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Research Paper

Bioleaching of gold, copper and nickel from waste cellular phone PCBs and computer goldfinger motherboards by two *Aspergillus niger* strains

Jorge Enrique Madrigal-Arias[1], Rosalba Argumedo-Delira[2], Alejandro Alarcón[4], Ma. Remedios Mendoza-López[2], Oscar García-Barradas[2], Jesús Samuel Cruz-Sánchez[2], Ronald Ferrera-Cerrato[4], Maribel Jiménez-Fernández[3]

[1]Facultad de Ingeniería y Ciencias Químicas, Universidad Veracruzana, Veracruz, México.
[2]Unidad de Servicios de Apoyo en Resolución Analítica, Universidad Veracruzana, Veracruz, México.
[3]Instituto de Ciencias Básicas, Universidad Veracruzana, Veracruz, México.
[4]Área de Microbiología, Postgrado de Edafología, Colegio de Postgraduados, Estado de México, México.

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Abstract

In an effort to develop alternate techniques to recover metals from waste electrical and electronic equipment (WEEE), this research evaluated the bioleaching efficiency of gold (Au), copper (Cu) and nickel (Ni) by two strains of *Aspergillus niger* in the presence of gold-plated finger integrated circuits found in computer motherboards (GFICMs) and cellular phone printed circuit boards (PCBs). These three metals were analyzed for their commercial value and their diverse applications in the industry. Au-bioleaching ranged from 42 to 1% for *Aspergillus niger* strain MXPE6; with the combination of *Aspergillus niger* MXPE6 + *Aspergillus niger* MX7, the Au-bioleaching was 87 and 28% for PCBs and GFICMs, respectively. In contrast, the bioleaching of Cu by *Aspergillus niger* MXPE6 was 24 and 5%; using the combination of both strains, the values were 0.2 and 29% for PCBs and GFICMs, respectively. Fungal Ni-leaching was only found for PCBs, but with no significant differences among treatments. Improvement of the metal recovery efficiency by means of fungal metabolism is also discussed.

Key words: bioleaching, PCBs, *Aspergillus*, gold, WEEE.

Introduction

The use of electrical and electronic equipment (EEE) has significantly increased in recent decades throughout the world, generating large amounts of waste electrical and electronic equipment (WEEE) (He *et al.*, 2006; Gavilán-García *et al.*, 2009). WEEE contains toxic components such as Pb, Cd, Hg, Cr VI, and polybrominated biphenyls but also valuable materials such as plastic, Fe, Cu, Al, Au, Ag, Pd, and other metals (Sum, 1991; Sodhi *et al.*, 2001; Huang *et al.*, 2009). Among WEEE, the gold-plated finger integrated circuits found in computer motherboards (GFICMs) and the printed circuit boards of cellular phones (PCBs) are a rich secondary source of metals such as Cu, Au, Pd and Ag (Lee *et al.*, 2007). Their recovery is typically achieved by pyrometallurgical and hydrometallurgical processes, which have certain energy and environmental disadvantages (Cui and Zhang, 2008; Weidenhamer and Clement, 2007). Therefore, some microbiological processes have been proposed as alternatives to chemical processes for treating such electronic waste. For instance, bacteria such as *Thiobacillus ferrooxidans* and *T. thiooxidans* and fungi such as *Aspergillus niger* and *Penicillium simplicissimum* are able to mobilize high percentages of Cu, Sn, Al, Ni, Pb and Zn powder from WEEE (Brandl *et al.*, 2001). In addition, *Chromobacterium violaceum* may solubilize Au and Cu as dicyanoaurate and dicyanocuprate ($[Au(CN)_2]$ and $[Cu(CN)_2]$, respectively) from manually cut PCBs under in vitro systems (Brandl and Faramarzi, 2006). *Acidithiobacillus ferrooxidans* is also capable to solubilize copper from PCBs; moreover, the Cu concentra-

