Chapter 13
Bioleaching of Selected Metals from E-Waste Using Pure and Mixed Cultures of *Aspergillus* Species

Amber Trivedi and Subrata Hait

Abstract Printed circuit board (PCB) is an essential part present in electronic waste (e-waste). Rich metallic content including base, precious, and toxic metals makes PCB a secondary metal reservoir. Recycling of PCB is necessary to conserve natural resources and to protect the environment. Bioleaching process is preferred over the conventional metallurgical techniques for metal recycling from e-waste due to its better efficiency and environmental compatibility. Metal bioleaching from e-waste employing heterotrophic microorganisms like fungi is the comparatively less explored area. In this context, bioleaching of selected metals such as Cu, Ni, and Zn from e-waste in the form of desktop PCB using pure and mixed cultures of *Aspergillus* species was attempted. *Aspergillus niger* was chosen for its organic acid production ability to presumably help in bioleaching of metals. As the metals are usually embedded in the polymer matrix in the PCB, *Aspergillus tubingensis* was selected for its polymer-degrading ability. The bioleaching experiments were performed using pulverized waste PCB (WPCB) in the particle size range of 0.038–1 mm for a period of 33 days at 1 g/L of pulp density. Results showed that the pure culture of *Aspergillus niger* was able to leach a maximum of 71% Cu, 32% Ni, and 79% Zn. Similarly, the corresponding maximum metal leaching efficiency employing a pure culture of *Aspergillus tubingensis* was 54% Cu, 41% Zn, and 14% Ni. Using mixed cultures of *Aspergillus* species, there was a marginal increase in metal bioleaching for Cu and Ni with extraction efficiency of 76 and 36%, respectively. Extraction efficiency of 63% for Zn was observed using the mixed culture. Results indicated the practical feasibility of fungal bioleaching using pure and mixed cultures of *Aspergillus* species for metal recycling from e-waste for prospective beneficiation.

Keywords Electronic waste · Printed circuit board · Metals · Fungal bioleaching · Pure and mixed cultures · *Aspergillus* spp

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13.1 Introduction

Sustained technological developments in electrical and electronic equipment (EEE) have increased the demand for EEE like computers, mobile phones, televisions (Awasthi et al. 2017; Iannicelli-Zubiani et al. 2017). The increased use of EEE along with their shorter shelf life has led to a significant increase in the generation of waste EEE (WEEE) or electronic waste (e-waste) globally. It has been reported that around 44.7 Mt of e-waste was generated in the year 2016 and is expected to grow to 52.2 Mt by 2021 (Baldé et al. 2017). In India, 1.95 Mt of WEEE is generated in the year 2016 (Baldé et al. 2017). Printed circuit board (PCB) or printed wiring board is an essential part of any EEE comprising around 6% (wt/wt) of EEE (Das et al. 2009). Metallic content of around 27–30% and non-metallic content with 70–73% makes PCB matrix complex and heterogeneous in nature (Zhou and Qiu 2010). Rich metallic content of PCBs up to 24% Cu, 6.3% Pb, and 3% Ni, which is considerably high in comparison with natural reservoirs, makes e-waste to be categorized as a metal-rich secondary reserve (Zhou and Qiu 2010). However, e-waste containing toxic metals such as Cr, Hg, Ni, and Pb beyond permissible limit turns it hazardous (Li et al. 2004; Priya and Hait 2017). Thus, e-waste management for metal recycling is a necessity not only for resource circulation but also for the environmental point of view (Niu and Li 2007). However, conventional metal recycling techniques such as pyro- and hydro-metallurgy techniques are associated with secondary environmental pollution and high-energy requirements (Cui and Zhang 2008; Ilyas et al. 2010; Pant et al. 2012).

