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Sequential biologically assisted extraction of Cu and Zn from printed circuit boards (PCB)

Arevik Vardanyan ^{a,b}, Narine Vardanyan^a, Nelli Abrahamyan^a, Mohamed Aatach^b and Stoyan Gaydardzhiev^b

^aDepartment of Microbiology, SPC “Armbiotechnology” of the National Academy of Sciences of Armenia, Yerevan, Armenia; ^bGeMME - Minerals Engineering, Materials & Environment, University of Liege, Liege, Belgium

ABSTRACT

The increased production and use of electrical and electronic equipment leads to obsolescence and disposal problems, necessitating materials recovery and recycling. This paper reports results on metal bioleaching from printed circuit boards (PCBs) using chemolithotrophic bacteria isolated at different sulphide ore biotopes in Armenia. Different ways of generating lixivants were investigated, namely using combination of *Acidithiobacillus ferrooxidans* 61 and *Acidithiobacillus thiooxidans* SO-1 bacteria generating biogenic Fe₂(SO₄)₃ and biogenic H₂SO₄. The sequence between these leaching agents permitted design of a 2-step process based on acidolysis and redoxolysis to leach non-ferrous metals from PCBs. To compare the efficiency of the sequential bioleaching of PCBs, several experimental runs were realised under the six modes at 10% pulp density. The flasks-based tests have witnessed almost complete recovery of Cu with the rest of the metals reaching extraction degree above 80%.





KEYWORDS

PCBs; chemolithotrophs; bioleaching; acidolysis; redoxolysis; recovery

Introduction

Environmental pollution with heavy metals presents a serious threat to living organisms in the ecosystems [1–3]. The heavy metals, acute environmental pollutants, may originate from discarded solid wastes and industrial wastewater. The heavy metals can accumulate in living organisms and then be transmitted via the food chains and pose human health risks [4].

Different organic pollutants and metals are not degradable and remain in the environment for a long time. Remediation using conventional physical and chemical methods is often uneconomical and associated with generation of large volumes of chemical-type waste [5,6]. On the other hand, the use of microorganisms is known as an environment-friendly and cost-effective alternative to recover metals from mineral-based substrates [7,8]. Microorganisms have a variety of mechanisms of metal sequestration and possess high metal biosorption capacities. In particular, microorganisms have the ability to

CONTACT Arevik Vardanyan  arevik.vardanyan@asnet.am  Department of Microbiology, SPC “Armbiotechnology” of the National Academy of Sciences of Armenia, 14 Gyurjyan Str, Yerevan 0056, Armenia; Stoyan Gaydardzhiev  s.gaydardzhiev@uliege.be  GeMME - Minerals Engineering, Materials & Environment, University of Liege, Allée de la découverte, 9. Bât. B52/3, Sart-Tilman, Liege 4000, Belgium

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degrade, detoxify, and even accumulate harmful organic as well as inorganic compounds. Different types of microorganisms are suggested to remove and recover metals and metalloids from industrial waste streams.

Among the different types of secondary post-consumption wastes (e-waste, spent batteries, slags) [9], *e-wastes* or waste electrical and electronic equipment (WEEE) represent the fastest growing and most problematic waste stream in the world (geographically scattered, poorly understood ‘mineralogy’).

In 2019, the world generated a striking 53.6 Mt of e-waste, an average of 7.3 kg per capita. Thus, the global generation of e-waste grew by 9.2 Mt since 2014 and is projected to grow to 74.7 Mt by 2030 – almost doubling in only 16 years [10].

Generally, e-waste contains between 3% and 5% in volume printed circuit boards (PCBs), which present the most valuable part of WEEE. Typically, PCBs from a PC can contain about 7% Fe, 5% Al, 20% Cu, 1.5% Pb, 1% Ni, 3% Sn, and 25% organic compounds, together with about 250 ppm Au, 1000 ppm Ag, 110 ppm Pd, and trace amounts of As, Sb, Ba, Br, and Bi. Cu and Au in computer derived PCBs could, respectively, be found in concentrations 20–40 and 25–250 times higher than in natural ores [11].

In order to support the transition towards a circular economy, the European Commission has established an EU action plan [12]. This plan requires including secondary raw materials such as waste electrical and electronic equipment (WEEE) as a source of metals from ‘urban-mine’ raw materials. Besides, existing EU legislation encourages the recycling of electronic waste through mandatory targets. However, it is acknowledged that only high-quality recycling can ensure the recovery of all raw materials [13]. Nevertheless, the management of WEEE is still to be improved since in Europe *approximately a mere 35%* of the collected stream is recycled, and the rest is land-filled [14].

Thus, the development of flexible, energy- and resource-saving bio-based technologies for extraction of valuable metals from WEEE is important. Biohydrometallurgical approaches could permit extraction of valuable and precious metals from PCBs, and the remaining metal-depleted streams could be valorised in sectors such as civil engineering (most of the depleted metal streams) or safely sent to dumping (a negligible amount).