Send correspondence to R. Argumedo-Delira. Unidad de Servicios de Apoyo en Resolución Analítica, Universidad Veracruzana. Luis Castelazo Ayala s/n, Col. Industrial Animas, 91190, Xalapa, Veracruz, México. E-mail: rargumedo@uv.mx.

tion in the solution is significantly increased by an Fe-enriched culture medium (Choi *et al.*, 2004). Pham and Ting (2009) showed that applying a bio-oxidation pretreatment with *A. ferrooxidans* to electronic wastes resulted in improved Cu-removal (80% in average), with Au-bioleaching/recovering being achieved by *C. violaceum* inoculation. In addition, Chi *et al.* (2011) reported that *C. violaceum* increases Au and Cu leaching from the PCBs of cellular phones from 7.78% to 10.8% and from 4.9% to 11.4%, respectively, during 8 days of incubation. In contrast, knowledge of metal bioleaching from WEEE by filamentous fungi is still scarce. Therefore, this study evaluated the tolerance to gold of four strains of *Aspergillus* and the bioleaching efficiency of gold, copper and nickel from PCBs and GFICMs by *Aspergillus* strains with higher tolerance to gold.

Materials and Methods

Fungal isolates

Aspergillus niger MX7 and *Aspergillus* sp. MX9 were isolated from metal-contaminated soil around a landfill located at Tronconal, Xalapa, Veracruz, Mexico. *Aspergillus niger* MXPE6 and *Aspergillus* sp. MXPE8 were isolated from an electronic board found at the same location.

Tolerance of *Aspergillus* strains to gold

The fungal strains were grown in Petri dishes containing potato dextrose agar (PDA, Baker®) at 28 °C for 5 days. Afterwards, individual PDA disks (7 mm diameter) with each fungal strain were extracted and placed on new Petri dishes with PDA. Gold was supplied in the culture medium by the addition of ($AuCl_3$, Sigma-Aldrich ®) 50, 150 or 300 mg L^{-1} at pH 4.0. The Petri dishes were incubated at 28 ± 2 °C for 11 days, and the fungal growth was assessed by measuring the diameter of each fungal colony every 24 h. Petri dishes without gold were used as controls.

Dismantling and downsizing of PCBs and GFICMs

Cellular phones were dismantled, and the metal parts were separated from the plastic components to obtain PCBs, which were hand-cut to a size of approximately 1x1 cm (~200 mg in weight). In the case of GFICMs, the samples were cut to a size of 0.850 mm. Both materials were washed with a solution of 1% NaClO and five rinses with ethanol. Subsequently, 200 mg of each material were separately digested with aqua regia for 8 h, and the dissolved samples were analyzed using an ICP optical emission spectrometer (Varian® Model 725-725-ES) to determine the contents of Au, Cu and Ni. This research focused on analyzing Au, Cu and Ni due to their commercial value and diverse applications in industries such as electricity, electronics, chemical, aerospace, and automotive. Additionally, these metals are the main components in electronic wastes (Richardson, 1997; Corti and Holliday, 2004; Osorio-Hernández, 2009; Tuncuk *et al.*, 2012).

Bioleaching culture conditions

Aspergillus strains with a high tolerance to gold were grown in Petri dishes with potato dextrose agar (PDA, Merck®) at 28 °C for 5 days; agar disks with fungal mycelium (7 mm diameter) were then used as inoculum for each fungal strain. A 15 mL aliquot of mineral liquid medium (g L^{-1}; 0.1 $CaCl_2$, 0.5 KH_2PO_4, 1.5 NH_4Cl, 0.025 $MgSO_4.7H_2O$, 50 glucose, pH 4.4) was added to 50 mL vials, and 200 mg of the previously washed and dried materials of PCBs or GFICMs was added. After that, a single PDA disk of fungal mycelium (~0.0017 g fungal dry weight) was added, and the treatments were incubated at 28 ± 2 °C at 280 rpm for 14 days. Previous experiments showed that the single inoculation of *A. niger* MX7 did not exert significant effects on the bioleaching of gold (Madrigal-Arias unpublished data). Thus, this fungal strain was only assessed in combination with *A. niger* MXPE6.

