Biotechnology for Metal Recovery from End-of-Life Printed Circuit Boards with Aspergillus niger

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Received: 23 July 2020; Accepted: 10 August 2020; Published: 11 August 2020

Abstract: The growing production and use of electric and electronic components has led to higher rates of metal consumption and waste generation. To solve this double criticality, the old linear management method (in which a product becomes waste to dispose), has evolved towards a circular approach. Printed circuit boards (PCBs) are the brains of many electronic devices. At the end of their life, this equipment represents a valuable scrap for the content of base metals such as Cu and Zn (25 and 2 wt %, respectively) and precious metals such as Au, Ag, and Pd (250, 1000, and 110 ppm, respectively). Recently, biotechnological approaches have gained increasing prominence in PCB exploitation since they can be more cost-efficient and environmentally friendly than the chemical techniques. In this context, the present paper describes a sustainable process which uses the fungal strain Aspergillus niger for Cu and Zn extraction from PCBs. The best conditions identified were PCB addition after 14 days, Fe⁺³ as oxidant agent, and a pulp density of 2.5% (w/v). Extraction efficiencies of 60% and 40% for Cu and Zn, respectively, were achieved after 21 days of fermentation. The ecodesign of the process was further enhanced by using milk whey as substrate for the fungal growth and the consequent citric acid production, which was selected as a bioleaching agent.

Keywords: printed circuit boards; biotechnologies; circular economy; Aspergillus niger; copper; zinc; food waste

1. Introduction

In recent years, the production of electrical and electronic equipment (EEE) has substantially increased with the development of science and technology [1,2]. At the same time, the average lifetime of electronic products has also been drastically reduced (to around 2 years), resulting in a massive generation of waste from electrical and electronic equipment (WEEE) (around 44.7 million tons in 2016) [3,4]. Printed circuit boards (PCBs) represent about 3–5% of the total WEEE collected every year [5]. They are composed of metals (around 40 wt %), ceramics (around 30 wt %), and plastics (about 30 wt %) [3,6–9]. The metal fraction includes 20% Cu, 5% Al, 1% Ni, 1.5% Pb, 2% Zn, and 3% Sn (w/w) [2,4,10,11]. The presence of considerable amounts of metals represents a very critical issue for their possible release into the environment in the case of incorrect management [12]. Nevertheless, this aspect also represents an opportunity for a transition towards a circular approach following the principle “resource–product–regenerated resource”, where the waste is converted into a resource (urban mining) [13,14]. The most conventional options used by industries to extract metals from PCBs are pyrometallurgy and hydrometallurgy [6,15]. Pyrometallurgical approaches produce pollutant emissions (dioxins and furans), and they usually involve high operation costs. Hydrometallurgy is a low energy-cost process which needs large amounts of chemical agents.
Alternatively, biohydrometallurgy is often simple, environmentally friendly, and economical, responding to the sustainability principles essential for the development of a circular economy [15–17]. Several studies have described metal extraction from PCBs using bacteria, mainly *Leptospirillum ferrooxidans*, *Acidithiobacillus thiooxidans*, *A. ferrooxidans*, and *Sulfobacillus thermosulfidooxidans*, principally for the recovery of Cu and other metals such as Zn, Sn, Pb, and Ni [18–30]; *Chromobacterium violaceum*, *Pseudomonas fluorescens*, and *Bacillus megaterium* have also been used for Au recovery [31–35]. On the other hand, fungal bioleaching has several advantages since fungi show a greater ability to tolerate toxic materials, a faster leaching action than bacteria, and the ability to grow in both alkaline and acidic mediums [36]. *Penicillium simplicissimum*, *P. chrysogenum*, and *Aspergillus niger* are the most common eukaryotic microorganisms used for metal leaching from different solid residues such as electronic scraps [10,15,20,36], contaminated soil [37], spent catalyst [38–40], fly ash [41–43], and red mud [44,45]. Citric, oxalic, and gluconic acids are the organic acids produced in the highest quantities by *A. niger* and are used for waste exploitation [37,46,47]. In detail, bioleaching with *A. niger* uses PCBs [10,20] or batteries [15,36] as substrate for metal extractions. High leaching efficiencies of 60% and 100% for Cu and Zn, respectively, were achieved after 21 days of fermentation with low pulp density of 0.1–0.5% (w/v).

