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Biotechnological prospecting for copper recovery in electro-electronic waste (memory board)

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ABSTRACT

The recovery of electro-electronic waste is linked to the fifteenth goal of the Sustainable Development Goals (SDGs) which aims to recover and promote the sustainable use of terrestrial ecosystems. One of the main components of waste electrical and electronic equipment is the printed circuit board (PCBs) which has in its composition valuable metals that can be recovered including copper. In this context, the aim of the present study was to investigate the potential of bioleaching of bacteria in the recovery of copper in the disposal of Printed Circuit Boards (PCBs). Three bacteria (Bacillus subtilis UCP 1594, Pseudomonas fluorescens UCP 1514 and Geobacillus stearothermophilus UCP 1520) were used. Initially, the bacteria were acclimatized in PCBs crushed into small particles and then they were transferred to medium (nutrient agar) added of 0.02g, 0.08g and 0.1g.L of crushed PCBs. Then, the acclimatized bacteria were submitted to the bioleaching process. The bioleaching process occurred in T&K medium added to a 2 cm PCBs, in addition to the inoculum of 10% (107CFU/mL). The process occurred under orbital agitation of 180rpm, during 15 days and 37ºC. The identification of the potential of the bacteria in the removal of copper from the PCBs was evaluated through electrical conductivity, atomic absorption, Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS). According to the results obtained, all bacteria grew in solid medium at the maximum concentration of PCBs tested (0.1g/L) with an uncountable number of colonies. In addition, the maximum concentration (18.98 S/m, 17.58 S/m and 17.31 S/m,) of electrolytes in the bioleached solution occurred after 15 days of cultivation of Pseudomonas fluorescens, Geobacillus stearothermophilus and Bacillus subtilis, respectively. The bacterium with the greatest potential to extract copper from PCBs was Geobacillus stearothermophilus with recovery of 13.9% of the copper adsorbed in the biomass. In addition, from the results of scanning electron microscopy - SEM it was possible to evidence the formation of corrosion pits by all strains studied. Therefore, the bacterium Geobacillus stearotermophillus showed promise for copper extraction from electro-electronic waste with economic potential to stimulate the reuse and reinsertion of metallic copper in the production chain, contributing to the circular economy, sustainability and recycling of electrical and electronic waste.

Keywords: Bacteria, Bioleaching, Motherboard, electro-electronic waste.

1 INTRODUCTION

Electro-electronic waste accounts for the largest part of the total waste produced and has the highest growth rate per year, estimated at 3% to 5%, representing about 20 to 50 million tons discarded (ZHAO, 2023).

The unconscious or incorrect use of technology can generate several consequences, one of them being electronic pollution that, with its chemical components causes pollution in the environment and damage to health. With the growth of electronics sales and rapid technological evolution arise two problems: first, an environmental problem, because electronics are made up of heavy metals that are discarded into the environment; second, lack of raw material, because with the production of electronics in vogue more raw material is needed (FERREIRA; DA SILVA; GALDINO, 2010).

The procedure for recycling waste begins with the collection and sorting of equipment, when those that have conditions of use are separated from those that cannot be reused. The recycling of PCBs is still limited due to the heterogeneity of the constituent materials and the complexity of their production. Therefore, the study of the recycling of these materials by mechanical, thermal and chemical processing or the combination of these are ongoing (CALGARO et al., 2014).

The continuous emissions of heavy metal particles can be absorbed by plants and animals, causing poisoning at all levels of the food chain, characterizing them as significant environmental pollutants, due to their toxicity, being a problem of increasing importance generating great negative impacts on human health and the environment (GIESE et al., 2021).

Biohydrometallurgy is the term used to describe the biotechnological processes that involve the interactions between microorganisms for the recovery of valuable metals. Bioleaching is a method linked to biohydrometallurgy for recovery of metals in WEEE. This method has advantages such as stimulating by biological treatment the rapid extraction of valuable metals, such as copper, present in unused printed circuit boards and at the same time contribute to the circular economy safely, efficiently and in compliance with sustainability standards (HENNEBEL et al., 2015).

Copper is found in nature in the form of minerals (such as chalcopyrite, calcocite, covelite, azurite, malachite, etc.). And because copper is in the form of minerals, metallurgical processing is necessary to obtain the metallic copper extracted from these minerals. Due to ductility, malleability and good electrical conductivity, copper is a metal widely used in the manufacture of wires, cables, and metal alloys (AZEVEDO AND CHASIN, 2013).