Determining the bioleaching ability, pH and fungal biomass

After incubation, fungal mycelium was separated from the culture medium by vacuum filtration and dried at 45 °C for 48 h to determine the fungal dry weight. The filtered culture medium was analyzed for measuring the pH and for quantifying the content of dissolved Au, Cu and Ni using an ICP optical emission spectrometer (Varian® Model 725-ES).

Statistical analysis

To assess the fungal tolerance to gold, a 4x3 factorial experiment was set up in a completely randomized experimental design (four *Aspergillus* strains and three metal doses). The bioleaching assay was established using a 2x2 factorial experiment in a completely randomized design, including two levels of fungal strains and two levels of materials. Each treatment for each assay had three replicates. The data were analyzed using an analysis of variance and a mean comparison test (Tukey, $\alpha = 0.05$) using the SAS statistical program (SAS Institute, 2010).

Results and Discussion

Tolerance of *Aspergillus* strains to gold

The presence of 50 mg Au L^{-1} did not significantly ($p < 0.001$) inhibit the growth of any of the four species of *Aspergillus*, for all sampling times (Figure 1 and Figure 2). However, the doses of 150 mg Au L^{-1} and 300 mg Au L^{-1} caused growth inhibition of *Aspergillus* sp. MX9 and *Aspergillus* sp. MXPE8 (Figures 2c, 2d). Overall, *A. niger* showed a high tolerance to doses of 150 mg L^{-1} and 300 mg L^{-1}, and *A. niger* MXPE6 was more tolerant to Au than *A. niger* MX7 (Figures 2a, 2b).

Furthermore, some studies have found that the presence of trace amounts of Au^0 and Au^{3+} ions does not affect the growth of microorganisms because Au ions are often deposited in cell walls and periplasmic membranes (Biryuzova *et al.*, 1987). This could explain why some of the *Aspergillus* strains showed no significant growth inhibition at a dose of 50 mg Au L^{-1}. Additionally, the Au tolerance observed for the four species of *Aspergillus* was higher than that reported by Karamushka and Gadd (1999) for *Saccharomyces cerevisiae*, whose growth was inhibited at doses of 39.4 mg Au L^{-1}.

Content of gold, copper and nickel from samples of PCBs and GFICMs and recovery through fungal bioleaching

The GFICMs and PCBs samples analyzed by ICP optical emission spectrometry showed that the contents of dissolved Au and Cu were greater from the GFICMs than the PCBs materials (Table 1). In contrast, the content of dissolved nickel was similar for both materials.

In the case of induced Au-bioleaching due to fungal activity, significant differences ($p \leq 0.001$) were observed between the fungal strains. The Au-bioleaching from PCBs materials with the consortium of both *Aspergillus* strains doubled the amount of Au in the culture medium when compared to the single inoculation of *A. niger* MXPE6 (Figure 3a). Thus, the recovery of Au from PCBs samples was 87% for the combination of the two *Aspergillus* strains, whereas the recovery for *A. niger* strain MXPE6 was 42% (Figure 3a). In the case of GFICMs, the amount of Au-bioleaching was 28 times greater with the combination of both *Aspergillus* strains than that obtained with only *A. niger* MXPE6 (Figure 3b).

There were significant differences ($p \leq 0.001$) in the ability of the fungi to induce Cu-leaching from the PCBs and GFICMs materials. Figures 3c-d show that the amount of Cu-leaching was higher from PCBs than GFICMs, and the recovery of Cu from PCBs was greater with the single inoculation of *A. niger* MXPE6 (5%) when compared to the combination of both *Aspergillus* strains (0.2%) (Figure 3c).

In contrast, the recovery of Cu from GFICMs was 26% in average in both fungal treatments but was greater with the combination of both *Aspergillus* strains (29%) in comparison to *A. niger* MXPE6 (24%). No significant differences were found for Ni-bioleaching; moreover, fungi showed limited ability to dissolve this metal, especially from the PCBs material. Thus, the recovery of Ni in the culture medium with the combination of both *Aspergillus* strains was 0.6%, while for *A. niger* MXPE6 inoculation this recovery was 0.8%.

