International Biodeterioration & Biodegradation 65 (2011) 600–604

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Co-metabolic degradation of pyrene by indigenous white-rot fungus *Pseudotrametes gibbosa* from the northeast China

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ARTICLE INFO

Article history:
Received 1 November 2010
Received in revised form
13 March 2011
Accepted 15 March 2011
Available online 14 April 2011

Keywords:
Co-metabolism
Biodegradation
PAHs
White-rot fungus
Laccase (Lac)

ABSTRACT

Pyrene as high molecular weight polycyclic aromatic hydrocarbons (PAHs) is found in most terrestrial environment. However, the biodegradation of pyrene has been limited due to its bioavailability and toxicity. So it is vital to study degradation-capable microorganism and suitable co-substrate. In this study, the indigenous white-rot fungus *Pseudotrametes gibbosa* isolated from ChangBai Mountain located in northeast China was used to degrade pyrene, and 6 co-substrates were selected as co-metabolic carbon and energy sources. The results showed that *P. gibbosa* was able to utilize pyrene as sole carbon and energy source. The degradation efficiency achieved 28.33% within 18 days. Meanwhile, co-substrate wheat bran extract could stimulate laccase (Lac) production significantly by *P. gibbosa*, compared with other co-substrates and control (without co-substrate). In the presence of co-substrates, the biodegradation efficiency of pyrene ranged from 34.23% to 50.64% which was enhanced due to co-metabolism except for salicylic acid (25.91%) and phthalic acid (21.64%).

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are comprised of two or more fused aromatic rings. They originate generally from natural and anthropogenic pyrolysis of organic matter such as forest fires, automobile exhaust, coal-refining processes and the oil industry. Due to their teratogenicity, carcinogenicity and mutagenicity to creature, PAHs have been studied thoroughly in recent years. Microbial biodegradation, as a friendly and effective method of removing these compounds from polluted sites, has been studied extensively (Valentín et al., 2006; Sayara et al., 2010; Wang et al., 2010). Compared to bacteria, white-rot fungi are better to colonize soil and compete with the autochthonous microflora due to their extracellular degradation characteristics (Li, 1996). The extracellular enzyme systems: lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac), secreted by white-rot fungi play a key role in lignin degradation. These non-specific enzymes cannot only catalyze lignin degradation, but also a wide range of organic pollutants, such as PAHs (Eggen and Majcherczyk, 1998), pesticides (Fragoeiro and Magan, 2008), polychlorinated biphenyls (PCBs) (Kordon et al., 2010), synthetic dyes (Zhao et al., 2006), etc.

There have been few reports on organisms capable of degrading the high molecular weight (HMW) polycyclic aromatic hydrocarbons (PAHs), for example pyrene and benzopyrene, as sole carbon and energy sources for growth (Veignie et al., 2004; Silva et al., 2009). The main causes are that the HMW PAHs solubility decreases and hydrophobicity increases with an increase in number of fused benzene rings (Juhasz and Naidu, 2000). Another reason why HMW PAHs cannot be degraded efficiently by microorganism is the lack of catabolic enzyme induction. So the appropriate co-substrates may be useful for the bioremediation of sites polluted with PAHs since they induce the secretion of catabolic enzymes and then promote the degradation of HMW PAHs. Many studies have been conducted to enhance the biodegradation of HMW PAHs by co-metabolism (Eggen, 1999; Hwang and Cutright, 2002, 2003; Tekere et al., 2005). As an important technique, co-metabolism has been extensively applied to the bioremediation of persistent organic pollutants (POPs) (Rentz et al., 2005; Xie et al., 2009).

The purpose of this study is to develop an optimal co-substrate to apply white-rot fungus *Pseudotrametes gibbosa* for pyrene co-metabolism degradation. In addition, the capability of *P. gibbosa* to degrade pyrene as a sole carbon and energy source was also investigated.

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doi:10.1016/j.ibiod.2011.03.003

2. Materials and methods

2.1. Chemicals

Pyrene with 98–99% purity was purchased from Alfa Aesar (Lancaster, United States), which was used as contaminants. All other reagents were AR grade unless otherwise stated.

2.2. Microorganism

White-rot fungus *P. gibbosa* from Mountain ChangBai in northern China was used in this study as PAHs-degrader. The organisms were stored on potato dextrose agar (PDA) plates at 4 °C.

2.3. Culture medium

Liquid medium: NH_4Cl 0.44 g L^{-1}, KH_2PO_4 0.2 g L^{-1}, $MgSO_4 \bullet 7H_2O$ 0.05 g L^{-1}, $CaCl_2$ 0.01 g L^{-1}, Tween 80 1.0 g L^{-1}, inorganic solution (1 mL L^{-1}) and vitamin solution (0.5 mL L^{-1}).

Potato Dextrose Agar (PDA) solid medium, Inorganic solution and Vitamin solution were same with previous study (Chen et al., 2008).

2.4. Culture condition

Three agar plugs(10 mm diameter) cut out from the margin of a 7-day mycelium grown on PDA solid medium were carried out in 150 mL conical flasks contained 50 mL of liquid medium and incubated for 22 days in a rotary shaker (120 rpm) at 28 °C.

