Bioleaching of Cu and Pb from printed circuit boards by *Rhizopus oligosporus* and *Aspergillus niger*

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**Abstract**

The purposes of this study was to evaluate the ability of *Aspergillus niger* and *Rhizopus oligosporus* for one-step bioleaching of Cu and Pb in printed circuit boards (PCBs) scrap from e-waste recycling shops, compared to acidic extraction with citric and lactic acids. The fungal spore suspension was cultivated in potato dextrose broth with dried PCBs and a shaker for 42 days. Every 7 days the leachates were analyzed for Cu and Pb concentrations using atomic absorption spectroscopy. The Cu and Pb concentrations in PCBs in e-waste recycling shops were 152.81 ± 26.54 and 25.62 ± 8.33 g kg\(^{-1}\) PCBs, respectively. The leaching experiment showed that 0.05 M citric acid was the most efficient leaching pure acid: more than 54.59% of Cu and 79.55% of Pb was released into solution. Heavy metal leaching by the lactic acid was less efficient. The best metal bioleaching efficiency was achieved by *A. niger* fungus, which extracted approximately 46.92% of Cu, and almost 30.63% of Pb from PCBs. *R. oligosporus* leached only 8.53 and 19.61% of Cu and Pb, respectively.

**Key words:** *Aspergillus niger*, bioleaching, Cu, Pb, printed circuit boards, *Rhizopus oligosporus*.

**Abbreviations:** PCBs, printed circuit boards; e-waste, electronic waste; PDA, potato dextrose agar; PDB, potato dextrose broth.

**Introduction**

The fast pace of technological innovation and ever shortening product life expectancy are among the factors contributing to the growing amount of electronic waste (e-waste). E-waste is any refuse created by discarded electronic devices, such as mobile phones, computers, washing machines and refrigerators etc. Printed circuit boards (PCBs) are found in practically all e-waste, and are the basis of the electronics equipment. Common materials found in PCBs are hazardous metals (such as Pb, Hg and Cd etc.) and precious metals (such as Cu, Au, Ag, Sn etc.) that can be recycled (Khanna et al. 2014). Previous studies showed that higher copper (Cu) and lead (Pb) levels are present in PCBs (Olubanjo et al. 2015). Cu and Pb concentrations in PCBs of computer central processing units (CPU) have very high levels, reaching 83 100 to 70 5300 Cu mg kg\(^{-1}\) and 18 060 to 400 650 Pb mg kg\(^{-1}\).

Leaching of metals from PCBs using chemicals is rapid and highly efficient, but has an environmental impact on air, water and land. Recently, bioleaching has been developed as a low-cost and eco-friendly technology for the removal of heavy metals from PCBs (Mishra et al. 2005). There are three groups of microorganisms that have been used for heavy metal leaching: autotrophic bacteria, heterotrophic bacteria and heterotrophic fungi (Abdullah et al. 2017). Bioleaching by fungi occurs through the excretion of organic acids, which provide a source of protons (acidolysis), ligands (complexolysis), and electrons (redoxolysis; Ceci et al. 2015). Some saprophytic filamentous fungi such as *Rhizopus oligosporus* and *Aspergillus niger* are also used to produce high amounts of various useful organic acids for food industries. The organic acid producer *R. oligosporus* can convert several alternative carbon sources to lactic acid, acetic acid and citric acid (De Reu et al. 1995), while citric acid and gluconic acid are mostly produced by fungal fermentation using *A. niger* (Show et al. 2015). The main organic acid produced by *A. niger* for heavy metal leaching was shown to be citric acid, which was produced at concentrations of approximately 0.057 M after 14 days and reached a relatively stable concentration level of citric acid (~0.05 M; Aung, Ting 2005; Amiri et al. 2012). Organic acids investigated for use in e-waste treatment include acetic acid, oxalic acid, lactic acid, and citric acid. These acids have been found to be effective in removing heavy metals by forming stable chelate complexes with the heavy metals (Park et al. 2013).

The aim of this study was to evaluate the ability of filamentous fungi *R. oligosporus* and *A. niger* to bioleach metals (Cu and Pb) in the PCBs by application of one-step leaching under static cultivation conditions during a relative long period.

**Materials and methods**

*E-waste collection, preparation and metal concentration analysis in samples*

PCBs were obtained using the quartering splitting method (Gerlach et al. 2002) from six e-waste recycling shops in Nakhon Sawan province, Thailand. The samples were
Results and discussion

Characteristics of printed circuit boards

The PCBs from six e-waste recycling shops in Nakhon Sawan province had high concentrations of Cu and Pb with values of 152.81 ± 26.54 and 25.62 ± 8.33 g kg⁻¹ PCBs, respectively, which constituted 17.84% of the PCBs total weight (Table 1). Concentrations of Cu and Pb in PCBs observed in this study were higher than those in some other countries such as Brazil (Bizzo et al. 2014), Japan (Hino et al. 2009) and Europe (Marco et al. 2008). However, the results were lower than the mean of 191.90 Cu g kg⁻¹ PCBs and 10.10 Pb g kg⁻¹ PCBs reported by Yoo et al. (2009) and 196.60 Cu g kg⁻¹ PCBs and 39.30 Pb g kg⁻¹ PCBs observed by Wang et al. (2005).