Results indicate that by using a fungal consortium, the recovery of Au from GFICMs or PCBs is significantly increased when compared to the single fungal inoculation. However, comparisons of our results were not possible because scientific information about Au-bioleaching from PCBs and GFICMs materials using filamentous fungi is still lacking.

Some organic acids such as citric acid produced by *A. niger* may cause the leaching of Cu, Cd, Zn, Mn, Pb, Cr and Al from red mud and ashes derived from the incineration of municipal waste (Singer *et al.*, 1982; Vachon *et al.*, 1994; Bosshard *et al.*, 1996). Similarly, *A. niger* is also capable of leaching Cu (60%) from mine waste, and Cu (68%), Zn (46%) and Ni (34%) from low-grade oxide ores (Mulligan *et al.*, 1999; Mulligan and Kamali, 2003; Mulligan *et al.*, 2004).

Nonetheless, studies on the leaching of Cu and Ni from WEEE by *A. niger* or other filamentous fungi are scarce. Brandl *et al.* (2001) reported that *Aspergillus niger* is able to recover 41% (80,000 mg L^{-1}) of Cu and 80% (15,000 mg L^{-1}) of Ni from WEEE powder. These results are in agreement with our findings for Cu- and Ni-bioleaching but also suggest that fungal bioleaching ability may depend on the type of material, particle size, and fungal growth condition.

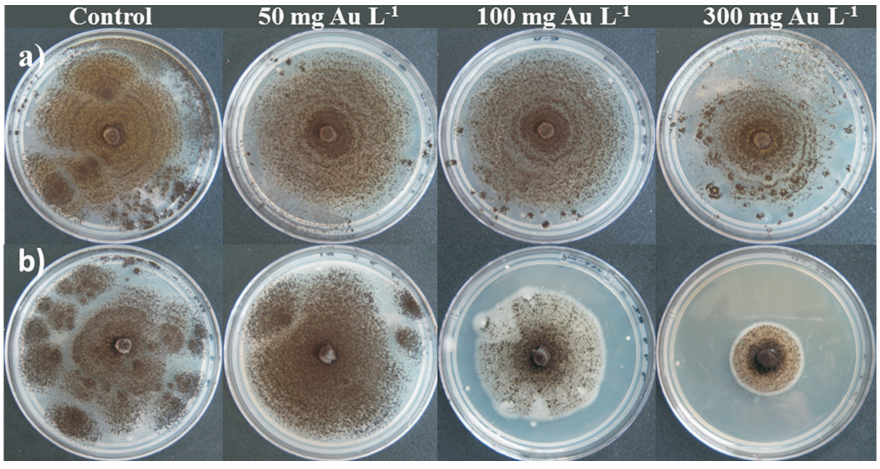

Figure 1 - Growth response of two *A. niger* strains exposed to four doses of $AuCl_3$ (mg L^{-1}) for 11 days. a) *A. niger* MXPE6 and b) *A. niger* MX7.

