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Gold Bioleaching from Printed Circuit Boards of Mobile Phones by *Aspergillus niger* in a Culture without Agitation and with Glucose as a Carbon Source

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Abstract: Hydrometallurgical and pyrometallurgical processes to recover gold (Au) from cell-phone printed circuit boards (PCBs) have the disadvantage of generating corrosive residues and consuming a large amount of energy. Therefore, it is necessary to look for biological processes that have low energy consumption and are friendly to the environment. Among the biological alternatives for the recovery of Au from PCB is the use of cyanogenic bacteria and filamentous fungi in cultures with agitation. Considering that it is important to explore the response of microorganisms in cultures without agitation to reduce energy expenditure in the recovery of metals from PCB, the present investigation evaluated the capacity of *Aspergillus niger* MXPE6 and a fungal consortium to induce Au bioleaching from PCB in a culture medium with glucose as a carbon source and without agitation (pH 4.5). The results indicate that the treatments with PCB inoculated with the fungal consortium showed a considerable decrease in pH (2.8) in comparison with the treatments inoculated with *A. niger* MXPE6 (4.0). The fungal consortium showed a significantly higher Au bioleaching (56%) than *A. niger* MXPE6 (17%). Finally, the use of fungal consortia grown without agitation could be an alternative to recover metals from PCB, saving energy and material resources.

Keywords: metal bioleaching; energy saving; filamentous fungi; fungal consortium; electronic wastes

1. Introduction

Obsolete cell phones are a component of waste electrical and electronic equipment (WEEE) due to the rapid release of cell phones with new technological improvements [1]. The useful lifetime of these electronic devices is approximately one to two years, and millions of obsolete cell phones are, therefore, discarded each year, producing large amounts of electronic waste [2,3]. Cell phones are complex electronic devices containing printed circuit boards (PCB) that act as their brain [4]. These devices are composed of valuable materials such as plastics (13%), glass-ceramics (24%), and metals (63%) including Cu, Fe, Al, Au, Ag, In, and Pd, as well as toxic elements such as Pb, Cd, As, Hg, and Br [5]. In addition to polymers (polyamide, polyethylene terephthalate, and polyethylene naphthalate) and ceramics, PCBs contain approximately 16% Cu, 4% Fe, 3% Ni, 2% Ag, 0.05% Au, and 0.03% Pd [6,7].

With the current trend toward resource sustainability, wasted cell phones are valuable secondary sources of metals and plastics, with the main driver of cell-phone recycling being their Cu and other

precious metal content [8–10]. Either hydrometallurgical or pyrometallurgical methods are commonly utilized for recovering metals from PCB. Hydrometallurgical processes consist of inducing acid or alkaline leaching of solid finely separated material; subsequently, leached metals are isolated and concentrated throughout solvent extraction, leaching precipitation, cementation, electro-oxidation, ion exchange, or filtration and distillation [11,12]. Pyrometallurgical processes include incineration, casting, sintering, and inducing reactions in the gas phase at elevated temperatures [13,14]. Both types of processes have environmental consequences, due to the generation of dust and emissions of combustion gases during pyrometallurgical processing, while hydrometallurgical processing may be associated with large volumes of highly corrosive leaching agents [12,15,16].

Biological alternatives emerged to reduce the negative environmental impacts caused by the application of the two described chemical processes [17]. These alternatives are based on utilizing bacteria such as *Acidithiobacillus ferrooxidans* and *A. thiooxidans* [18,19] which are able to induce the leaching of metals from sulfidic and non-sulfidic minerals like CuS [20,21]. These bacteria are also utilized for bioleaching Al, Co, Mo, and Ni from exhausted catalysts; in this regard, *A. ferrooxidans* was more efficient on inducing the leaching of Al, Co, and Ni, whereas *A. thiooxidans* leached Mo more efficiently after 30 days [22]. Moreover, Wang et al. [23] indicated that *A. ferrooxidans* and *A. thiooxidans* recovered Cu, Zn, and Pb from smaller particles of PCB.