To recover metals from e-waste, pyrometallurgical [15,16], hydrometallurgical [17,18] and biometallurgical [19,20] techniques are traditionally employed. The first and second are required the use of either high temperatures or aggressive chemicals to melt or degrade the metal-containing matrix, resulting in significant economic cost and large environmental impact. Bioleaching, however, could be an environment-friendly metals recycling option. It is well tested, particularly in the extraction of metals from low-grade ores [21] and recently from e-waste [22–26]. Bioleaching is based on the ability of microorganisms to regenerate leaching agents, which are responsible for metal extraction [27–29]. For Cu extraction, the process is based on the action of Fe(III), which oxidises metallic Cu in the input material, transforming it into soluble Cu(II) accompanied by the ferric iron reduced to Fe(II). The role of the microorganisms is to re-oxidise Fe(II) back to Fe(III) and accomplish the cyclic process.

In contrast to natural ores bioleaching, in case of WEEE treatment, an iron or eventually a sulphur source has to be provided in addition to the nutritive medium. This is usually realised in the form of ferrous sulphate, with acid provided directly via pH

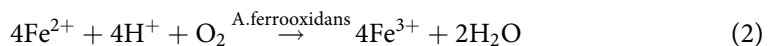
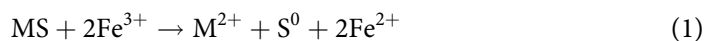
control through sulphuric acid or the addition of elemental sulphur. Such an approach increases process operating costs, however. Furthermore, 'urban-mine' raw materials are often highly acid-consuming and therefore require significant pH buffering agents to maintain an acidic environment [26,30–33].

Recently, studies done at the University of Liege, Belgium [34,35] have revealed that leaching of WEEE materials is inhibited by the presence of toxic elements in the substrate itself. In such a way, the toxicity is considered as a key factor which limits pulp density and leaching efficiency [36,37]. To overcome these problems, staggering lixiviant production and adding the feed in a two-step mode have been tried, which necessitates the production of ferric iron in a primary tank. The biolixiviant is then mixed with the 'urban-mine' substrate in a separate tank where metal dissolution occurs. Under this configuration, there is no need for the microorganisms to be in contact with the materials, thus allowing work at increased pulp densities [38]. Such attempts eliminate the toxicity in the leaching step, and no regeneration of the ferric iron or acid (proton lixiviant) occurs since they are consumed by the material to be leached. As a result, the system may be limited in terms of ferric iron availability for the leaching step.

Acidolysis (formation of acids) and redoxolysis (microbially driven or catalysed oxidation and reduction processes) are among the principal metal mobilisation mechanisms [39–41]. The dissolution of metals by biogenic acids occurs through acidolysis [42]. Acidolysis is driven by a variety of autotrophic sulphur oxidisers (e.g. *Acidithiobacillus thiooxidans*, *Acidithiobacillus caldus*, *Sulfobacillus thermosulfidooxidans* etc.), as well as heterotrophic (*Bacillus*, *Pseudomonas*, *Chromobacterium*) and fungal cells (*Aspergillus*, *Penicillium*) [43–45]. In the leaching systems, Fe^{3+} is one of the most used redoxolysis agent, which is then reduced to Fe^{2+} in the course of the bioleaching. Further on, Fe^{2+} is re-oxidized to Fe^{3+} by iron oxidisers (*Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Sulfobacillus thermosulfidooxidans*) [46,47]. Chemolithotrophic iron and sulphur oxidising bacteria are among the well-known bioleaching consortia which can perform the above described activity.

A. ferrooxidans is one of the most important microorganisms for Cu recovery [48]. Several studies focus on the tolerance of *A. ferrooxidans* to different metals [49] evaluating and reporting resistivity to high concentrations of Cu (up to 800 mM CuSO_4) [50] and other metals such as Zn, Ni, Fe, Cd, Cr, Mo, Ag and As [51].

Currently, it is accepted that the oxidation of sulphide minerals occurs by indirect mechanism involving a Fe(III) ion (Equation 1) with the process efficiency being limited by the activity of the microorganisms and their ability to regenerate the leaching agent – Fe(III) (Equation 2).



Depending on the mode of regeneration of the oxidising agent (Fe(III)), one could distinguish an indirect 'non-contact' and a 'contact' mechanism [52]. In case of a 'non-contact' mechanism, ferric iron is regenerated in the liquid phase by free 'swimming' planktonic bacteria. The indirect 'contact' mechanism is based on the attachment of bacteria to the surface of the sulphide mineral. Once adhered to the

surface of the mineral, bacteria produce extracellular polymeric substances (EPS) which mediate attachment of cells on the substrate surface. In case of 'contact' mechanism, the electrochemical processes that result in mineral dissolution take place in the bacterium-mineral interface [53,54]. Overall, it is accepted that the oxidation of minerals is realised by the Fe(III) ions associated with extracellular polymeric substances.

Depending on the conditions being practised, Cu bioleaching efficiency from PCBs has been reported to vary from 50% to 100%, with leaching times of up to 5 days and pulp densities in the range of 1–3%. Several investigations have shown that sulphur- and ferrous iron-supplemented media are efficient for bioleaching [19,37,55]. Wu et al. 2018 [56] investigated Cu extraction from PCBs using bacteria-free culture supernatant. They showed that nearly 100% of Cu could be recovered during 2 h at pulp density of 5%. In the same work, it is also implied that the non-contact mechanism was predominant in bioleaching of Cu from PCBs.

