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Optimization of hydrogen peroxide-to-hemoglobin ratio for biocatalytic mineralization of polycyclic aromatic hydrocarbons (PAHs)-contaminated soils

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HIGHLIGHTS

- PAHs removal in a contaminated soil was greater at H_2O_2-to-Hb mass ratio ≥ 3.
- Greater high molecular weight PAHs were removed at higher H_2O_2-to-Hb mass ratios.
- The optimal H_2O_2-to-Hb mass ratio for BaP removal in soil was determined to be 3.
- BaP removal in the presence of only Hb can be attributed to the BaP capture by Hb.
- BaP removal and CO_2 generation was positively correlated supporting mineralization.

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ABSTRACT

This study investigates the efficiency of hemoglobin (Hb)-catalyzed biocatalytic reactions for removal of polycyclic aromatic hydrocarbons (PAHs) in a historically PAHs-contaminated soil and of benzo(*a*)pyrene (BaP) in an artificially BaP-contaminated soil. PAHs removal tests at various H_2O_2-to-Hb mass ratios (0−3.7) showed that the PAHs removal was greater at H_2O_2-to-Hb mass ratio of ≥ 3. This was attributed to the greater removal of high molecular weight PAHs at higher H_2O_2-to-Hb mass ratios. The BaP removal increased from 36% to 85% with increasing H_2O_2-to-Hb mass ratio from 1 to 3, and further increase in H_2O_2-to-Hb mass ratio decreased the BaP removal. Thus, the optimal H_2O_2-to-Hb mass ratio for BaP removal was determined to be 3 in the artificially BaP-contaminated soil. The BaP removal in the presence of only Hb can be attributed to the capture of BaP by Hb. The increased BaP removal in the presence of H_2O_2 is likely due to BaP mineralization as the BaP removal and the CO_2 generated showed a strong positive correlation ($R^2 = 0.999$). Overall, this study shows that the Hb-catalyzed biocatalytic reactions can effectively remove PAHs in soil.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) consist of two or more benzene rings. PAHs are generated during pyrolysis at high temperatures or by incomplete combustion of organic materials. But they are not readily decomposed under natural conditions making PAHs common pollutants. Some PAHs (e.g., benzo(a)pyrene (BaP), benz(a)anthracene, benzo(b)fluoranthene) are carcinogenic (Mumtaz and George, 1995). The U.S. Environmental Protection Agency (US EPA) selected 16 compounds belonging to PAHs based on human health risk, and seven compounds among the 16 PAHs were classified as carcinogenic compounds. Due to their harmful potential, carcinogenicity, and mutagenesis to humans and eco-systems, PAHs are recognized as serious environmental contaminants, and PAHs-contaminated soils has been treated by chemical oxidation, biological treatment, and photo-oxidation (Rivas, 2006). Among PAHs, BaP has five benzene rings and low water solubility (0.0038 mg L^{-1} at 25 °C) (Sims and Overcash, 1983). Under EPA's Guidelines for Carcinogen Risk Assessment, BaP was classified to be carcinogenic to human (Group 1) based on strong and consistent evidences in animals and humans. BaP is a common byproduct of combustion processes, and can also be produced naturally by forest fires and volcanoes. BaP is commonly used as an environmental

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indicator for PAHs. In Korea, BaP is being regulated by the Soil Environment Conservation Act of 2009 (Ministry of Environment (Korea), 2009).

PAHs-contaminated soils are often treated by using chemical oxidation reactions such as Fenton reaction, heme-catalyzed reaction, and hemoglobin (Hb)-catalyzed reaction (Gan et al., 2009; Chung et al., 2014; Kang et al., 2015a). The hydroxyl radicals (OH•) generated by the chemical reaction between hydrogen peroxide (H_2O_2) and iron ion (i.e., Fenton's reagent) were used to treat PAHs-contaminated soils (Rivas, 2006; Usman et al., 2012). Although Fenton's reagent can treat non-degradable compounds by biological treatments (Ravikumar and Gurol, 1994; Nam et al., 2001), the reaction is optimal at low pH conditions (pH 2–3), which may destroy the soil environment (Watts et al., 1990). In addition, Fenton's reagent is less effective for high-molecular weight PAHs (HMW-PAHs) (i.e., four to six fused aromatic rings) than low-molecular weight PAHs (LMW-PAHs) (i.e., two or three fused aromatic rings) (Jonsson et al., 2007). But recent studies showed that HMW-PAHs can be removed by fungi (Shi et al., 2017). The lignin-degrading enzyme activities of white rot fungi can simultaneously degrade lignin and various organic pollutants through the peroxidase catalytic cycle (Acevedo et al., 2011; Lee et al., 2014). PAHs can be degraded in the presence of the lignin-degrading enzymes such as lignin peroxidase and manganese dependent peroxidase and H_2O_2-forming enzymes (Acevedo et al., 2011).

