Journal of Hazardous Materials 183 (2010) 669–676

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

An efficient PAH-degrading *Lentinus* (*Panus*) *tigrinus* strain: Effect of inoculum formulation and pollutant bioavailability in solid matrices

Stefano Covino[a], Monika Čvančarová[b,c], Milan Muzikář[b], Kateřina Svobodová[b], Alessandro D'annibale[a], Maurizio Petruccioli[a], Federico Federici[a], Zdena Křesinová[b,c], Tomáš Cajthaml[b,*]

[a] *Department of Agrobiology and Agrochemistry, University of Tuscia, Via San Camillo De Lellis s.n.c., 01100 Viterbo, Italy*
[b] *Laboratory of Environmental Biotechnology, Institute of Microbiology, v.v.i., Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ-142 20 Prague 4, Czech Republic*
[c] *Institute for Environmental Studies, Faculty of Science, Charles University in Prague, Albertov 6, 128 43 Prague 2, Czech Republic*

ARTICLE INFO

Article history:
Received 22 March 2010
Received in revised form 15 July 2010
Accepted 20 July 2010
Available online 27 July 2010

Keywords:
Biodegradation
White rot fungi
Polycyclic aromatic hydrocarbons
Fungal growth
Lentinus (*Panus*) *tigrinus*
Irpex lacteus

ABSTRACT

This study comparatively investigated the PAH degradation ability of *Lentinus tigrinus* and *Irpex lacteus* in a historically polluted soil and creosote-impregnated shavings. With this regard, the effect of type of inoculum carrier (*i.e.*, wheat straw, corn cobs and commercial pellets) and contaminant bioavailability was thoroughly determined. Although degradation performances of *L. tigrinus* were not significantly affected by the type of the support, they were invariably better than those of *I. lacteus* on both the polluted soil and the creosote-impregnated shavings. Although degradation efficiencies of all fungal microcosms were highly and significantly correlated with bioavailability, certain PAHs, such as chrysene and benzo[a]pyrene, were removed by *L. tigrinus* from the polluted soil at amounts that exceeded about 2.3-fold their respective bioavailabilities. Degradation of PAHs was negatively correlated with their organic carbon sorption coefficients (K_{oc}) and hydrophobicity ($\log P$). The strength of linear association with the latter parameter, however, was not affected by the type of contaminated matrix in *L. tigrinus*-based microcosms while it was significantly larger in the historically polluted soil than in the creosote-impregnated shavings in *I. lacteus* ones.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

In the last decades, the biological degradation of PAHs has aroused significant interest since these ubiquitous contaminants have been included among priority pollutants owing to their toxic, mutagenic and, in some cases, carcinogenic properties [1]. PAHs arise from the incomplete combustion of organic matter and are found as major components of creosote and coal tar. From a structural viewpoint, these contaminants exhibit two or more fused benzene rings arranged either in a linear, angular or cluster mode. It has been suggested that the persistence of PAHs in the environment might be due to their low susceptibility to nucleophilic attack owing to the presence of dense clouds of π-electrons on both sides of the ring structures [2]. In addition, some physical properties of PAHs, such as low aqueous solubility and high solid/water distribution ratios, hamper microbial degradation of these compounds thereby resulting in their accumulation in soils and sediments [1].

Due to these objective constraints, there is the need to develop bioremediation protocols as environmentally sustainable approaches to the remediation of PAH-contaminated matrices in alternative to physico-chemical treatments. Although several bacterial and actinomycetes species have been shown to degrade PAHs in soil [2], some peculiarities exhibited by white rot fungi (WRF) make them particularly attractive for this purpose [3]. In this respect, WRF are endowed with an extracellular, non-specific and radical-based ligninolytic machinery that confers them the ability to degrade a wide range of contaminants, including high molecular mass PAHs which are seldom prone to bacterial attack [4,5]. Moreover, the filamentous growth mode of WRFs provides them with the ability to diffusely penetrate into soil aggregates and to act as dispersion vectors of resident pollutant-degrading bacteria [6].