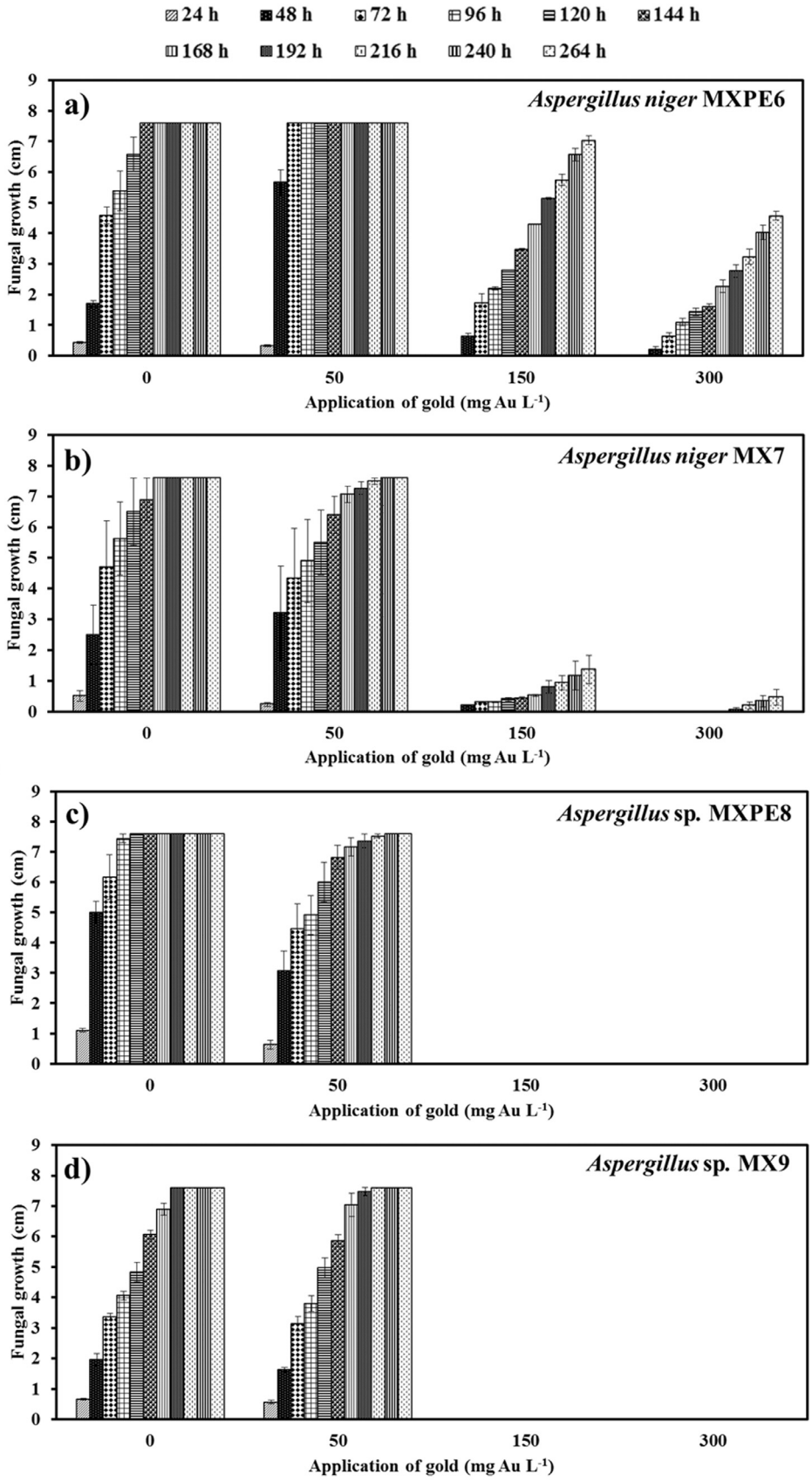

Figure 2 - Growth response of four *Aspergillus* strains exposed to four doses of $AuCl_3$ (mg L^{-1}) for 11 days. a-b) Fungal growth of *A. niger* MXPE6 and *A. niger* MX7; c-d) fungal growth of *Aspergillus* sp. MXPE8 and *Aspergillus* sp. MX9.

Table 1 - Content of gold (Au), copper (Cu) and nickel (Ni) from samples of printed circuit boards of celullar phones (PCBs) and the gold-plated finger integrated circuits found in computer motherboards (GFICMs).

Metal type	Metal content (% w/w)	
	PCBs	GFMBCs
Au	0.0038 ± 0.0002	0.0607 ± 0.0001
Cu	21.4 ± 0.04	48.3 ± 0.05
Ni	0.51 ± 0.003	0.51 ± 0.001

Means ± Standard error.

