New BIOTECHNOLOGY xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

New BIOTECHNOLOGY

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

Full length Article

PCB in the environment: bio-based processes for soil decontamination and management of waste from the industrial production of *Pleurotus ostreatus*

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ARTICLE INFO

Keywords:
Actinobacteria
α Subunit (RHDα) aromatic ring hydroxylating dioxygenases
Dynamic biopile
Firmicutes
Laccase
Pleurotus ostreatus
Polychlorinated biphenyls
Spent mushroom substrate

ABSTRACT

Polychlorinated biphenyls (PCBs) are hazardous soil contaminants for which a bio-based technology for their recovery is essential. The objective of this study was to validate the exploitation of spent mushroom substrate (SMS), a low or null cost organic waste derived from the industrial production of *P. ostreatus*, as bulking agent in a dynamic biopile pilot plant. The SMS shows potential oxidative capacity towards recalcitrant compounds. The aim was consistent with the design of a process of oxidation of highly chlorinated PCBs, which is independent from their reductive dehalogenation. Feasibility was verified at a mesocosm scale and validated at pilot scale in a dynamic biopile pilot plant treating ten tons of a historically contaminated soil (9.28 ± 0.08 mg PCB/kg soil dry weight). Mixing of the SMS with the soil was required for the depletion of the contaminants. At the pilot scale, after eight months of incubation, 94.1% depletion was recorded. A positive correlation between Actinobacteria and Firmicutes active metabolism, soil laccase activity and PCB removal was observed. The SMS was found to be exploitable as a versatile low cost organic substrate capable of activating processes for the oxidation of highly chlorinated PCBs. Moreover, its exploitation as bulking agent in biopiles is a valuable management strategy for the re-utilisation of an organic waste deriving from the industrial cultivation of edible mushrooms.

Introduction

Polychlorinated biphenyls (PCBs) have been utilized for decades in many industrial applications. Because of their adverse health effects in man, their manufacture, processing and distribution have been banned since the 1990s. However, PCB detection in the environment is still ubiquitous due to their high chemical stability and affinity for hydrophobic organic solids [1].

Bacterial PCB biodegradation in natural compartments has been reported [2–4]. Generally, PCB congeners with four or more chlorine atoms undergo bacterial anaerobic reductive dechlorination. Lower-chlorinated PCB congeners are subject to co-metabolic aerobic oxidation mediated by dioxygenases, encoded by the *bph*A gene family [5,6]. Fungal PCB biodegradation has also been reported. Fungi with the capacity to transform several PCB congeners in liquid medium were described [7–13]. A few studies investigated fungal transformation capacity in soils [14–17]. These were based mainly on the bioaugmentation of Basydiomyctes, among others *Pleurotus ostreatus*, massively inoculated in the presence of a fungal growth substrate (e.g. lignocellulosic matrices) to transform PCBs in spiked and actually contaminated soils [14,15]. Myco-augmemtation for the treatment of contaminated matrices derives from the capacity of fungi to produce substrate-unspecific extracellular and intracellular oxidoreductases, laccases and Mn dependent and independent peroxidases [18–20], enabling them to transform organic compounds belonging to different chemical classes comprising PCBs [12]. *Pleurotus ostreatus* produces ligninolytic enzymes described for their capacity to transform a plethora of waste substrates [21–23] including PCBs [24]. The basidiomycete is also an edible mushroom whose industrial cultivation is affected by the production of significant amount of spent mushroom substrate (SMS), a lignocellulosic cellulosic matrix, that has to be disposed of, creating a bottleneck in the production chain for mushroom farmers [25]. The exploration of new applications for re-utilisation of SMS are desirable. However, the SMS was reported as being characterised by high levels of residual extracellular oxidoreductases produced by the still metabolically active mycelium of the basidiomycete,

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http://dx.doi.org/10.1016/j.nbt.2017.08.011
Received 13 November 2016; Received in revised form 25 August 2017; Accepted 28 August 2017

and by an active microbial community composed of fungi and bacteria colonising the lignocellulosic matrix [26–28]. Thus, SMS exploitation in bioremediation processes was explored and the ability of the SMS and its inherent microbiota to transform different contaminants was reported in relation to treatment of soils [27–30]. In more detail, the SMS from *P. ostreatus* was previously validated as a low cost organic substrate exploitable for its oxidative potential for the treatment of polycyclic aromatic hydrocarbons (PAH) contaminated soil, when exploited as lignocellulosic bulking agent in biopile reactors, sites of oxidative processes where the amendment of a bulking agent increases aeration of the soil. Thus, the SMS was exploited as the carrier of both the mycelium of *P. ostreatus* and of a non-identified microflora, comprising an uncharacterized fungal and bacterial load inoculated into the contaminated matrices and eventually involved in the oxidation of the contamination [27,28].

In this context, our aim was to verify the efficiency of SMS from *P. ostreatus* as a bulking agent in biopile reactors for the depletion of PCBs from a historically contaminated soil derived from an industrially dismissed area in Italy, where coolant fluids had been produced for 50 years. A mesocosm scale experimental design was initially set up and successively validated at a pilot scale in a dynamic biopile treating ten tons of contaminated soil. PCB depletion was measured in parallel with the quantitative measurement of changes in the microbial community structure, with reference to both metabolically active fungi and bacteria. The laccase enzymatic activity and the levels of transcription of different bacterial biphenyl dioxygenases were also determined.