In the case of cyanogenic bacteria, Chi et al. [24] demonstrated that *Chromobacterium violaceum* leached Au (10.8%) and Cu (11.4%) from PCB pulp obtained from cell phones over eight days of incubation. Meanwhile, Natarajan and Ting [25] reported that bioleaching of Au by *C. violaceum* is a function of pH, recovering 18% Au at pH 9, 22.5% at pH 9.5, and 19% at pH 10, when using pulp of PCB (0.5%). The development of a strain of metabolically designed *C. violaceum* was reported, which was capable of producing more than 70% cyanide and recovered twice the amount of Au of electronic waste compared with bacteria that did not undergo any modification [26,27]. Other reports mention that *Pseudomonas aeruginosa* and *Bacillus megaterium* are able to recover up to 37% Au from PCB pulp [28,29]. In the case of filamentous fungi, there is little information, and it is mentioned that the *Aspergillus niger* consortium is capable of leaching Au (87%) from PCB in a culture with agitation [30].

Most of the studies mentioned involve agitated cultures, where a stirring device that consumes energy and generates a cost is necessary. However, if they were scaled at the pilot plant level, the agitation would generate a cost that could be determinant in the viability of the biological process. Therefore, it is necessary to look for alternatives for the culture which reduce the production costs on an industrial scale; such is the case of "cultivation without agitation" that will not generate energy costs, as it does not require agitation. It is also essential to search for microorganisms that have a good recovery of metals from PCBs in cultures without agitation. Thus, the present study evaluated the capacity of Au bioleaching by either *A. niger* or one fungal consortium (*A. niger* MXPE6 + *A. niger* MX7) in a culture medium with glucose as a carbon source, with an excess of manganese and without agitation.

2. Materials and Methods

2.1. Fungal Isolates

The *Aspergillus niger* MX7 strain was isolated from metal-contaminated soil around a landfill located at Tronconal, Xalapa, Veracruz, Mexico, and *A. niger* MXPE6 was isolated from an electronic board collected at the same location. Strain *A. niger* MX7 grows faster (five days) than strain *A. niger* MXPE6 (seven days) when they are cultivated in a Petri dish with potato dextrose agar (PDA) at 25 °C. Another difference is that *A. niger* MXPE6 produces a yellow pigmentation when grown on PDA and *A. niger* MX7 does not produce any pigmentation. Both fungal strains were selected from previous bioassays focused on testing their tolerance to high concentrations of gold [30].

2.2. Dismantling Cell Phones and Quantification of Au in PCB

Cell phones were dismantled, and their PCBs were cut up and powdered; this material was sterilized with ethanol for 4 h and dried for 24 h at 40 °C. Subsequently, four representative samples (1 g each) were digested with aqua regia (3:1 HCl/HNO_3) for 48 h at 220 °C, and the obtained solution was filtered and brought to 50 mL with deionized water. Dissolved samples were analyzed in an inductively coupled plasma optical emission spectrometer (ICP-OES, Varian®Mod. 725-EN, Texcoco-Estado de México, México) to determine the content of Au in the PCB.

2.3. Cultivation Conditions

Plastic bioreactors (5 L of capacity) were prepared with 1.6 L of the following mineral medium ($g{\cdot}L^{-1}$): 1.5 $(NH_4)_2SO_4$, 0.5 NaH_2PO_4, 0.1 $CaCl_2$, 0.1 $MgCl_2$, and 20 glucose, pH 4.4. The addition of glucose to the culture medium was based on its effect of inducing high levels of citric acid production by *A. niger* [31,32]. Subsequently, 11 g of powdered PCB (previously sterilized) was added to each bioreactor.

Aspergillus niger MXPE6 was grown in Petri dishes with potato dextrose agar (PDA, Bioxon®, Cuautitlán Izcalli-Estado de México, México) at 28 °C for 10 days. Then, 38 individual discs of 7 mm in diameter containing fungal mycelia were transferred to the bioreactors. The same procedure was used for inoculating the fungal consortium (*A. niger* MXPE6 + *A. niger* MX7), but 19 individual discs of PDA containing the mycelia of each fungal strain were used for inoculating the corresponding bioreactors.

