we concluded that bioleaching is a possible alternative for extracting metals from PCB.

The chemical analysis of the PCB powder was performed using two solvents: aqua regia and nitric acid. The combination of the two solvents provided more accurate results for the chemical analysis.

The present study revealed the effectiveness of the natural microbial consortium obtained from bauxite and pyrite ore samples in terms of leaching efficiency compared with the conventional use of pure cultures or a defined consortium. The microbial consortium used for metal solubilization was efficient, as 96.93% copper and 93.33% zinc solubilization was achieved in 240 h (with 10 g/l PCB powder). More importantly,

the consortium used in the present study would be competitive under the non-sterile conditions of the commercial bioleaching process.

These studies provide evidence for the presence of Sn and Pb in the precipitate. Hence, further studies should be conducted to avoid the precipitate formation. For this purpose, a consortium growing at $\text{pH} < 2.0$ could be useful because increased acidic conditions minimize precipitate formation. Using a consortium growing at $\text{pH} < 2.0$ might lead to an increase in leaching efficiency.

An alternative strategy needs to be developed to deal with the problem of 'early cementation.'

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