

# *In Vitro* Removal of Electronic and Electrical Wastes by Fungi Isolated from Soil at Annaba Area in Northeast of Algeria

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## ABSTRACT

Electronic and electrical wastes (EEW) have increased exponentially in recent years due to technological progress. The uncontrolled incineration of these wastes causes pollution of air, soil, and water that has dangerous effects on health of human beings and other living organisms. This work isolated fungi that are capable of degrading some of these electronic wastes. In this study, fungi isolated from soil polluted by EEW were grown on potatoes dextrose agar (PDA) medium. The estimation of the biodegradation was achieved by inoculation of both rechargeable batteries and printed circuit boards on a minimum solid and liquid medium with selected fungal strains. During the process of biodegradation on solid medium, microscopic observation was done, and on liquid medium the production of keratinolytic enzymes was evaluated using a colorimetric method after incubation with keratine powder. After 30 days, the obtained results showed that *Geotrichum candidum* is capable of degrading battery and circuit boards with rates of 23% and 71%, respectively, while *Rhizopus stolonifer* reduced battery weight by 7% and printed circuit boards by 60%. Microscopic observations showed no morphological modification in *Geotrichum candidum*, while there was sporocyst formation in *Rhizopus stolonifer*. The detection of enzymatic production indicated that there is a relation between the biodegradation process of electronic wastes and keratinolytic enzymes in *Geotrichum candidum*.

## 1. INTRODUCTION

The use of electrical and electronic equipment (EEE) is nowadays increasing rapidly and significantly in all fields, which has led to the accumulation of their wastes in the environment (Gavilán-García et al., 2009; Hall and Williams, 2007). EEW are rich in highly toxic metals such as Cd, Hg, Pb, As, Cr, Cu, Ni, and Zn (Huang et al., 2009; Sodhi and Reimer, 2001; Sum, 1991). These wastes are toxic due to their complexed primary substance, and their effect on the environment. The composition fully justifies the specific management of the wastes resulting from these equipments once used. Moreover, these wastes represent an interesting and a respectable source of raw materials (Cui and Zhang, 2008). Faced with this

situation, chemical processes have been used for the bioremediation and recovery of metals from EEW, but the residues from these processes are highly polluting and corrosive and also consume large amounts of energy (Cui and Zhang, 2008; Weidenhamer and Clement, 2007).

Recently, microorganisms provide new pathways for eliminating the accumulated electronic wastes. In view of the environmental problems caused by these metal extraction processes, a very limited amount of research have been realized about the microorganisms, including filamentous fungi, for the biodegradation of EEW and the recovery of metals from these wastes by several methods. This limited research focuses on species of the genera of *Aspergillus*,

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*Penicillium* and *Rhizopus* and their ability to recover metals from these wastes. These fungal strains are able to perform several mechanisms to biodegrade and remove these wastes. Hydrolysis as the first stage is defined as the reaction with water where the water molecules lead to cleavage of chemical bonds within a material followed by the esterification, dehydrogenation, hydroxylation, and dioxygenation in order to obtain simple molecules that are easy to use by fungal cells and other cells (Brandl et al., 2001).

In absence of sufficient research on this subject, the objective of this work is to isolate potential filamentous fungi from polluted soil that are able to biologically degrade EEW without any risk to the environment, and to estimate the production of certain enzymes during this process, since filamentous fungi are the preferred source of industrial enzymes due to their excellent capacity to produce extracellular proteins (Kubicek et al., 2009; Schaffner and Toledo, 1991). Because of their consistent morphology and versatile metabolic ability, fungi play crucial roles as degraders and symbionts in the environment as a whole, including soil and aquatic habitats (Tomer et al., 2020). The use of fungi isolated from natural sources that are able to remediate these wastes is more effective and safer as they do not harm the environment, and the research can be expanded and applied in all areas to eliminate these wastes which are harmful to humans in short and long term.