Bioreactors were incubated at room temperature (19–23 °C) for 32 days. In addition, two control bioreactors were included: (1) a biotic control bioreactor inoculated with the corresponding fungal inoculum without PCB powder, and (2) an abiotic control bioreactor with PCB powder, but without any fungal inoculum. Each treatment included three replicates. During incubation, three samples were collected every 96 h to determine pH values, total sugars [33], fungal protein content [34], total dry biomass, and the amount of Au leached by using optical emission spectrometry (ICP-OES, Varian®Mod. 725-ES, Cuautitlán Izcalli-Estado de México, México).

Coefficients of biomass yield ($Y_{X/S}$) or gold bioleaching ($Y_{CAu/S}$) per glucose consumed were calculated on the basis of Equation (1).

$$Y_{X/S} = -(dx/ds), \tag{1}$$

where X, CAu, and S represent the biomass content, gold bioleaching, and the substrate, respectively, and d indicates the differential of such parameters.

The fungal growth rate (k) was calculated with Equation (2) [35].

$$W^{1/3} = {W_0}^{1/3} + kt, \tag{2}$$

where W is the biomass content, W_0 is the initial biomass concentration, and t represents the period of experimentation.

2.4. Accumulation of Au in Fungal Biomass

From the total dry fungal biomass obtained in each treatment, four samples of the dry biomass (0.5 g) were collected. Each sample was individually digested with 10 mL of aqua regia (previously described) at 220 °C for 48 h. The resulting solution was filtered and brought to 50 mL. The dissolved samples were analyzed via optical emission spectrometry (ICP-OES, Varian®Mod. 725-EN, Texcoco-Estado de México, México) to determine the content of Au in the fungal dry biomass.

2.5. Experimental Design and Statistical Analysis

A 2 × 2 factorial experiment was set in a completely randomized design, including two levels of fungal inoculation: individual inoculation and inoculation with consortium and two levels of electronic

waste (with PCB and without PCB). The resulting four treatments had three replicates. Data were analyzed using an analysis of variance and the mean comparison test (Tukey, $\alpha = 0.05$) using the SAS statistical program [36].

3. Results and Discussion

3.1. Fungal Growth in Culture Media with or without PCB

Treatments inoculated with *A. niger* MXPE6 and with the fungal consortium (*A. niger* MXPE6 + *A. niger* MX7) showed high glucose consumption in the first four days (Figure 1A,B). However, no significant differences ($p \leq 0.001$) were observed between treatments with PCB residues and their corresponding biotic controls, at all sampling times. Our results are similar to those reported by Madrigal-Arias et al. [30] for *A. niger* MXPE6 grown in culture media amended with PCB waste (but without agitation) for 14 days. Findings of both studies highlight the need for carbon sources to provide energy to support fungal metabolic functions and fungal biomass production under stressful conditions [37,38].

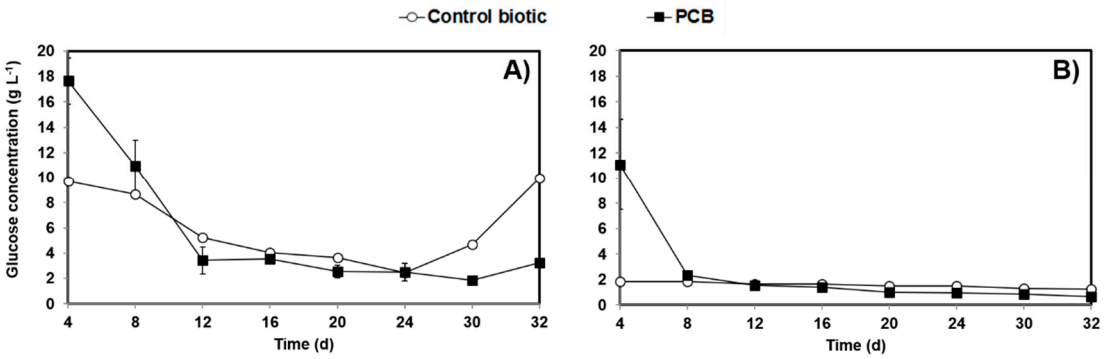


Figure 1. Glucose concentration in the culture medium of treatments with and without printed circuit boards (PCBs): (**A**) *A. niger* MXPE6; (**B**) fungal consortium (*A. niger* MXPE6 + *A. niger* MX7). Data are provided as means ± standard error; $n = 3$.