Materials and methods

Chemicals and soil

Chemicals used were of analytical grade and purchased from Sigma-Aldrich (Milan, Italy). PCB congeners were purchased by Ultrascientific (USA). The soil derived from a former industrial area producing coolant fluids in the north of Italy. The texture of the soil was sandy–loam (38% silt, 52% sand, and 10% clay) with total phosphorous, 1.6%; total organic carbon, 2.3%; total nitrogen 1.5%; pH 7.2; PCB contamination up to 9.28 ± 0.08 mg PCB/kg soil dry weight (dw). Any contamination by total petroleum hydrocarbons and polycyclic aromatic hydrocarbons was detected. The heavy metal contamination is reported in Table 1.

Table 1
Soil heavy metal content.

Heavy metals	mg/kg soil d.w.
Sb	1,1
As	2,5
Be	1,1
Cd	0,3
Co	7,0
Cr	15
CrVI	0,2
Fe	16,4
Mn	651
Hg	0,2
Mo	8
Ni	16
Pb	47
Cu	50
Se	1,5
Sn	7,5
Tl	1,2
Te	1,3
V	24,5
Zn	114

Experimental conditions

A total of 9 experimental mesocosms, each containing 50 kg of contaminated soil (9.28 ± 0.08 mg PCB/kg soil dw) were prepared and maintained in a temperature controlled (21 ± 1 °C) dark chamber at 60% of the soil maximum water holding capacity (WHC_{max} = 17.4% dry mass). Three mesocosms out of 9 were mixed with 10% on a weight base ratio with the SMS from the industrial production of *P. ostreatus*. Three microcosms were mixed with 10% on a weight base ratio with autoclaved SMS (121 ± 1 °C, 1 Atm, 1 h). Three mesocosms were not mixed with the SMS. All mesocosms were routinely mechanically mixed every 3 d of incubation and checked for water content. Soil samples were collected every 2 months. In pilot scale experiments a total of 10 tons of contaminated soil was mixed in a tank (length x width x height, 4,2 × 1,8 × 1,2 m) with 10% on a weight base ratio of the SMS. The tank was prepared in March and closed in November and managed as a dynamic biopile as described in [27]. Collection of representative samples for chemical (PCB content), molecular (taxonomical and functional molecular markers) and biochemical (laccase activity) data was performed as described in [27] every two months.

PCB extraction and analytical procedures

Analyses were performed on 15 PCB congeners including six indicator PCBs that are the set of PCBs used to estimate total PCBs to simplify analytical approaches [31]. A total of 10 g of soil samples deriving from the different technical replicates collected were divided into two parts and separately extracted and analysed. One part was qualitatively and the other was quantitatively analysed for PCB content. Soils were dried in a vented oven at 25 °C for 24 h. All samples were extracted using a soxhlet apparatus with dichloromethane as the solvent. Qualitative and quantitative analysis of PCBs was performed with an Agilent 6890 GC-5975B Series MS system in the selective ion monitoring mode following the methodology described in [32].

Molecular techniques

Total RNA from soil was purified using the MoBio RNA power soil total RNA isolation kit (MoBio Laboratories Inc., USA) following the manufacturer's instructions. Potential DNA contamination was eliminated with DNase I treatment using the RiboPure bacteria kit (Ambion, USA) according to manufacturer's instructions. RNA quantity, quality, and purity were analysed using gel electrophoresis on a 1% (w/v) agarose gel stained with ethidium bromide and viewed spectrophotometrically with an Implen nanophotometer (Implen GmbH, Germany). To produce the cDNA template for PCR amplification of the *bph*A genes and the ribosomal 16S and 18S retrotranscripts, reverse transcription was performed on the total community RNA extracted, using the RNase H activity-less RevertAid premium reverse transcriptase (Fermentas, Lithuania) according to the manufacturer's instructions. The absence of contaminating DNA was confirmed by using nontranscribed RNA as template in PCR amplification. Primers used for the reverse transcription and the quantification of the transcripts for *bphA*, the total 16S rcDNA and the total 18S rcDNA are listed in Table 2. Taxa specific 16S rcDNA primers were used for quantification of the Actinobacteria, Acidobacteria, α- and β-Proteobacteria, Bacteroidetes and Firmicutes. Primers used are described in Table 2. The qPCR reactions were carried out as described in [32]. For the *bphA* transcripts, the standard DNA template was the LB400 *bphA* genes cloned into the pGEM-T (10^2 to 10^9 copies). Amplification efficiencies were calculated from the slopes of the standard curves [33]. The amplification levels of the retrotranscribed taxa-specific genes and *bphA* genes were first normalized by the amplification level of 16S rcDNA [34,35]. The fractional copy numbers of the amplified genes were calculated as the ratio between the normalized amplification levels of the gene in SMS amended soil and in soil amended with autoclaved SMS and the