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Bio-modification of carbonaceous matter in gold ores: Model experiments using powdered activated carbon and cell-free spent medium of *Phanerochaete chrysosporium*

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ABSTRACT

Carbonaceous matter in refractory gold ore is known to be one of the primary causes of gold recovery loss. Model experiments were conducted to simulate the bio-modification of carbonaceous matter using powdered activated carbon (PAC) as a surrogate and cell-free spent medium (CFSM) of *Phanerochaete chrysosporium*. The CFSM was used because of the lignin peroxidase and manganese peroxidase secreted by the microbe during its incubation. In the present work, an investigation was conducted to determine the physical and chemical alterations in PAC after enzymatic treatment and its effect on $Au(CN)_2^-$ uptake. Characterization of the solid residues of PAC by ^{13}C NMR and N_2 adsorption after bio-modification revealed that the treatment had decomposed poly-aromatic carbons into aliphatic carbons and also reduced the specific surface area from 1430 m^2/g to 697 m^2/g in 14 days. As a result, $Au(CN)_2^-$ uptake decreased from 100% (0.048 mmol/g) to 43% within 12 h primarily due to the enzyme treatment and adsorption of CFSM components. It further decreased to 26% due to surface passivation by bio-chemicals derived from CFSM and/or decomposed aliphatic hydrocarbons from aromatic carbons between 7 days and 14 days. These findings may contribute to efforts to decrease preg-robbing in hydrometallurgical processing of refractory gold ores.

1. Introduction

Refractory gold ores (RGO) are widely distributed in the world. They can be found in several gold mining regions, including the Prestea, Tarkwa and the Ashanti goldfields of Ghana, the Sao Bento Mineracao in Brazil, the Carlin Gold Mine of Nevada in USA, the California mother lode in USA, the Kerr Addison Mines in Canada, the Bakyrchik mine in Kazakhstan and Natalkinsk mine in Russia, the Ratatotok district, North Sulawesi in Indonesia, and Shaanxi in China (Osseo-Asare et al., 1984; Abotsi and Osseo-Asare, 1986; Afenya, 1991; Turner et al., 1994; Miller et al., 2005; Marsden and House, 2006; Yang et al., 2013). This ore type has a poor leaching efficiency, attributable to the presence of sulfides (mainly pyrite and arsenopyrite) and carbonaceous matter (CM). To improve gold extraction, a pre-cyanidation step is typically included in the processing of RGO. For instance, a gaseous (roasting) and/or aqueous oxidation stage is employed to destroy the sulfides and carbonaceous matter.

The difficulty with sulfide minerals arises from encapsulation of tiny gold grains, making them inaccessible to the cyanide lixiviant. On the other hand, the CM in RGO tends to adsorb gold cyanide ($Au[CN]_2^-$) in the pregnant solution, leading to a decrease in gold recovery, a process dubbed as "preg-robbing". The negative impact of CM can be reduced through chemical or biological decomposition (Osseo-Asare et al., 1984; Abotsi and Osseo-Asare, 1986; Afenya, 1991; Amankwah et al., 2005; Marsden and House, 2006; Nanthakumar et al., 2007; Ofori-Sarpong et al., 2010, 2013a, 2013b; Yang et al., 2013). Biological pre-treatment has gained prominent attention because it can be safely and flexibly applied. Fungi-mediated pre-treatment of RGO has attracted recent attention as an alternative to the well-established bacteria-based methods because of the highly oxidative conditions created by some white-rot fungal species and also their tolerance to metal ions (Baldrian, 2003; Ofori-Sarpong et al., 2010, 2013a, 2013b). Favourable outcomes were achieved when RGO was treated by cell-free spent medium (CFSM) of *Phanerochaete chrysosporium* presumably containing secreted enzymes (Ofori-Sarpong et al., 2013b). However, further examination is required to elucidate the complete interaction between RGO and CFSM. The present work seeks to quantify the activities of

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released enzymes and also investigate the enzyme effects on RGO by using powdered activated carbon (PAC) as a surrogate for the CM.