## 2. METHODOLOGY

### 2.1 Isolation and identification of fungal strains

The fungal strains were isolated and screened from a soil rich in electronic and electrical wastes located at the Wilaya of Annaba in Algeria, in this site, EEW have accumulated for more than 20 years. After, 5 cm of the surface layer of the soil is removed using a sterile spatula, 100-150 g of soil were collected in a sterile flask and transported to the laboratory at 4°C (Pochon and Tardieux, 1962).

A 10 g aliquot of soil was diluted in 90 mL of sterile normal saline, then decimal dilutions were prepared until  $10^{-6}$  (Clark et al., 1983). The isolation was carried out on PDA with gentamicin antibiotic in order to inhibit the growth of bacteria (Botton et al., 1990). PDA was prepared using extracts of 200 g of Potatoes, 20 g of Dextrose and 15 g of Agar in 1 L of distilled water then autoclaved at 120°C for 20 min. Surface, 1 mL of each dilution was inoculated onto the PDA medium, plates were incubated at 25°C for 7 days (Botton et al., 1990).

For purification of the isolated fungi, a small piece of agar was taken where the hyphal end is located and transfer to another agar plate.

Fungi were identified by their morphology in culture and their mycelium and spores under the optical microscope with magnification of 40X and 60X.

### 2.2 Screening of the isolated fungal strains

The isolated and identified fungi were transferred to PDA medium with electronic and electrical wastes. The diameter of the isolated fungal mycelium were measured in order to screen the fungal strain for the biodegradation test.

### 2.3 Preservation of the screened strains

The most common method for preserving fungal strains consists of seeding the strains into PDA slants agar, these cultures were kept for one week at 25°C, then they are stored at 4°C to promote their viability and limit the possibility of variations (Botton et al., 1990).

### 2.4 Preparation of EEW

EEW namely, rechargeable batteries (Sony) and printed circuit boards (Sony) have been cut into small pieces with a known weight (0.3 g); they were then disinfected separately with ethanol (Figure 1).

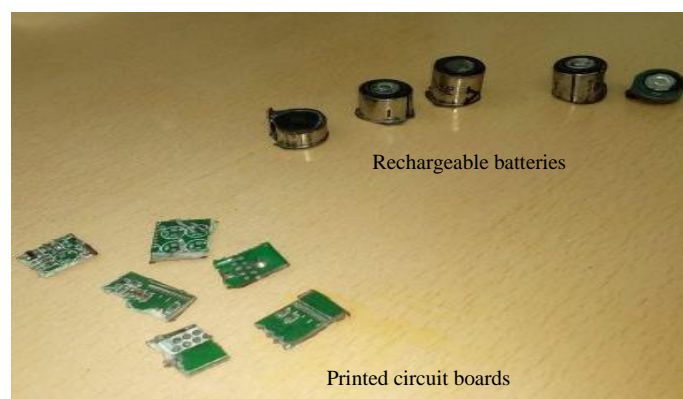
### 2.5 Test of biodegradation

#### 2.5.1 In liquid medium

In this method, a piece of EEW was placed in a 1 L flask containing 800 mL of the basal medium which consists of (g/mL):  $K_2HPO_4$  (6.4),  $KH_2PO_4$  (0.8),  $(NH_4)_2SO_4$  (0.4),  $MgSO_4$  (0.16), NaCl (0.08),  $FeSO_4 \cdot 7H_2O$  (0.016),  $CuSO_4$  (0.0004),  $ZnSO_4$ ,  $CaCO_3$  (0.4) with pH=7 (Nakajima-Kambe et al., 1999). EEW pieces were added to an erlenmeyer flask (250 mL) containing 100 mL of the basal medium described above. Agar disks with fungal mycelium (7 mm diameter) were then used to inoculate each fungal strain, a control was also prepared which was free of EEW pieces, after inoculation, the media were incubated in a shaker incubator (100 rpm) at 27°C for 1 month. The weight of EEW was measured before and after to evaluate the weight variation.

