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ENVIRONMENTAL MICROBIOLOGY - RESEARCH PAPER

Bioleaching of copper and nickel from mobile phone printed circuit board using *Aspergillus fumigatus* A2DS

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Abstract

The recovery of metals from electronic waste was investigated by using fungal strain *Aspergillus fumigatus* A2DS, isolated from the mining industry wastewater. Fifty-seven percent of copper and 32% of nickel were leached (analyzed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES)) by the organism after one-step leaching at a temperature of 30 °C (shaking condition for 7 days). Maximum % of copper and nickel were obtained at a pH of 6 (58.7% Cu and 32% Ni), the temperature of 40 °C (61.8% Cu and 27.07% Ni), a pulp density of 0.5% (62% Cu and 42.37% Ni), and inoculums of 1% (58% Cu and 32.29% Ni). The XRD pattern of PCB showed 77.6% of copper containing compounds. XRD analysis of the leachate residue showed only 23.2% Euchorite ($ASCu_2H_7O_8$) and 9.4% other copper containing compounds, indicating the leaching property of the fungus. HPLC analysis of the spent medium showed the presence of different acids like citric,

Highlights • Aspergillus fumigatus ability to leach metals from PCB of mobile phone has not been reported. The strain isolated showed genotypic differences from the already reported strains of Aspergillus fumigatus, so the isolates was named as Aspergillus fumigatus A2DS.

• Fifty-seven percent of copper and thirty-two percent of nickel were leached by the organism after one-step leaching at a temperature of 30 °C (shaking condition for 7 days). Effects of various factors like pH, temperature, inoculum %, and pulp density conditions were also analyzed. Maximum percentage of copper and nickel were obtained at a pH of 6 (58.7% Cu and 32% Ni), temperature of 40 °C (61.8% Cu and 27.7% Ni), a pulp density of 0.5% (62% Cu and 42.37% Ni), and inoculum of 1% (58% Cu and 32.29% Ni).

• ICP-OES of the leachate obtained using spent media (containing organic acids like citric acid, succinic acid, and fumaric acid, confirmed by HPLC and FTIR) indicate acidolysis of the metal. Sixty-one percent of the copper and 35% of nickel were leached out after seven days of incubation at shaking condition and 57% of copper and 32.8% of nickel at static condition confirming acidolysis property of the strain.

• The main highlight of the study is that the prolonged incubation of the strain until 30 days did not show much leaching ability. The variation in pH and biomass were noted throughout the 20 days. pH decreased up to the sixth day and there after the pH remained constant, confirming the correlation between acid metabolites and leaching.

• TEM and SEM–EDX of the residue obtained after leaching confirmed the ability to absorb and adsorb metals on the mycelium. Ten percent copper was adsorbed by the mycelium. Metals like copper and nickel were also absorbed and adsorbed by the fungal mycelium, which will provide an economical alternative for removing toxic heavy metals from industrial wastewater and aid in environmental remediation.

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succinic, and fumaric acid. The FTIR spectrum showed a decrease in carboxylic stretching in the leachate produced after bioleaching using spent medium. ICPOES of the leachate obtained using spent medium showed that 61% of the copper and 35% of nickel were leached out after seven days of incubation at shaking condition and 57% of copper and 32.8% of nickel at static condition confirming acidolysis property of the strain. *A. fumigatus* A2DS metal absorption and adsorption ability were observed using transmission electron microscopy (TEM) and scanning electron microscopy energy dispersive X-ray (SEM-EDX) respectively. The results thus indicate that bioleaching of Cu and Ni is bioleached by *A. fumigatus* A2DS.

Keywords Absorption · Acidolysis · Adsorption · *Aspergillus fumigatus* · Bioleaching · Copper · FTIR · HPLC · Nickel · XRD

Introduction

The growing amount of e-waste is mainly fueled by higher consumption rates of EEE (electrical and electronic equipment), short life cycles, and few repair options. Asia generated the highest quantity of e-waste in 2019 at 24.9 Mt, followed by America (13.1 Mt) and Europe (12 Mt), while Africa and Oceania generated 2.9 Mt and 0.7 Mt, respectively. Europe ranked first worldwide in terms of e-waste generation per capita, with 16.2 kg per capita. Oceania was second (16.1 kg per capita), followed by America (13.3 kg per capita), while Asia and Africa generated just 5.6 and 2.5 kg per capita, respectively (Vanessa [33]. Most printed circuit boards (PCBs) of e-waste contain various kinds of metals [35]. Copper (19.91%), aluminum (7.06%), nickel (5.35%), iron (3.56%), tin (2.03%), lead (1.01%), and also precious metals like silver and gold are present in PCB [38]. Landfilling, burning, and incineration are common methods used for the disposal of e-waste. Pyrometallurgy, hydrometallurgy, and pyro-hydrometallurgy are used to extract heavy metals from ores. The major disadvantage of these methods is that large amounts of hazardous wastes are generated at the end. The bioleaching method generates very little quantity of waste. Four different stages like (i) acidolysis, (ii) complexolysis, (iii) redoxolysis, and (iv) bioaccumulation [8] are involved in bioleaching. There are certain approaches in bioleaching of metals using fungi,one step, two step, and spent medium are the main procedures which are reported by several researchers [1, 18]. In a one-step approach, the WPCBs are added into the medium at the first moment of incubation,in a two-step approach, they are added when a sudden drop in pH is detected (3rd–5th days), and eventually, in spent medium approach, leaching experiments took place after 14 days of fungal activation in fungus free acidic filtrates. Each of the approaches has its own benefits and may lead to different outputs. Many reports are available on the bioleaching ability of bacteria from mobile phone printed circuit board (PCB) [24, 25]. A few researchers have studied the impact of fungi on the recycling of metals from cellular phone PCBs. Solubilization through fungi is by the formation of organic or inorganic acids. There is various advantage of using fungi for bioleaching. The major advantage include (i) they can to grow under harsh environmental condition (ii) leaching manner with a shorter lag phase,and (iii) secondary metabolites (organic acids) produced by fungi, interact with metal ions and form complexes with metal ions, thus lowering the toxicity of the metabolites to the biomass [7]. Studies on fungi like *Aspergillus niger* and *Penicillium simplicissimum* on the bioleaching ability were carried out by Brandl et al. [5] and by Faraji et al. [10]. However, no reviews are available in the capacity of *Aspergillus fumigatus* to leach metals from cellular PCBs. The main aim of the present work is to study the capacity of *Aspergillus fumigatus* A2DS to leach out metals by acidolysis and also the ability of fungal mycelium to adsorb and absorb metals present inside the PCB of a cellular phone.