Therefore, the objective of the work was to apply sustainable biological treatment using bacteria to recover the copper present in the Printed Circuit Boards (PCBs) of the waste electrical and electronic equipment (WEEE).

2 MATERIAL AND METHODS

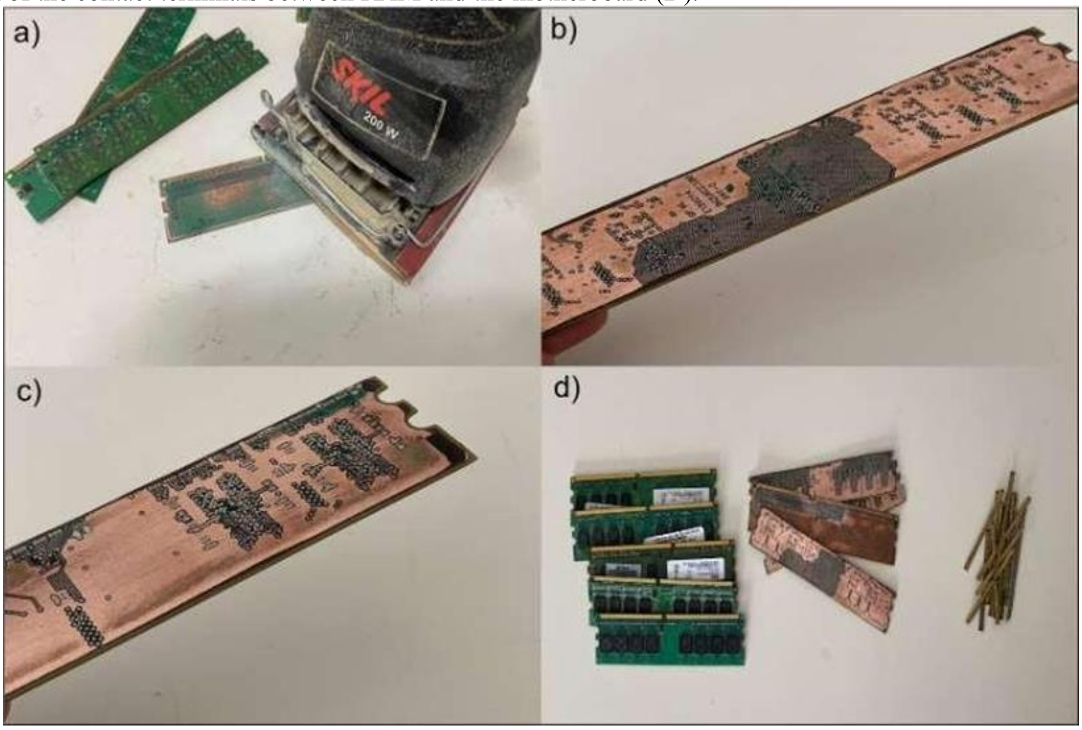
2.1 COLLECTION AND DISMANTLING OF PRINTED CIRCUIT BOARDS

The Printed Circuit Boards (PCBs) were acquired from unused computers in the Department of Electronic and Systems Engineering of UFPE (Federal University of Pernambuco). The electronic components present in the Printed Circuit Boards, capacitors and resistors, were removed manually by undocking with the aid of tweezers and pliers for total removal. All the dismantling was carried out on a bench with the use of personal protective equipment - PPE's.

2.2 REMOVING THE SOLDER MASK FROM PCIS

The first step to remove the solder mask present in the (printed circuit boards), there is the use of the mechanical process through orbital palm sander as shown in (Fig 2A). It is decided to sand the welding mask from the outermost level, remaining the mask on the innermost levels (deeper) as shown in Fig. 2B and Fig 2C. Still regarding the mechanical procedures, the contact terminals between the RAM and the motherboard are separated (cut) from the rest of the board as shown in Fig 2D. Then, a physicochemical method was applied by immersing the PCBs in caustic soda at 60°C under agitation during the min until the complete removal of the weld mask.

Figure 2 - Removal of the solder mask from the printed circuit boards using the orbital palm sand paper (A, B, C) and separation of the contact terminals between RAM and the motherboard (D).



2.3 STANDARDIZATION OF PLATE SIZE AND STERILIZATION

The dismantled circuit boards initially had their dimensions reduced by manual guillotine. A part of this material was reduced to approximately the size of $2cm^2$, while the other part was crushed were exposed to UV light for 30 minutes.