Now-a-days, bioleaching technique or bioextraction of metals from e-waste using diverse microorganisms are gaining attention worldwide due to its eco-friendly nature. Hydrometallurgy coupled with biotechnology by employing microorganisms is known as biometallurgy. Bioleaching is an eco-friendly, effective, and low-cost metal recycling technique (Beolchini et al. 2012; Brandl et al. 2001; Priya and Hait 2018). Numerous microorganisms consisting of mostly autotrophic bacteria have been reported to be used for bioleaching of metals from electronic waste (Brandl et al. 2001; Priya and Hait 2017, 2018). Microorganisms produce a diverse range of metabolites and biomass during bioleaching, which aids the metal extraction from the solid matrix (Lundgren et al. 1986). However, metal bioleaching employing heterotrophic fungi is the comparatively less explored area. Fungi have a shorter lag phase and faster leaching rate by showing more tolerance to toxic constituents. Further, fungi can grow in acidic as well as in alkaline conditions. Furthermore, the fungi excreted metabolites like organic acids assist to leach the metals (Wu and Ting 2006). Fungal species like *Aspergillus spp., Penicillium spp.*, and yeast like *Y. lipolytica* are well known for the production of organic acids (Magnuson and Lasure 2004; Sauer et al. 2008; Singleton 2001). Fungal bioleaching of metals from e-waste is mostly limited to the pure culture of *Aspergillus* spp. and *Penicillum* spp. The specific attribute of fungal species like the polymer-degrading ability of *Aspergillus tubingensis* is yet to be explored for fungal bioleaching of metals from e-waste as metals are embedded in the polymer matrix of PCB. Moreover, a combination or consortia of fungal cultures with different attributes are yet to be explored for metal bioleaching from e-waste. *Aspergillus tubingensis*, a polymer degrading fungal species in combination with *Aspergillus niger*, known for producing organic acids can be explored for bioleaching of metals from PCB. In this context, the bioleaching of selected metals, viz. Cu, Ni, and Zn from the waste PCB (WPCB) of obsolete computer was comparatively assessed employing pure and mixed fungal cultures of *Aspergillus tubingensis* and *Aspergillus niger*.

13.2 Materials and Methods

13.2.1 WPCB Collection and Sample Preparation

WPCBs (Fig. 13.1) of obsolete computer were collected and brought to the laboratory from local EEE repairing shops in Bihta, Patna. After removing the mounted parts manually, WPCBs were cut into smaller parts. Smaller WPCB parts were then pulverized using a cutting mill (SM200, Retsch, Germany) and separated into particle size fraction of 0.038–1 mm using a sieve shaker (AS200, Retsch, Germany).

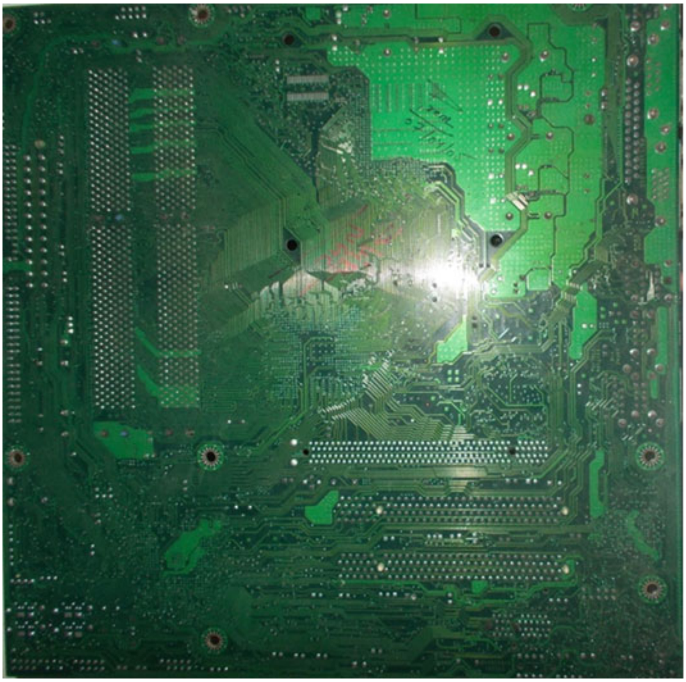

Fig. 13.1 Typical WPCB of obsolete computer used in the present study

Particle size fraction ranging from 0.038–1 mm was subsequently used for metallic content quantification, morphological characterization, and bioleaching experiment.

13.2.2 Fungal Cultures

In bioleaching study, pure and mixed fungal cultures of *Aspergillus* spp. i.e., *Aspergillus niger* (MTCC-281) and *Aspergillus tubingensis* (ATCC-76608) were employed. *Aspergillus niger* was chosen for its organic acid production ability to presumably help in bioleaching of metals. As the metals are usually embedded in the polymer matrix in the PCB, *Aspergillus tubingensis* (ATCC-76608) was selected for its polymer-degrading ability. The pure culture of the *Aspergillus niger* and *Aspergillus tubingensis* was obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), India and American Type Culture Collection (ATCC), USA, respectively. Fungal cultures were inoculated in separate 1000 mL conical flasks containing 500 mL of potato dextrose broth (PDB) medium in each upon autoclaving. The flasks were then incubated in an incubated shaker (SIF 5000R, Jeio Tech, South Korea) for 48 h at 200 rpm and 30 °C.

