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Optimization of bioleaching conditions for metal removal from CCA-treated wood by using an unknown Polyporales sp. KUC8959

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A B S T R A C T

The purpose of this study was to investigate the effect of extraction conditions (i.e., culture filtrate concentration, extraction temperature, and extraction time) on the removal of metals from chromated copper arsenate (CCA)-treated wood particles by using an unknown Polyporales sp. KUC8959. As the first research, a 20-run central composite design using response surface methodology was applied to optimize the system and construct the models, which predicted metal removal by bioleaching. The coefficients of determination of fitted models were 0.874–0.989, which indicated that the models can predict the metal removal yield accurately under various conditions. The Cu removal model suggested that the following conditions, culture filtrate concentration of 45.8%, extraction temperature of 34.2 °C, and extraction time of 20.6 h, were required for maximal removal of Cu (82.1%). The model predicted that extraction conditions of increased severity would result in complete removal of Cr and As from CCA-treated wood particles. In order to confirm actual metals removal efficiency, metals extraction was subsequently conducted under optimal bioleaching condition evaluated in this study. By applying the model, we demonstrated 83.9% Cu, 96.0% Cr, and 99.3% As removal from treated wood particles.

1. Introduction

Chromated copper arsenate (CCA)-treated wood has been implicated in environmental contamination and human toxicity because it contains toxic metals. Chromium, copper, and arsenic can leak out of CCA-treated wood used in public structures, thereby leading to environmental accumulation and biological impact (Brown et al., 2003; Kim et al., 2007; Lebow and Foster, 2005; Solo-Gabriele et al., 2003; Townsend et al., 2005). In particular, arsenic and chromium (VI) are highly toxic to humans; however, a small amount of chromium (III) is essential as a nutrient (Bissen and Frimmel, 2003; Dayan and Paine, 2001). Although many countries have restricted or banned the use of CCA and CCA-treated wood products, incineration and landfill disposal of such products remain a concern to environmental and human health. Landfill disposal can release toxic CCA components to the environment (Dubey et al., 2009; Moghaddam and Mulligan, 2008; Saxe et al., 2007), and incineration of the treated wood can emit arsine-containing gases or produce metal-containing fly ash (Helsen and Van den Bulck, 2004).

To alleviate environmental concerns, recent studies have investigated alternative disposal methods, including chemical extraction, bioleaching, and liquefaction (Kartal et al., 2004a; Kim et al., 2004, 2009; Sierra-Alvarez, 2007). The method of bioleaching with a fungus-produced organic acid is one of the most promising disposal alternatives. The fiber sources and metals separate during the bioleaching process and can be recovered and recycled for additional use (Janin et al., 2009a,b). Furthermore, bioleaching does not cause secondary environmental contamination by anthropogenic chemicals, unlike chemical extraction methods (Clausen et al., 2006; Kazi and Cooper, 2006). Several basidiomycetes such as *Fomitopsis palustris*, *Coniophora puteana*, and *Laetiporus sulphureus* and ascomycetes such as *Aspergillus niger* were applied for bioleaching of CCA-treated wood wastes (Kartal et al., 2004a,b). However, low removal efficiencies for Cr and Cu by these isolates became a main drawback of the bioleaching process, while a high reduction in As level was demonstrated by *F. palustris* (100%) and *A. niger* (97%).

Recently, we established a brown-rot fungus, an unknown Polyporales sp. KUC8959 (GenBank accession number, EU024961; Old ID, LAS6497), as a prominent fungal species for the bioleaching of CCA-treated wood wastes (Kim et al., 2009). This fungus, which was newly identified as an unclassified species in an order of

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Polyporales, was shown to produce more oxalic acid than the other fungi tested including *Antrodia albida*, *Daedalea dickinsii*, and *F. palustris*. When the fungus was used in a bioleaching process, it removed more than 90% of the copper, chromium, and arsenic from CCA-treated wood sawdust. However, affecting factors of the bioleaching process remained to be optimized for practical application, since the previous work aimed at screening of prominent fungus for bioleaching process.

Removal efficiencies of metals by the fungus might be affected by several factors. Previous studies demonstrated that oxalic acid concentration, extraction temperature, and extraction time play a key role in metal extraction from CCA-treated wood by various chelating agents (Kartal et al., 2004a; Kim et al., 2004, 2009; Sierra-Alvarez, 2009). The objectives of this study were to investigate the effect of extraction conditions (i.e., culture filtrate concentration, extraction temperature, and extraction time) on the removal of metals and to determine the optimized conditions for removing metals from treated wood by using an unknown Polyporales sp. KUC8959. Several recent studies have used central composite design (CCD) with response surface methodology (RSM) to develop an optimization model and/or a practical application for bioremediation of various environmental pollutants or to trigger enzyme and organic acid production by microorganisms (Aghaie et al., 2009; Amini et al., 2009; Roriz et al., 2009; Sharma et al., 2009). CCD with RSM is considered a robust mathematical and statistical technique for use in models that alleviate problem when multiple parameters may influence responses. Consequently, CCD with RSM was used to model the optimized model in the present study.