Materials and methods

Chemicals

All the chemicals used for the study were of analytical grade. Sucrose, $NaNO_3$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, and KCl were procured from S.D. Fine Chem. Limited (Mumbai, India) and yeast extract from Hi Media, Mumbai, India.

Collection and processing of PCB of mobile phone

Mobile phones for the present study were obtained from e-waste recyclers and the students of Kadi Sarva Vishwavidyalaya (KSV). The PCBs of mobile phones were separated from other parts of mobile phones carefully. The PCBs were made into a fine powder using an instrument called File (it is an instrument used for grinding) at the Department of Mechanical Engineering, KSV Gandhinagar, Gujarat. The larger particles were separated using a sieve (pore size 100µm). The fine powder (Fig. 1A) thus obtained was used for studying the bioleaching ability of fungi.

Analysis of the particle size of PCB powder

Wet dispersion unit SUCELL and the compact laser diffraction sensor HELOS/BR (carried out at SCICART, Gujarat) were used for analyzing the particle size.

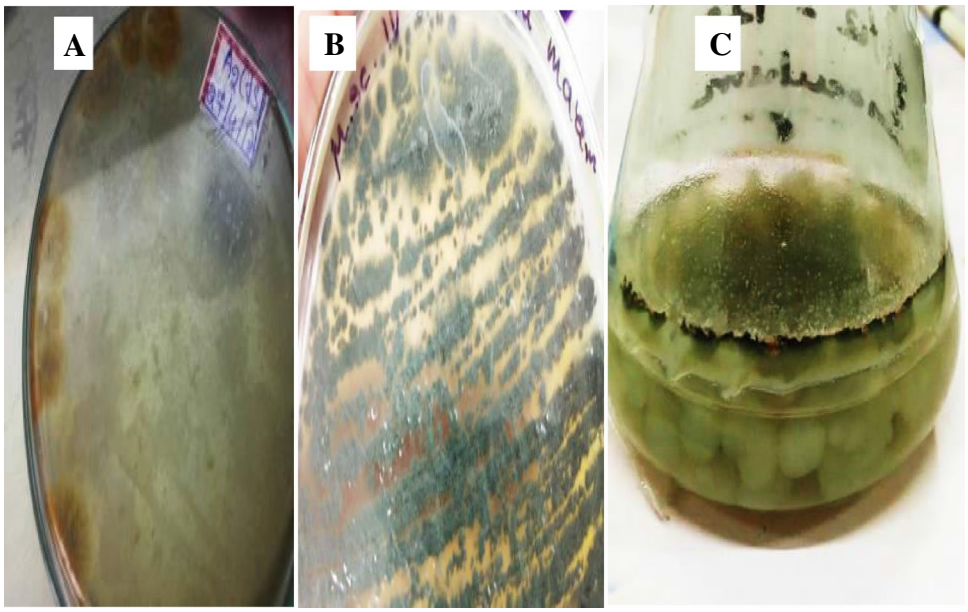


Fig. 1 **A** *A. fumigatus* A2DS growth on sucrose medium (2% PCB). **B** Sucrose medium + 1% PCB. **C** *A. fumigatus* A2DS forming balls (shaking condition in sucrose medium)

Acid digestion and ICP-OES analysis of PCB (mobile phone)

Forty-milliliter aqua regia (3 parts hydrochloric acid to 1 part nitric acid) was used for digesting 1 g of PCB [10]. Using ICP-OES (Perkin Elmer, USA, Optima 3300 RL), the metal concentration of digested samples was then analyzed (Carried out at SCICART, Gujarat).

Collection of metal containing effluent samples

Metal resistant organisms were isolated from different effluent/soil samples. The selection of area was based on the assumption that these samples contain heavy metals and thus heavy metal resistant organisms. The area included mining, textile effluent, motor garages soil sample, and steel store soil samples. Textile effluent was from Tadkeshwar district, Surat. Mine effluent from Rajparadi district Bharuch, motor garages soil sample and steel store soil sample from Gandhinagar (Samples were collected before the wastewater treatment and contain metals like copper and nickel less than 10 mgl^{-1}). Collected samples were transported to the laboratory and were used for the study.

Isolation of metal resistant organisms from contaminated soil and water samples

Sucrose broth medium consisted of (g/L) the following: sucrose (100), $NaNO_3$ (1.5), KH_2PO_4 (0.5), $MgSO_4{\cdot}7H_2O$ (0.025), KCl (0.025), and yeast extract (1.6) [4] that was used for the isolation of the fungi. Sucrose media with different concentrations of PCB (1, 2, and 3%) were prepared followed by the addition of 10 mL of soil suspension/effluent sample (10% w/v). For the growth of fungi, the flasks were kept at 30 °C at 120 rpm for 7 days [5]. To obtain individual colonies, the above enriched samples were spread onto the sucrose agar medium (agar added in sucrose broth medium with composition same as above) and incubated at 30 °C. The plates were observed for colonies after 48–72 h of incubation. The isolated colonies were purified by sub-culturing on to sucrose agar medium supplemented with 1% PCB.

Phenotypic and Genotypic Identification of fungal strain

Lacto phenol cotton blue mounting technique was used for observing the morphology of the isolated fungi. Cultural characteristics and the microscopic view were used for phenotypic identification. Internal Transcribed Space Sequencing (ITS) of 18 srRNA gene (rDNA) was used for genotypic identification (performed at Xcelris Labs Ltd, Ahmedabad, and Gujarat). The phylogenetic tree was constructed using the Mega software.