#### 2.5.2 On solid medium

The pieces of EEW with equal weights were put on the surface of Petri plates, then the basal solid medium supplemented with Tween 80 (0.6 mL/L) was



**Figure 1.** Preparation of EEW

added (Brunner et al., 2018). Agar disks with fungal mycelium were inoculated into the solid medium. The plates were incubated at 27°C for one month.

### 2.6 Microscopic observation

This part was carried out since the growth of fungal strains on unconventional carbon sources could modify the mycelium morphology, these modifications are observable with a light microscope (Bourzama et al., 2019). The mycelium parts were spread out on a glass slide with sterile normal saline, and observed under a light microscope in order to observe the modifications shown on hyphal tips in comparison with the observation of the same fungal strain on conventional carbon source (grown on PDA).

### 2.7 Keratinolytic activity

For keratinolytic activity determination, keratin powder (20 mg) was incubated with 3.0 mL of phosphate buffer (pH 7.8) and 2.0 mL of the culture filtrate for 1 h at 37°C in a shaking water-bath at 160 rev/min. 2.0 mL of 10% trichloroacetic acid (TCA) was added to stop the enzyme reaction. Samples were stored on a refrigerator at 4°C for 30 min. They were then centrifuged for 15 min at 10,000 g in a cooled

centrifuge. The absorbance of the supernatant fluid at 280 nm was measured using a UV-Visible spectrophotometer (Takiuchi et al., 1982). During the biodegradation process, keratinolytic activity of the screened strains was tested and compared to a control, i.e., the fungal strain growing on a conventional source of carbon (PDA).

## 3. RESULTS

### 3.1 Isolation and strain identification

Four fungal strains were isolated and identified to be *Aspergillus niger*, *Geotrichum candidum*, *Penicillium italicum*, and *Rhizopus stolonifer*. The fungal morphology was studied macroscopically by observing the colony characteristics (color, shape, size, and hyphae), and microscopically by a microscope with a digital camera (Table 1) (Gaddeyya et al., 2012).

### 3.2 Screening of the EEW-degrading fungal strains

Among the four identified fungal strains, only two (*Geotrichum candidum* and *Rhizopus stolonifer*) were selected because their growth was faster when compared to the others based on their mycelium diameter (Table 2).

**Table 1.** The characteristics of isolated fungal strains

Fungal strain	Macroscopic characteristics	Microscopic characteristics
<i>Aspergillus niger</i>	The conidial heads to be globose and dark brown in color that have been shown to divide into a number of columns.	The carbon black/dark brown color of the spores from their conidial heads (biserite). Hyphae are septate.
<i>Geotrichum candidum</i>	Flat, white to cream, dry and finely suede-like with no reverse pigment.	Hyphae are hyaline, septate, branched and break up into chains subglobose to cylindrical arthroconidia.
<i>Penicillium italicum</i>	Small colonies, greens dark to yellow	Very abundant conidia produced in a chain at the end of the phialids on penicilles larges, triverticillé.
<i>Rhizopus stolonifer</i>	A grayish fluffy mass, a body unbranching sporangiophores.	Black spores and are similar to cotton candy are also visible.

**Table 2.** Diameter of the isolated fungal mycelium on PDA at 27°C for 7 days

Fangual strain	Diameter (mm)
<i>Aspergillus niger</i>	15
<i>Geotrichum candidum</i>	63
<i>Penicillium italicum</i>	31
<i>Rhizopus stolonifer</i>	74