Some bioleaching studies are performed in a single step only, where PCBs and inoculum are employed together in the bioleaching system. Because WEEE is heterogeneous with more than 60 elements being present during a bioleaching process, metals different from the metal of interest could also be brought into solution, including toxic metals. These toxic metals could affect the biological activity of the chemolithotrophic bacteria [22,57–59].

Based on the open questions and the process challenges briefly summarised above, the main aim of this study is to investigate the ability for metal recovery from PCBs using a sequential two-step bioleaching including combination of acidolysis and redoxolysis. In the first stage, generation of oxidising agent (Fe^{3+} or H^+) by iron and/or sulphur oxidising bacteria is performed, then in the second stage the biogenic solution of Fe^{3+} or H_2SO_4 is used for bioleaching of PCBs. As a result, all the metals targeted for extraction are excluded from direct contact with the biomass. In order to optimise bioleaching process and metallurgical recovery, we have applied a sequential bioleaching process using lixivants obtained by *A. ferrooxidans* and *A. thiooxidans*. Thus, fresh bio-based reagents are used making the process economical and environment-friendly. The bioleachate obtained from the PCBs dissolution could be reused via re-oxidising Fe^{2+} by iron oxidising bacteria to Fe^{3+} in a continuous cycle. The metals in the bioleachate which could affect bacterial activity could also be recirculated but purged away once their concentration exceeds the tolerable limits.

Materials and methods

Preparation of PCBs

In this work, the researchers used multi-layer printed circuit boards originating mainly from discarded laptops made by ASUS. The depopulated PCB samples were cut by scissors into pieces (1.5×1 cm) which were further used in the experiments. Before bioleaching, the existing passive layer (green solder mask) of the PCBs was removed by boiling the fragmented PCBs in 10% NaOH for 15 minutes. Afterwards, the samples were thoroughly washed with deionised water, dried and preserved for leaching. The samples

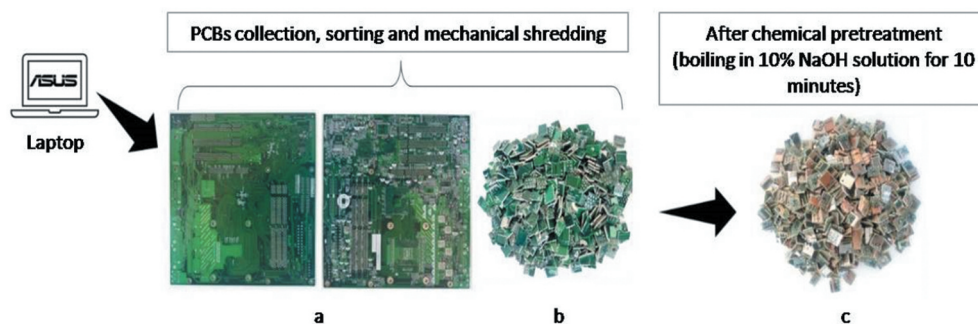


Figure 1. View of depopulated PCBs after: dismantling and components removal (a), being cut (b) and chemical pre-treatment (c).

were weighed before and after each of the above-mentioned pre-treatment procedures. Figure 1 schematically shows the procedure for preparing the PCBs for leaching.

Culture growth and lixiviant preparation

A. thiooxidans SO-1 (KP455985) were grown at 30°C in a modified 0 K medium consisting in $(\text{NH}_4)_2\text{SO}_4$ -0.5 g/L, KCl-0.05 g/L, K_2HPO_4 -0.5 g/L, $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ -0.5 g/L, $\text{Ca}(\text{NO}_3)_2$ -0.01 g/L, and elemental sulphur – 10 g/L. The elemental sulphur has served as a source of sulphuric acid after its conversion by the microorganisms. Upon reaching a logarithmic phase of growth, the culture liquid was filtered using a 589/2 Whatman filter to remove the remaining elemental sulphur and used for bioleaching. For receiving a bacteria-free supernatant (biogenic H_2SO_4), the bacterial lixiviant was filtered through 0.2 μm membrane under vacuum. The above steps have aimed to generate a biogenic H_2SO_4 used in the leaching process – acidolysis.

A. ferrooxidans 61 (KM819692) was grown at 30°C in a modified 9 K medium containing 44.2 g/L $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$. Similar to the *A. thiooxidans*, once the logarithmic growth phase was reached, the culture liquid was used for bioleaching. For obtaining a bacteria-free lixiviant (biogenic $\text{Fe}_2(\text{SO}_4)_3$), the culture liquid was filtered using a 0.2 μm membrane under vacuum. This step is redoxolysis, given the biogenic Fe^{3+} used as the main oxidising agent.

Cells' enumeration was determined by direct counting under a microscope (Optica B-810, Italy) using a Thoma Chamber. The initial cell number was determined as 2.5×10^8 and 3.0×10^8 cells/mL for *A. ferrooxidans* 61 and *A. thiooxidans* SO-1, respectively.