Considering the current end-of-life PCB availability and the relevant content of Cu and Zn, the present paper aims to improve the sustainability of the process compared to the current state of the art. The possibility to increase the quantity of treated PCBs makes the treatment more attractive for stakeholders and suitable for industrial scale-up. Many conditions were investigated, including the possible inclusion of food industry waste as fungal growth substrate to improve the environmental sustainability of the treatment.

2. Materials and Methods

2.1. Preparation of Waste Printed Circuit Boards (PCBs)

PCBs used in this paper were obtained from computer devices. They were shredded by stainless steel blades and pliers after manually removing the main parts of electronic components (e.g., capacitors, batteries, and resistors). Finally, the residue was crushed to obtain a granulometry smaller than 0.5 mm, suitable for the bioleaching experiments. The metal fraction was separated from the plastic and flame retardants by density and the PCB powder was washed with NaCl-saturated water. The resulting PCBs had mean metal concentrations of 25% Cu and 2% Zn.

2.2. Microorganisms and Inoculum

Fungal microorganisms, classified as *A. niger*, were isolated in the laboratory from environmental samples. The inoculation of fungi was carried out inside sterile Petri dishes with a diameter of 100 mm in YPD broth (10 g/L yeast extract (Y), 20 g/L peptone (P), and 20 g/L D-glucose (D)), where 1.5% agar was added. The medium, before being used, was stirred and heated to 60 °C to achieve a homogeneous amber color and subsequently autoclaved. Finally, 100 mg/L of antibiotic (rifampicin) was added. The inoculated plates were incubated at room temperature for about 7–10 days. One-milliliter aliquots of the prepared inoculums were inoculated to 100 mL of the glucose medium. The glucose medium was prepared with the following composition: solution A was composed of 2.5 g of (NH₄)₂SO₄, 0.25 g of MgSO₄·7H₂O, and 0.025 g of KH₂PO₄ dissolved in 450 mL of distilled water; solution B was composed of 1 g of yeast in 50 mL of distilled water; solution C was composed of 150 g of D-glucose dissolved in 500 mL of distilled water; and solution D was composed of 1 g/L ZnSO₄·7H₂O, 0.05 g/L MnSO₄·H₂O, and 0.1 g/L FeSO₄·7H₂O. The solutions A, B, and C were mixed and autoclaved, and 1 mL of the solution D was added to the resulting solution. The pH of solution was adjusted to 6.5 in the first day and readjusted to pH 3 during the bioleaching experiments. The bioleaching tests were carried out in 250 mL Erlenmeyer flasks which were incubated at 30 °C and shaken at 120 rpm. Each treatment was performed in duplicate. The pH was recorded by a pH meter inoLab Multi 720 (WTW).
2.3. Bioleaching Experiments

The bioleaching processes were conducted to verify the effect of two factors: the PCB addition at different fermentation times and the addition of two oxidant agents (Fe$^{3+}$ or Mn$^{7+}$). The first factor was monitored by adding PCBs at three different times: at the beginning, after 7 days, or after 14 days of fermentation. The bioleaching process was carried out for 7 days after the PCB addition. In the case of PCB addition at the beginning, the longest time of 14 days allowed the fungal growth and acid production. The pH of medium was continuously monitored and readjusted to 3 using a 2 M NaOH solution. The second factor (the oxidant agent) was tested by adding Fe$^{3+}$ (40.67 g/L of Fe$_2$(SO$_4$)$_3$) or Mn$^{7+}$ (6.14 g/L of KMnO$_4$) simultaneously with the PCB addition at all tested conditions. The Fe$^{3+}$ and Mn$^{7+}$ amounts were determined by stoichiometric ratio with Cu, following Equations (1) and (2) [8,48,49]:

$$\text{Cu}^0 + 2 \text{Fe}^{3+} \rightarrow \text{Cu}^{2+} + 2 \text{Fe}^{2+} \quad (1)$$

$$5 \text{Cu}^0 + 2 \text{Mn}^{7+} \rightarrow 5 \text{Cu}^{2+} + 2 \text{Mn}^{2+} \quad (2)$$