**3.3 Test of biodegradation**

**3.3.1 In liquid medium**

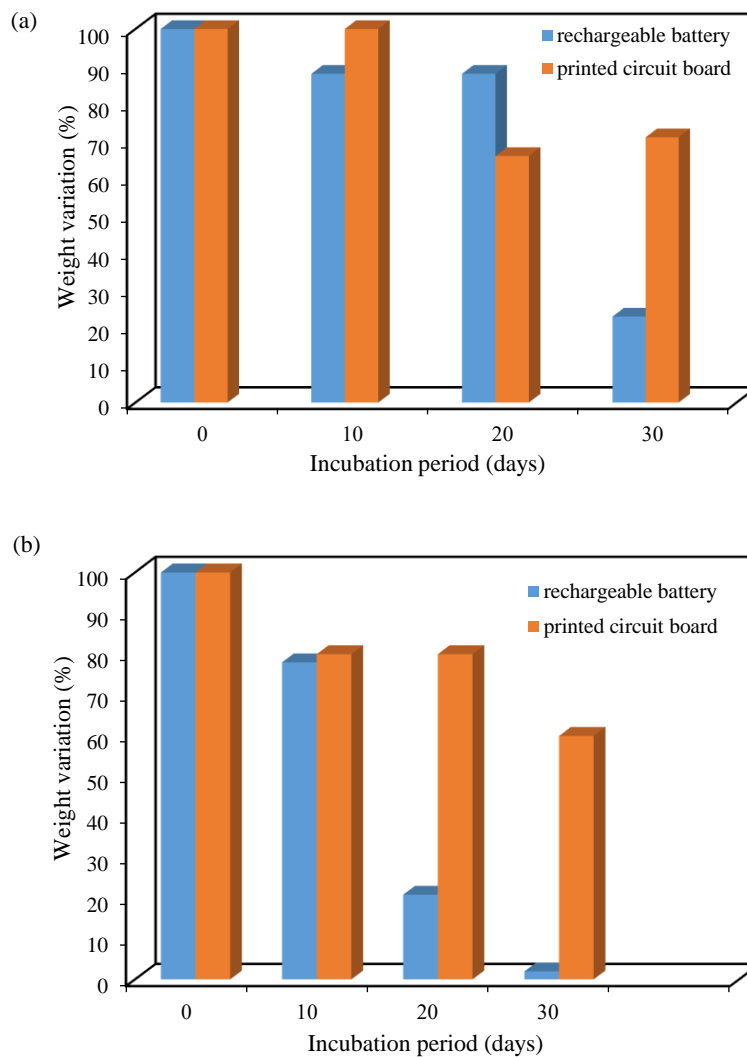
After the first 10 days, *Geotrichum candidum* caused a decrease in the weight of battery pieces while the weight of the boards remained constant. After 30 days, the weight of battery continued to decrease (23%) and the weight of board was highly reduced (71%) (Figure 2(a)).

After 10 days, *Rhizopus stolonifer* gave a

decrease in the weights of both EEW types. In addition, at day 30, the weight of the battery and the board decreased to 7% and 60%, respectively (Figure 2(b)).

**3.3.2 On solid medium**

The results of this test showed that the tested strain *Geotrichum candidum* grew on the minimum medium containing a piece of EEW with a diameter of 2 cm after 30 days. While *Rhizopus stolonifer* grew on the same medium with a diameter of 7.1 cm after 30 days (Table 3). This growth on a minimum medium that does not contain any carbon source except two electronic wastes (EEW) could be explained by the ability of both strains to degrade all wastes into simple carbon compounds and use their carbon content for continuing their vegetative development but without the production of CO<sub>2</sub>.



**Figure 2.** Weight variation of EEW during the incubation period (30 days), (a)=in presence of *Geotrichum candidum*, (b)=in presence of *Rhizopus stolonifera*

**Table 3.** Growth of *Geotrichum candidum* and *Rhizopus stolonifer* on a minimum solid medium with the parts of EEW after 30 days

Fungal strain	Diameter with rechargeable batteries (mm)	Diameter with printed circuit boards (mm)
<i>Geotrichum candidum</i>	20	19
<i>Rhizopus stolonifer</i>	68	71