13.2.3 Metallic Content and Morphology of Pulverized WPCB and the PCB Residue

Morphology of the pulverized WPCB and the PCB residue after bioleaching was magnified using a scanning electron microscope (SEM) (EVO 50, Carl Zeiss, Germany). To compute the bioleaching efficiency, the content of selected metals i.e. Cu, Ni, and Zn in the pulverized WPCB sample was quantified following the acid digestion as per the USEPA method 3052 (Magnuson and Lasure 2004) and filtration using 0.22 µm filter with appropriate dilution. The diluted samples were then analyzed using inductively coupled plasma mass spectrometer (ICP-MS) (7800, Agilent, USA) for metal quantification.

13.2.4 Bioleaching Experiments

For bioleaching experiments, 100 mL of autoclaved PDB medium with adjusted pH of 5 in 250 mL conical flasks were added with the pulverized WPCB sample in the size fraction of 0.038–1 mm at an e-waste pulp density of 1 g/L. In order to comparatively investigate the metal leaching efficiency of two *Aspergillus* spp., bioleaching experiments were conducted in three sets. In the first and second sets, 1 mL spore suspension each of pure culture of *Aspergillus niger* and *Aspergillus tubingensis* was inoculated in the autoclaved flasks separately. In the third set, 1 mL each of spore suspension of both the fungal spp. was inoculated in the autoclaved flasks. All the inoculated flasks were then incubated in an incubated shaker (SIF 5000R, Jeio Tech, South Korea) at 170 rpm and 30 °C. Control reactors without the addition of fungal culture were also maintained under similar experimental conditions. The dissolution of the selected metals such as Cu, Zn, and Ni from the pulverized WPCB matrix in the bioreactors and control reactors was analyzed at every 3 days by analyzing metal concentration in the leachate using ICP-MS (7800, Agilent, USA). All bioleaching experiments were conducted in triplicate and average values along with standard deviations were reported.

13.2.5 Analytical Measurements

The microscopic structure of the pulverized WPCB particles and the PCB residue upon bioleaching was magnified using SEM (EVO 50, Carl Zeiss, Germany). Elemental analysis of the WPCBs of obsolete computer for the targeted elements i.e. Cu, Zn, and Ni was performed using ICP-MS (7800, Agilent, USA) in the digestate prepared using following the microwave-assisted acid digestion as per the USEPA 3052 method (USEPA 1995). Bioleachate samples collected from all three sets of bioleaching reactors employing pure and mixed fungal cultures along with control reactors were measured for the targeted metals using ICP-MS following filtration through a filter having pore size of 0.22 µm for removal of fungal mycelium and suspended particles followed by dilution with deionized (DI) water. Analyses were done in triplicate.

13.3 Results and Discussion

13.3.1 Metallic Content and Morphology of Pulverized WPCB and the PCB Residue

The metal quantification of the pulverized WPCBs of obsolete computer revealed that Cu, among the targeted metals, is found to be the highest in abundance with a content of 21.5%, followed by 0.10% Zn and 0.08% Ni. Since the presence of the base metals like Cu, Zn, and Ni was found to be in abundance, the pulverized WPCB of obsolete computer can be considered as a secondary metal reservoir.

Morphology of pulverized WPCBs in the particle size fraction of 0.038–1 mm and the PCB residue obtained upon bioleaching was observed under SEM. The SEM image of the pulverized PCBs (shown in Fig. 13.2a) revealed the non-uniformity in particle shapes and sizes. The pulverized WPCB samples were observed to consist of predominantly rod- and lumps-shaped agglomerated fiberglass small particles along

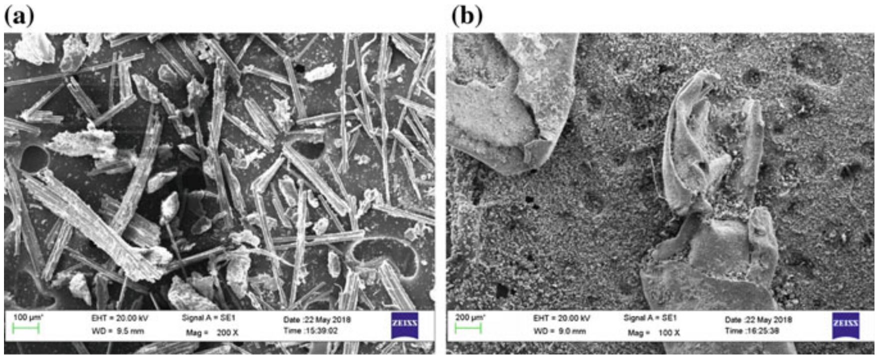

Fig. 13.2 SEM-micrograph of **a** raw pulverized WPCB, and **b** PCB residue upon fungal bioleaching