Characterization of the CM is central to defining its interactions with *P. chrysosporium*. RGO may contain up to 7 wt% of CM which usually exists as finely disseminated particles in the ore (Osseo-Asare et al., 1984; Abotsi and Osseo-Asare, 1986; Afenya, 1991; Marsden and House, 2006; Yang et al., 2013). Extraction of CM and the subsequent gold adsorption experiments indicated that elemental carbon and organic acids were most responsible for preg-robbing (Abotsi and Osseo-Asare, 1986). These components of RGO may have been formed from precursors such as ligninolytic biomass by processes similar to coal formation (Hauck, 1975). Lignin can be actively metabolized by *P. chrysosporium* into smaller organic molecules (Tien and Kirk, 1984; Kirk and Farrell, 1987; Kersten and Cullen, 2007) and this ability of the microbe could be related to the observed reduction in preg-robbing by RGO and carbon materials like anthracite and activated carbon (Ofori-Sarpong et al., 2013a). This is, however, mostly qualitative understanding and there is the need to determine the prominent pathways leading to reduction in $Au(CN)_2^-$ uptake.

Fundamental understanding of the mechanisms associated with the interaction between RGO and CFSM derived from *P. chrysosporium* is necessary for designing an effective pre-treatment method. It has been proposed that the microbe affects gold uptake by carbonaceous matter through a combination of aromatic bond cleavage by enzymes, surface passivation by extracellular polymeric substances (EPS) and pore blockage by spores (Ofori-Sarpong et al., 2013a). These digestive enzymes may, however, be significantly influenced by factors like substrate specificity, pH, temperature, metal toxicity and immobilisation (Tien and Kirk, 1984; Kirk and Farrell, 1987; Tien, 1988; Wariishi et al., 1992; Fu and Viraraghavan, 2001; Mielgo et al., 2003; Kersten and Cullen, 2007; Zeng et al., 2013). For example, enzyme selectivity for activated carbon can be either reduced, enhanced or unaffected over the time course of treatment. The current lack of such information necessitates this investigation on the products formed via PAC biodegradation.

2. Experimental

2.1. Materials

All reagents and powdered activated carbon (PAC) used were of special grade and supplied by Wako Chemicals, Japan. PAC was selected as a surrogate for natural carbonaceous matter due to its amorphous crystal structure (unpublished XRD data), which shares some resemblance with the elemental carbon in RGO (Afenya, 1991). Additionally, activated carbon has been extensively applied on the industrial scale for the adsorption of $Au(CN)_2^-$ after cyanidation. Screening analysis of as-received PAC revealed a particle size distribution of 97% below 57 μm. Gold foil with approximately 94% purity (94.3% Au and 5.6% Ag) was provided by Imai Kinpaku Co., Ltd., Japan. Ultra-pure water produced by Millipore synergy unit was used throughout the experiment.

2.2. Growth culture and CFSM harvesting

A white rot fungus *Phanerochaete chrysosporium* was provided for this study by the courtesy of Dr. Ming Tien (Penn State University, University Park, PA, USA). It was regularly maintained on a solid agar with a chemical composition of 10 g glucose, 1.18 g succinic acid, 0.2 g ammonium tartrate, 1 mg thiamine, 0.4 mmol veratryl alcohol, 2 g KH_2PO_4, 0.5 g $MgSO_4$ and 0.1 g $CaCl_2 \cdot 2H_2O$ per 1 L in addition to 70 mL trace element solution (3 g $MgSO_4 \cdot 7H_2O$, 1 g NaCl, 0.5 g $MnSO_4 \cdot 5H_2O$, 0.1 g $FeSO_4 \cdot 7H_2O$, 0.1 g $CuSO_4$, 10 mg boric acid per 1 L) (Tien, 1988).