Study on bioleaching ability of *A. fumigatus* A2DS by one-step bioleaching

A suspension of *A. fumigatus* A2DS (10^6 spores/mL) was prepared. One milliliter of the suspension was added into two sets (n=3) of 100 mL sucrose broth containing 1% PCB of mobile phone in 250-mL Erlenmeyer flasks. Flasks (medium+culture+PCB) were incubated in an orbital shaker at 120 rpm at 30 °C. One set of the flask was incubated for 7 days [37], and the other set of flask was kept for 30 days [14]. After incubation, the flasks were kept idle for 5 min, and 5–8 mL of leachate was collected separately from each

flask. The larger particles present in the leachate was filtered using Whatman No 1 filter paper (pore size: 11µm). The fungal spores/hyphae present in leachate were also removed by centrifugation at 10,000×*g* for 10 min. The metal present in the leachate was analyzed using ICP-OES method (Perkin Elmer, USA; Optima-3300 RL). To nullify the effect of evaporation on bioleaching, a control set without any inoculum (PCB+medium) was also used. The above procedure was repeated for static conditions. The following formula was used to find out the % of metal leached by the strain (using the mean value obtained (n=3)), where c1 is the concentration of metal in the leachate and c0 is the initial concentration of metal in PCB.

$$\%ofmetalleached = \frac{c1}{co} * 100$$

Optimization of various factors involved in one-step bioleaching

The bioleaching ability of the fungus is affected by different factors like pH, temperature, percentage inoculums, and percentage pulp density. Using the same procedure mentioned above, the effects of pH (4, 6, 7, 8), temperature (20, 30, 40 °C), inoculum size (spore suspension of 0.5, 1, 2% (v/v)), and the pulp density (0.5, 1, 2% (w/v)) on bioleaching ability of *A. fumigatus* were also studied. For studying all factors mentioned above, the flasks were incubated at 120 rpm for 7 days. Sucrose medium containing PCB without fungal inoculum was kept as a control in all studies. The percentage of metal leached was found out by using the formula mentioned above.

XRD spectrum of bioleached residue and PCB of mobile phone

X-ray diffraction (XRD) analysis was used to study the structural changes in PCB. The bioleached residues obtained after one-step bioleaching experiment were treated with deionized water. After removing the moisture content, the pellet was made into a fine powder using mortar and pestle and analyzed using XRD (carried out at SCICART, Gujarat) (model: Xpert MPD; Make, Philips, Holland). XRD analysis of the PCB powder was also carried out (control). The variation in the diffraction pattern (between test and control) was observed using Match 3 Software.

Study on the organic acid production by A. fumigatus A2DS

The ability of *A. fumigatus* to produce organic acid was studied by inoculating 1 mL of spore suspension (10^6 spores/mL) of *A. fumigatus* A2DS into 100 mL sucrose broth (without PCB) containing flasks. The flasks were incubated at 30 °C at 120 rpm. The changes in pH and biomass were noted till 20 days. Variations in pH and biomass were noted throughout the 20 days (data not shown). pH decreased up to the sixth day, and thereafter the pH remained constant. To confirm the production of acids, after the sixth day of incubation in sucrose broth, the content was filtered through the Whatman No 1 filter paper (pore size: 11µm) and centrifuged (Remi 2-24L) at 10,000 g for 10 min to remove fungal pellets. The presence of citric acid and oxalic acid in the sucrose medium without e-waste was estimated using titrimetric and spectrophotometric methods respectively. The presence of organic acid in the filtrate (spent medium) was also analyzed using FTIR (Spectrum GX Perkin Elmer, USA) (Outsourced at SCICART, Gujarat) and HPLC (Agilent, Model 1100 series, outsourced at AUM Research Laboratories). HPLC was carried out by using a C18, 5 micron column (250-mm length and 4.6-mm internal diameter), with a mobile phase of 25 mM K_2HPO_4, adjusted to pH 2.5. A 1.5 mL/min of the sample was injected for 15 min, and elutes were detected at λ 210 nm.

Role of acids in leaching of metals by A. fumigatus A2DS

The ability of acids to leach the metal was carried after confirmation of organic acid secretion by *A. fumigatus* A2DS strain. Five milliliters of the spent medium (obtained after growing fungus in sucrose broth) was added to the sucrose broth with PCB (1%) and kept for incubation at 30 °C for 7 days in shaking (120 rpm) and static condition. After incubation, the medium was centrifuged at 10,000x*g* for 10 min, and the medium was filtered through Whatman No 1 filter paper (pore size: 11µm), and the filtrate was analyzed for the presence of metal using ICP-OES. Organic acids present in the leachate (shaking condition) were analyzed using FTIR.

Metal absorption and adsorption ability of A. fumigatus A2DS analyzed using TEM and SEM-EDX

TEM (Tecnai 20, Philips, and Holland) and SEM-EDX (ESEM EDX XL-30, Philips, the Netherlands) analysis were used to establish the ability of fungi to absorb and adsorb metals respectively. The fungal culture of *A. fumigatus* A2DS was inoculated into a sucrose broth medium containing 1% PCB of mobile phone and incubated at 30 °C for 7 days. To obtain the biomass, the fungal containing medium was passed through a Whatman No 1 filter paper. The resulting biomass was washed with 0.02 phosphate buffer and dehydrated in a graded ethanol series for 5 min each. The fungal biomass was observed under TEM and SEM-EDX (carried out at SCICART, Gujarat).

Statistical analysis

Three sets of readings were taken for all the experiments. Data points in the figures represent means with error bars shown (±SD). Two-way ANOVA was used to analyze the significance of experimental results (with a 95% level of confidence, α=0.05) (Sundar and Richard, 2012).

Results

Particle size analysis using SUCELL and the compact laser diffraction sensor HELOS/BR

Particles with X_{90} of 114.77 µm and X_{10} of 3.45 µm were used for all studies. A reduction in the size of the particles after bioleaching (data not shown) was observed.

ICP-OES analysis of metals present in PCB of mobile phone

The ICP-OES analysis of the acid digested PCB mobile phone showed 242.3 $mg.g^{-1}$ of copper, 3.05 $mg.g^{-1}$ of lead, and 7.10 $mg.g^{-1}$ of nickel. Bhumika et al. [3] studies also showed similar results.

Isolation and identification of metal leaching organisms

Metal resistant individual colonies were observed on sucrose agar medium supplemented with 1% and 2% PCB (Fig. 1A) and not in 3% PCB. No growth was observed on media plated with soil samples. The isolated organisms were purified and maintained on a sucrose agar medium (1% PCB). On sucrose agar medium, the culture showed bluish grey-colored conidial spores (Fig. 1B). Fungi formed balls in shaking conditions in sucrose broth medium (Fig. 1C). Lacto phenol cotton blue mounted, fungal slides were observed under light microscope (×40). The macroscopic and microscopic view showed morphological characteristics similar to *Aspergillus.* Genotypic identification was carried out using ITS sequencing of 18 srRNA. Sequences were deposited into the Genbank (NCBI) DNA sequence database (accession no. MK424485) and also analyzed by BLAST. BLAST analysis showed 99% similarity with *Aspergillus fumigatus* and 1% different from reported *Aspergillus fumigatus*. So the isolate was named as *Aspergillus fumigatus* A2DS. Phylogenetic trees were constructed, and evolutionary history was inferred by using the maximum likelihood method and Kimura 2-parameter model [21]. The bootstrap consensus tree inferred from 500 replicates was taken to represent the evolutionary history of the taxa analyzed [11]. Phylogenetic analysis was conducted using MEGA X software. The phylogenetic tree was constructed using Mega software (Fig. 2).

