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Sorption of Electronic Waste through Fungi: A Bioremediation Technology

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Abstract—*Production and accumulation of electronic waste (e-waste) is now becoming a global growing waste problem. Different fungal cultures were evaluated for biosorption from electronic-waste (e-waste) under submerged conditions. Pleurotus florida showed maximum specific activity of laccase enzyme (6.98 U/mg) on 20th day of incubation in comparison to other microbial cultures. Maximum biosorption of copper and iron was observed in Pleurotus florida followed by Trametes versicolor spp. Ganoderma lucidum showed less biosorption ability in comparison to other fungi. Therefore, there is a need to develop and implement certain cost-effective processes for the extraction of metals from e-waste which in turn minimize the environmental hazards of toxic metal contaminants. However, very few studies have been reported by the use of microbial cultures as a biosorption tool for certain metals present in the e-waste. These results confirmed that there would be a need to select an appropriate biosorbent and its conditions along with an effective mechanism for the sorption of heavy metals.*

Introduction

Electronic waste (E-waste) consists of waste generated from utilized electronic devices and household appliances in the form of computers, cellular phones, stereos, refrigerators and air conditioners etc. which are being discarded for their original intended use and are sent for recovery, recycling or disposal (Lucier and Gareau 2019). It possesses different substances in the form of heavy metals, metalloids, flame retardants (precious, toxic and non-toxic elements). Some of the heavy metals are potentially toxic, if these are not handled in an environmentally sound manner which in turn deteriorates human health and environmental hazards (Sinha 2010). Moreover, some of the toxic compounds and heavy metals leach into the soil and affect the flora and fauna on the earth. Therefore, production of e-waste is now becoming the rapidly growing waste problem in the world.

There are different approaches for degrading e-waste that includes various physical, chemical and biological methods. However, these physico-chemical methods have their own drawbacks and limitations. Therefore, there is a need for alternate biological approach for the removal of toxic metals from e-waste which is an efficient, cost-effective and eco-friendly method. One of the biological approaches is biosorption that involves the ability of bacteria, algae, fungi and yeast to adsorb heavy metals from waste through uptake (Sivasubramanian 2006). These microorganisms possess efficient metabolic pathways which utilize these toxic metals as energy source for growth and development, electron acceptor during respiration, fermentation and co-metabolism (Igiri *et al* 2018). Moreover, microbes also possess certain characteristics degradative oxidoreductases enzymes including the laccase enzymatic system among prokaryotic bacteria and eukaryotic fungi for detoxification of toxic compounds by oxidative coupling. These oxidoreductases include laccase, manganese peroxidase and lignin peroxidase (Janusz *et al* 2017). These microbial enzymes undergo oxidation-reduction reactions which cleave the chemical bonds of the persistent toxic substances into non-toxic forms. Thus, the presence of unique enzymatic system of microorganisms are involved in leaching different metal species from e-waste followed by biosorption and accumulation inside the cell that would help in remediation of heavy metal components in e-waste in an efficient and eco-friendly manner. Therefore, the present study has been planned to screen microbial cultures for potential degradation or biosorption on electronic waste.

Material and Methods

Procurement and maintenance of cultures

Three cultures *Ganoderma lucidum*, *Pleurotus florida* and *Trametes versicolor* were procured from Department of Microbiology, PAU, Ludhiana. These fungal cultures were maintained on potato dextrose agar media at 25°C.

Collection of electronic waste and growth of fungal cultures on e-waste

Electronic waste were collected from a local electronic waste recycling unit and dumpyards and cut into small pieces with the help of cutter for experimental studies. These pieces were washed with distilled water and ethanol. After washing, these washed e-waste pieces were mixed with mushroom minimal media, autoclaved and further inoculated with standard inoculum of all cultures, incubated at 25°C and growth of all cultures were observed.

Measurement of laccase activity

These cultures were tested for their ability to produce laccase on suitable broth. The sterilized broth along with e-waste was inoculated with different culture inoculums, incubated at appropriate temperature (25±2°C). Control that is without e-waste was also inoculated with microbial cultures. Laccase activity was carried out as described by Turner (1974) and measured at the interval of 4 days upto 24 days of incubation.

Atomic absorption spectroscopy

Cultures were inoculated in liquid minimal media containing e-waste PCB chips. The estimation of laccase activity estimation was carried at the interval of 4 days followed by the AAS at the day of maximum activity for the estimation of Cu and Fe in mycelium. The samples were digested in aquaregia solution and analyzed for Cu and Fe concentrations using an atomic absorption spectrophotometer through Cu and Fe lamps.

