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

Tinnapan Netpae*, Sawitree Suckley

Faculty of Science and Technology, Nakhon Sawan Rajabhat University, Thailand

*Corresponding author, E-mail: tinnapan.n@nsru.ac.th

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 ground in the cutting mill to a particle size fraction lower than 2.5 mm. Ground PCBs samples were washed with deionized water and thereafter placed in an oven for 24 h at an 80 °C (Isildar 2016). The dried PCBs samples were kept in plastic zip lock bags prior to analysis.

The dried PCB samples were digested in concentrated acid. Acid extraction of heavy metals was accelerated at higher temperature as described by Ofudje et al. (2014). Briefly, the dried PCBs samples were digested using a mixture of 5 mL concentrated HNO_3, 10 mL concentrated HCl and glass beads in a flask containing 1 g of the sample. The mixed solution was evaporated on a hot plate until brown nitroxide fumes ceased to appear. The solution was then filtered through filter paper and diluted to 25 mL with deionized water and transferred to separate glass bottles prior to heavy metal analyses.

Bioleaching tests

The *A. niger* strains were isolated from Mae Tao creek sediments from the zinc ore area in Mae Sot District, Tak Province, Thailand (Netpae et al. 2015). The *R. oligosporus* strain was kindly donated by Biology and Biotechnology Program, Science and Technology Faculty, Rajabhat Nakhon Sawan University, Thailand. The fungi were maintained by subculturing onto Potato Dextrose Agar (PDA) medium. Fresh cultures of fungi were obtained from a 7-day-old culture grown on PDA slants and incubated at 28 ± 2 °C. The fungal spores were harvested from slant bottles by washing with sterile distilled water containing 0.8% Tween 80 and counted using a hemocytometer and diluted to give a spore suspension of 10^7 spores mL^{-1}.

The fungal spore suspension was cultivated in a 250 mL Erlenmeyer flask with 80 mL Potato Dextrose Broth (PDB) with dried PCBs 0.3 g at 28 ± 2 °C in a shaker at 150 rpm. Every 7 days during a leaching over a period of 42 days, the fungal mycelium of cultures was filtered by filter paper and the leachate solution were analyzed for pH and concentration of extracted metals (Cu and Pb) using a pH meter and AAS, respectively. The mycelium was dried at 70 °C in hot air oven until constant weight and this weight was recorded. Leaching efficiency of the biogenically produced acids was compared with pure commercial 0.05 M citric and 0.05 M lactic acids (analytical reagent grade), incubated under the same conditions as the bioleaching experiment.

Atomic absorption analysis

Cu and Pb was measured using an atomic absorption spectrophotometer (Perkin Elmer model PinAAcle 900T) with a flameless graphite system.

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 ($p < 0.05$).

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

E-waste recycling shops	Heavy metal concentration in PCBs samples (g kg^{-1})	
	Cu	Pb
1	158.54 ± 5.91 b	28.34 ± 1.67 c
2	169.35 ± 2.73 c	34.22 ± 3.95 d
3	121.38 ± 1.58 a	18.08 ± 1.02 b
4	122.21 ± 1.19 a	12.62 ± 0.46 a
5	152.71 ± 1.28 b	27.06 ± 2.65 c
6	193.51 ± 3.90 d	33.39 ± 1.13 d
Mean ± SD	152.81 ± 26.54	25.62 ± 8.33

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

Time	Citric acid (0.05 mol L^{-1})		*A. niger*		Lactic acid (0.05 mol L^{-1})		*R. oligosporus*	
(days)	Cu (g L-1)	Pb (g L^{-1})	Cu (g L^{-1})	Pb (g L^{-1})	Cu (g L^{-1})	Pb (g L^{-1})	Cu (g L^{-1})	Pb (g L^{-1})
7	1.43 ± 0.14 a	1.00 ± 0.18 a	0.82 ± 0.10 a	0.40 ± 0.03 a	0.14 ± 0.02 a	0.49 ± 0.04 a	0.46 ± 0.03 a	0.84 ± 0.07 a
14	8.13 ± 0.40 b	1.28 ± 0.09 a	7.21 ± 0.51 b	0.82 ± 0.06 b	1.95 ± 0.18 b	0.73 ± 0.13 b	1.33 ± 0.06 b	1.27 ± 0.06 b
21	19.15 ± 0.62 c	2.59 ± 0.05 b	9.83 ± 0.61 c	0.89 ± 0.10 b	6.36 ± 0.33 c	0.73 ± 0.02 b	2.23 ± 0.36 c	1.66 ± 0.18 c
28	23.26 ± 1.80 d	6.32 ± 0.15 c	13.79 ± 0.28 d	1.77 ± 0.10 c	10.81 ± 0.79 d	0.73 ± 0.07 b	3.25 ± 0.42 d	1.59 ± 0.07 c
35	27.92 ± 1.78 e	6.67 ± 0.23 d	24.13 ± 0.83 e	2.57 ± 0.30 d	10.24 ± 0.16 d	0.78 ± 0.02 b	4.28 ± 0.35 e	1.57 ± 0.23 c
42	28.08 ± 0.94 e	6.70 ± 0.18 d	23.63 ± 0.46 e	2.58 ± 0.03 d	10.13 ± 0.37 d	0.79 ± 0.05 b	4.39 ± 0.63 e	1.65 ± 0.07 c

influence on metal releasing from PCBs (Arwidsson, Allard 2010). After 21 days of cultivation, the pH of culture medium steadily increased and reached a maximum on day 42 of cultivation. pH is a well-known growth regulator of many fungi and affects fungal growth (Amiri et al. 2011).