ICP-OES analysis of the leachate after one-step bioleaching and optimization of various factors influencing bioleaching of PCB

The bioleaching ability of the isolated organism was analyzed using ICP-OES, and the results are presented in Table 1. Fifty-seven percent of copper and 32% of nickel were leached by the organism after the one-step leaching at a temperature of 30 °C (shaking condition (120 RPM) for 7 days) (Fig. 3A). Increasing the period of incubation of the strain along with PCB powder showed a slight variation in the biomass (data not shown). Incubating for 30 days also did not show much difference in the percentage of metals in the leachate (Fig. 3A); this might be due to the depletion of sucrose and thereby decrease in biomass production and acid production. Various factors influencing bioleaching were also analyzed. Effects of various factors like pH, temperature, inoculum %, and pulp density were also analyzed. Maximum % of copper and nickel were obtained at a pH of 6 (58.7% Cu and 32% Ni), inoculum of 1% (58% Cu and 32.29% Ni), the temperature of 40 °C (61.8% Cu and 27.07% Ni), and a pulp density of 0.5% (62% Cu and 42.37% Ni) (Fig. 3 B, C, D, and E, respectively). In the present study, maximum leaching was obtained at pH 6 (*A. fumigatus* A2DS showed maximum oxalic production (spectrophotometrically) at pH 6 (data not included) indicating the role of oxalic acid in bioleaching.

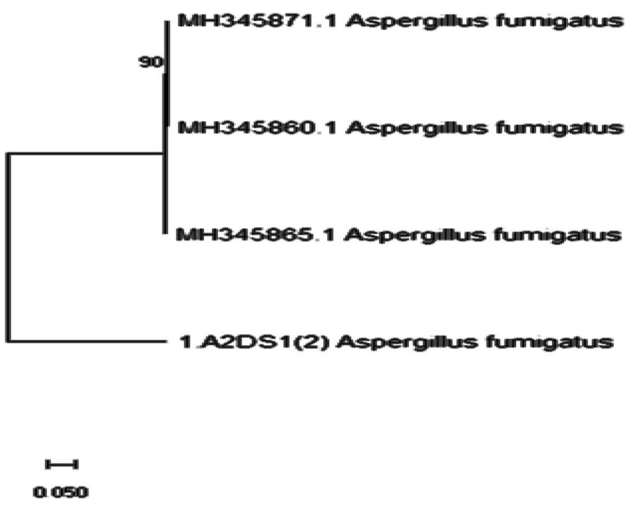


Fig. 2 Phylogenetic tree based on the internal transcribed spacer region nucleotide sequence of culture *A. fumigatus* A2DS strain (constructed using Mega software)

Table 1 Cu and Ni solubilized by *A. fumigatus* A2DS from PCB

Results of ICPOES solubilized by *Aspergillus fumigatus* A2DS			
Parameters		Cu (mg/g^{-1})	Ni (mg/g^{-1})
E-waste (1%)		242.3 ± 1	7.10 ± 0.1
Shaking 7 days		139.43 ± 1	2.34 ± 1
Static 7 days		118.85 ± 1	2.49 ± 1
Shaking 30 days		149.9 ± 5	2.18 ± 1
Static 30 days		139 ± 5	2.29 ± 1
pH	4	118.75 ± 1	1.99 ± 0.4
	6	142.43 ± 1	2.29 ± 0.1
	7	139 ± 5	1.92 ± 0.1
	8	111.4 ± 1	1.02 ± 0.1
Temp	40	149.75 ± 1	1.92 ± 2
	30	139.4 ± 5	1.02 ± 1
	20	124.3 ± 1	1.92 ± 2
% Inoculums	0.5%	114.3 + 1	2.02 ± 2
	1%	139 ± 5	2.29 ± 0.7
	2%	124.3 ± 2	2.30 ± 2.0
% Pulp density	0.5% Before leaching 192.31 (Cu)/4.27 (Ni)	120.7 ± 0.1	1.8 ± 0.1
	1% Before leaching 242.31 (Cu)/7.10 (Ni)	114.32 ± 5	2.29 ± 1
	2% Before leaching 302.3 (Cu)/9.86 (Ni)	111.4 ± 0.1	3.11 ± 0.1

XRD spectrum of PCB and bioleached residue after bioleaching

X-ray diffraction pattern of the bioleached (Fig. 4A) and non-bioleached (Fig. 4B) (control: without fungal inoculation) residues were varying. The XRD spectrum of PCB of mobile phone powder and the residue obtained after bioleaching showed 100% intensity at an angle of 43.444°. Figure 4B shows 77.6% of copper containing compounds and in Fig. 4A shows only around 23.2% Euchorite and 9.4% other copper containing compounds.

TEM and SEM–EDX analysis of leached residue

The metals absorbed/adsorbed by the cell were visualized by TEM and SEM respectively. The EDX analysis of the printed circuit board (control) showed maximum copper (13%), followed by aluminum (10.76%) (Fig. 5A). The % weight of the metal in the EDAX spectrum was calculated using eZAF algorithms. The EDX analysis of the residue after fungal bioleaching showed copper (1.38%) and aluminum (2.47%) were adsorbed onto the surface of mycelium (Fig. 5B). SEM analysis of leached residue was carried out to find out the ability of metal ions to be adsorbed onto the surface of fungal mycelium (Fig. 5C). The length of the metal absorbed on fungal mycelium as observed using TEM is represented in yellow color (Fig. 5D). The absorption and adsorption of the metal can be also confirmed by other methods also.