**3.4 Microscopic observation**

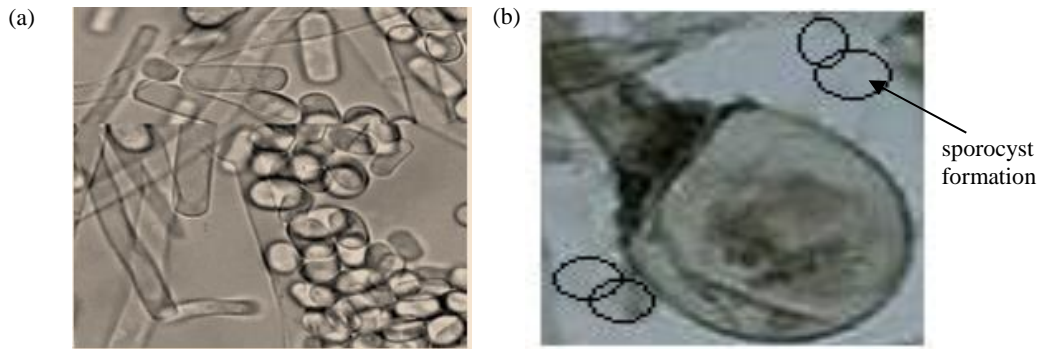
The following Figure 3(a) showed that there is no difference in microscopic observation between the fungal strain *Geotrichum candidum* after 30 days incubation on a minimum medium, and that on the conventional carbon source (PDA). While in *Rhizopus stolonifer* with EEW parts, sporocyst formation was observed at the tip which gives the black color which is darker than the normal and bursts to release a higher

number of spores, also the formation of spherical vesicles (Figure 3(a) and (b)) compared to the control on a conventional carbon source (on PDA) was noticed.

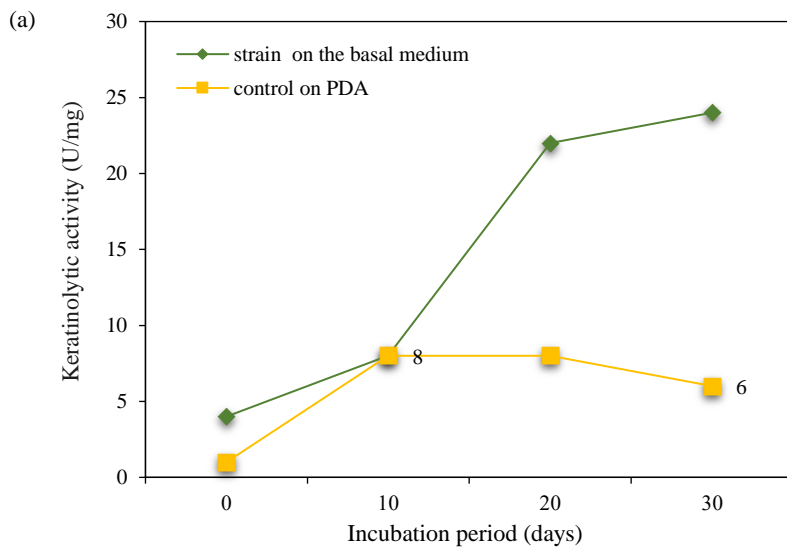
**3.5 Keratinolytic activity**

As shown in Figure 4(a), the highest values of enzyme secretion were obtained after 10 days of incubation and it decreased following the same pattern of the control. This may be explained by the presence of a relation between this enzyme secretion and the biodegradation process.

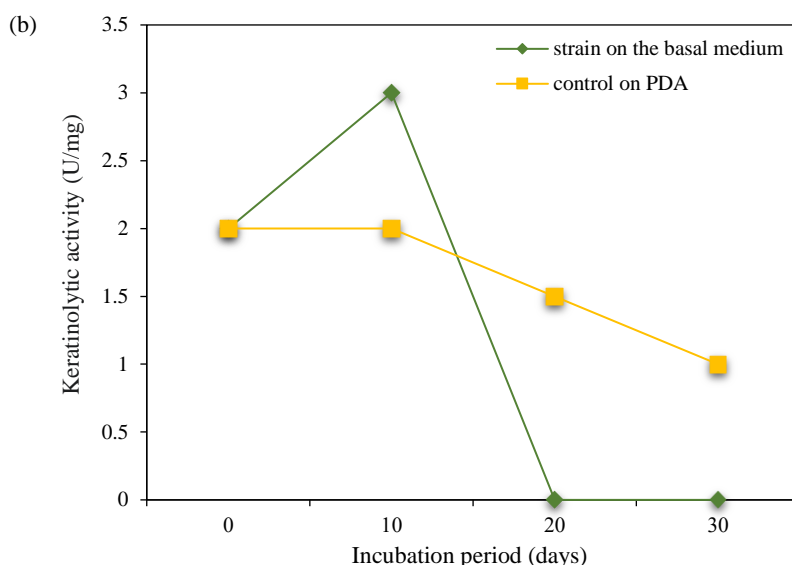
The same note was observed with *Rhizopus stolonifer* (Figure 4(b)) but after 20 days, keratinolytic activity was not detected. This means that there no relation between this enzyme and EEW biodegradation.