Bioleaching

The bioleaching experiments were performed in 250 mL Erlenmeyer flasks placed on an orbital shaker-incubator (Biosan, Latvia) run at 220 rpm and temperature of 40°C.

Tests were performed at 10% PCBs pulp density with pH of the lixiviant adjusted to 1.1 using a 10 N H_2SO_4 .

To compare the efficiency of the sequential bioleaching of PCBs, several experimental runs were realised under the six modes described below:

- (A) Single-stage bioleaching by biogenic H_2SO_4 .
- (B) Single-stage bioleaching by biogenic $\text{Fe}_2(\text{SO}_4)_3$.
- (C) Two-stage bioleaching: the initial step was realised using cell-free lixiviant (biogenic H_2SO_4) obtained from *A. thiooxidans* SO-1 and the 2nd stage by cell-free lixiviant (biogenic $\text{Fe}_2(\text{SO}_4)_3$) from *A. ferrooxidans* 61.
- (D) Two-stage bioleaching: the first step was done with a lixiviant obtained from *A. thiooxidans* SO-1 and the 2nd stage – lixiviant obtained from *A. ferrooxidans* 61.
- (E) Two-stage bioleaching: the first step was done with cell-free lixiviant obtained from *A. ferrooxidans* 61 and the 2nd stage – by the cell-free lixiviant from *A. thiooxidans* SO-1.
- (F) Two-stage bioleaching: the first step was done by the lixiviant obtained from *A. ferrooxidans* 61 and the 2nd stage by lixiviant obtained from *A. thiooxidans* SO-1.

Sampling for determination of pH, redox potential and metal concentration was performed at 1, 3 and 24 hours. Evaporation was compensated prior to each sampling using the respective leachates.

Chemical analysis

The metal concentration of the PCBs before and after bioleaching was determined by an Inductively Coupled Plasma Optical Emission Spectrometry (Agilent 5800ICP-OES) and atomic emission absorption (AES). The spent PCBs were acid digested before being subjected to ICP-OES analysis. To this end, 0.25 g of PCBs were heated at 150°C with 10 mL $\text{HNO}_3:\text{HCl}$ (3:1) for 15 minutes in a microwave digestion unit [29]. Concentrations of Fe^{3+} and Fe^{2+} were determined by the complexometric method with EDTA [60]. All experiments were performed in triplicate. The data presented here are the average values from the repeated experiments with $\pm 2\%$ variation.

Sulphate was detected by a trichloroacetic acid method [61] and measured at 360 nm by UV-6300PC double beam spectrophotometer (VWR).

Cu extraction rate was calculated during the first 2 hours of the leaching process as:

$$d[\text{Cu}^{2+}]/dt \quad (3)$$

where $d[\text{Cu}^{2+}]$ – is the change in concentration of Cu during 1 and 2 hours,
 dt – is the leaching period (hour).

The calculation of metal recovery has followed equation 4.

$$\text{MR}(\%) = \frac{\text{CMI} \times 50}{m \times \text{CMs}} \times 100 \quad (4)$$

where MR is metal recovery, %

CMI – concentration of leached metal in the pregnant leaching solution (PLS), %

CMs – metal concentration in the input solid, %

m – mass of used PCBs, g

50 – volume of the leachate inside the flasks, mL

The standard deviation was calculated through equation 5.

$$SD = \sqrt{\frac{\sum (x+\mu)^2}{N}} \quad (5)$$

where

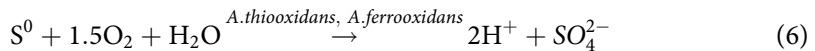
- SD – standard deviation,
- x – value in the data set,
- μ – mean of the data set,
- N – number of data points in the population.

Results and discussions

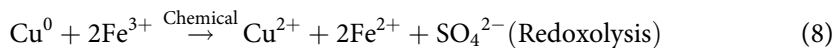
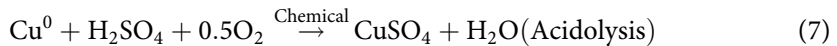
Table 1 shows the main metal content in the prepared PCBs.

It could be noted that Cu, Al, Ni and Pb present the principal non-ferrous metals of interest. Since Pb and Sn are known as non-soluble in sulphuric acid media, we decided to focus on the degree of bringing Cu, Zn, Al, and Ni in solution only.

Provided the bioleaching of PCBs has required the indirect leaching mechanism by biogenic H_2SO_4 and ferric iron Fe(III), we could assume that the role of *A. thiooxidans* SO-1 in the process is to catalyse the oxidation of the elemental sulphur to sulphuric acid through dissolved oxygen involvement (Equation 6). On the other side, the main role of *A. ferrooxidans* 61 in the process is the oxidation of Fe(II) to Fe(III) (Equation 2), the latter being a strong oxidising agent.



Accordingly, the biogenic H_2SO_4 and Fe^{3+} mobilise zero valent Cu from WEEE under two routes shown in equations 7 and 8, respectively.



It is assumed that during the PCB bioleaching, the bacterial attachment on the substrate surface is not essential, because the main oxidising agent responsible for the leaching is Fe (III). Hence, it can be hypothesised that the bioleaching of PCBs takes place by indirect ‘non-contact’ mechanism. In our study, we have used two types of lixivants: culture-bearing lixiviant containing iron and sulphur oxidising bacteria and corresponding cell-free liquids.