2.4 MICROORGANISMS

The bacteria used in the process were Bacillus subtillis UCP 1594, Pseudomonas fluorescens UCP 1514 and Geobacillus stearothermophilus UCP 1520 isolated from the waters of the Formoso River in the state of Pernambuco (Brazil), provided by the UCP Culture Collection - Catholic University of Pernambuco, located at the Center for Research in Environmental Sciences and Biotechnology (NPCIAMB), UNICAP, Recife, Pernambuco, Brazil.

2.5 ACCLIMATIZATION OF BACTERIA

The culture medium used for acclimatization of the bacteria was the Nutrient Agar medium (1 g/L of meat extract, 5 g/L of peptone, 2 g/L of yeast extract, 5 g/L of sodium chloride and 15 g/L of agar) added with different concentrations of the crushed printed circuit boards (0.02, 0.08 and 0.1g/L). The growing conditions were temperature of 37ºC incubated for 24 hours.

2.6 INOCULUM

The bacteria were grown in the Nutrient Broth (NB) medium consisting of meat extract 1.0g/L, yeast extract 2.0g/L, peptone 5.0g/L and sodium chloride 5.0g/L for 24h at a temperature of 37ºC. Then, aliquots of the culture, of each bacterium, were removed for analysis of microbial growth by optical density (Spectrophotometry at 600nm) until obtaining 107cel/mL.

2.7 BIOLEACHING PROCESS

Initially, the T&K culture medium was prepared from buffer solutions (A and B). Buffer A has the following composition: 0.625 g/L (NH4)2SO4, 0.625 g/L K2HPO4, 0.625 g/L MgSO4.7H2O. Then, solution B was prepared (166.5 g/L FeSO4.7H2O). The pH of the solutions was adjusted to 7. Solution B was sterilized by filtration in Millipore (0.22 µm) and solution A was sterilized in autoclave at 121 °C. Then, the two solutions (A and B) in the ratio of 4:1 were joined in 250 mL Erlenmeyer vials containing 72 mL of solution (72 mL of solution A and 18mL of solution B) to obtain the medium T&K. To this medium (T&K) was added a printed circuit board in the size of $2cm^2$. The bioleaching test started after the addition of the bacterial inoculum (10 % v/v) for 15 days, temperature of 37°C and 180 rpm. For the control, a printed circuit board in the size of $2cm^2$ was added to the T&K medium, but without the presence of the bacterial inoculum.

Table 2 - Composition of T&K medium for bioleaching process of WEEE plates by bacteria

SOLUTION A	CONCENTRATION (g L^{-1})
$(NH_4) SO_4$	0.625
$K_2 HPO_4$	0.625
$MgSO_4 .7H_2O$	0.625
SOLUTION B	**CONCENTRATION (g L^{-1})**
$FeSO_4 .7H_2O$	166.5

2.8 SEPARATION OF SUPERNATANT FROM BIOMASS

After the bioleaching process, the supernatant was separated from the biomass to identify the possible presence of copper. For this, the supernatant was transferred to Falcon tubes for centrifugation at 8000 rpm for 20 min at 10ºC.

2.9 DETERMINATION OF THE FINAL PH

To confirm the pH constancy, during the whole process, aliquots were removed for pH evaluation with the aid of the METTLER TOLEDO pH meter.

2.10 DETERMINATION OF SEM-EDS

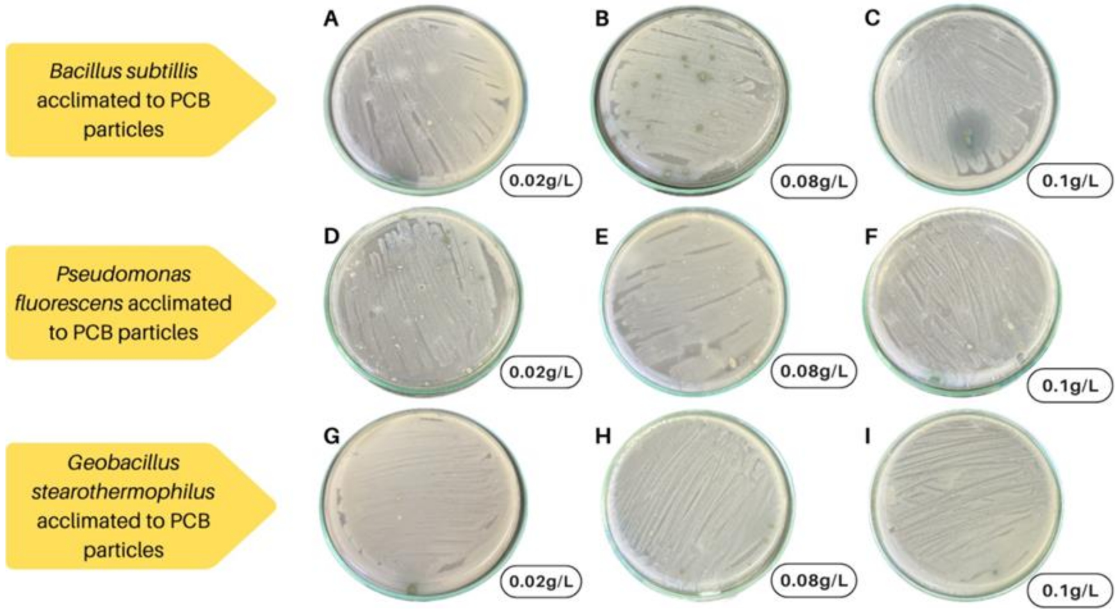
Scanning Electron Microscopy (SEM) coupled to energy dispersive X-ray spectroscopy (EDS) integrated with the Scanning Electron Microscope (SEM) was performed to identify the presence of copper in biomass after bioleaching process.