The PCB concentration in bioleaching experiments was 2.5% (w/v). At regular time intervals, both citric acid production (2, 7, 14, and 21 days) and metal concentration (2, 4, 7, 10, 14, 16, 18, and 21 days) were monitored.

A chemical control test was carried out at the same bioleaching conditions to confirm the effect of the citric acid with or without oxidant agents. The operative conditions were PCB concentration 2.5% (w/v), pH 3.0, 30 °C, and 7 days. The citric acid concentration chosen was the same as that of the organic acid produced by A. niger in the bioleaching experiments (15 g/L), and the same amounts of Fe$^{3+}$ and Mn$^{7+}$ were used.

Additional tests were carried out to test the possibility of producing citric acid using an alternative carbon source to reduce the environmental load due to the glucose consumption for the fungal growth. Two kinds of agriculture and food residues were used, olive wastewater and milk whey (a cheese production residue). Both kinds of waste were used without or with an ozonation pretreatment (30 min and a flux of 7 gO$_3$/L·h). The choice of these residues was due to their high COD content (around 150 g COD/L). Furthermore, these waste flows represent a relevant management problem in the Mediterranean area [50,51]. Their use allows for the solution of a double problem by decreasing the consumption of raw materials and the reducing the amount of food waste disposed, in agreement with the circular economy pillars.

2.4. Analytical Determination

The concentrations of Fe (Fe$^{3+}$ and Fe$^{2+}$), Mn$^{2+}$, Zn$^{2+}$, and Cu$^{2+}$ were periodically analyzed in the leaching solutions. The concentrations of Mn, Zn, and Cu were measured by an atomic absorption spectrophotometer (Techcomp, AA6000). On the other hand, the quantification of the Fe content was performed by a UV/VIS spectrophotometer by the colorimetric thiocyanate method (Jasco Model 7850). The total Fe concentration was determined by oxidizing Fe$^{2+}$ to Fe$^{3+}$ with potassium permanganate, and consequently the Fe$^{2+}$ concentration was calculated as the difference between total Fe and Fe$^{3+}$ concentrations. The concentration of citric acid produced by A. niger in the medium was quantified by the Water HPLC instrument.

2.5. Statistical Analysis

In order to verify the effect of oxidant agents (Fe$^{3+}$ or Mn$^{7+}$) and the best time for both PCB and oxidant agent addition (at the beginning, after 7 days of fermentation, or after 14 days of fermentation), a two-way analysis of variance (ANOVA) was carried out. When significant differences were observed, an SNK post hoc comparison test ($\alpha = 0.05$) was also performed. An additional statistical analysis was also conducted aimed at confirming the leaching role of both citric acid and oxidant agent.
3. Results

3.1. Bioleaching Experiments

Figure 1 shows the Cu and Zn leaching profiles in the bioleaching tests with PCBs. These results demonstrated that the leaching efficiency for both Cu and Zn increased when the PCBs and oxidant agent were added 14 days after the beginning of fermentation. This was achieved thanks to the highest citric acid production by *A. niger* when PCBs/oxidant agent were added at the end of fungal metabolism (Figure 2). In detail, the citric acid reached the concentration of 13.8 ± 4.5 g/L at these conditions, even though 0.033 ± 0.002 and 1.6 ± 0.7 g/L of citric acid were produced when PCBs and oxidant agent were added at the beginning of fermentation and after 7 days after the start of fermentation starting, respectively. The lowest citric acid production by *A. niger* was due to both the metal toxicity and the PCB inhibition on fungal metabolism due to the high substrate concentration [5,10,20].