Results

Different compounds such as persistent organic pollutants, polycyclic aromatic hydrocarbons, pesticides and electronic wastes influence the stability of ligninolytic enzymes in the extracellular environment. The e-waste basically consists of heavy metals viz. Cu, Fe, Cd, Pb, etc which may alter the regulation of metabolic activity of microorganisms by enhancing the enzyme activity. *Pleurotus florida* , *Ganoderma lucidum* and *Trametes versicolor* were used in order to detect their ability to produce laccase at weekly intervals upto four weeks in minimal broth (pH 6.5) with e-waste as inducer under submerged culture conditions at 25°C.

The results of the laccase activity are presented in Table 1. It was observed that laccase activity was found to be maximum in *Pleurotus florida* (6.98 U/mg of protein) followed by *Trametes versicolor* (5.96 U/mg of protein) on 20th day of incubation in minimal medium supplemented with e-waste Time dependent increase in enzymatic activity was observed in all the microbial cultures.

Table 1: Laccase specific activity of fungal cultures in the presence and absence of e-waste.

Organism	Specific Enzyme Activity (U/mg)						
	Time (day)						
	0	4	8	12	16	20	24
Pleurotus florida							
Without e-waste	0.52	1.52	2.56	2.82	3.22	3.23	2.98
Treated with e-waste	2.89	3.70	4.69	5.79	5.97	6.98	6.64
Ganoderma lucidum							
Without e-waste	0.56	1.34	2.16	2.55	2.67	2.92	1.98
Treated with e-waste	1.79	2.47	4.61	4.78	4.82	4.86	4.64
Trametes versicolor							
Without e-waste	0.45	1.42	2.55	2.65	2.87	2.93	2.76
Treated with e-waste	2.97	3.48	4.59	5.83	5.96	5.96	5.67
CD (5%) for treatment	0.101 (significant)						

* Average of three replicates with Critical Difference (5%)

ESTIMATION OF BIOSORPTION OF COPPER (Cu) AND IRON (Fe) THROUGH ATOMIC ABSORPTION SPECTROSCOPY (AAS)

Biosorption by mycelium were analyzed through AAS in the presence of e-waste in the broth for all cultures on 20th day of incubation. Two heavy metals: copper (Cu) and iron (Fe) were detected through Atomic Absorption Spectroscopy on 20th day of incubation. Biosorption of copper and iron were analyzed from mycelium /microbial growth through Atomic Absorption Spectroscopy on 20th day of incubation when maximum laccase activity and growth was observed. As shown in Table 2. *Pleurotus florida* showed maximum biosorption ability (97.26 mg/g of copper and 94.13 mg/g of iron) while *Ganoderma lucidum* showed less biosorption ability in comparison to other species.

Table 2: Biosorption of fungal cultures for copper and iron from e-waste by Atomic Absorption Spectroscopy (AAS)

Elements	Cultures	Mycelium/Microbial growth; mg/g (Biosorption/uptake)
Copper	*Pleurotus florida*	97.26
	Ganoderma lucidum	49.40
	Trametes versicolor	76.66
Iron	*Pleurotus florida*	94.13
	Ganoderma lucidum	57.86
	Trametes versicolor	82.13

Discussion

Leaching of metal ions from e-waste resulted in increased microbial growth which in turn leads to increased broth turbidity and hence enzymatic expression was upregulated as observed by Lang *et al* (2000). Different fungus responded differently with different inducers which plays an important role in enhancing the biomass production and hence enzymatic activity. It was observed by Gnanamani *et al* (2006) that copper sulphate has found to be the best inducer for the production of laccase in comparison to veratryl alcohol and cycloheximide in *Phanerochaete chryrosporium* NCIM 1197. Baldrian and Gabriel (2002) found that copper addition upto 5mM increased the laccase expression at eight folds in *P. ostreatus.* Supplementation of Cu in the basal medium, induced laccase production in *Trametes versicolor* under solid state fermentation (SSB; Xin and Geng 2011) whereas it has been observed by Akpinar and Urek (2017) that iron supplement was found to be less effective to induce laccase activity in *Pleurotus eryngii* under SSB condition as these ions may hinder the electron transport system of laccase and substrate conversion. The time dependent effect of inducers might be very crucial for inducing laccase activity in *Pleurotus ostreatus* as observed by Zhu *et al* (2011).

Microbes can also absorb metals through biosorption that involves the binding of metals from e-waste on the cell surface from which they can be absorbed into the cell (Danis *et al* 2008). The higher growth rate with huge biomass leads to absorption of large amount of heavy metals from contaminated sites by sequestration after chelation or accumulating these metals inside their membranes and later eject them using carrier or channel protein (Amutha and Abhijit 2015). This statement is in agreement with our findings. The sorption studies of different concentration of Cu and Pb by *Aspergillus* spp. were studied by Price *et al* (2001) which provide the ability of *Aspergillus* spp. to absorb large amount of heavy metals rapidly. It has been found that *P. tuber-regium* absorbs heavy metals from crude oil contamination site and can be used as bioremediator for heavy metals. It has been observed by Du Pleiss *et al* (1995) that the process of degradation leads to an increase in metal or elemental mobility.

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