Table 1. Main metal concentration in the input PCBs.

Metal, %	Concentration
Cu	21.53
Al	6.95
Pb	3.2
Zn	0.78
Ni	5.12
Fe	3.86
Sn	1.98

Figure 2 shows the sequence of the two-stage PCB leaching involving biogenic H_2SO_4 (acidolysis) and biogenic $\text{Fe}_2(\text{SO}_4)_3$ (redoxolysis) process.

Figures 3–7 in the next section show the results from the comparative bio-assisted extraction for Cu, Zn, Al, and Ni, following the six bioleaching modes combining single and two-stage steps.

The data shown in Figure 3 suggest that the extraction of Cu by biogenic H_2SO_4 (mode A) and biogenic $\text{Fe}_2(\text{SO}_4)_3$ (mode B) reaches about 10.2% and 42.3%, respectively, after 24 h. The addition of biogenic $\text{Fe}_2(\text{SO}_4)_3$ during the second stage (1st stage – 24 h, 2nd stage – 24 h) resulted in about 83% of Cu reported in the PLS (mode C). In case of addition of the corresponding culture bearing liquids in the second stage (mode D), almost 74% of Cu (10% in the 1st stage and 64% in the 2nd stage) was leached. In such a way, the beneficial effect from the sequential leaching of Cu – modes C and D could be demonstrated. It is obvious that the Cu extraction degree under this two-stage leaching is largely superior to the one achieved during the single stages (A and B) only, which has brought cumulatively about 52.5% Cu recovery. However, it should be noted that Cu extraction degree by the culture bearing liquids composed of *A. thiooxidans* SO-1 (1st stage) and *A. ferrooxidans* 61 (2nd stage) (D) is lower compared to the case when the corresponding cell-free lixiviant was used.

Thus, taking into consideration the kinetics of Cu leaching through sequence involving biogenic H_2SO_4 and biogenic ferric iron, it can be concluded that the ferric iron is the oxidising agent with the greatest significance for the efficient Cu recovery.

Since Cu is the most economically important metal influencing largely the PCBs' intrinsic value, only the leaching kinetics of Cu are presented. Table 2 shows the rates of

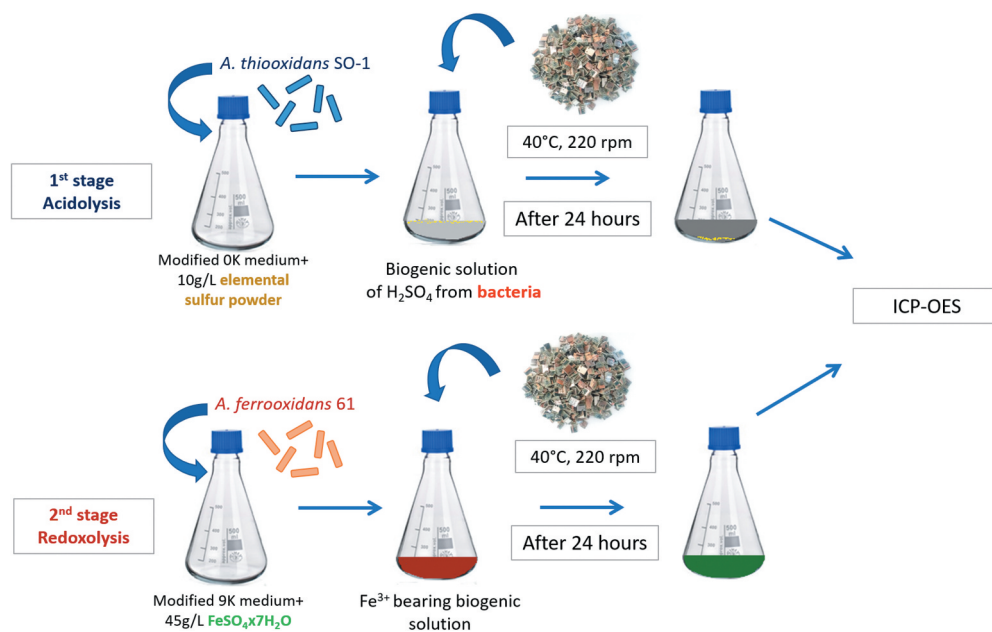


Figure 2. A hypothetical two-stage PCBs bioleaching sequence.