Fungal dry biomass and changes in culture medium pH

The amount of dry fungal biomass decreased significantly in the presence of either GFICMs or PCBs; however, this reduction was dependent on the treatment (Figure 4). For instance, PCBs resulted in stronger growth inhibition for the combination of *A. niger* MX7 and *A. niger* MXPE6 when compared to the growth of *A. niger* MXPE6. However, both fungal treatments showed promising recovery of Au or Cu from PCBs, as shown in Figures 3a and 3c. In contrast, the presence of GFICMs resulted in a similar production of dry biomass in both fungal treatments (Figure 4), though the recovery of Au or Cu was limited. Due to the lack of research on the effects of GFICMs or PCBs on fungal growth, we are unable to explain our data.

Regardless the presence of PCBs or GFICMs, the pH of the *A. niger* MXPE6 culture media did not show significant variations (4.4 in average) (Figure 5). In contrast, the application of the GFICMs material with *A. niger* MXPE6 resulted in a significant increase of pH (6.6) when compared to the control or to the presence of PCBs (4.4 in average). The increase in pH due to the application of GFICMs agrees with that reported by Brandl *et al.* (2001), who explained that *A. niger* increases the pH of the culture medium if the metal concentration in the WEEE is high.

Our data suggest that certain fungal strains may be useful for recovering precious metals from WEEE, as demonstrated for some bacterial strains (Chi *et al.*, 2011). Research on this subject may result in fundamental information for generating low-cost and environmentally sound microbial biotechnologies for the recovery of precious metals from WEEE.

In conclusion, Au tolerance of *Aspergillus* species could be a good indicator for selecting filamentous fungi able to cause bioleaching of gold from WEEE. Additionally, the use of a fungal consortium, as shown in this study, increases the bioleaching of Au from PCBs and GFICMs derived from WEEE.