![Figure 1](image)

**Figure 1.** Cu (a,c,e) and Zn (b,d,f) leaching efficiency time profile in the bioleaching tests with *A. niger* and with the printed circuit boards (PCBs) and/or oxidant agent (ferric iron or potassium permanganate) added at the beginning (a,b), after 7 days (c,d), or after 14 days (e,f) of the fermentation period.

The results proved the positive effect of Fe\(^{3+}\) as an oxidant agent, with an increase of both Cu and Zn leaching (Figure 1). On the other hand, the Mn\(^{7+}\) were not relevant at all to the tested conditions. In further detail, Cu leaching rose from 19.0 ± 0.2% when Fe\(^{3+}\) was added at the fermentation beginning to 57.0 ± 3.0% when it was added after 14 days. The same trend was observed for Zn leaching efficiency, from 5.6 ± 0.1% to 36.5 ± 4.8%. The highest recovery efficiency of PCBs and Fe\(^{3+}\) added after 14 days was explained by the highest citric acid concentration, which increased the Fe dissolution from 10.6 ± 2.6% to 60.5 ± 5.3% (Figure 3). Moreover, the Fe speciation demonstrated that the total dissolved Fe reacted with PCB powder at the highest citric acid concentration [52]. Therefore, at the end of the experiment, Fe was completely in the reduced form (Fe\(^{2+}\)) due to the reaction with Cu and Zn (Equation (1)). In the other tested conditions, around 50% of the dissolved Fe reacted with PCBs to leach metals. The statistical analysis (ANOVA) confirmed the positive effects of both the PCB and the oxidative agent (Fe\(^{3+}\)) addition after 14 days with a P value lower than 0.05. The Fe\(^{3+}\) use produced an additional...
advantage. In the tests without $\text{Fe}^{3+}$, the Zn leaching efficiency after 2 days decreased from 2.8 ± 0.5%, 10.3 ± 0.7%, and 51.5 ± 0.5% to around 0% when PCBs were added at the beginning, after 7 days, or after 14 days of the fermentation period, respectively. Fe created a stable complex able to prevent the Zn precipitation in oxalate form [53,54]; this was due to the oxalic acid present as a by-product of citric acid synthesis by *A. niger* metabolism [36,46].

Figure 2. Citric acid concentration in the bioleaching experiments with *A. niger*.

Figure 3. Fe dissolution and speciation in the bioleaching experiments: PCBs and $\text{Fe}^{3+}$ added at the beginning (a), after 7 days (b), or after 14 days (c) of the fermentation period.
3.2. Chemical Controls and Statistical Analysis

To verify that both the Cu and Zn extractions were due to the citric acid produced by A. niger and to the oxidant agent added (Fe$^{3+}$ or Mn$^{7+}$), chemical controls were carried out reproducing the bioleaching conditions. The results in Figure 4 confirmed the positive effect of Fe$^{3+}$ addition with final yields of 59.4 ± 0.9% and 24.6 ± 5.0% for Cu and Zn, respectively. The statistical analysis, carried out to compare the chemical results with the corresponding bioleaching ones, demonstrated that the results were not statistically different, with a P value higher than 0.05 for both the metal targets (Table 1). These results confirmed that the Cu and Zn leaching from PCBs were due to the concurrent effects of citric acid and oxidant agent and excluded the possible effect of glucose or other organic acids (such as oxalic or gluconic acid) produced by A. niger.

![Figure 4](image_url)  
**Figure 4.** Cu (a) and Zn (b) leaching efficiency in the chemical control carried out with citric acid (C.A.) and oxidant agents (Fe$^{3+}$ or Mn$^{7+}$).

| Treatment         | Statistical Analysis (ANOVA) | df | Cu       | Zn       | Cu       | Zn       |
|-------------------|-----------------------------|----|----------|----------|----------|----------|
|                   |                             |    | MS F P   | MS F P   | MS F P   | MS F P   |
| C.A.              |                             | 1  | 2.98 3.74 0.19 | 2.06 50.75 0.05 |
| C.A.+Fe$^{3+}$    |                             | 1  | 5.74 0.46 0.57 | 140.6 2.98 0.23 |
| C.A.+Mn$^{7+}$    |                             | 1  | 0.58 0.33 0.62 | 1.48 8.74 0.10 |