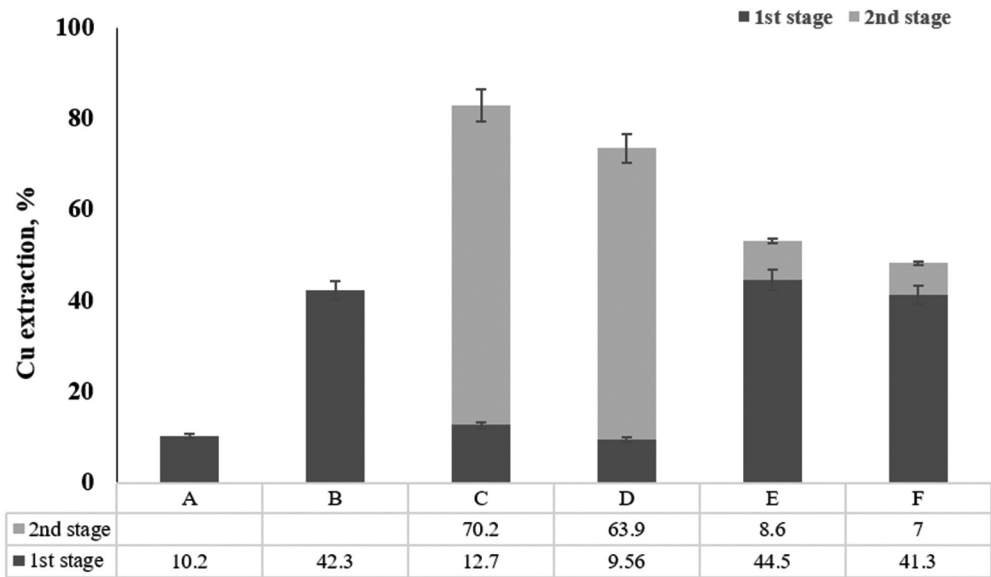


Figure 3. Cu extraction degree achieved under the 6 different bioleaching modes (duration of each stage –24 hours).

Table 2. Rate of Cu leaching and Fe^{3+} reduction (g/l h).

No.	Mode of treatment	Leaching rates of Cu, g/L h		Reduction rates of Fe^{3+} , g/L h	
		V1	V2	V1	V2
1	Biogenic $\text{Fe}_2(\text{SO}_4)_3$ (E)	4.4	0.23	7.2	0.42
2	Biogenic $\text{Fe}_2(\text{SO}_4)_3$ + <i>At. ferrooxidans</i> 61 (F)	4.5	0.2	7.4	0.39

Cu leaching and Fe^{3+} reduction during the 1 hour (V1) and the following 2 h (V2) of the process.

As can be seen from Table 2, the highest rate of Cu leaching is observed during the first hour of PCBs' leaching with biogenic ferric iron (V1) both in the presence and absence of bacterial cells. Over the next 2 hours, the leaching rate drops sharply to 0.2 g/L h (V2).

As seen from the graph shown in Figure 4, after 3 hours of PCBs' leaching, the amount of Fe^{3+} decreases to 0.78 g/L, and the amount of Cu transferred into the leachate is only 6.5 g/L (30%). After 24 hours, the amount of leached Cu increases reaching about 9.1 g/L, corresponding to nearly 41% recovery in the PLS, with almost complete absence of ferric iron in the leaching solution.

It is assumed that ferrous iron resulting from the chemical reaction between the PCBs and ferric iron is continuously oxidised to ferric iron in the presence of bacteria, which, in turn, in accordance with the above-mentioned Equation 9, promotes Cu dissolution and increases its concentration in solution.

As seen from Table 2 and Figure 4, the Cu leaching seems to be conjugated with the reduction of ferric to ferrous iron according to reaction 8. Moreover, a correlation between the Cu leaching rates and ferric iron reduction is observed which can be described by equation 9:

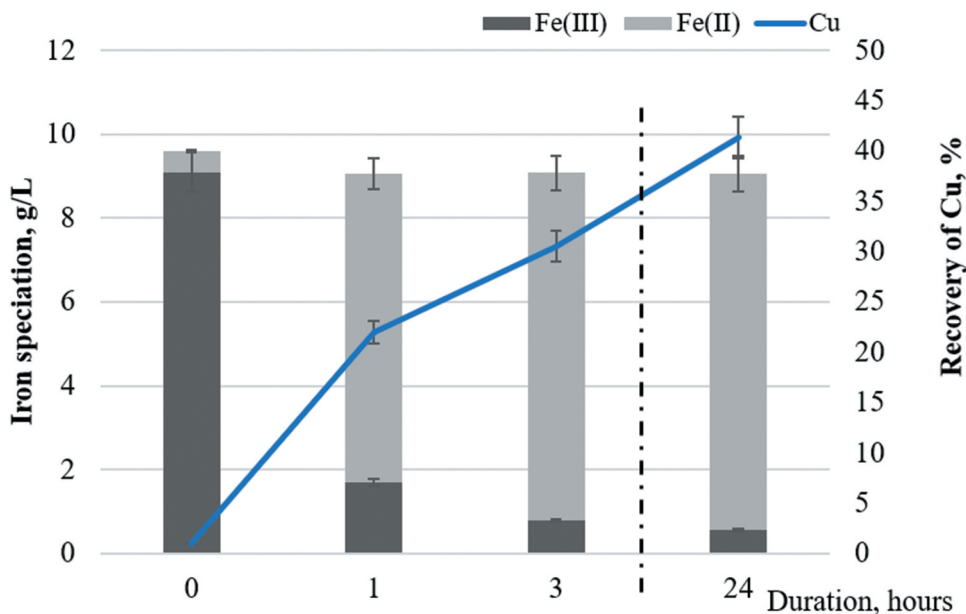


Figure 4. Kinetics of Cu extraction from PCBs and iron speciation in the biogenic $\text{Fe}_2(\text{SO}_4)_3 + \text{At. ferrooxidans}$ 61 system (F).

$$\frac{d[\text{Cu}^{2+}]}{dt} = -\frac{d[\text{Fe}^{3+}]}{2 \times dt} \quad (9)$$

The data presented in Figure 5 show that the degree of Zn brought into solution by cell-free biogenic H_2SO_4 (A) and cell-free biogenic $\text{Fe}_2(\text{SO}_4)_3$ (B) reaches 29% and 18%, respectively. During the two-stage leaching, a slightly higher extraction of Zn is observed under modes E (54.3%) and F (48.4%), with the 1st stage done using ferric iron.