**Figure 3.** Microscopic observation of the screened strains, (a) *Geotrichum candidum*, (b) *Rhizopus stolonifer* after 30 days of incubation on a basal medium solid with EEW (60X).



**Figure 4.** Relation between the keratinolytic activity (U/mg) and the degradation of EEW during the incubation period (days) in comparison with the control grown on a conventional source of carbon (PDA) for each screened strain, (a)=*Geotrichum candidum*, (b)=*Rhizopus stolonifer*.



**Figure 4.** Relation between the keratinolytic activity (U/mg) and the degradation of EEW during the incubation period (days) in comparison with the control grown on a conventional source of carbon (PDA) for each screened strain, (a)=*Geotrichum candidum*, (b)=*Rhizopus stolonifera*. (cont.)

#### 4. DISCUSSION

Recent chemical research aiming to recycle some components of EEW have been carried out to eliminate these dangerous wastes (Mozos et al., 2020). Thus, the aim of the current study is to safely remove some types of EEW using microorganisms. Tomer et al. (2020) exposed several mechanisms for biodegradation in fungi. Microorganisms achieve this process directly by the cells or indirectly by the extracellular enzymes (Muthu, 2014). Fungi have a large exo-enzymatic arsenal capable of degrading organic macromolecules that are unable to bio-assimilate due to their high molecular weight (Internship report IUT, 2013), like in *Geotrichum candidum* in our study. Many fungi excrete enzymes into the environment and can be used as producers of keratinolytic enzymes. A specific class of proteolytic enzymes includes the keratinases which catalyse the hydrolysis of keratins. The microorganisms responsible for biodegradation are different from each other and they have their own optimal growth conditions. The biodegradation is generally considered to be enzyme-catalyzed hydrolysis and non-enzymatic hydrolysis (Wackett and Hershberger, 2001).

EEW are rich mainly in metals and heavy metals: Cu, Fe, Cd, Cr, Pb, etc., and with lower rate, plastic substances (Huang et al., 2009; Sodhi and Reimer, 2001; Sum, 1991). In our study, we used printed circuit boards (PCBs) which are flat bases generally made of phenolic resin reinforced with glass fibre that functions as an electrical insulator. These boards are made up mainly of metallic and non-

metallic parts and organic compounds (Szałatkiewicz et al., 2014). The metallic parts include 13% Cu, 5% Fe, and 2% Al (Hall and Williams, 2007). Rechargeable lithium-ion batteries have also been used, consisting of lithium, Mn, Ni, Cd, Zn, and Cu. This leads us to the fact that *Geotrichum candidum* and *Rhizopus stolonifera* are also able to absorb/adsorb heavy metals from rechargeable batteries.

However, only few studies have been carried out on the use of fungi in the biodegradation of EEW. Some studies focused on the biodegradation of EEW by two species of fungi, *Aspergillus niger* and *Penicillium* sp. to extract lithium and cobalt from spent batteries by the production of organic acids (oxalic acid and citric acid). These acids have the effect of leaching and extracting up to 85% of lithium and 48% of cobalt from the cathodes of the batteries (Cunningham and Harwood, 2016). Similarly, Navaneethakrishnan et al. (2020) used fungi, namely, *Ustilago trichophora* and *Aspergillus niger*, for recycling lithium ion batteries. The acids change the status of several metals such as copper in EEW (Brandl et al., 2001). Citric acid produced by *A. niger* is the main leaching agent for PCB heavy metals (Aung and Ting, 2005). Because of the lack of reports on other species of fungi, we have chosen to extrapolate our study on other species which are *Rhizopus* sp. and *Geotrichum* sp.