FTIR and HPLC analysis of spent medium (fungus grown on sucrose broth) and filtrate obtained after bioleaching using spent medium (medium+1% PCB+spent medium)

The production of organic acid was further confirmed using HPLC and FTIR. The FTIR spectrum of the spent medium (*A. fumigatus* A2DS+sucrose broth) showed stretching corresponding to acid groups (FTIR 1401.3 Ft (cm^{-1})). The FTIR spectrum of spent medium (Fig. 6A and Table 2) (without any PCB) was compared with the FTIR of the leachate (spent medium + PCB) obtained. The stretches corresponding to active functional acid groups were present in the spent medium, but the height of the peak decreased in the leachate, showing utilization of acids in bioleaching of PCB (Fig. 6B and Table 2). HPLC chromatogram showed the presence of different acids like citric, succinic, and fumaric acid in the spent medium (Fig. 6C). To confirm the acidolysis of the metal by the fungal metabolites, the ICP-OES analysis of the leachate obtained after bioleaching using spent medium was carried out. Sixty-one percent of copper and thirty-five percent of nickel in the leachate after 7 days of incubation at shaking condition and 57% of copper and 32.8% of nickel at static condition were obtained

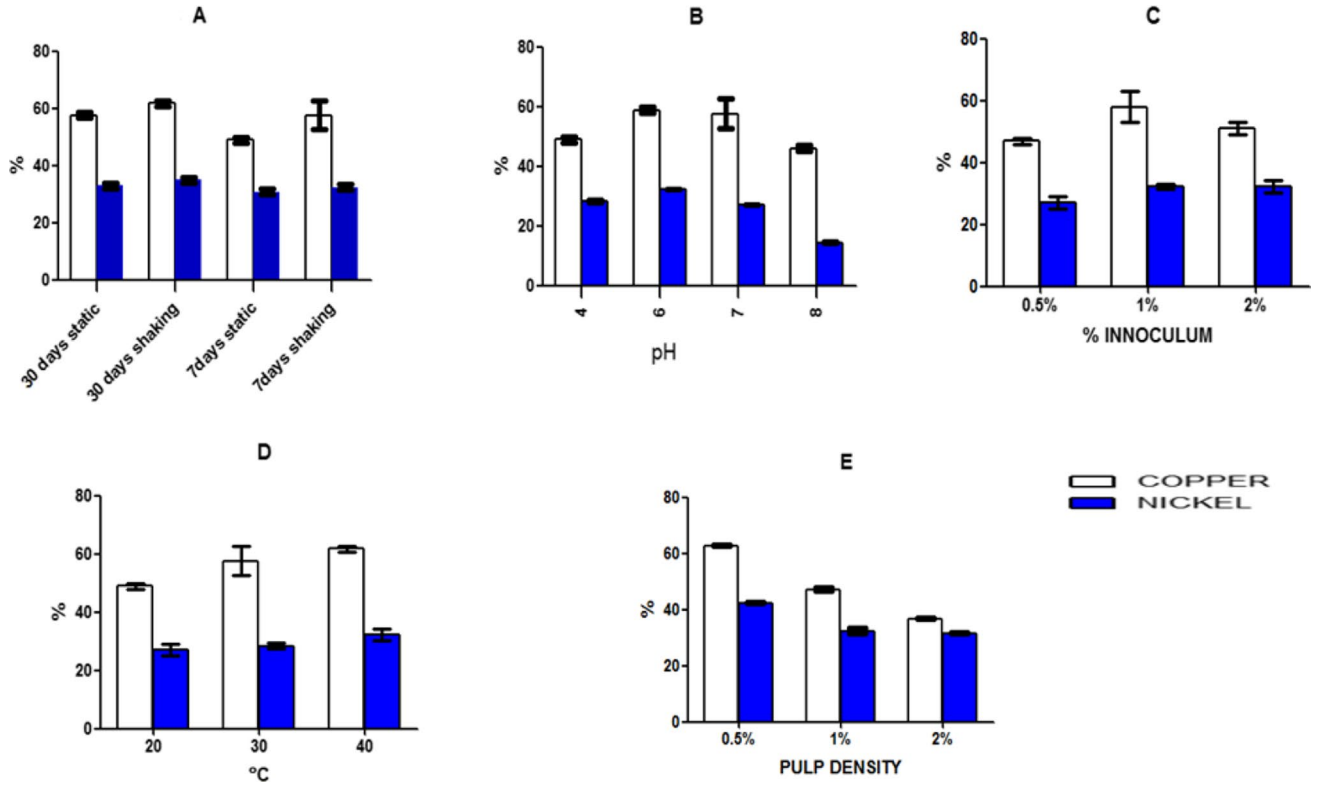


Fig. 3 Role of various factors in bioleaching (one-step bioleaching) from PCB of mobile phone by *A. fumigatus* A2DS strain. **A** Shaking and static conditions at varying time period (after 7 days and after 30 days) (P value < 0.0001; all values were significant when compared to control and also between groups). **B** Varying pH like 4, 6, 7, and 8 (P value < 0.0001; all values were significant when compared to control and also between groups). **C** Varying % inoculums like 0.5%, 1%, and 2% (P value < 0.0005; all values were significant when compared to control and also between groups). **D** At different temperatures (P value < 0.0001; all values were significant when compared to control and also between groups). **E** Varying pulp density (P value < 0.0001; all values were significant when compared to control and also between groups). The data presented in this figure presents the mean of values obtained from triplicate experiments at a significant level (P < 0.05) based on two-way ANOVA analysis

confirming the role of fungal metabolites in the leaching process (Fig. 7).

Discussion

Young and Veasey [39] have reported that "particle size has a negative correlation with the metal removal efficiency and high particle sizes may lead to poor metal extraction efficiency." Particles with X_{90} of 114.77 µm and X_{10} of 3.45 µm were used for all studies, as the surface area increases with decreasing particle size, the contact between the metal-bearing waste particles and the leachate in the leaching medium increases, thus leading to higher metal extraction efficiency [29]. A reduction in the size of the particles after bioleaching (data not shown) was observed. Similar outcomes were obtained by Faraji et al. [10] using *Aspergillus niger*. The metallic parts of the PCBs were dissolved during the bioleaching experiment while the non-metallic parts remained undissolved [10]. The ICP-OES analysis of the acid digested PCB mobile phone showed 242.3 $mg.g^{-1}$ of copper, 3.05 $mg.g^{-1}$ of lead, and 7.10 $mg.g^{-1}$ of nickel. Bhumika et al.'s [3] studies also showed similar results. Brandl et al. [5] had reported the role of microbes to mobilize metals from electronic waste materials. But there are limited data available on bioleaching studies using a mobile phone.