with metallic particles. Further, the rough surface of the pulverized PCB was featured with several shiny spots indicative of metal abundance. The variations in the surface texture of particles can be ascribed to the response of metal diversity, glass fibers, etc. when passed through the different forces during sample preparation using milling operation (Murugan et al. 2008). Upon pulverization, the adherence of fungus to the pulverized particles is likely to aid the bioleaching of metals. The SEM image of the PCB residue (shown in Fig. 13.2b) obtained from the mixed fungal culture bioreactor showed degraded surface featured with fine particulates in the backdrop suggesting metal dissolution from the pulverized PCB particles.

13.3.2 Bioleaching of Targeted Metals from E-Waste Using Pure and Mixed Fungal Cultures

Bioleaching results revealed that the metal leaching from pulverized PCB in the pure cultures of *Aspergillus tubingensis* and *Aspergillus niger* differed significantly at 1 g/L of e-waste pulp density with better dissolution in case of *Aspergillus niger*. The metal extraction was observed to increase gradually during the initial period and showed an asymptotic trend after 30 days of bioleaching in the pure and mixed cultures of *Aspergillus tubingensis* and *Aspergillus niger* (Fig. 13.3). The maximum metal leaching employing a pure culture of *Aspergillus tubingensis* was observed to be 54% Cu, 41% Zn, and 14% Ni. Similarly, the corresponding maximum metal leaching efficiency employing a pure culture of *Aspergillus niger* was 71% Cu, 32% Ni, and 79% Zn at the pulp density of 1 g/L.

Using the mixed cultures of *Aspergillus tubingensis* and *Aspergillus niger*, there was a marginal increase in metal bioleaching for Cu and Ni with extraction efficiency of 76 and 36%, respectively. For Zn, the maximum extraction efficiency of 63% was observed using the mixed culture. However, the maximum Zn extraction efficiency in mixed culture was observed to be more than that in the pure culture of

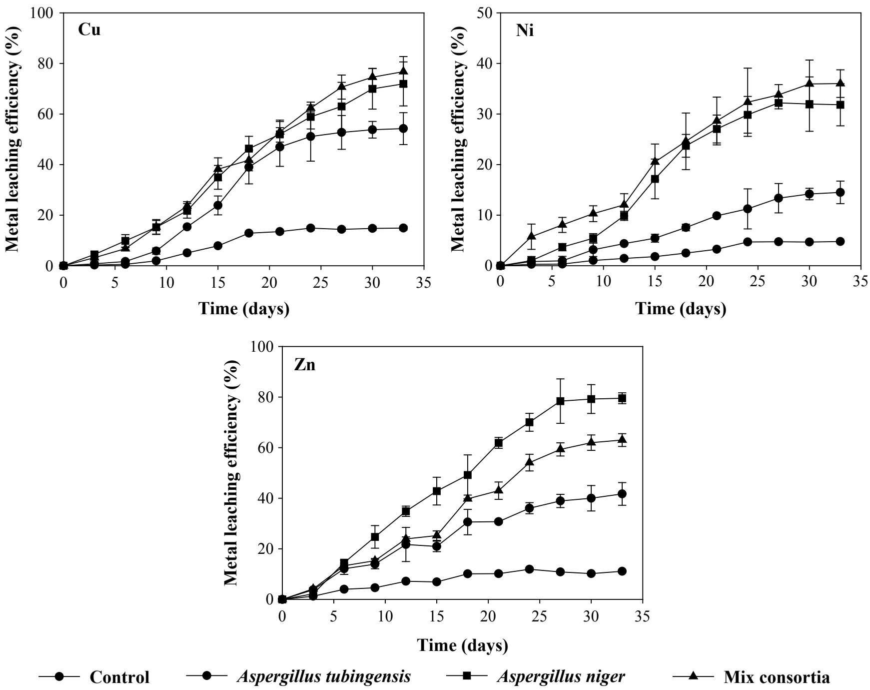

Fig. 13.3 Bioleaching of selected metals from WPCB of obsolete computer using pure and mixed cultures of *Aspergillus niger and Aspergillus tubingensis*