Liquid cultures of *P. chrysosporium* for CFSM production were prepared with the same composition as the solid agar cultures. This microbe was grown in a 1 L volume of medium at pH 4.0 ± 0.1 in a 2 L-flask. All flasks were covered by porous caps to allow for aeration and incubated at 37 °C under a stationary condition. After 7 days of fungal growth, the fungal mass and spores were separated from the CFSM by a sterilized 0.22 μm stericup filter unit. The enzymatic activities of lignin peroxidase (LiP) and Mn peroxidase (MnP) in the filtrate were determined by veratryl alcohol (Tien, 1988) and Mn^{2+} assay (Wariishi et al., 1992). One unit (1 U) of enzyme activity is defined as the amount of enzyme required to convert 1 μmol of substrate per minute. CFSM was used within a day of harvesting for PAC treatment. The total dissolved organic carbon (TOC) in the CFSM was determined using a Shimadzu VCSH analyser (Kyoto, Japan) in triplicate.

2.3. Enzymatic treatment

25 mg and 250 mg of PAC were separately added to 100 mL flasks with a porous cap and then sterilized by autoclave before enzyme treatment. Hereafter, they are named as PAC 5 and PAC 50, respectively. 50 mL of fresh CFSM was added to respective flasks and the pH was adjusted to 4.0 using either 1 M HCl or 1 M NaOH. After pH adjustment, the mixtures were shaken for enzymatic reaction at 30 °C and 120 rpm for 14 days.

Separately, 50 mL of CFSM was autoclaved at 120 °C, 1 bar for 20 min and then allowed to cool down. Afterwards, its MnP and LiP activities were determined before it was used to react with 25 mg PAC for observation of the physicochemical effects of CFSM on PAC. Hereafter the solid residue of this PAC sample is named as control PAC 5.

All experiments of bio-degradation with CFSM were conducted in duplicates. The filtrate and bio-modified PAC residue were collected periodically for characterization. The residual enzyme activity and total dissolved organic matter concentration of were also determined. The bio-modified solid was subjected to washing with ultrapure water assisted by ultra-sonication prior to solid analysis. Afterward, a portion of these bio-modified solids were supplied for various washing techniques using simple alcohols to remove the bio-chemicals on the surface of PAC after CFSM treatment. The optimal procedure was washing with 2-propanol at 50 °C for 5 min under ultra-sonication.

2.4. Characterization of pristine and bio-modified PAC

The chemical alterations in CFSM-treated PAC following the enzyme treatment were examined using X-ray diffraction (XRD), Fourier transformed infrared spectroscopy (FTIR) and ^{13}C-nuclear magnetic resonance spectroscopy (^{13}C NMR), and measurement of zeta potential. The physical changes in the solid were determined by N_2 adsorption and scanning electron microscopy (SEM).

XRD patterns of the pristine and bio-treated PAC were collected on an Ultima IV diffractometer (RIGAKU, Akishima, Japan) using Cu Kα radiation (40 kV, 40 mA) at a scanning speed of 2° min^{-1} and scanning step of 0.02°.

FTIR spectra were collected in DRIFT method by a FT/IR-670 Plus spectrometer (JASCO, Tokyo, Japan) in the transmission mode using 0.5 wt% sample in KBr crystal (spectacle grade, JASCO Corporation, Tokyo, Japan) under the following conditions: accumulation, 100 times; resolution, 16 cm^{-1}; detector, triglycine sulfate (TGS); range of wavenumbers, 4000–400 cm^{-1}.

Solid ^{13}C-CPMAS NMR spectra were collected on an ECA400 (JEOL, Akishima, Tokyo, Japan) equipped with a 3.2-mm CPMAS probe. The experiments were carried out using a standard 4 mm double resonance MAS probe spinning at 15 kHz, π/2-pulse length of 3.2 μs, and a recycle delay of 5 s. Resonance frequency and magnetic field strength used were 100.53 MHz and 9.39 T, respectively. The pristine powdered activated carbon (super special grade, Wako) was used as a standard for ^{13}C NMR. Chemical shifts were referenced externally to tetramethyl silane (TMS) at δ 0 ppm using the methyl signal of hexamethyl benzene at δ 17.36 ppm as the secondary standard.

The BET specific surface area and pore size distribution of PAC 5 samples before and after enzymatic treatment were determined. The samples were pre-treated in two steps: vacuum degassing for 90 min at

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