2.5. The degradation of pyrene

Pyrene degradation by *P. gibbosa* was conducted in 150 mL conical flasks by inoculating three agar plugs(10 mm diameter) into 50 mL of liquid medium(as above) supplemented with various co-substrates (5 g L^{-1} corn flour, 5 g L^{-1} wheat bran, 5 g L^{-1} sawdust, 5 g L^{-1} glucose, 150 mg L^{-1} salicylic acid, 150 mg L^{-1} phthalic acid) and 10 mg L^{-1} pyrene. The conical beaker was performed on a rotary shaker (120 rpm) at 28 °C. The control experiment was conducted without co-substrates under the same conditions. After 22 days cultivation, Lac activity, biomass and biodegradation efficiency of pyrene were measured. The glucose consumption was conducted in the medium supplemented with 5 g L^{-1} glucose and 10 mg L^{-1} pyrene after inoculation. The biodegradation efficiency of pyrene and the glucose concentration were measured every three days.

All experiments were carried out in triplicate, and the results were expressed as the mean values.

2.6. Extraction of pyrene

The pyrene residues from the liquid medium were extracted twice with 50 mL of cyclohexane. The pyrene residues from fungus mycelium were extracted twice with 20 mL of cyclohexane–acetone solution (1:1, v/v) in an ultrasonic bath extractor. The both extracts from above were combined and concentrated to 1 mL for GC–FID.

Ultrasonic extraction method is one of PAHs extraction methods recommended by the U.S. EPA. This method is suitable for extracting stable compounds such as PAHs and can break cells and extract PAHs in vivo. Also the method is simple, fast, less solvent, high extraction efficiency (Capelo et al., 2005).

2.7. Analytical methods

2.7.1. Assay for Lac activity

The activity of Lac was measured as described by Bourbonnais and Paice (1990). One unit (U) of Lac is defined as the amount of enzyme oxidizing 1 μmol of ABTS per minute.

2.7.2. Assay for glucose concentration

Glucose concentration was measured as described by Gao et al. (2008). All samples were centrifuged at 9000 rpm for 10 min, and the supernatant was analyzed.

2.7.3. Gas chromatography (GC) analysis of PAHs

The gas chromatograph equipped with a flame ionization detector and an Agilent 19091J-413 HP-5 5% Phenyl Methyl Siloxane (30.0 m in length; 0.25 μm in diameter) was used for pyrene analysis. The column temperature was programmed as follows: 200 °C for 3 min, increasing to 260 °C at 5 °C min^{-1}. The injection volume was 1 mL and the split ratio was 50:1.

2.7.4. Assay for biomass

Biomass was analyzed by dry weight. The mycelium filtered from the Liquid medium was dried at 105 °C for 4 h, and kept in desiccators until constant weight.

3. Results and discussion

3.1. The biodegradation of pyrene as sole carbon and energy source

To identify whether white-rot fungus *P. gibbosa* can consume HMW PAHs as sole carbon and energy sources, we used pyrene to represent an HMW PAH to conduct the research. As shown in Fig. 1(a), *P. gibbosa* was able to utilize pyrene as sole carbon and energy source for growth and then degrade it. The degradation

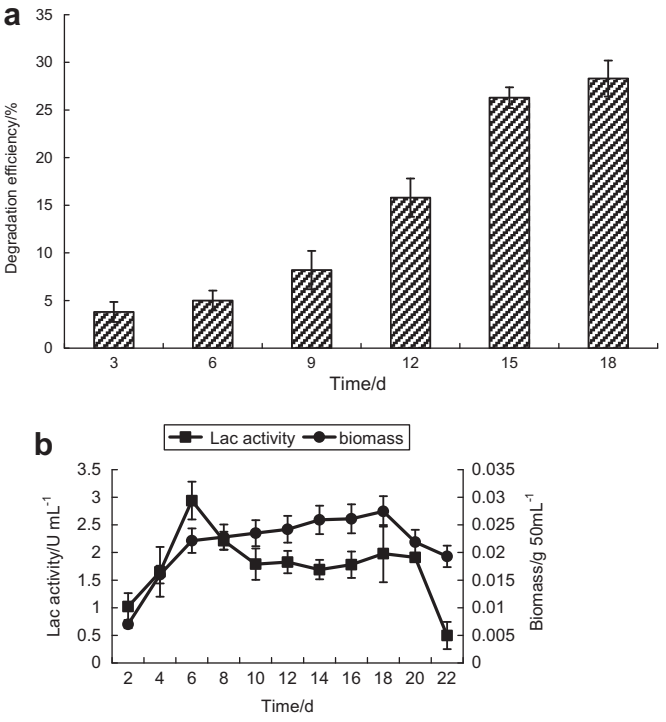

Fig. 1. (a) The degradation of pyrene by white-rot fungi without co-substrates; (b) Lac activities and growth curves of *Pseudotrametes gibbosa* without co-substrates.