Aspergillus tubingensis and less than that in the pure culture of *Aspergillus niger*. A compilation of literature on the bioleaching of metals from e-waste using different fungal species along with the maximum metal extraction efficiency and bioleaching period is provided in Table 13.1. It is evident from Table 13.1 that the maximum metal extraction efficiency achieved for the selected metals in the present study is comparable with the results reported in the literature. Owing to the organic acid production ability of *Aspergillus* spp., the inoculated fungi were thriving on the PDB medium for the production of various organic acid like citric acid and oxalic acid which further kept pH of the solution low (Singleton 2001). Some other studies have also shown that citric and oxalic acids are the predominant organic acid produced by *Aspergillus* spp. facilitating metal bioleaching (Kim et al. 2016; Horeh et al. 2016; Santhiya and Ting 2005).

13.3.3 Future Implications

The present study has shown that the pure and mixed fungal culture of *Aspergillus tubingensis*, a polymer-degrading species and *Aspergillus niger*, an organic acid

Table 13.1 Bioleaching of metals from e-waste using various fungal species

E-waste	Fungal species	Duration (day)	Maximum metal extraction efficiency (%)	References
E-waste scrap	*A. niger and P. simplicissimum*	21	Al: 95, Cu: 65, Ni: 95, Pb: 95, Zn: 95	Brandl et al. (2001)
Lithium ion batteries	*A. niger*	30	Al: 65, Cu: 100, Li: 95, Ni: 38	Kim et al. (2016)
Catalyst	*A. niger*	70	Al: 55, Ni: 65	Santhiya and Ting (2005)
Converter	*P. simplicissimum*	13	Zn: 93	Schinner and Burgstaller (1989)
Hydrocracking catalyst	*P. simplicissimum*	30	Al: 25, Mo: 92.7, Ni: 66.43	Amiri et al. (2011)
Fluid catalytic cracking catalyst	*A. niger*	14	Al: 30, Ni: 9, Sb: 64, V: 36	Aung and Ting (2005)
Electronic scrap	*A. niger*	42	Cu: 68.3, Pb: 27.9	Kolenčík et al. (2013)
Electronic scrap	*P. chrysogenum*	24	Al: 96, Cu: 48, Ni: 73, Zn: 25	Ilyas et al. (2013)
PCB	*A. niger*	3	Cu: 80	Jadhav and Hocheng (2015)
PCB	Mixed culture	27	Al:15, Pb: 20, Sn: 8, Zn: 49	Xia et al. (2018)
Computer PCB	*A. niger*	21	Cu: 85%, Ni: 80, Zn: 100	Faraji et al. (2018)
Computer PCB	*A. tubingensis and A. niger*	33	Cu: 76%, Ni: 36, Zn: 63	This study

producing species can efficiently bioleach metals from waste PCB at a pulp density of 1 g/L in 33 days. This highlights the practical feasibility of fungal bioleaching of metals from e-waste using the pure and mixed cultures of *Aspergillus tubingensis* and *Aspergillus niger*. Therefore, it is essential to improvise the bioleaching efficiency of metals from WPCB by employing different fungal species in pure and mixed forms along with the addition of suitable organic ligands to develop a hybrid process. Acclimatized fungal species with prior exposure to the e-waste environment may lead to enhanced metal bioleaching. Moreover, the metal extraction rate can be increased by optimizing the different factors governing the fungal bioleaching process like pH, inoculum size, continuous supply of nutrients, etc.

13.4 Conclusions

The metal extraction from e-waste employing pure and mixed forms of *Aspergillus* fungal strains revealed that *Aspergillus niger* was able to leach out more metals as compared to *Aspergillus tubingensis*. At a pulp density of 1 g/L, the pure culture of *Aspergillus niger* showed maximum metal extraction of 79% Zn, followed by 71% Cu and 32% Ni. At the same pulp density, the corresponding metal extraction efficiency for Cu, Zn, and Ni using *Aspergillus tubingensis* was 54, 41, and 14%, respectively. Except for Zn, the mixed culture of *Aspergillus niger* and *Aspergillus tubingensis* showed a marginal increase in the metal bioleaching reaching extraction efficiency of 76 and 36% for Cu and Ni, respectively at an e-waste pulp density of 1 g/L. In the present study, the metal extraction efficiency for Zn using the mixed fungal culture of *Aspergillus* strains was 63%. However, the metal extraction rate and the time required for bioleaching can be further enhanced by employing a consortium of fungal strains along with the addition of suitable organic ligands to develop a hybrid process.

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