3 RESULTS AND DISCUSSION

3.1 GROWTH POTENTIAL OF BACTERIA IN ACCLIMATIZED MEDIUM WITH PCI PARTICLES

All tested bacteria were effectively able to grow in culture medium containing increasing concentrations (0.02, 0.08 and 0.1 g/L) of PCBs particles as shown in Figures 3A, 3B and 3C for Bacillus subtillis, as well as Figures 3D, 3E and 3F for Pseudomonas fluorescens and Figure 3G, 3H, 3I for Geobacillus stearothermophilus. Thus, it is evidenced that the tested bacteria have the ability to adapt their physiology to survive the exposure of metals such as copper and other metals present in the PCBs.

Figure 3 - Acclimatization of Bacillus subtillis, Geobacillus stearothermophilus and Pseudomonas fluorescens at different concentrations of copper present in PCBs



3.2 ELECTRICAL CONDUCTIVITY AS A TOOL TO IDENTIFY THE PRESENCE OF METALS IN THE BIOLEACHED SOLUTION

Studies show that the higher the conductivity, the better electrical conductor the material will be and the lower the conductivity value, the better electrical insulator the material will be. The maximum electrical conductivity was detected after 336h (15 days) with values of 18.98 S/m, 17.58 S/m and 17.31 S/m of electrolytes in the solution bioleached by Pseudomonas fluorescens, Geobacillus stearothermophilus and Bacillus subtilis, respectively.

Table 3 - Evaluation of electrical conductivity after bioleaching of metals present in printed circuit boards (PCBs) by Bacillus subtilis, Pseudomonas fluorescens and Geobacillus stearothermophilus

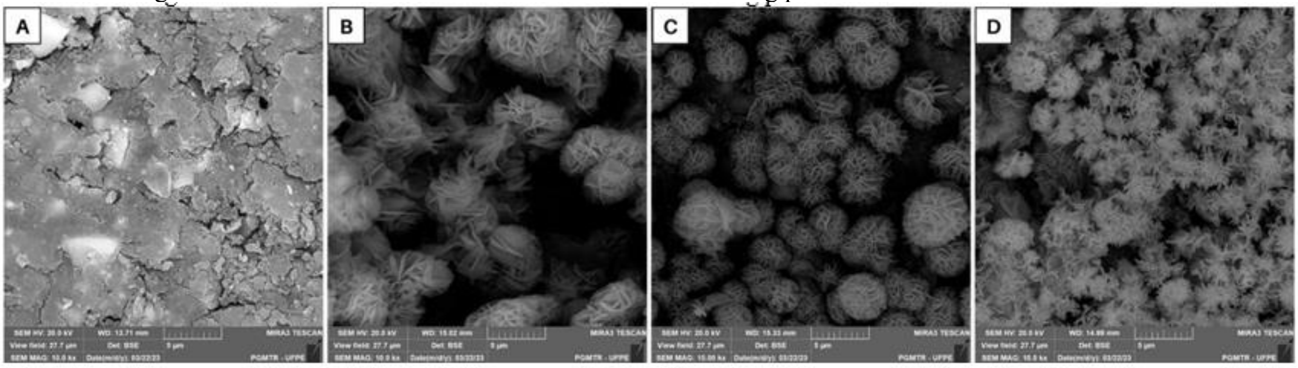
TIME (h)	***Bacillus subtillis* (S/m)**	***Pseudomonas fluorescens* (S/m)**	***Geobacillus stearothermophilus* (S/m)**
0	6.50	5.12	6,10
24	9.70	8.88	9,30
48	10.88	9.51	11,16
72	11.78	9.60	11,60
96	11.70	10.20	11.80
120	12.50	12.61	12.40
144	13.80	17.92	12.96
168	15.30	17.80	13.45