The protein content in *A. niger* MXPE6 showed a significant increase at 12 h (1.25 mg·g^{-1}) when compared to the control (0.8 mg·g^{-1}) and exhibited a recovery at 28 and 32 h in the presence of PCB (Figure 2A). In contrast, the protein content of the fungal consortium ranged from 1 to 2 mg·g^{-1}, but no significant differences were observed in comparison to the biotic control (Figure 2B). Due to the lack of scientific information concerning the effects of filamentous fungi on electronic wastes, it was not possible to compare our results to other research ones.

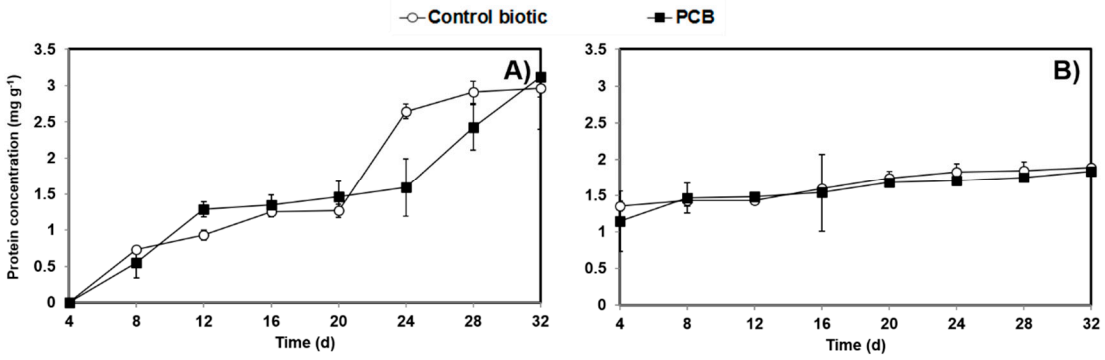


Figure 2. Concentration of protein in the culture medium of treatments with and without PCB: (**A**) *A. niger* MXPE6; (**B**) fungal consortium (*A. niger* MXPE6 + *A. niger* MX7). Data are given as means ± standard error; $n = 3$.

Table 1 shows the coefficients of either fungal biomass yield ($Y_{X/S}$) or gold bioleaching ($Y_{CAu/S}$) per consumed substrate. Results show that the biomass yield was higher in the biotic control (*A. niger* MXPE6 + *A. niger* MX7) than the remaining treatments. The fungal growth rate (k) was slightly greater in presence of PCB than fungal treatments without this electronic residue (Table 1). More importantly, the single inoculation of *A. niger* MXPE6 resulted in almost triple the amount of Au bioleached per glucose consumed when compared to the two *Aspergillus* strains (Table 1).

Total fungal dry biomass in presence of PCB was significantly ($p \leq 0.001$) increased when compared to respective controls (Figure 3). Results are opposite to those findings by Madrigal-Arias et al. [30], who reported a diminished dry biomass of two strains of *A. niger* grown in culture media with WEEE. The variability in the metallic composition of the printed circuit boards could have been a factor that influenced the growth of the fungi, because PCBs of different brands and models of cell phones were used.

3.2. Changes in pH of Culture Media

The culture medium inoculated with *A. niger* MXPE6 in the presence of PCB exhibited higher pH (~4.0) than the control (Figure 4A). In contrast, when the fungal consortium was inoculated in the medium with PCB, no significant differences were detected in the pH when compared to the control (Figure 4B). Overall, the addition of PCB to the culture media without fungal inoculation resulted in a high pH level (8.0).