According to Sparringa et al. (2002), mycelial growth of *R. oligosporus* is optimal at pH 5.5 to 5.8 and is lower at higher pH of media. Kolencik et al. (2013) reported 12 days of incubation as the end of the active growth phase, after which a significant decrease in citric acid concentration in culture broth was detected. This could be due to an increase of resorption of produced organic acids by *A. niger*, which is probably related to the observed significant increase of the pH of liquid medium at day 14 of incubation.

Cu and Pb bioleaching from PCBs by fungus

Prior to the one-step bioleaching experiment, the 0.3 g PCB samples were digested with acid. The average Cu and Pb concentrations were 51.44 ± 1.40 and 8.42 ± 0.34 g L^{-1}, respectively. The results showed that 0.05 M citric acid was the most efficient leaching pure acid, as more than 28.08 ± 0.94 g L^{-1} (54.59%) of Cu and 6.70 ± 0.18 g L^{-1} (79.55%) of Pb was released into solution. The heavy metal leaching by lactic acid was lower (percentage leaching of Cu and Pb from PCBs was 19.69 and 9.38%, respectively). The best metal bioleaching efficiency was reached by *A. niger*, which extracted approximately 24.13 ± 0.83 g L^{-1} (46.92%) of Cu, and almost 2.58 ± 0.03 g L^{-1} (30.63%) of Pb from PCBs. *R. oligosporus* leached 8.53 and 19.61% of Cu and Pb, respectively, in the one-step bioleaching (PCBs within culture medium was directly inoculated with microorganism) with pro-longed static cultivation in the light under laboratory conditions (Table 2 and Fig. 2).

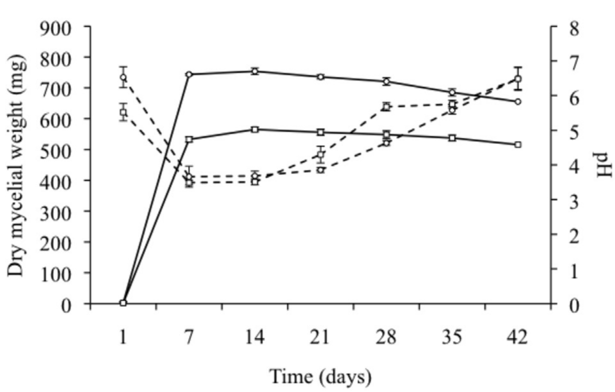

Fig. 1. Growth of mycelium and changes of medium pH during PCBs leaching by *Rhizopus oligosporus* (circles) and *Aspergillus niger* (squares) between 0 to 42 days incubation.

The fungus Aspergillus sp. is among the most commonly used microorganisms in bioleaching of metals from PCBs (Brandl et al. 2001; Madrigal-Arias et al. 2015; Argumedo-Delira et al. 2019). Copper and lead precipitation, which might affect the leaching efficiency, is a complex process including a broad range of interactions of microbial surface and metabolites with dissolved metals in culture medium, including changes in pH during fungal incubation (Kolencik et al. 2013). Citric acid produced by *A. niger* is the main leaching agent of heavy metals, which is produced at concentrations of approximately 57 mM after 14 days (Aung, Ting 2005). Lactic acid, acetic acid and citric acid are the major organic acids produced by *R. oligosporus* (De Reu et al. 1995). These acids serve as lixiviant and aid in solubilization of various base metals from PCBs (Dave et al. 2018). The acids induce the leaching of metals from waste PCBs by acidolysis, complexolysis and redoxolysis mechanisms (Ceci et. al. 2015).

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.

In view of some other similar experiments, the one-step bioleaching should be considered as insufficient (Brandl et al. 2001; Kolencik et al. 2013). However, Santhiya and Ting (2005) suggested that the effect of more than one-step bioleaching may be over-exaggerated, and that the leaching process is more significantly affected by the pulp density and particle size. Considering that bioleaching is uncomplicated, cost-effective and environmentally friendly, the application of bioengineered microorganisms with enhanced organic

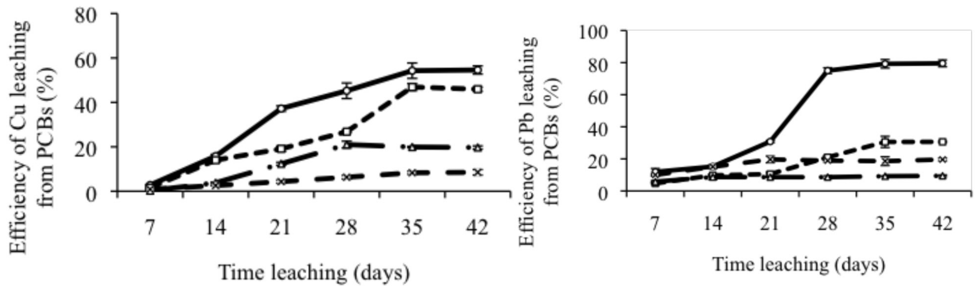

Fig. 2. Time dependences of the efficiency of Cu (A) and Pb (B) leaching from PCBs with 0.05 mol L^{-1} citric acid (circles), 0.05 mol L^{-1} lactic acid (triangles), *Aspergillus niger* (squares) and *Rhizopus oligosporus* (diamonds) at leaching period from 1 to 42 days.

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.

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