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Comparison of three culture media for one-step and two-step bioleaching of nickel and cadmium from spent Ni-Cd batteries by *Aspergillus niger*

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ABSTRACT

Among the biological processes, bioleaching is the most widely employed method for metal extraction from electronic waste (e-waste). The purpose of this research was to evaluate the ability of one-step and two-step bioleaching under different culture media for nickel (Ni) and cadmium (Cd) removed from spent Ni-Cd batteries by the fungus *Aspergillus niger*. In this result, the concentrations of Ni and Cd in battery scraps from e-waste recycling shops were respectively 578.13 ± 7.02 mg Ni g^{-1} and 128.35 ± 11.42 mg Cd g^{-1}. During the bioleaching process, both heavy metal removals were higher in Richards's Broth (RB) followed by the Potato Dextrose Broth (PDB) and Malt Extract Broth (MEB), respectively. The maximum citric acid production (96.99±3.05 mM) and lower pH values were obtained in the RB medium. The bioleaching experiment showed that the recovery for the two-step leaching method was higher than the one-step bioleaching method. Following an incubation period of 21 days between the spent Ni-Cd batteries and RB medium, the two-step bioleaching experiment suggested that the citric acid products of *A. niger* were the best leaching agent for Ni and Cd bioleaching.

1. 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). The environmental risk increases due to heavy metal contamination caused by uncontrolled e-waste recycling activities, drawing increasing attention worldwide. Almost half the e-wastes of Thailand are dumped as landfills [1], while e-wastes ending up as landfills are described as a toxic time bomb. They may be released into the environment after several years by natural means, and there is a possibility of leaching heavy metals. These may reach the soil, groundwater, freshwater sources, and living organism [2,3,4]. It has had a negative impact on human health and caused serious environmental pollution [5]. The nickel-cadmium (Ni-Cd) battery is a type of e-waste often found in mobile phones, laptops, tablets, and other electronic devices. Cadmium is a known human carcinogen, and its exposure causes damage to the kidneys and skeleton [6]. Nickel is a micronutrient essential for human beings; however, it is also one of the most common allergenic metals, and approximately 10 to 15% of the world population suffers from nickel allergy. A higher concentration of Ni is carcinogenic, and when inhaled in large amounts, it can cause damage to the brain, liver, muscles, and kidneys [7].

The leaching of metal from e-waste using chemicals is rapid and highly efficient, but it has an environmental impact on the air, water, and land [8]. On the other hand, bioleaching is a simple and effective technology that utilizes microorganisms to recover metals from e-waste. Filamentous fungi have the potential to be used in the bioleaching process. It was reported that the *Rhizopus* sp. consortium is capable of leaching Cu (85%) [9] from chalcopyrite and Fe (82.42%) from Silica-Sand in a culture with agitation [10]. Media containing carbohydrate and nitrogen sources are required for the organic acid production of fungi at a pH range of 5 to 6 and a room temperature range from 15 to 37˚C [11]. *Aspergillus* sp. is the most common fungal used for the heavy metal leaching

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from e-waste [10,12]. The filamentous fungus *Aspergillus niger* is an established microorganism used to produce mostly two organic acids (citric acid & oxalic acid), and the pH is one of the most significant factors influencing this process [13]. The earlier studies indicated that the bioleaching process could be achieved in two ways: (i) one-step bioleaching, that is incubating microorganism with e-waste materials at the beginning of the bioleaching, and (ii) two-step bioleaching, that is preculturing microorganism for a few days before adding the e-waste materials into the culture media [14,15].The purposes of this research were to examine the citric acid and oxalic acid production of *A. niger* in the bioleaching of Ni and Cd from spent Ni-Cd batteries by application of one and two-step leaching under different culture media.