Table 1. Coefficients of fungal biomass yield ($Y_{X/S}$), gold bioleaching ($Y_{CAu/S}$), and fungal growth rate (k) per glucose consumed, for two strains of *Aspergillus niger* cultured in liquid media with or without printed circuit boards (PCBs) of cell phones under non-stirring conditions, after 32 days.

Treatments	$Y_{X/S}$ (mg·g^{-1})	$Y_{CAu/S}$ (mg·g^{-1})	k ((mg·L^{-1})$^{1/3}$ d^{-1})
A. niger MXPE6	6.33		0.20
A. niger MXPE6 + PCB	7.79	0.29	0.32
A. niger MXPE6+*A. niger* MX7	32.71		0.19
A. niger MXPE6+*A. niger* MX7 + PCB	9.59	0.11	0.19

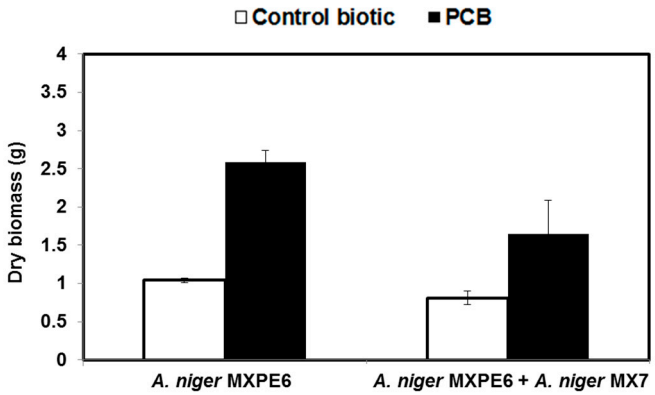


Figure 3. Dry biomass of treatments with and without PCB. Data are given as means ± standard error; $n = 3$.

The treatments inoculated with *A. niger* MXPE6 in the presence of PCB lowered the pH to 3.5 (Figure 4A), and the inoculation with the fungal consortium resulted in an even lower pH (2.8) (Figure 4B).

Aspergillus niger produces high concentrations of organic acids (e.g., citric acid, oxalic acid, glutamic acid) that contribute to the acidification of culture media [39,40]. This may partly explain the pH values recorded in controls with *A. niger* MXPE6 or with the fungal consortium (2.8 on average). Moreover, the pH in the culture medium slightly raised due to the addition of PCB, and this effect was also

described by Madrigal-Arias et al. [30] for *A. niger* MXPE6 and for the same fungal consortium (*A. niger* MXPE6 + *A. niger* MX7) in the presence of PCB under stirring culture conditions. Brandl et al. [41] reported an increase in pH in treatments amended with powder from electronic wastes and inoculated with *Thiobacillus thiooxidans*, *T. ferrooxidans*, or *A. niger*. Pham and Ting [42] reported a similar pH increase due to bacterial inoculation (*Chromobacterium violaceum* and *Pseudomonas fluorescens*), indicating that leaching of metals leads to an increase in pH in the culture medium in the presence of WEEE.

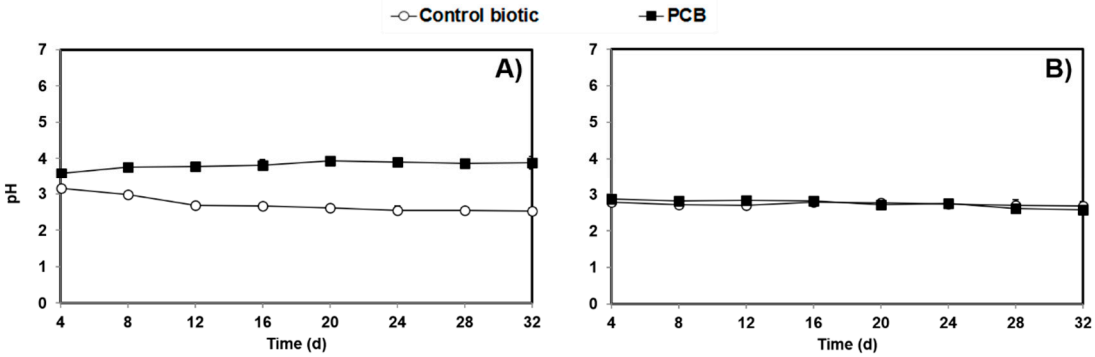


Figure 4. Behavior of pH in the culture medium of treatments with and without PCB: (**A**) *A. niger* MXPE6; (**B**) fungal consortium (*A. niger* MXPE6 + *A. niger* MX7). Data are given as means ± standard error; $n = 3$.