Primary ores are less complex in nature, whereas electronic waste is in a complex and concentrated form [28]. The PCB added effluent, favored only the growth of the metal resistant organisms. Lacto phenol cotton blue mounted, fungal slides were observed under the light microscope (×40); sequencing techniques and bioinformatics tool confirmed that the organism is *Aspergillus fumigatus* A2DS. The bioleaching ability of the isolated organism was analyzed using ICP-OES. Fifty-seven percent of copper and 32% of nickel were leached by the organism after a one-step leaching at a temperature of 30 °C (shaking condition (120 RPM) for 7 days. Studies on *Aspergillus niger* MXPE6 by Jorge et al. [20] showed that 24 and 5% of Cu was bioleached from gold-plated finger integrated circuits found in computer motherboards

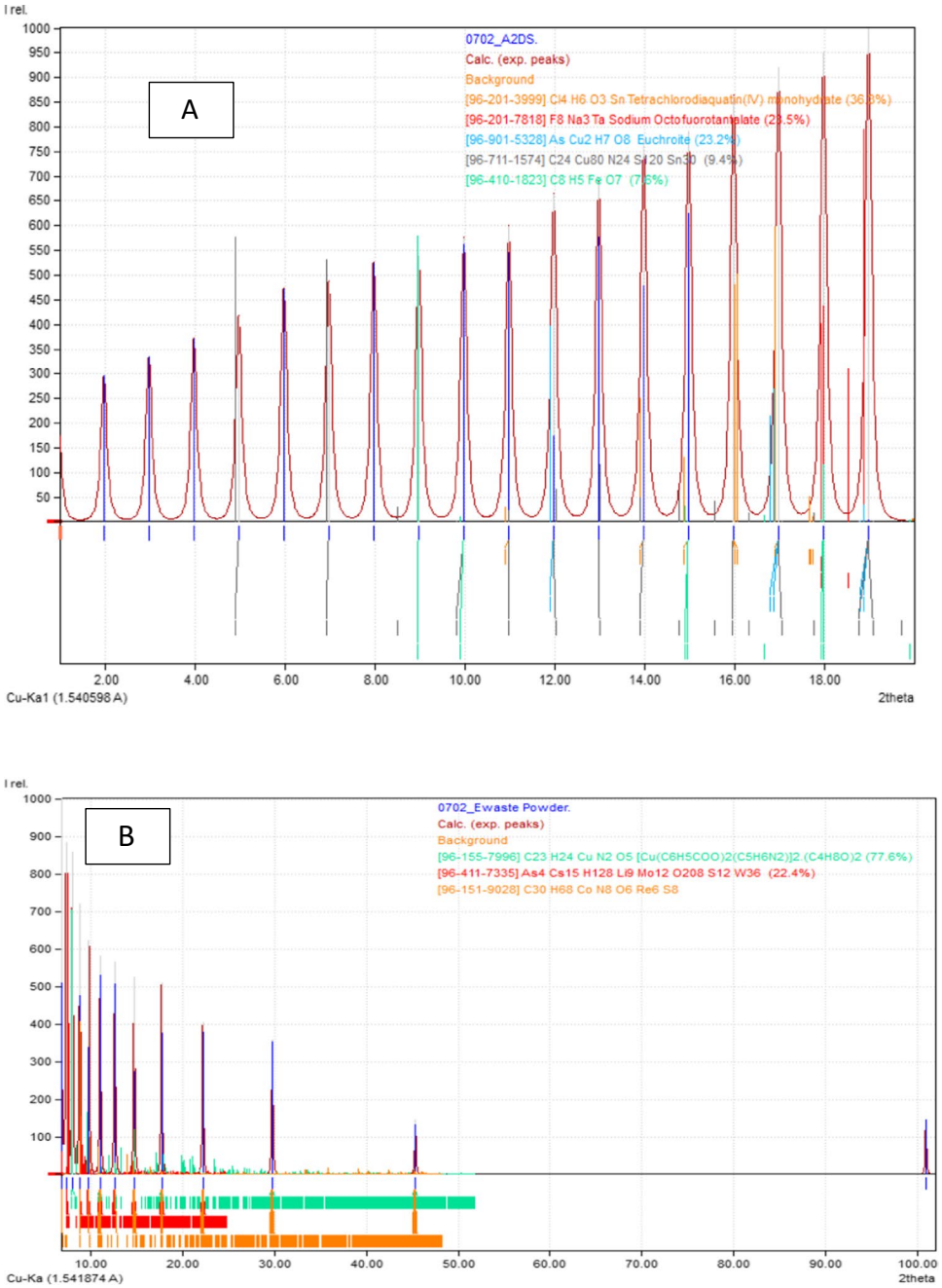


Fig. 4 XRD pattern of sample. **A** After bioleaching. **B** Before bioleaching

(GFICMs) and cellular phone printed circuit boards (PCBs), respectively. When compared to *Aspergillus niger* MXPE6, *Aspergillus fumigatus* A2DS has more bioleaching ability. The effects of various factors like pH, temperature, inoculum %, and pulp density were also analyzed. Maximum % of copper and nickel were obtained at a pH of 6 (58.7% Cu and 32% Ni), inoculum of 1% (58% Cu and 32.29% Ni), the temperature of 40 °C (61.8% Cu and 27.07% Ni), and a pulp density of 0.5% (62% Cu and 42.37% Ni). The results are in accordance with studies carried out by Nwagu and Okolo [27]. The studies of Walaszczyk et al. [34] on *Aspergillus niger* showed that oxalic production is maximum at pH 6. The released organic acids such as oxalic and citric acids which in turn reduced the host metal oxides/hydroxides to their lower valence states, and thus dissolving the base metals following the indirect mechanism. In acidolysis, the protonation of oxygen atoms covering the surface of metallic compounds. The protons and oxygen are associated with water; thus, the metal is detached from the surface (Eq. (1)). In complexolysis, metal complexes and chelates are formed, leading to the solubilization of the metal