The fungi are non-chlorophyllous, therefore they are incapable of performing photosynthesis. They need to find their carbon in organic compounds. In spite of the use of a minimum medium that is free of organic matter for biodegrading EEW with our fungal

strains, they were still alive. This suggests that they have used some of the components of the EEW as a source of carbon.

The growth of the fungal strains supplemented with the EEW was very fast because the tested fungi already contained the biodegradation enzymes, as they were isolated from a soil rich with these class of wastes.

*Rhizopus* sp. produces several organic acids (De Reu et al., 1995). However, in PCB biodegradation, the produced acids facilitate the elimination of the metallic components of PCBs via solubilization (Dave et al., 2018). The acids induce leaching of metals from PCB wastes through acidolysis and complexolysis mechanisms (Ceci et al., 2015). After extensive research, only a few studies were found on the ability of *Geotrichum candidum* to biodegrade the EEW. Therefore, the current study will be a first step in this field. This work is only a preliminary step and further studies are intended regarding the application of these fungal strains in EEW removal and the role of keratinolytic enzymes in the biodegradation process as well as to understand the nature of sporocyst formation in *Rhizopus stolonifer*. This application can be by insertion of nonspecific extracellular enzymes such as keratinolytic enzymes directly in the natural environments for providing degradation of EEW, or by formation of fungal biofilm mainly in the aquatic media.

## 5. CONCLUSION

The results of this research are very encouraging, considering that the isolated fungal strains *Geotrichum candidum* and *Rhizopus stolonifer* showed a significant capacity to biodegrade EEW, which was more remarkable with *Geotrichum candidum*. This study also affirmed a role of the keratinolytic enzymes in *Geotrichum candidum* and sporocyst formation in *Rhizopus stolonifer* during the biodegradation process of electronic wastes.

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## REFERENCES

Aung KMM, Ting YP. Bioleaching of spent fluid catalytic cracking catalyst using *Aspergillus niger*. Journal of Biotechnology 2005;116(2):159-70.