3.3. *Gold Bioleaching by the* Aspergillus niger Strains *from PCB Residue*

Gold bioleaching showed significant differences ($p \leq 0.001$) between treatments inoculated with either *A. niger* MXPE6 or the fungal consortium. The fungal consortium resulted in greater Au bioleaching at all sampling times, and maximum bioleaching (56%) was recorded between 12 and 16 h (Figure 5). In contrast, the maximum Au bioleaching (17%) for *A. niger* MXPE6 occurred at 4 h and 32 h (Figure 5). However, the Au bioleaching yield per glucose consumed was greater with the inoculation of *A. niger* MXPE6 in comparison to the fungal consortium (Table 1).

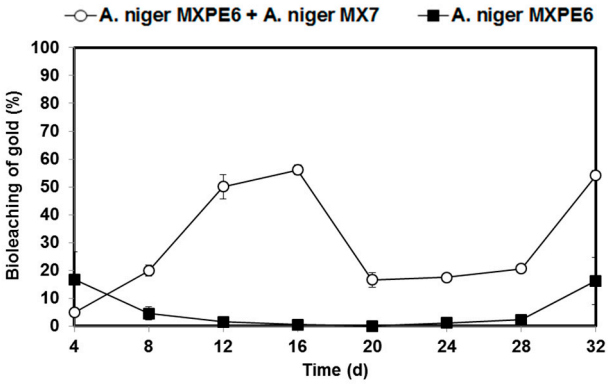


Figure 5. Leaching of gold at room temperature (19–23 °C) for 32 days. Data are given as means ± standard error; $n = 3$.

Metal bioleaching induced by *A. niger* was previously reported by Ren et al. [43], indicating that this fungal species removed 56%, 100%, 30%, and 19% of Cu, Cd, Pb, and Zn, respectively, from culture media amended with contaminated soil. The ability of fungi to remove metals from solid matrixes is explained by the release of organic acids [44]. Sayer et al. [45] showed that the organic acids produced by *A. niger* may bind Co and Zn more efficiently than commercial organic acids. Furthermore, *A. niger* is able to solubilize 68% of Cu, 46% of Zn, and 34% of Ni from low-grade ores [46]. Brandl et al. [41] observed that *A. niger* causes the leaching of Cu, Sn, Al, Ni, Pb, and Zn from powdered WEEE. In regard

to precious metals from WEEE, Madrigal-Arias et al. [30] reported Au bioleaching from PCB of cell phones by *A. niger* MXPE6 (42%) and an *Aspergillus* consortium (87%) in a culture without agitation.

Our results indicate that the degree of Au bioleaching induced by either *A. niger* MXPE6 or the fungal consortium in a culture without agitation was low when compared to previous scientific reports. However, we provide further scientific evidence about the effectiveness of *Aspergillus* strains on inducing Au bioleaching from PCB in a culture without agitation.

Our experimental conditions were performed based on the limited information about the ability of *A. niger* to induce Au bioleaching in non-stirring environments. Microbial growth, microbial byproduct biosynthesis, and bioleaching processes require high oxygen availability, which is often favored in cultures with agitation [47]. However, static cultures may also induce good microbial biomass that correlates to contaminant biodegradation but depends on cultural conditions, as well as on microbial genotypes [48–50].