192	15.50	17.85	13.80
216	14.98	18.20	13.95
240	15.40	18.30	14.10
264	15.57	18.50	14.75
288	16.20	18.46	14.97
312	16.67	18.50	16.20
336	**17.31**	**18.98**	**17.58**

3.3 CHARACTERISTICS OF PCBSAFTER BIOLEACHING EVALUATED BY SCANNING ELECTRON MICROSCOPY (SEM)

Figure 4 shows the Printed Circuit Boards (PCBs) visualized by scanning electron microscopy (SEM) after bioleaching. In Figure 4A it is possible to identify the characteristics of the surface of the control plate (without bioleaching treatment), while in Figure 4B it is observed the PCBs plate after bioleaching by Bacillus subtilis, in Figure 4C the PCBs plate after bioleaching by Pseudomonas fluorescens and in Figure 3D the PCBs plate after bioleaching by Geobacillus stearothermophilus.

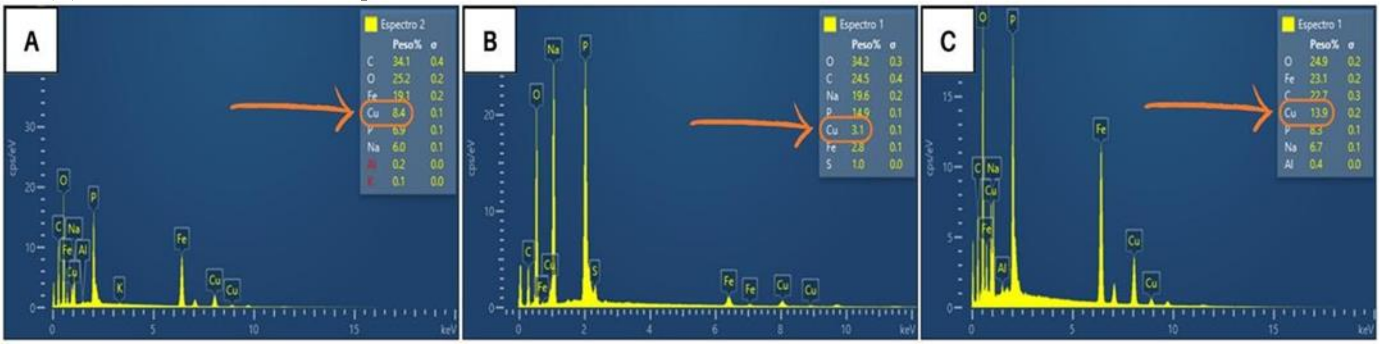
Figure 4 – PCBs surface characteristics after the bioleaching process visualized on the 10kx lens



3.4 IDENTIFICATION BY ENERGY DISPERSIVE SPECTROSCOPY (EDS) OF THE ADSORBING CAPACITY OF THE BIOMASS OF BACILLUS SUBTILIS, PSEUDOMONAS FLUORESCENS AND GEOBACILLUS STEAROTHERMOPHILUS

Figure 5 shows the potential that the bacteria had to adsorb the copper present in the PCBs. Significant copper adsorption values were obtained by Bacillus subtillis (8.4%) (Figure 5A) and Pseudomonas fluorescens (3.1%) (Figure 5B) after 15 days. However, the maximum adsorption capacity of copper (13.9%) occurred by the biomass of Geobacillus stearothermophilus (Figure 5C).

Figure 5 - Energy dispersive spectroscopy (EDS) analysis of biomass: (A) *Bacillus subtillis*, (B) *Pseudomonas fluorescens* and (C) *Geobacillus stearothermophilus*



4 CONCLUSION

The bilixiviation bioprocess performed from the submerged culture promoted the maximum adsorption capacity of copper (13.9%) by the biomass of Geobacillus stearothermophilus after 15 days of cultivation. In addition, the result of scanning electron microscopy - SEM showed the formation of corrosion pits by all strains studied indicating the phenomenon of bioleaching. Thus, the bacterium Geobacillus stearotermophillus showed promise for copper extraction from electro-electronic waste with economic potential to stimulate the reuse and reinsertion of metallic copper in the production chain, contributing to the circular economy, sustainability and recycling of electrical and electronic waste.

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