2. Materials and methods

2.1. Fungal isolate and oxalic acid production

A brown-rot fungus, an unknown Polyporales sp. KUC8959, was obtained from the Research Institute for Sustainable Humanosphere of Kyoto University, Japan, and used in this study. The fungus was previously identified as a notable fungus for the bioleaching of CCA-treated wood wastes (Kim et al., 2009). The fungus was transferred from feedstock to 2% malt extract (Difco) agar (Showa) (MEA) media and allowed to grow for 1 week at 27 °C. And then 10 fungal disks (5 mm in diameter), which were removed from the pre-cultured media, were inoculated in 100 mL of 2% malt extract liquid media and cultured for 7 days at 150 rpm on a rotary shaker at 27 °C. After incubation, fungal hyphae were removed from the liquid fermentation broth by filtering and then washed thoroughly with sterile deionized water. Oxalic acid was produced by the fungus in liquid media according to Choi et al. (2010). Briefly, 4.74 mg of the fungal hyphae was inoculated into 100 mL of 2.4% malt extract liquid media and was agitated for 229 h at 150 rpm on a rotary shaker at 27 °C. As a reference, initial pH values of the liquid media were 4.97–5.00. After cultivation, the fungal hyphae were filtered out of the liquid media, and the culture filtrate was used as the extracting solution for metal bioleaching from CCA-treated wood particles. The final concentration of oxalic acid in the culture filtrate, 4.0 g/L, was determined by high performance liquid chromatography (Varian 940-LC, Varian, USA) according to Kim et al. (2009).

2.2. Bioleaching process and metal determination

Radiata pine (*Pinus radiata* D. Don) sapwood lumber (4 × 10 cm in cross-section and 30 cm in length) were treated with 2% CCA-C (CrO_3, 44.5–50.5%; CuO, 17.0–21.0%; As_2O_5, 30.0–38.0%) using a full-cell process to 4 kg/m^3 retention level. After treatment, the treated wood was wrapped in plastic bags for 48 h at 60 °C to allow complete fixation of CCA elements. And then the treated wood was air-dried at room temperature for 2 weeks, and hammer-milled to 6–16 mesh. Ten grams of CCA-treated wood particles were placed in a flask that contained 100 mL of the diluted culture filtrate of unknown Polyporales sp. KUC8959 (5–95%). To prepare each concentration of a culture filtrate calculated by CCD (Table 1), a culture filtrate of the fungus was diluted with deionized water on a volume basis. The bioleaching process was carried out by agitating the flasks at 150 rpm on a rotary shaker at various extraction temperatures (20–60 °C) and lengths of time (5–45 h).

Following each bioleaching process, the wood particles were washed thoroughly with sterile deionized water and oven-dried for 48 h at 60 °C. The particles were then ground to 40 mesh and digested to facilitate the analysis of copper, chromium, and arsenic levels by using the peroxide-nitric acid method (AWPA, 2005a). The amounts of each metal were determined by using inductively coupled plasma-optical emission spectrometer (Vista Pro, Varian Inc., USA) according to AWPA A21-00 (2005b). The percentage of metals removal from each sample was calculated based on the initial amount of metals in the un-remediated particles.

2.3. Central composite design and response surface methodology

A 20-run CCD using RSM was used to determine the optimal conditions for the removal of metal from CCA-treated wood particles with a culture filtrate of unknown Polyporales sp. KUC8959. Table 1 shows the range and levels of coded and assigned variables. Three variables, culture filtrate concentration, extraction temperature, and extraction time, were chosen and the range of variables was determined by preliminary experiments. According to a second-order CCD, a total of 20 experimental runs including 8 cube points (Runs 1–8), 6 star points (Runs 9–14), and 6 central points (Runs 15–20) were designed (Table 2): the number of experimental runs required are 2^n ($2^3 = 8$), $2n$ ($2 \times 3 = 6$), and 6 for cube point, star point, and central point, respectively (n, the number of factors). And then cube points were regularly coded as -1 or 1. Central points were coded as 0. The low and high factors of star point were coded as -1.68 or 1.68.

The experimental response was described by the following empirical second-order polynomial:

$$Y = \beta_0 \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

where Y is the predicted response, β_0 is the constant coefficient, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the interaction of the coefficient, and x_i and x_j are the coded factors (Aghaie et al., 2009).

SAS version 9.1.3 (SAS Institute Inc., USA) was used for statistical analysis and to generate the three-dimensional (3D) response surface plots and two-dimensional (2D) contour plots.

Table 1
Coded and assigned variables of different levels of the central composite design to determine optimal conditions for metal removal from treated wood particles using a culture filtrate of an unknown Polyporales sp. KUC8959.

Factors	Levels				
	−1.68	−1	0	1	1.68
Culture filtrate concentration, %	5	23.3	50	76.8	95
Extraction temperature, °C	20	28.1	40	51.9	60
Extraction time, h	5	13.1	25	36.9	45