2. Materials and methods

2.1. Ni-Cd batteries collection, preparation, and metal concentration analysis sample

The Ni-Cd batteries were obtained using the quartering splitting method [9] from six e-waste recycling shops in the Nakhon Sawan province, Thailand. The samples were ground in a cutting mill to a particle size fraction lower than 2.5 mm. The ground battery samples were washed with deionized water and later placed in an oven for 24 hours at 80ºC [15]. The dried battery samples were kept in plastic zip-lock bags prior to analysis. The acid extraction of heavy metal from the battery samples was accelerated at a higher temperature, as described by Ofudje *et al.* [16]. The samples were digested by using a mixture of 5 ml of conc. HNO_3, 10 ml of conc HCl, and a glass bead in a flask that contained 1 g of the sample. The solution was evaporated on a hot plate until the brown fumes disappeared. The digested solution was then filtered through Whatman number 42 filter paper, diluted to 25 ml with deionized water, and transferred to separate glass bottles prior to analyses. Ni and Cd concentrations in the samples were determined by atomic absorption spectrometry (AAS) via PerkinElmer's PinAAcle 900T model by using the flameless method of a graphite system.

2.2. Bioleaching tests

The fungus *A. Niger* strain ATCC 1015 was kindly donated by the Biology and Biotechnology program, Faculty of Science and Technology, Rajabhat Nakhon Sawan University, Thailand. The fungi were maintained by subculturing onto a Potato Dextrose Agar (PDA) medium. Fresh cultures of fungi were obtained from a 5-day old culture grown on PDA slants and then incubated at 28±2°C. The fungal spores were then harvested from the slant bottles by washing with sterile distilled water containing 0.8% Tween 80 and enumerated using a haemocytometer to give a spore suspension of 10^7 spores ml^{-1}. Two methods of bioleaching for the Ni and Cd recovery were carried out. In one-step bioleaching, the fungal spore suspension was cultivated in a 250 ml Erlenmeyer flask with 80 ml of broth media with 0.3 g dried Cd-Ni battery samples at 28±2°C; it was agitated at 150 rpm in an incubator shaker for 42 days. The two-step bioleaching first cultured the fungus in broth media without the Cd-Ni battery samples for seven days (beginning of organic acid production); then, the sterilized, dried battery samples were added. The broth media used in the bioleaching experiments were Potato Dextrose Broth (PDB), Malt Extract Broth (MEB), and Richards's Broth (RB); the broth media without fungal that was incubated under the same conditions as the bioleaching experiment served as the control. The fungal mycelium of these cultures was filtered using Whatman No.1 filter paper every seven days, and the pH value, acid quantities, and concentration of extracted Ni and Cd in the broth media or leachate solution were determined. The residual of both the heavy metal ions and pH were analyzed using AAS and a pH meter, respectively. The quantity of citric acid and oxalic acid were examined in high-performance liquid chromatography (Shimadzu Corporation, LC-20AD), equipped with a UV/VIS diode array detector-DAD at 210 nm [7]. The column was an Aminex HPX-87H with a size of 300x7.8 mm. Sulfuric acid 5 mM at 0.6 cm^3/min was used as the eluent.

2.3. Statistical analysis

All the experiments were triplicated. The mean values were used in the analysis of data by using the analysis of variance (one-way ANOVA) and Post Hoc. Duncan test ($p<0.05$).

3. Results and discussion

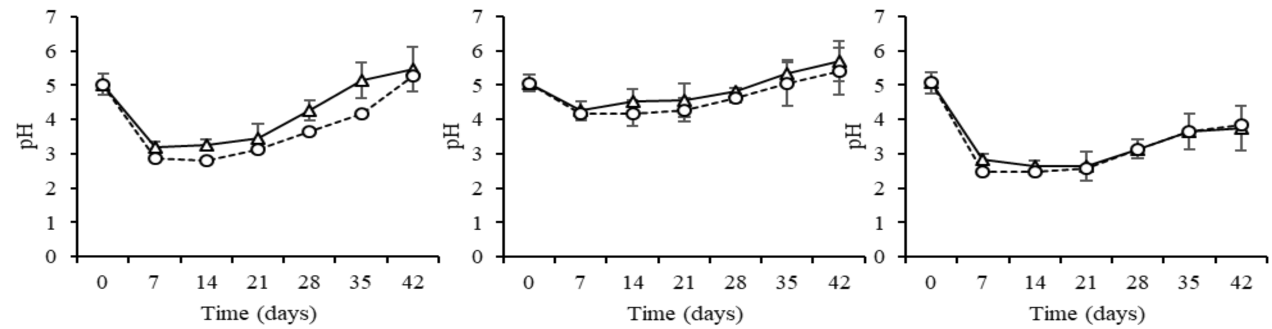
3.1. Ni and Cd concentrations in Ni-Cd batteries from e-waste recycling shops