3.3. Citric Acid Production Using Alternative Carbon Sources

When food wastes (olive wastewater or milk whey) were used for the fungal growth, only 0.13 ± 0.01 g/L of citric acid was produced (Figure 5). The main problems were the toxic effect of phenols on fungal metabolism in the case of olive wastewater [55–57] and the low availability of lactose as a carbon source for fungal metabolism in the milk whey experiment [58–62]. The additional pretreatment by ozonation allowed reducing the concentration of phenols and decomposing lactose in a more available saccharide such as glucose, galactose, or fructose. After the pretreatment step, A. niger produced around 6.1 ± 0.1 and 13.7 ± 4.4 g/L of citric acid after 14 fermentation days with olive wastewater and milk whey, respectively. These quantities were enough to complete the leaching of the two metal targets. The decrease of the final COD concentration in both food wastes simplified the final sludge management.
4. Discussion and Conclusions

This work proposed a bioleaching process for metal extraction from end-of-life PCBs with *A. niger*. The results prove the possibility to increase the treated PCB amount from 0.5% to 2.5% w/v without Cu efficiency decrease [10,20]. The relevance of the proposed approach is confirmed by two main reasons: (i) Cu is the main metal of interest in PCBs, after precious metals (Au and Pd). The economic sustainability of its extraction from these scraps is also connected to its high concentration [4,7,63]. (ii) Cu is the main interferent in Au leaching; therefore, its previous extraction allows for a significant increase of both efficiency and purity in the Au recovery [32,33,64]. The present paper represents an example of success in the implementation of circular economy principles described by the New European Circular Economy Action Plan [65]. Indeed, PCBs are included within the list of key products in the documents (electronics and ICT). Furthermore, the biotechnological implementation allows the substitution of hazardous chemicals to protect citizens and the environment. The chance to give value to the waste (both PCBs and food waste for the citric acid production) pushes the market towards the creation of a secondary raw materials market, while avoiding export to non-European countries, in agreement with the modern circular policies. More comprehensively, the biotechnological approach using the *A. niger* strain allowed the exploitation of end-of-life PCBs at a low temperature, reducing the consumption of chemical agents. High efficiencies, around 60% and 40% for Cu and Zn, respectively, were achieved at the best selected conditions: addition of PCBs and Fe$^{3+}$ (oxidant agent) 14 days after the start of fermentation (when *A. niger* reached the exponential growth phase and produced the maximum amount of citric acid (around 15 g/L)), 30 °C, 7 days leaching time, and 2.5% (w/v) PCB concentration. A further recovery process allows for the new metal’s placement on the market, while avoiding the depletion of raw materials. Moreover, the environmental sustainability of the treatment was enhanced by the use of food wastes (milk whey and olive wastewater) for *A. niger* metabolism and was able to replace the glucose from primary sources, solving the criticalities connected to their management.

**Author Contributions**: Conceptualization, A.B. and F.B.; methodology, A.B. and F.B.; software, A.B.; validation, A.B. and D.K.; formal analysis, A.B. and G.M.; investigation, A.B., D.K., and G.M.; resources, F.B.; data curation, A.B. and F.B.; writing—original draft preparation, A.B. and D.K.; writing—review and editing, A.B. and F.B.; visualization, A.B. and G.M.; supervision, F.B.; project administration, F.B.; funding acquisition, F.B. All authors have read and agreed to the published version of the manuscript.

**Funding**: This research received no external funding.

**Acknowledgments**: This work has been carried out within a subcontract of the H2020-760792 FENIX Project. Part of the work has been realized thanks to Doctorate exchange and Post-Doc within S.U.N.B.E.A.M. Project—Erasmus.
Conflicts of Interest: The authors declare no conflict of interest.

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