Mycelial growth and changes in pH of culture medium during bioleaching

Exponential growth phases were characteristic for each of the fungal strains (Fig. 1). The mycelial growth curves showed a log phase that occurred in the period from 0 to 14 days for both fungi. After that, a sharp decrease in mycelial mass until 42 days was observed, possibly because the nutrient had been consumed, leading to the death phase (Melgar et al. 2013). Moreover, the fungi biomass growth pattern suggests adaptation of the fungi to the heavy metals in PCB leachate, by which growth is reduced as the metal leaching concentration increases.

The pH of an aqueous solution is one of the important factors that plays a key role in bioleaching processes. The optimum pH for organic acid production by most filamentous fungi is acidic (Poole 1999). We observed similar effect of pH of PDB media during incubation of R. oligosporus and A. niger in presence of PCBs (Table 2). The initial exponential fungal growth phase during 7 days of cultivation was associated with a rapid decrease of the pH of culture medium, most likely due to extensive production of acidic secondary metabolites that had considerable

Table 1. Concentration of Cu and Pb in PCBs from six e-waste recycling shops in Nakhon Sawan Province, Thailand. Means within a column with the same letter are not significantly different (p < 0.05), significantly at a higher b > c > d

<table>
<thead>
<tr>
<th>E-waste recycling shops</th>
<th>Heavy metal concentration in PCBs samples (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cu</td>
</tr>
<tr>
<td>1</td>
<td>158.54 ± 5.91 b</td>
</tr>
<tr>
<td>2</td>
<td>169.35 ± 2.73 c</td>
</tr>
<tr>
<td>3</td>
<td>121.38 ± 1.58 a</td>
</tr>
<tr>
<td>4</td>
<td>122.21 ± 1.19 a</td>
</tr>
<tr>
<td>5</td>
<td>152.71 ± 1.28 b</td>
</tr>
<tr>
<td>6</td>
<td>193.51 ± 3.90 d</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>152.81 ± 26.54</td>
</tr>
</tbody>
</table>

Statistical analysis

All of the experiments were conducted in triplicate. Mean values were used in the analysis of data using analysis of variance (one-way ANOVA) and the Post Hoc Duncan test (p < 0.05).
Table 1. Concentration of Cu and Pb in solution after leaching from 0.3 g PCBs by pure acids and organic acids from fungus between 7 to 42 days incubation. *Means within a column with the same letter are not significantly different (p < 0.05), significantly at a higher b > c > d > e. **The 0.3 g PCBs contained 51.44 ± 1.40 g L⁻¹ and 8.42 ± 0.34 g L⁻¹ for Cu and Pb, respectively.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Citric acid (0.05 mol L⁻¹)</th>
<th>A. niger</th>
<th>Lactic acid (0.05 mol L⁻¹)</th>
<th>R. oligosporus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (g L⁻¹)</td>
<td>Pb (g L⁻¹)</td>
<td>Cu (g L⁻¹)</td>
<td>Pb (g L⁻¹)</td>
<td>Cu (g L⁻¹)</td>
</tr>
<tr>
<td>7</td>
<td>1.4 ± 0.14 a</td>
<td>1.00 ± 0.18 a</td>
<td>0.82 ± 0.10 a</td>
<td>0.40 ± 0.03 a</td>
</tr>
<tr>
<td>14</td>
<td>8.13 ± 0.40 b</td>
<td>1.28 ± 0.09 a</td>
<td>7.21 ± 0.51 b</td>
<td>0.82 ± 0.06 b</td>
</tr>
<tr>
<td>21</td>
<td>19.15 ± 0.62 c</td>
<td>2.59 ± 0.05 b</td>
<td>9.83 ± 0.61 c</td>
<td>0.89 ± 0.10 b</td>
</tr>
<tr>
<td>28</td>
<td>23.26 ± 1.80 d</td>
<td>6.32 ± 0.15 c</td>
<td>13.79 ± 0.28 d</td>
<td>1.77 ± 0.10 c</td>
</tr>
<tr>
<td>35</td>
<td>27.92 ± 1.78 e</td>
<td>6.67 ± 0.23 d</td>
<td>24.13 ± 0.83 e</td>
<td>2.57 ± 0.30 d</td>
</tr>
<tr>
<td>42</td>
<td>28.08 ± 0.94 e</td>
<td>6.70 ± 0.18 e</td>
<td>23.63 ± 0.46 e</td>
<td>2.58 ± 0.03 d</td>
</tr>
</tbody>
</table>

The ability of fungus was limited in PCBs leaching when compared to chemical leaching because of the handling of a live system was difficult, as the organisms failed to remain viable for a longer time due to the toxicity of heavy metals (Dave et al. 2018; Losa, Bindschedler 2018). Thus, there is an urgent need to focus on development of a better strain, process optimization, scale-up of the current process for e-waste management and treatment.
acid production and purified or preconcentrated microbial extracts should not be considered as alternatives.

Conclusions

One kilogram of PCBs from e-waste recycling shops in Nakhon Sawan Province, Thailand contained 152.81 ± 26.54 g Cu, and 25.62 ± 8.33 g Pb. Following an incubation period of 42 days, the best metal bioleaching (leaching percentage of Cu and Pb were 46.92 and 30.63% from PCBs) was by organic acidic products of A. niger. R. oligosporus leached 8.53 and 19.61% for Cu and Pb, respectively.

Acknowledgements

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References


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