The results showed that the Ni-Cd batteries from six e-waste recycling shops in Nakhon Sawan province had high concentrations of Ni and Cd. The Ni and Cd concentrations in the batteries were 578.13 ± 7.02 mg Ni g^{-1} and 128.35 ± 11.42 mg Cd g^{-1}, respectively. However, the high concentration of both heavy metals in the Ni-Cd batteries in this study was lower than the mean of 697.93 mg Ni g^{-1} and 230.52 mg Cd g^{-1} observed in rechargeable batteries from the Korea battery recycling association, South Korea [12].

3.2. pH and organic acid variations in difference leaching experiments

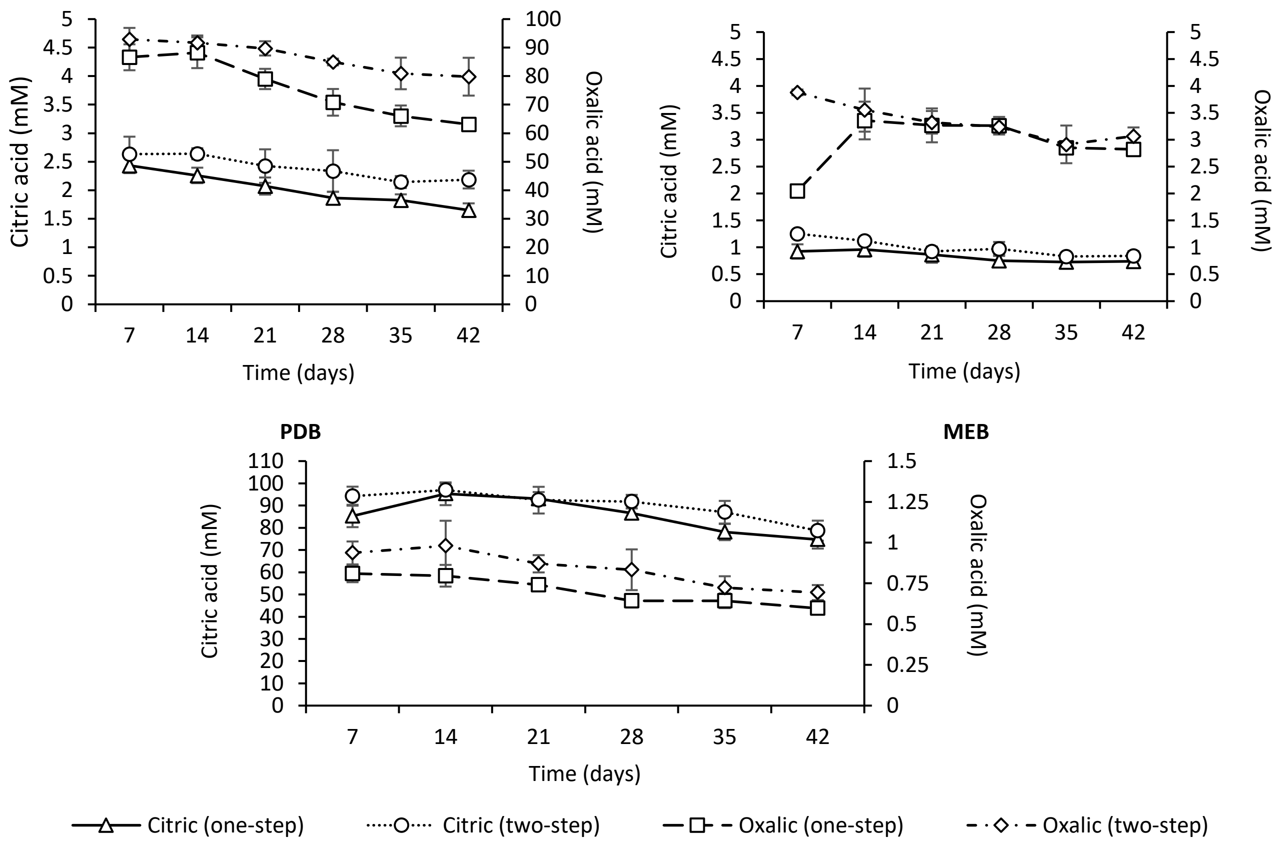
The changes of pH, citric acid, and oxalic acid in the broth media after a long incubation period of *A. Niger* in broth media with spent battery samples are presented in Figures 1 and 2. After 21 days of experiments with both bioleaching methods, the pH of the culture medium steadily increased and reached its maximum on the 42^{nd} day of cultivation. However, the pH value increased more dramatically in the one-step process than in the two-step process at the beginning. The lower pH values in the bioleaching process could be explained by the higher concentration of organic acids produced for extract Ni and Cd from the spent batteries.