The biogenic H_2SO_4 was found to be more effective for Al extraction (Figure 6). The Al recovery under A and B modes was 21% and 10%, respectively, suggesting that the biogenic H_2SO_4 is the better option for Al recovery, not the biogenic $\text{Fe}_2(\text{SO}_4)_3$. The highest Al recovery has been reached under D mode (54%) with cell-free lixivants obtained by sulphur and iron oxidising bacteria.

The results shown in Figure 7 suggest that during sequential leaching of Ni from PCBs, the influence of biogenic H_2SO_4 and biogenic $\text{Fe}_2(\text{SO}_4)_3$ is nearly equal, the recoveries being 43.4% (C) and 39.8% (E) regardless of the sequence of lixiviant addition.

Table 3 shows the variation of pH and redox potential during the leaching experiments.

In our previous experiments (data not shown), we have studied metal extraction from PCBs in the range of pH 1.0–2. It has been shown that the lower the pH, the higher the metal extraction yield, which is consistent with other similar studies [26,62]. The presented data show that pH increases from its initial value of 1.1 to about 1.6–1.9 in all the tested modes. It is worth mentioning that, in the course of

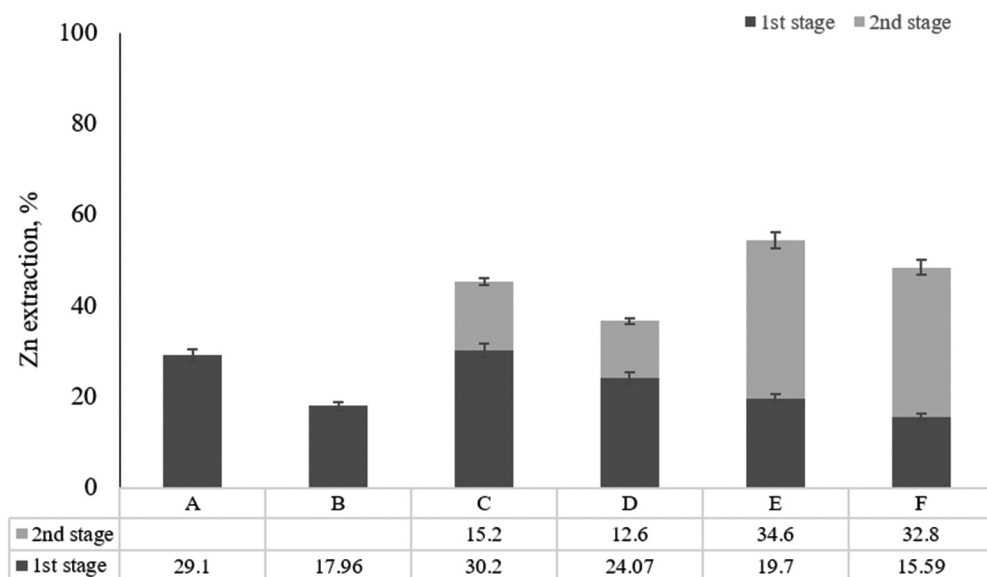


Figure 5. Zn extraction degree during 6 different modes of bioleaching (duration of each stage –24 h).

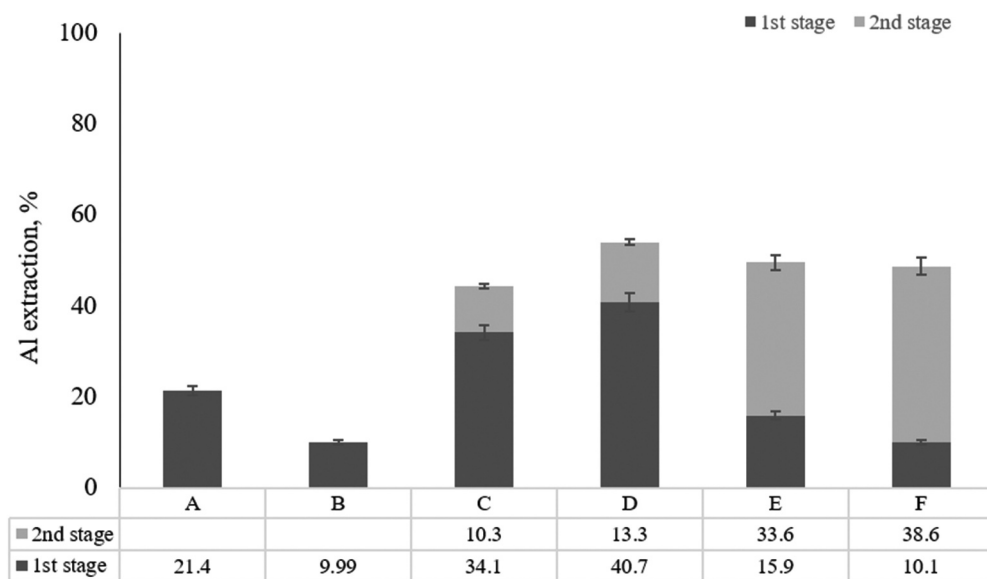


Figure 6. Al extraction degree during 6 different modes of bioleaching (duration of each stage –24 h).