The colonization ability of WRF on a given contaminated matrix, however, might be hampered by both competition with the resident microbiota and adverse physico-chemical characteristics of the matrix itself [7] thus resulting in reduced degradation performances [5,8,9]. With this regard, the use of lignocellulosic wastes in inocula formulation [10,11] increased the antagonistic potential and, therefore, the growth capacity of WRF in remediation applications [12–15]. The large variability of results, however, highlights the importance of lignocellulosic waste/fungus interactions thus suggesting the need for further investigations.

Although the PAH degradation ability of WRF has been suggested to be less affected than that of bacteria and actinomycetes by the mass transfer rate of these contaminants from the solid to the

* Corresponding author. Tel.: +420 241062498; fax: +420 214062384.
E-mail address: cajthaml@biomed.cas.cz (T. Cajthaml).

0304-3894/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2010.07.078

liquid phase, the low bioavailability of these contaminants, which tends to increase with aging [16], remains a key-constraint to the fungal clean-up of PAH-contaminated matrices [17]. Consequently, it is of paramount importance to relate fungal degradation results to the extent of the bioavailable fraction in order to devise appropriate mycoaugmentation protocols.

Among WRF, *Lentinus* (*Panus*) *tigrinus* has recently attracted research interest due to its ability to clean-up PAH-contaminated liquid and solid matrices even under saline conditions [18]. Recently, the degradation ability of *L. tigrinus* CBS 577.79 towards PAHs has been shown in N-rich and N-limited standard liquid media under axenic conditions [19].

Therefore, main objective of the present study was to assess the PAH-biodegradation potential of *L. tigrinus* CBS 577.79 on real solid matrices derived from a wood treatment plant in view of its possible use in mycoremediation applications. In particular, degradation performances of the strain under study were investigated in both a historically polluted soil (HPS) and creosote-impregnated shavings (CIS) and compared with those of *Irpex lacteus* CCBAS 238/617, the degradation capacity of which had been extensively demonstrated [20,21]. In order to gain insights into the matrix effect, attempts were done to establish a relationship between the biodegradation efficiency and the contaminant bioavailability estimated with sequential supercritical fluid extraction. Moreover, both growth ability on the two contaminated matrices and PAH degradation performances were evaluated as a function of three carriers (*i.e.*, wheat straw, corn cobs and commercial pellets) employed to formulate fungal inocula. The thorough determination of the impacts of both bioavailability and inoculum formulation on fungal degradation addressed in this study might represent a significant and positive contribution to the mycoremediation general understanding.

2. Materials and methods

2.1. Materials

Wheat straw (WS), corn cobs (CC), chopped into approx. 1.5 cm pieces, and commercial pellets (0.8 mm Ø) (CP, *ATEA Praha*, Prague, Czech Republic) were used as carriers for fungal inocula. The last carrier is a wheat straw-based composite material employed for domestic-heating purposes with a density of 1.34 kg dm^{-3}, a moisture of 6.4% (w/w) and a C/N ratio of 45:1.

The sandy-loamy HPS, collected from the same wood treatment plant in Soběslav (southern Bohemia, CZ) where CIS had been impregnated, was air-dried for 7 d at room temperature and then passed through a 2 mm-sieve. Its main properties were as follows: water-holding capacity 20%, pH 5.1, total organic carbon 0.5%, total organic matter 0.9%. Organic carbon was determined with hot chromic acid digestion and the total organic estimation was based on combustion at 650 °C. It contained several metals (mg kg^{-1}): As 17.5, Cd 0.4, Co 1.74, Cr 9.41, Cu 6.8, Fe 322, Hg 5.7, Pb 4.5 and Zn 75.6. Granulometric composition was: sand 58.2%, fine sand 27.7%, silt 8.1%, clay 6.0%. CIS from oak wood sleepers, provided by the company Eko-Bio Vysočina, Ltd., Czech Republic, were air-dried for 7 d at room temperature, mechanically ground and then passed through a 2 mm-sieve. The sandy-loamy soil (MBU) was used as the non-contaminated control. Its main properties were as follows: total organic carbon 0.8%, total organics 1.4%, pH 5.3, water-holding capacity 31% and granulometric composition was: sand 50.9%, fine sand 31.2%, silt 10.8%, clay 7.1%. All solvents of p.a. quality, trace analysis quality or gradient-grade were purchased from Merck (Darmstadt,
Germany).