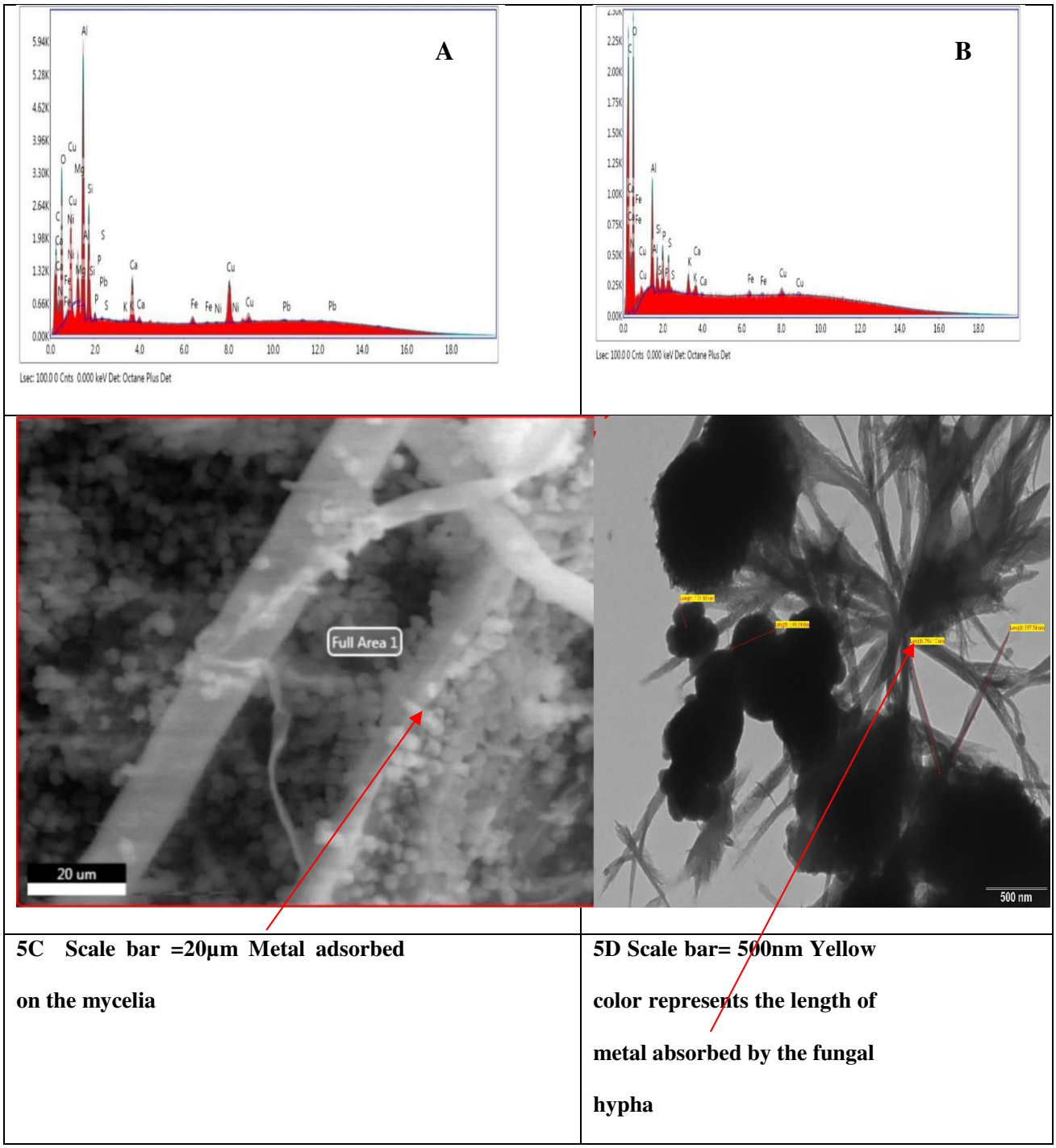
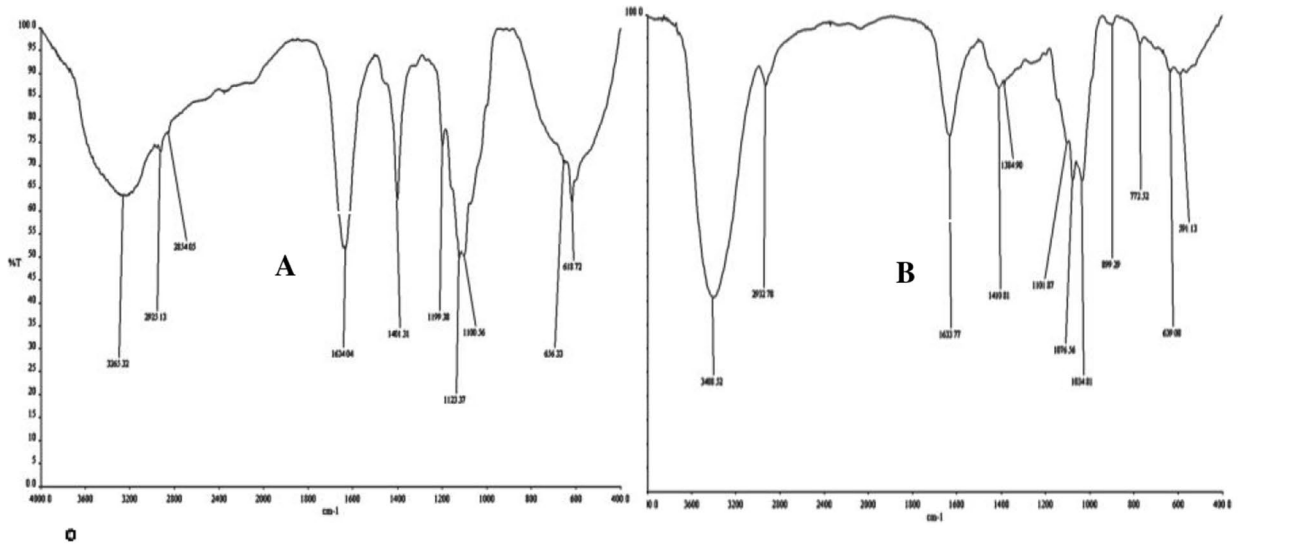


Fig. 5 TEM and SEM- EDX analysis of the PCB of the mobile phone powder and the residue obtained after leaching. **A** EDX spectrum of PCB of mobile phone powder. **B** EDX spectrum of the metal adsorbed on *A. fumigatus* A2DS mycelium. **C** SEM view (scale bar = 20 nm) of the *A. fumigatus* A2DS mycelium. **D** TEM (scale bar = 500 nm) view of *A. fumigatus* A2DS mycelium with metal absorbed (yellow color)

ions (due to the complex capacity of a molecule). For example, a complex of oxalic acid with aluminum, iron and magnesium, a complex of citric acid with magnesium and calcium and a complex of tartaric acid with iron, calcium, silica, magnesium, and aluminum. Equation (2) is an example of complexolysis reaction that produces nickel citrate (Asghari et al. 2012):



VWD: Signal A, 214 nm
Results

Name	Retention Time	Area	Area %
	4.13	538846	7.86
	4.73	3565808	52.03
	6.12	1016455	14.83
	6.65	131510	1.92
	7.36	139657	2.04
	8.77	297970	4.35
	10.88	145787	2.13
	14.80	824210	12.03
	16.23	193510	2.82

Fig. 6 FTIR spectrum. **A** The FTIR spectrum of spent media. **B** FTIR of the leachate obtained using spent media. **C** HPLC chromatogram of spent media (sucrose broth fungal mycelium) (1, citric acid; 2, succinic acid; 3, fumaric acid)