3.4. Gold Accumulation in Fungal Biomass

Accumulation of Au in the biomass obtained from the fungal consortium was 10% greater than that from *A. niger* MXPE6 (Figure 6). Nevertheless, fungal treatments had an average gold accumulation of 8.5 $mg \cdot g^{-1}$ of biomass. *Aspergillus niger* is able to accumulate Au and Ag from target solutions containing cyanide [51].

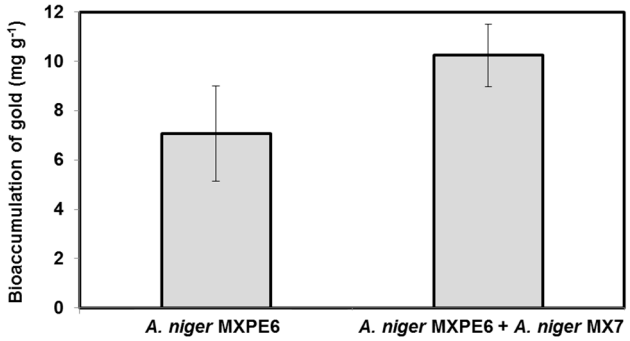


Figure 6. Accumulation of gold in the fungal biomass of PCB treatments. Data are given as means ± standard error; $n = 3$.

4. Conclusions

In the present study, the bioleaching of Au from PCBs of cellular telephones by *Aspergillus niger* MXPE6 and the fungal consortium formed by *A. niger* MXPE6 + *A. niger* MX7 in a culture medium with glucose as a source of carbon and without agitation was evaluated. The results indicate that the treatments inoculated with *A. niger* and the fungal consortium are capable of bioleaching Au under the conditions tested and accumulate it in the biomass. The following conclusions can be drawn: (1) Au bioleaching from PCBs of cell phones in a culture without agitation is possible by *Aspergillus niger* strains; (2) Au bioleaching from PCB is significantly increased by the use of the fungal consortium in the culture without agitation; (3) the decrease in pH was determinant in the treatments inoculated with the fungal consortium to increase Au bioleaching from PCB; and (4) the accumulation of Au in biomass was also favored in the treatments inoculated with the fungal consortium. Finally, the use of fungal consortia in a culture without agitation could be an alternative for the recovery of Au from electronic waste with a lower energy expenditure than in cultures with agitation. Additionally, there are environmental benefits that could be obtained by using this type of process in comparison with conventional physicochemical methods for the recovery of Au from electronic waste.

5. Recommendations and Perspectives

In preliminary experiments, it was observed that the shape and size of the PCB plate influences the growth of microorganisms. In the case of filamentous fungi, particles smaller than 0.0706 mm of PCB affect the growth of fungi such as *A. niger*, *Trichoderma harzianum*, *T. viride*, *Fusarium oxysporum*, and *F. solani*. It was also observed that inoculation with mycelium discs presents higher percentages of bioleaching for metals than inoculation with spores. Therefore, it is suggested to use PCB sizes greater than 0.0706 mm and inoculate with mycelium discs for metal bioleaching tests with filamentous fungi.

The main disadvantages presented by the Au bioleaching processes from PCB are the low percentages of recovery (on average 34%), the contamination of cultures, and the time spent in the process [24,25,28–30]; compared to Au leaching processes that have higher yields (on average 90%) of recovery, they do not present contamination and the reactions are carried out in relatively short times [52–55]. However, bioleaching has the advantage of generating less toxic residues than leaching processes.

In addition, studies report that acidophilic bacteria such as *Acidithiobacillus ferrooxidans* and *A. thiooxidans* are widely used in the bioleaching of metals such as Cu, Al, Co, Mo, Zn, Pb, and Ni from PCBs of cell phones or computers [18–23]. In the case of Au, it was reported that these bacteria were used to give a pretreatment to the PCB, removing up to 80% Cu and other base metals, thereby increasing the gold/copper ratio in the residual solid. Subsequently, the bacterium *C. violeaceum* was cultivated with the PCB pretreated for the bioleaching of Au [56]. The pretreatment reduced the toxicity of the PCB, and promoted the growth of the bacteria *C. violeaceum*.

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Conflicts of Interest: The authors declare no conflict of interest.

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