- Botton B, Breton A, Fevre M, Gauthier S, Guy P, Larpent JP. Useful and Harmful Mold: Importance Industrial (Moisissure Utiles et Nuisibles: Importance industrielle). Paris, France: Elsevier Masson; 1990. p. 11-190.
- Bourzama G, Ennaghra N, Soumati B, Benoune S, Atriche N. Effect of zinc metal at high concentration on secondary metabolic pathways in *Penicillium chrysogenum* strain. Journal of Microbiology, Biotechnology and Food Sciences 2019;9(2):307-13.
- Brandl H, Bosshard R, Wegmann M. Computer-munching microbes: Metal leaching from electronic scrap by bacteria and fungi. Hydrometallurgy 2001;59:319-26.
- Brunner I, Fischer M, Ruthi J, Stierli B, Frey B. Ability of fungi isolated from plastic debris floating in the shoreline of a lake to degrade plastics. PLOS ONE 2018;13(8):e0202047.
- Ceci A, Kierans M, Hillier S, Persiani AM, Gadd GM. Fungal bioweathering of mimetite and a general geomycological model for lead apatite mineral biotransformations. Applied and Environmental Microbiology 2015;81:4955-64.
- Clark EM, White JF, Patterson RM. Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of in vitro culture of the fungus. Journal of Microbiology Methods 1983;1:149-55.
- Cui J, Zhang L. Metallurgical recovery of metals from electronic waste: A review. Journal of Hazardous Materials 2008;58: 228-56.
- Cunningham JA, Harwood V. Fungi recycle rechargeable lithium-ion batteries. American Chemical Society [Internet]. 2016 [cited 2020 Aug 21]. Available from: <https://www.sciencedaily.com/releases/2016/08/160821093037.htm>.
- Dave SR, Sodha AB, Tipre DR. Microbial technology for metal recovery from e-waste printed circuit boards. Journal of Bacteriology and Mycology 2018;6(4):241-7.
- De Reu JC, Rombouts FM, Nout MJR. Influence of acidity and initial substrate temperature on germination of *Rhizopus oligosporus* sporangiospores in tempe fermentation. Journal of Applied Bacteriology 1995;78:200-8.
- Gaddeyya G, Niharika PS, Bharathi P, Kumar PKR. Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. Advances in Applied Science Research 2012;3:2020-6.
- Gavilán-García A, Román-Moguel A, Almada-Calvo F, Aburto-Mejía S. Electronic waste generation and technology for an appropriate management in México. Boletín de la Sociedad Química de México 2009;3(2):85-92.
- Hall WJ, Williams PT. Processing waste printed circuit boards for material recovery. Circuit World 2007;33:43-50.
- Huang K, Guo J, Xu Z. Recycling of waste printed circuit boards: A review of current technologies and treatment status in China. Journal of Hazardous Materials 2009;164:399-408.
- Kubicek CP, Mikus M, Schuster A, Schmoll M, Seiboth B. Metabolic engineering strategies for the improvement of cellulose production by *Hypocrea jecorina*. Biotechnology Biofuels 2009;2:19.
- Mozos EA, Bilbao AR, Díaz-Martín F. WEEE Recycling and circular economy assisted by collaborative robots. Applied Science 2020;10(14):4800.
- Muthu SS. Roadmap to Sustainable Textiles and Clothing: Environmental and Social Aspects of Textiles and Clothing Supply Chain. New York, USA: Springer Singapore; 2014.
- Nakajima-Kambe T, Shigeno-Akutsu Y, Nomura N, Onuma F, Nakahara T. Microbial degradation of polyurethane, polyester

- polyurethanes and polyether polyurethanes. *Applied Microbiology and Biotechnology* 1999;51(2):134-40.
- Navaneethakrishnan V, Pavithra K, Vanitha V. Recycling of lithium ion batteries using fungi based organic acids. *Materials Science and Engineering* 2020;872:12-3.
- Internship report IUT. Nîmes on Study of the biodegradation of biodegradable polymers (Etude de la biodégradation de polymères biodégradables). Paris, France: Ecole des Mines d'Alès; 2013.
- Pochon J, Tardieux P. Soil microbiology analysis technique (Technique d'analyse en microbiologie du sol). Edition of Tourtourielle. France: Saint-Mandé; 1962.
- Schaffner DW, Toledo RT. Cellulase production by *Trichoderma reesei* when cultured on xylose-based media supplemented with sorbose. *Biotechnology and Bioengineering* 1991;37:12-6.
- Sodhi MS, Reimer B. Models for recycling electronics end-of-life products. *OR Spektrum* 2001;23:97-115.
- Sum E. The recovery of metals from electronic scrap. *Journal of Metallurgy* 1991;43:53-61.
- Szałatkiewicz J, Szewczyk R, Budny E, Missala T, Winiarski W. Measurement and control system of the Plasmatron Plasma reactor for recovery of metals from printed circuit board waste. *Advanced Intelligent* 2014;267:687-95.
- Takiuchi I, Higuchi D, Sei Y, Koga M. Isolation of an extracellular proteinase (keratinase) from *Microsporum canis*. *Sabouraudia* 1982;20:281-8.
- Tomer A, Singh R, Singh SK, Dwivedi SA, Reddy CU, Keloth MRA, et al. Role of Fungi in Bioremediation and Environmental Sustainability. Switzerland: Springer Nature; 2020. p.1-20.
- Wackett LP, Hershberger CD. Biocatalysis and Biodegradation: the Microbial Transformation of Organic Compounds. Washington, USA: ASM Press; 2001.
- Weidenhamer JD, Clement ML. Leaded electronic waste is a possible source material for lead-contaminated jewelry. *Chemosphere* 2007;7:1111-5.