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Lignocellulosic materials as solid support agents for *Bjerkandera adusta* SM46 to enhance polycyclic aromatic hydrocarbon degradation on sea sand and sea water media

Ade Andriani[a,b], Sanro Tachibana[c,*]

[a] *The United Graduate School of Agricultural Sciences, Ehime University, 3-5-7 Tarumi Matsuyama, Ehime 790-8566, Japan*
[b] *Research Centre for Biotechnology, Indonesian Institute of Sciences, Jl. Raya Bogor, km. 46, Cibinong, Bogor 16911, Indonesia*
[c] *Department of Applied Bioscience, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan*

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ABSTRACT

The utilization of white rot fungi (WRF) as degrader agents in extreme environments is still limited by their specific growth requirements. This study examined the ability of *Bjerkandera adusta* SM46 (GenBank accession number: KU055648), a recently isolated WRF from Saragamine mountain, Japan, to treat sea sand and sea water contaminated with polycyclic aromatic hydrocarbons (PAHs) for potential applications in the bioremediation processes. Several PAHs (2–5 rings) were used as pollutants under saline-alkaline stress conditions. Among four lignocellulosic materials, i.e., wood meal, kapok fibre, rice straw, and pulp waste, rice straw was a lignocellulosic material selected as the most suitable support based on the fungal growth, ligninolytic enzymes production, and degradation rates of PAHs after inoculation with *B. adusta* SM46. Rice straw-immobilized *B. adusta* (RSIB) showed faster growth and colonization, and increased laccase (Lac), manganese peroxidase (MnP), and lignin peroxidase (LiP) activity. The optimum granule size of rice straw as an immobilizing agent for *B. adusta* was 840 µm. Lac, MnP, and LiP activities were monitored for 15 and 30 d. Low-molecular-weight PAHs (LMW-PAHs, 2–3 rings) were the most extensively degraded by RSIB. When grown on high-molecular-weight PAHs (HMW-PAHS, 4–5 rings), degradation rates varied between 16% and 63% on contaminated sea sand and between 22% and 61% on contaminated sea water. RSIB may also be used as an alternative method to more effectively and efficiently produce ligninolytic enzymes than the submerged culture method.

1. Introduction

White rot fungi (WRF) have been widely investigated as degrader agents for many toxic compounds such as industrial textile dyes, persistent organic pollutants (POPs), petroleum crude oil, and polycyclic aromatic hydrocarbons (PAHs) (Eichlerova et al., 2007; Chen et al., 2010; Behnood et al., 2014; Sari et al., 2014). Their ability to degrade pollutants has been associated with production of ligninolytic enzymes, such as laccase (Lac), manganese peroxidase (MnP), and lignin peroxidase (LiP). Ligninolytic enzymes degrade a large number of xenobiotic compounds into less toxic compounds such as carbon dioxide due to their low substrate specificity, non-stereoselectivity, and strong oxidative abilities (Kotterman et al., 1998; Asgher et al., 2014). However, previous studies reported that the application of WRF as biodegrader agents in wastewater treatment is limited. WRF require specific growth conditions, have poor mechanical strength and low resistance, and may be inhibited by particular pollutant concentrations (Huang et al., 2015). These operational stability characteristics limit the practical and multipurpose applications of WRF to various sectors of current industrial processes (Asgher et al., 2014).

Fungal immobilization using a suitable solid support is one technique used to increase the competitive characteristics of WRF in contaminated environments. This method enhances long-term use, reusability, and the stimulation of degrading-related enzymes produced by fungi. The solid support may also be used by fungi as a shield from unfavorable environmental conditions, including high pollutant concentrations and predators (Huang et al., 2015). Previous studies reported that immobilized fungi have several advantages over free-living fungal cells. In a comparison with free-living cells, immobilized microbial cells were found to not only promote the biodegradation process, but also had a number of other advantages, such as high cell density, the potential for continuous processing, cell stability, and

* Corresponding author.
E-mail addresses: adea002@lipi.go.id (A. Andriani), tatibana@agr.ehime-u.ac.jp (S. Tachibana).

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lower costs of recovery (Daâssi et al., 2013; Chen et al., 2014).

Immobilization may be conducted using various solid methods such as adsorption, covalent binding, entrapment, cross-linking, membrane confinement, and nanomaterial use as well as the combination of these methods. Hydrogels of natural polymers such as gelatin, alginate, chitosan, pectin, agarose, carrageenan, and cellulose have been commonly used as solid support materials for this process (Hidayat and Tachibana, 2012; Gassara-Chatti et al., 2013; Zawawi et al., 2016). Nanobiocatalytic-like nanofibrous polymers and biocers (a class of nanocomposite materials) have been attracting attention for their application as biocatalysts. On the other hand, most high-tech biocatalysts involve a complex process and high costs for their practical application (Asgher et al., 2014). Therefore, the use of natural support materials for cell immobilization such as lignocellulosic materials have resulted in a satisfactory pollution removal process becoming more feasible (Mohammadi and Nasernejad, 2009; Sari et al., 2014). Lignocellulosic materials exist in large amounts in nature and are inexpensive. These materials are the most abundant raw materials obtained from hardwood, softwood, grasses, and agricultural residues. Lignocellulose is a complex substrate that is composed of a mixture of carbohydrate polymers (cellulose and hemicellulose) and lignin. These materials act as organic supports that allow for the growth of fungi by providing nutrition, an infrastructure, and protection from various environmental stresses. Lignocellulosic materials were also resistant to attack by indigenous pathogens, because lignin contents are involved in bacterial growth inhibition (Rubilar et al., 2011). Lignocellulosic materials, as natural substrates for WRF, support fungal growth and induce the ligninolytic system, and, as a consequence, pollutant degradation (Dzul-Puc et al., 2005; Mohammadi and Nasernejad, 2009; Rubilar et al., 2011; Sari et al., 2014). Previous studies investigated the use of wheat grains as solid substrate agents for the degradation of pentachlorophenol by *Anthracophylum discolor* in Kirk medium and soil (Rubilar et al., 2011). Mohammadi and Nasernejad (2009) reported the significant enhancement of anthracene degradation by sugarcane bagasse-immobilized *Phanerochaete chrysosporium* in nitrogen-limited synthetic growth medium (pH 5). However, the application of the lignocellulosic material-immobilized WRF, *B. adusta* to the degradation of various PAH compounds in contaminated complex medium (under saline-alkaline stress conditions) such as sea sand and sea water has not been conducted.