2.2. Microorganisms and inocula preparation

L. tigrinus 577.79 and *I. lacteus* 238/617 were from the CBS (Baarn, NL) and CCBAS (Institute of Microbiology AS CR, Prague, CZ) culture collections, respectively. The strains were maintained at 4 °C and periodically sub-cultured on MEG agar plates containing (g l^{-1}): malt extract, 5; glucose, 10; agar, 15 g; pH 5.0.

Fungal pre-inocula were grown under stationary conditions for 7 d at 28 °C in 250 ml Erlenmeyer flasks containing 20 ml MEG liquid medium. Cultures were then homogenized with the Ultraturrax-T25 (IKA-Labortechnik, Staufen, Germany) and 1.0 ml aliquots of the mycelial suspension were added to 16 cm × 3.5 cm test-tubes either containing WS (2 g) or CC (6 g) or CP (10 g), the moisture contents of which had been previously adjusted to 70% (w/w) and subsequently sterilized by autoclaving (121 °C, 45 min). After inoculation, the cultures were covered with cotton-wool stoppers and then grown for 2 weeks at 28 °C under stationary conditions.

2.3. Fungal treatment of polluted matrices

The moisture contents of the two soils (*i.e.*, HPS and MBU) and of the CIS were previously adjusted to 15 and 25% (w/w) respectively, with sterile deionized water. Then, the test-tubes containing immobilized inocula were added with a layer of either PAH-polluted matrices (25.0 and 5.0 g of HPS and CIS, respectively) or MBU control soil (25 g). Due to the different apparent densities of the colonized carriers and the contaminated matrices, different weights of them were overlaid in order to ensure uniform heights of the resulting bed. Incubation controls were prepared by adding the polluted matrices to heat-inactivated (121 °C, 30 min) immobilized inocula. Each microcosm, namely the combination of inoculum carrier, fungal strain and target-matrix, was prepared in triplicate (controls included) and incubated for 60 d at 28 °C. Throughout the incubation, sterile deionized water was periodically added in order to keep constant the moisture content of each microcosm.

2.4. Extraction and analyses of ergosterol and aromatic pollutants

Total ergosterol was extracted and analyzed as described by Šnajdr et al. [22]. Samples (0.5 g) were sonicated at 70 °C for 90 min with 3 ml of a methanolic solution of KOH (10%, w/v). After the addition of 1 ml distilled water, each sample was extracted for three times with 2 ml of cyclohexane. The solvent was evaporated under a nitrogen stream and the solid residue dissolved in 1 ml methanol. These samples (20 μl) were then analyzed isocratically by reversed-phase high performance liquid chromatography (RP-HPLC) equipped with a LiChroCart column filled with LiChrospher® 100 RP-18 (250 mm × 4.0 mm; particle size 5 μm; pore size 100 Å) equilibrated with 100% methanol at a flow rate of 1 mL min^{-1}. The elution profile was monitored at 282 nm.

Extraction of aromatic pollutants was performed with an ASE 200 System (Dionex, Voisins-le-Bretonneux, France). Either 10 g of HPS or 2 g of CIS were loaded into the extraction cell (11 ml) and subsequently extracted with hexane–acetone (3:1, v/v) mixture (HAM). Static heating was applied to the vessel (150 °C, 5 min) and subsequent extraction was performed for 7 min at the same temperature under 103.4 bar. The cell was then flushed with 7 ml HAM and finally the solvent was purged from the cell by nitrogen for 60 s. For each sample, this extraction cycle was performed twice. From here onwards, the resulting organic extracts, air-dried under vacuum at room temperature and finally dissolved in acetonitrile (5 and 20 ml for HPS and CTS, respectively) are referred to as contaminants extract. RP-HPLC analyses were performed using a system consisting of a 2695 Separations Module (Waters, Milford, MA) equipped with a LiChroCart column filled with LiChrospher® PAH (250 mm × 5 mm; particle size 5 μm; pore

Download English Version:

https://daneshyari.com/en/article/579576

Download Persian Version:

https://daneshyari.com/article/579576

Daneshyari.com