The highest concentration of both organic acids was observed in the two-step leaching experiment within 14 days of cultivation ($p<0.05$). The maximum production of citric acid (96.99±3.05 mM) and oxalic acid (91.75±2.50 mM) were obtained with the RB medium and PDB medium, respectively. The optimum for organic acid production by most filamentous fungi are produced more efficiently under acidic conditions [17]. This initial increase in pH occurs simultaneously with sucrose hydrolysis, implying that the fungus is adapting to the medium and is at the beginning of the growth phase [14]. The pH of an aqueous solution is one of the important factors which plays a key role in the bioleaching processes. It is a well-known growth regulator in many fungi and its effect on fungal growth is recognized [18].



PDB MEB RB

Fig. 1. Changes in the pH of nutrient broths with battery scraps during one-step (—△—)and two-step (…o…) bioleaching by the *A. niger* between 1 to 42 days incubation.



RB

Fig. 2. Changes in the organic acids of nutrient broths with battery scraps during one-step and two-step bleaching by the *A. niger* between 1 to 42 days incubation.

3.3. Comparison of different experiment of Ni and Cd leaching

The Ni and Cd concentrations in the one-step and two-step bioleaching experiments after the incubation of *A. Niger* and spent Ni-Cd batteries with different media broth are presented in Tables 1 and 2. The results showed that the two-step bioleaching experiment was more efficient for Ni and Cd leaching than one-step bioleaching. The extracted amounts of both heavy metals by *A. Niger* were higher in the RB medium than in the PDB and MEB media. Since citric acid was found in large quantities in the RB medium, it could be considered as the best leaching agent in this study. Therefore, the *A. Niger*, which generates citric acid, could then be used as an alternative to heavy metal leaching agents.

Table 1. Ni and Cd concentrations in nutrient broths during one-step bioleaching by the *A. Niger* between 1 to 42 days incubation.

Time (days)	Cd						Time (days)	Ni					
	PDB		MEB		RB			PDB		MEB		RB	
	Mg/g	%	Mg/g	%	Mg/g	%		Mg/g	%	Mg/g	%	Mg/g	%
7	59.04^{d}	46.00	41.74^{f}	32.52	100.52^{b}	78.32	7	381.57^{c}	66.00	159.16^{g}	27.53	435.45^{c}	75.32
14	69.12^{c}	53.85	47.28^{e}	36.84	105.12^{ab}	81.90	14	392.38^{d}	67.87	164.25^{ef}	28.41	452.16^{a}	78.21
21	69.42^{c}	54.09	46.15^{e}	35.96	105.29^{a}	82.03	21	389.25^{d}	67.33	169.85^{e}	29.38	448.34^{a}	77.55
28	68.14^{bc}	53.09	46.76^{e}	36.43	105.48^{a}	82.18	28	385.84^{dc}	66.74	167.14^{ef}	28.91	446.26^{ab}	77.19
35	69.64^{c}	54.26	46.51^{ef}	36.24	106.80^{a}	83.21	35	387.52^{d}	67.03	168.24^{e}	29.10	451.06^{a}	78.02
42	69.45^{c}	54.11	46.18^{ef}	35.98	106.93^{a}	83.31	42	389.08^{d}	67.30	166.73^{ef}	28.84	444.64^{ab}	76.91

* Means within a column with the same letter are not significantly different ($p < 0.05$), significantly at a higher b>c >d>e>f>g.