Table 2 Analysis of FTIR plot of spent media (sucrose broth + *A. fumigatus* A2DS strain) and leachate (spent media + media with PCB)

S.N	Plot (Fig. 6A) analysis of spent media(after growth of *A. fumigatus*)		Plot (Fig. 6B) analysis of leachate obtained using spent media	
	cm^{-1}	Stretching	cm^{-1}	Stretching
1	3265.52.1	-O–H stretching	3408.52	-O–H stretching
2	2925.13, 2854.05	C–H stretching	2932	C–H stretching
3	1634.06	-C = C stretching - C = O stretching	1633.7	-C = C stretching - C = O stretching
4	1401.3	-COO stretching	1410.8	–COO stretching
5	1199.38	-C–N stretching due to amines	1384.90	N = O stretching
6	112.37, 1100.56	Si–O stretching, clay minerals	1034.81	Si–O stretching, clay minerals

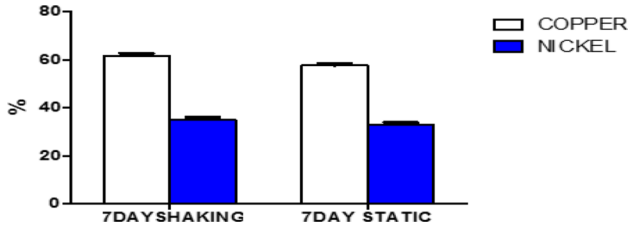


Fig. 7 Role of *A. fumigatus* A2DS spent media in leaching of the copper and nickel from e-waste (P value < 0.0001; all values were significant between control and other groups)

$$NiO + 2H+ \rightarrow Ni^{2+} + H_2O \quad (1)$$

$$Ni^2 + C_6H_8O_7 \rightarrow Ni\left(C_6H_5O_7\right) + 3H^+ \quad (2)$$

Furthermore, metal ions solubilized into solution by acidolysis are stabilized in complexolysis [7]. In redoxolysis, the oxidation-reduction processes help the fungal leaching. In the present study, maximum leaching was obtained at pH 6 (*A. fumigatus* A2DS showed maximum oxalic production (spectrophotometrically) at pH 6: data not included) indicating the role of oxalic acid in bioleaching. Maximum citric acid (5.76 gm %) production by *A. fumigatus* A2DS was at a pH of 7, at a temperature of 50 °C, and in the medium containing sucrose (10%) (data not shown). *A. fumigatus* A2DS was able to produce maximum oxalic acid at a pH of 8, temperature of 30 °C, and at static condition (0.071 mg/mL) (data not shown). The production of organic acid was further confirmed using HPLC and FTIR. The stretches corresponding to active functional acid groups were present in the spent medium, but the height of the peak decreased in the leachate, showing utilization of acids in bioleaching of PCB. Similar results were reported by Bullen et al. [6], Fischer et al. [12], Grube et al. [15], Smidt and Schwanninger [30], and Willner [36]. HPLC chromatogram showed the presence of different acids like citric, succinic, and fumaric acid in the spent medium. This finding is in agreement with those of Burgstaller and Schinner [7] and Bosshard et al. [4]. The role of citric, fumaric, succinic, and malic acid in bioleaching has been reported by Vanessa et al. [32]. To confirm the acidolysis of the metal by the fungal metabolites, the ICP-OES analysis of the leachate obtained after bioleaching using spent medium was carried out. Sixty-one percent of copper and thirty-five percent of nickel in the leachate after 7 days of incubation at shaking condition and 57% of copper and 32.8% of nickel at static condition were obtained confirming the role of fungal metabolites in the leaching process.

X-ray diffraction pattern of the bioleached and non-bioleached (control: without fungal inoculum) residues were varying. In addition, comparing the diffraction patterns, after bioleaching experiments the patterns have become much noisier and the intensities of peaks have been decreased. [17]. The structural pattern was changed due to the leaching of metal ions. Similar results were obtained by Faraji et al. [10] using *Aspergillus niger*.

Kuyucak and Volesky [23] and Kratochvil and Volesky [22] had stated that "chemi-sorption mechanisms include complexation, chelation, micro precipitation, and microbial reduction, while physical sorption mechanisms generally involve electrostatic forces and ion exchange." Biological processes like biosorption, bioreduction, bio-mineralization, and bio-precipitation are used for the recovery of metals [16]. Metal absorption and adsorption are the property of microbes like bacteria, eukaryotic cells of the phyla algae, fungi, and yeast [9]. It is typically carried out by inactive biomass. The positive charges of the amine group present on the cell wall attract ions and get adsorbed on the surface [26]. The metals absorbed/adsorbed by the cell were visualized by TEM and SEM respectively. (The property of absorption/adsorption has to be again confirmed by other methods). Fifty-seven percent of copper and 32.8% of nickel were leached by the organism, and rest of the metal was absorbed/adsorbed by the fungal mycelium.

Conclusion

Fifty-seven percent of copper and thirty-two percent of nickel were leached by the organism after one-step leaching at a temperature of 30 °C (static condition for 7 days). Maximum % of copper and nickel were obtained at a pH of 6 (58.7% Cu and 32% Ni), the temperature of 40 °C (61.8% Cu and 27.07% Ni), a pulp density of 0.5% (62% Cu and 42.37% Ni), and inoculums of 1% (58% Cu and 32.29% Ni). The XRD peak patterns of leachate residue and e-waste show much difference indicating the solubilization of copper from PCB. The ability of the fungus to absorb and adsorb metals was carried out by TEM and SEM-EDX respectively. The SEM-EDX analysis of the printed circuit board (control) showed maximum copper (13%), followed by aluminum (10.76%). The EDX analysis of the residue after fungal bioleaching showed copper (1.38%) and aluminum (2.47%) adsorbed on to mycelium. FTIR of the leachate (sucrose medium+*A. fumigatus* A2DS) also indicated the use of acid metabolites in the bioleaching of PCB. HPLC chromatogram showed the presence of different acids like citric, succinic, and fumaric acid in the spent medium. ICP_OES analysis of leachate obtained using spent medium showed that 61% of copper and 35% of nickel after 7 days of incubation at shaking condition and 57% of copper and 32.8% of nickel at the static condition also confirmed the role of fungal metabolites in the leaching process. The results thus showed that the strain *A. fumigatus* A2DS can leach copper and nickel from

the PCB of the mobile phone as well as absorb and adsorb metals.

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