Heme in lignin peroxidase produced by white rot fungi catalyzes H_2O_2 degradation and reactive moieties generated during this reaction can degrade organic contaminants such as pentachlorophenol (PCP) and polychlorinated biphenyls (PCBs) (Chen et al., 1999, 2006; Chung et al., 2014; Kang et al., 2015b). Similarly, heme-containing Hb can be used as a catalyst (i.e., Hb-catalyzed biocatalytic reaction) (Kang et al., 2015a). Hb-catalyzed cyclic reactions are shown in Eqs. (1)–(3) (Kang et al., 2015a). Fe^{3+} in Hb (Hb-Fe^{+3}) is oxidized to $\text{Hb-Fe}^{+4\bullet}$ (Eq. (1)), which reacts with organic pollutants (RH) generating $R^{\bullet}$ (Eq. (2)). $R^{\bullet}$ is oxidized to R_{OX} and CO_2 as $\text{Hb-Fe}^{+4\bullet}$ is reduced to Hb-Fe^{+3} (Eq. (3)). Hb-catalyzed biocatalytic reaction was used to remediate total petroleum hydrocarbons- and crude oil-contaminated soils (Keum et al., 2015; Jho et al., 2016).

$$\text{Hb-Fe}^{+3} + H_2O_2 \rightarrow \text{Hb-Fe}^{+4\bullet} \quad (1)$$

$$\text{Hb-Fe}^{+4\bullet} + RH \rightarrow \text{Hb-Fe}^{+4} + R^{\bullet} \quad (2)$$

$$\text{Hb-Fe}^{+4} + R^{\bullet} \rightarrow \text{Hb-Fe}^{+3} + R_{OX} + CO_2 \quad (3)$$

In the previous study, the doses of heme and H_2O_2 were identified as the most important factors for PCP degradation in soil (Chen et al., 2006). Similarly, the doses of Hb and H_2O_2 were identified as the most important factors for degradation of organic pollutants in soil using Hb-catalyzed biocatalytic reaction (Kang et al., 2015a). However, the Hb-catalyzed biocatalytic reaction has not been used for PAHs-contaminated soil remediation. Therefore, this study aims at remediating PAHs-contaminated soil using the Hb-catalyzed biocatalytic reaction. In particular, the efficiencies of PAHs removal from a historically PAHs-contaminated soil and an artificially BaP-contaminated soil using Hb-catalyzed biocatalytic reactions were investigated. Also, the optimum conditions for BaP removal was determined.

2. Materials and methods

2.1. Historically PAHs-contaminated soil sample

Soil samples taken from the storage site of waste railroad ties (Uiwang station, South Korea) were air-dried and sieved through a 2 mm sieve. The sum of the concentrations of 16 PAHs (including naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, BaP, dibenz(a,h)anthracene, indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene) in the historically PAHs-contaminated soil was 259 ± 5.5 mg kg^{-1}. The soil texture was sandy loam (66% sand, 18% clay, 16% silt) with organic carbon content of 20% and pH of 6.3.

2.2. Artificially BaP-contaminated soil sample

Soil samples were air-dried and sieved through a 2 mm sieve. The soil texture was sandy clay loam (48% sand, 25% clay, 27% silt) with organic carbon content of 13% and pH of 6.6. The background BaP concentration was 0.82 ± 0.01 mg kg^{-1}. Artificially BaP-contaminated soil (10 mg kg^{-1}) was prepared using a BaP solution (1000 mg L^{-1}) prepared by dissolving BaP (>99.9%, Accustandard) in hexane (HPLC grade, J.T. Baker, USA).

2.3. PAHs degradation tests

The historically PAHs-contaminated soil sample (2 g) was placed in a 40 mL glass vial. A phosphate buffer (pH 7.0) with or without Hb and H_2O_2 was then added to have the solid-to-liquid ratio of 1:1. The lyophilized powder of Hb from bovine blood was purchased from Sigma-Aldrich (Sigma, USA). The Hb concentration was fixed at 0.015 g (g soil)$^{-1}$ and a range of H_2O_2-to-Hb mass ratios (0.7, 2, 2.7, 3, 3.3, and 3.7) was used to determine the effect of H_2O_2-to-Hb mass ratio on PAHs removal. The experiments were carried out for 24 h. The residual concentrations of 16 PAHs and individual compound of 16 PAHs were analyzed. The control samples without injection of both H_2O_2 and Hb were used to compare the PAHs removal of the samples. The control samples only contained a phosphate buffer (pH 7.0). Single experiment was carried out for each condition to observe the PAHs degradation trend with H_2O_2-to-Hb mass ratio.

2.4. Optimization of BaP degradation

The artificially BaP-contaminated soil was used to determine the optimum doses of Hb and H_2O_2 for degradation of BaP in the soil samples. The Hb-catalyzed biocatalytic reactions were carried out for 24 h using 2 g soil placed in a 40 mL glass vial. The degradation tests used a factorial design with two factors, each at six levels (i.e., 36 combinations were tested), and three replicates were used for each combination. The experimental design used a range of Hb concentrations from 0.000 to 0.025 g Hb (g soil)$^{-1}$ and a range of H_2O_2 concentrations from 0.000 to 0.050 g (g soil)$^{-1}$. The samples without both H_2O_2 (0.000 g H_2O_2 (g soil)$^{-1}$) and Hb (0.000 g Hb (g soil)$^{-1}$) contained only a phosphate buffer (pH 7.0).

2.5. BaP mineralization tests in micro-reactors

The effect of H_2O_2 concentration on BaP removal and CO_2 generation due to BaP mineralization was studied using a micro-reactor consisting of a reactor and a CO_2 collector. The soil sample (10 g) was placed in the reactor (250 mL), and the CO_2 produced from either microbial respiration or BaP mineralization was collected in 0.01 M KOH solution placed in the CO_2 collector. The H_2O_2 concentrations of 0.00, 0.02, 0.03, and 0.04 g (g soil)$^{-1}$ were used with the Hb concentration of 0.010 g (g soil)$^{-1}$. The samples without Hb (0.000 g Hb (g soil)$^{-1}$) were used as controls. The control samples and the samples without H_2O_2 (0.000 g H_2O_2 (g soil)$^{-1}$) contained only a phosphate buffer (pH 7.0). Triplicate