Coastal areas and offshore marine environments have been exposed to classes of toxic organic contaminants such as PAHs. PAHs are some of the most toxic organic pollutants, most of which exert carcinogenic effects in humans. The major sources of PAH contamination include crude oil leakage, forest fires, fires, and coal burning. Bioremediation of PAH-contaminated sea sand and water was previously reported. These environments are complex systems containing a number of factors that may affect the growth of WRF, strongly influence their viabilities, and inhibit degrading-related enzymes. The main factors inhibiting the pollutant degradation process in these systems are their saline-alkaline characteristics (high mineral content and pH) and the low bioavailability of pollutants. Saline-alkaline characteristics of these systems may inhibit the growth of WRF and, consequently, degrading-related enzymes such as the ligninolytic system (Rubilar et al., 2011; Nicolaus et al., 2015; Andriani et al., 2016). Therefore, this study was conducted in an effort to investigate the potential of applying lignocellulosic material-immobilized WRF as a solid support agent for the degradation of PAHs under saline-alkaline stress conditions.

In the present study, we investigated the use of several lignocellulosic materials: kapok fibers, rice straw, pulp waste, and wood meal, as WRF-immobilizing agents for the degradation of 4 PAHs with different ring aromatic rings: naphthalene (NAP), phenanthrene (PHE), chrysene (CHR), and benzo[a]prene (BaP). To the best of our knowledge, the potencies of these materials as solid support agents for the degradation of PAHs have not been examined to date.

The objective of the present study was to select an appropriate lignocellulosic material as a solid support for the growth and ligninolytic enzyme production of *B. adusta* SM46 in the sea sand and water. Comparison of reaction kinetics in different medium was also analyzed. We also investigated the effects of different granule size of rice straw on PAHs degradation by the fungus. Moreover, we also analyzed the effectiveness of partial purified-extracellular crude enzyme produced by rice straw immobilized *Bjerkandera adusta* SM46 for degradation PAHs under a shaking batch culture.

2. Materials and methods

2.1. Chemicals

NAP, PHE, CHR, and BaP were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The characteristics of these PAHs are shown in Table 1. Lignocellulosic biomasses (kapok fibers, rice straw, wood meal, and pulp waste) were obtained from the Faculty of Agriculture, Ehime University.

2.2. Microorganisms

The WRF strain used in this study was *B. adusta* SM46 (GenBank accession number: KU055648). The fungus was cultivated at 25 °C on 2% malt extract agar (MEA) for 7 days in a disposable plastic Petri dish and maintained at 4 °C prior to use.

2.3. Preparation of lignocellulosic material as a fungal solid support agent

Rice straw (± 0.5 cm length), wood meal, kapok fibers, and pulp waste were used as lignocellulosic materials. Ten grams of each of these materials, 10% (w/w) glucose, 15% (w/w) *shiitake no sato* (a kind of sugar-based nutrient from mushrooms), and 60% (v/w) distilled water were added to an autoclavable plastic tray and then autoclaved at 121 °C and 1 atm for 3 h. Several 5-mm disks of the growing fungus *B. adusta* SM46 in MEA medium were inoculated on the tray and incubated for approximately one month. Fungi were ready to be used in subsequent experiments when fully grown mycelia appeared on the tray.

2.4. Preparation of PAH-contaminated sea sand

The sea sand used in experiments was collected from a beach in Matsuyama city, Japan. Sea sand samples were air-dried and pretreated to remove stones. Thirty grams of sea sand, 10% (w/w) glucose, 15% *shiitake no sato* (a kind of nutrient), and 30% (v/w) distilled water were added to the autoclavable plastic tray (340 ml in size). Prior to use, sea sand samples were autoclaved at 121 °C and 1 atm for 3 h. Sea sand samples were spiked with a stock solution of PAHs (NAP, PHE, CHR, and BaP) diluted in dimethylformamide and 1% tween 80 (v/w) to reach concentrations of 50 ppm and maintained under laminar air flow for 4 h for solvent evaporation.

2.5. Selection of a suitable lignocellulosic material-immobilizing agent for B. adusta SM46

Selection was conducted using the 4 types of lignocellulosic material-immobilized *B. adusta* SM46 described above and free cell mycelia (without the addition of lignocellulosic materials). Sterile PAH-contaminated sea sand samples (only 1 PAH, BaP, was used in this screening) were inoculated with 3 g of lignocellulosic material-immobilized fungus for the immobilized fungal treatment and 4 ml of the fungal inoculum (free cell treatment). The inoculum was obtained from fungal cultures incubated at 25 °C and, after 7 days, homogenized in a sterilized blender for 10 min at 10,000 rpm. The fungal inoculum treatment was conducted as a control to investigate whether immobi-