the 24 h test duration, no formation and precipitation of jarosite (e.g. $\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$) were observed. The redox potential decreased in all tested modes as the leaching process progressed, accompanied by ferric iron reduction.

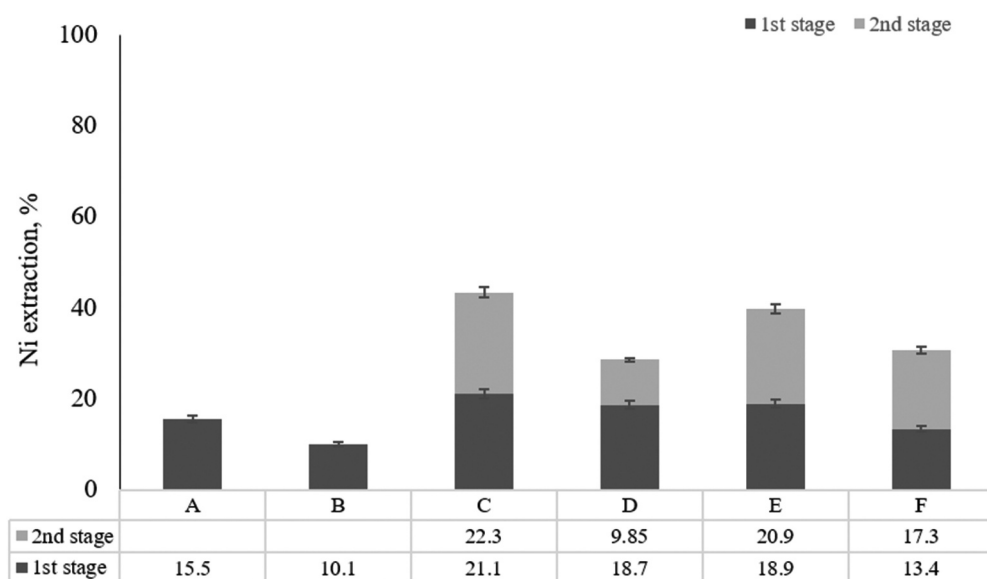


Figure 7. Ni extraction degree during 6 different modes of bioleaching (duration of each stage –24 h).

Table 3. Variation in pH, redox potential (vs. Ag/AgCl) and acid consumed during single-step and two-step bioleaching experiments (acid consumption: 5 ml of 10 N H₂SO₄ in 1000 ml medium).

N	Bioleaching modes	pH			Redox potential (mV)		
		0h	3h	24h	0h	3h	24h
1	A	1.1	1.4	1.85	634	472	421
2	B	1.1	1.75	1.98	680	570	489
3	C	1.1	1.3	1.61	634	464	397
4	D	1.1	1.23	1.56	654	464	375
5	E	1.1	1.35	1.75	680	524	499
6	F	1.1	1.31	1.7	688	520	460

Conclusions

A sequential two-step bioleaching by acidolysis and redoxolysis has proved efficient for recovery of non-ferrous metals contained in end-of-life (EoL) PCBs. For Cu, Ni and Zn, the best combination was found to be a biogenic H₂SO₄ obtained from *A. thiooxidans* SO-1 in the first stage and biogenic Fe₂(SO₄)₃ obtained from *A. ferrooxidans* 61 in the second. For Cu, Zn and Ni, altogether, the optimum lixiviant was a bacteria-free biogenic solution (mode C). For Al, however, a culture-bearing liquid with *A. thiooxidans* SO-1 and *A. ferrooxidans* 61 (mode D) was the more efficient option, not a bacteria-free lixiviant one.

It can be concluded that a sequential bioleaching with biogenic H₂SO₄ and biogenic Fe₂(SO₄)₃ is a promising process way and depending on the targeted metal to be recovered, a choice could be made between either single- or double-stage bioleaching involving *A. ferrooxidans* 61 and *A. thiooxidans* SO-1 bearing solutions, containing, respectively, biogenic Fe₂(SO₄)₃ and H₂SO₄. The sequence order could vary with the targeted metal.

It is clear that the sequential bioleaching is an environment-friendly approach with low energy demand and low CO₂ footprint and a reduced amount of inorganic acids. Further work will aim to increase the pulp density of the process and investigate the selective extraction of the non-ferrous metals in e-waste streams, by means of the simultaneous or sequential use of sulphur and iron oxidising bacteria or their consortia, as well as of heterotrophic bacteria.

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ORCID

Arevik Vardanyan  <http://orcid.org/0000-0001-8715-5870>

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