** The mean Ni and Cd concentrations in 1 g with battery scraps were 578.13 ± 7.02 mg and 128.35 ± 11.42 mg, respectively.

Table 2. Ni and Cd concentrations in nutrient broths during two-step bioleaching by the *A. Niger* between 1 to 42 days incubation.

Time (days)	Cd						Time (days)	Ni					
	PDB		MEB		RB			PDB		MEB		RB	
	Mg/g	%	mg/g	%	Mg/g	%		Mg/g	%	Mg/g	%	Mg/g	%
14	70.59^{c}	55	52.78^{e}	41.12	114.49^{b}	89.20	14	375.78^{c}	65	204.25^{g}	35.33	467.30^{b}	80.83
21	70.41^{c}	54.86	54.60^{de}	42.54	118.48^{a}	92.31	21	380.18^{cd}	65.76	197.89^{g}	34.23	470.66^{ab}	81.41
28	70.57^{c}	54.98	56.23^{d}	43.81	119.20^{a}	92.87	28	386.60^{e}	66.87	203.04^{fg}	35.12	473.60^{ab}	81.92
35	69.48^{c}	54.13	55.11^{de}	42.94	120.06^{a}	93.54	35	382.90^{d}	66.23	201.71^{f}	34.89	473.26^{ab}	81.86
42	70.96^{c}	55.29	54.88^{de}	42.76	119.44^{a}	93.06	42	384.23^{de}	66.46	202.98^{gf}	35.11	477.42^{a}	82.58

* Means within a column with the same letter are not significantly different ($p < 0.05$), significantly at a higher b>c >d>e>f>g.

** The mean Ni and Cd concentrations in 1 g with battery scraps were 578.13 ± 7.02 mg and 128.35 ± 11.42 mg, respectively.

*** For two-step bioleaching, the cultures were pre-cultivated for 7 days before the addition of sterilized battery scraps.

The maximum two-step bioleaching efficiency of fungus *A. Niger* during incubation in the RB medium induced the extraction of both heavy metals within 21 days: 81.41% for Ni and 92.31% for Cd. The recovery for the two-step leaching method was found to be higher than the one-step leaching method. These results agree well with what has been observed by previous reports [19,20]. Past research has postulated that the good growth of strains in the two-step bioleaching process is due to the higher tolerance of the mycelium to the leaching materials compared to the spores [21]. This result implied that the metabolic pathway of *A. niger* could be changed depending on the nutrients available. Biosynthesis of oxalic acid from glucose occurs by hydrolysis of oxaloacetate to oxalate and acetate catalysed by cytosolic oxaloacetate, whereas citric acid is produced as an intermediate in the tricarboxylic acid cycle involving a polysaccharide such as sucrose [22]. During the growth of *A. Niger*, the equations for the generation of energy and acceleration of reaction to extract heavy metals from e-waste are as follow [23]:

$$C_6H_{12}O_6 + 4.5O_2 \rightarrow 3C_2H_2O_4 \text{ (Oxalic acid)} + 3H_2O$$

$$C_6H_{12}O_6 + 1.5O_2 \rightarrow C_6H_8O_7 \text{ (Citric acid)} + 2H_2O$$

Aspergillus sp. is among the most commonly used microorganisms in the bioleaching of metals from spent batteries [12]. The efficiencies of Ni and Cd leaching by *A. niger* observed in this present work were higher than other research, such as Kim *et al.* [12] and Kolencik *et al.* [17]. This may be attributed to various effects, including selective and effective accumulation/sorption of leached metals by the fungus during incubation [19]. Heavy metal 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 [15]. Our findings represent a novel type of metal solubilization technique, which may be implemented to develop a one-stage recycle reactor system. Such systems may provide a more efficient and cleaner industrial application for the recovery of heavy metals from e-waste.

4. Conclusions

Ni-Cd batteries from e-waste recycling shops contained 578.13 ± 7.02 mg of Ni g^{-1} and 128.35 ± 11.42 mg of Cd g^{-1}. Two-step bioleaching showed the maximum efficiency of fungus during incubation in the RB medium within 21 days; the Ni and Cd concentrations in the nutrient broths during two-step bioleaching were 81.41% and 92.31%, respectively.

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