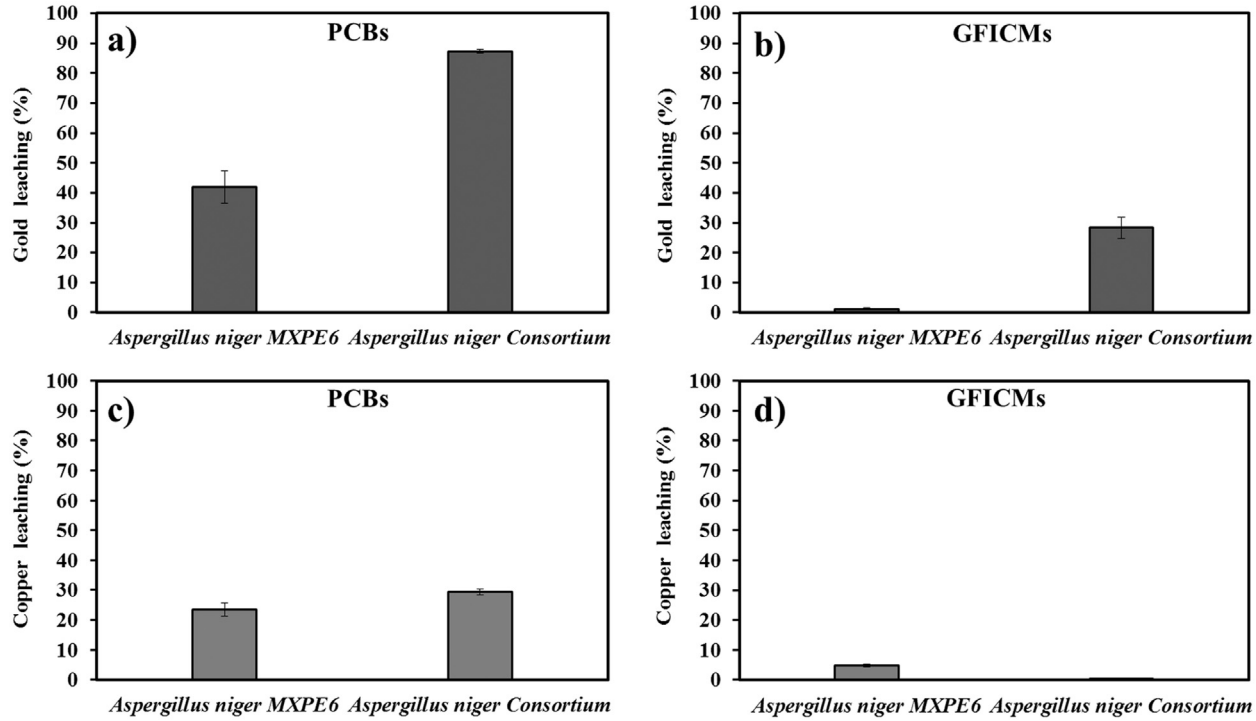

Figure 3 - Gold and copper leaching from electronic waste by an *Aspergillus* consortium (*A. niger* MXPE6 + *A. niger* MX7) and *A. niger* MXPE6 after 14 days at 28 °C. a) Au-bioleaching from printed circuit boards of cellular phones (PCBs), b) Au-bioleaching at gold-plated finger integrated circuits found in computer motherboards (GFICMs), c) Cu-bioleaching from printed circuit boards of cellular phones, and d) Au-bioleaching at gold-plated finger integrated circuits found in computer motherboards. n = 3, Means ± standard.

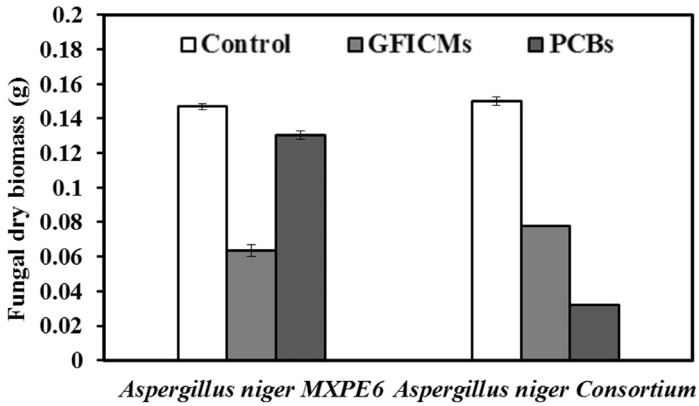

Figure 4 - Dry biomass of the *Aspergillus* consortium (*A. niger* MXPE6 + *A. niger* MX7) and *A. niger* MXPE6 exposed to printed circuit boards of cellular phones (PCBs) and gold-plated finger integrated circuits found in computer motherboards (GFICMs) after 14 days at 28 °C (n = 3, Means ± standard error).

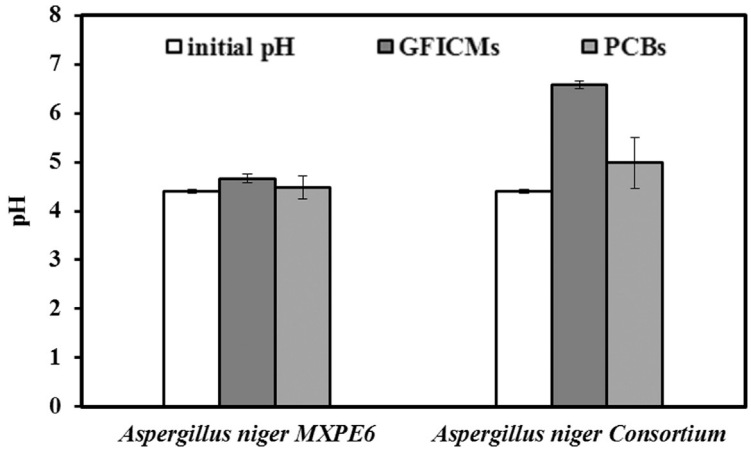

Figure 5 - Variations of pH induced by the *Aspergillus* consortium (*A. niger* MXPE6 + *A. niger* MX7) and *A. niger* MXPE6 exposed to printed circuit boards of cellular phones (PCBs) and gold-plated finger integrated circuits found in computer motherboards (GFICMs) after 14 days at 28 °C (n = 3, Means ± standard error).

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