Process Biochemistry 49 (2014) 2266–2271

Contents lists available at ScienceDirect

Process Biochemistry

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

Degradation of carbofuran by *Trametes versicolor* in rice husk as a potential lignocellulosic substrate for biomixtures: From mineralization to toxicity reduction

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ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 3 October 2014
Accepted 9 October 2014
Available online 22 October 2014

Keywords:
Degradation
Pesticide
Lignocellulosic waste
White-rot fungi
Toxicity

ABSTRACT

Biopurification systems for the treatment of pesticide-containing wastewaters contain a biomixture constituted by low-cost materials (soil, lignocellulosic substrates and humic components). In particular, the use of bioaugmented lignocellulosic materials as part of the biomixture components has been scarcely studied. The use of the fungus *Trametes versicolor* grown in rice husk (RH) was evaluated as an option of lignocellulosic bioaugmented substrate of potential application in biomixtures for carbofuran (CFN) elimination. RH supported growth and activity of the fungus, as evidenced visually and by laccase activity. The system was able to degrade 55.1% CFN in 34 d with a half-life of 29.9 d, and produced 3-hydroxycarbofuran (but not 3-ketocarbofuran) as a transformation product. This metabolite was successfully eliminated during the process. Moreover, a small fraction of CFN was mineralized (7.4%) within 64 d. Toxicological evaluation revealed a marked detoxification during the process, achieving an 89% decrease in the toxicity after 34 d. Overall performance of the bioaugmented substrate indicates that RH/*T. versicolor* is an interesting option to take into account in the design of biomixtures for pesticide degradation.

1. Introduction

Among anthropogenic activities, agriculture exerts a deep impact on the environment. Agricultural production includes the use of different agrochemicals and in many cases complex mixtures of products, which can be incorporated into different environmental compartments causing diffuse contamination due to runoff or leaching from farms to water bodies or events of point-source contamination as accidental pesticide spills, inadequate disposition of application residues or washing residues from application [1].

Despite the application of Good Agricultural Practices by the farmers, the sites designated for the handling of equipment for pesticide application and their wastes usually suffer from a major contamination impact [2,3]. That situation occurs over many cycles of agricultural production and results in the constant incorporation of agrochemicals into soil and water [2]. An evidence of this issue is the detection of pesticide residues on samples of freshwater and groundwater near farm fields [4,5].

A common pesticide employed as a broad spectrum systemic nematicide, insecticide and acaricide is carbofuran (CFN), which belongs to the family of carbamates. Toxicological issues linked to CFN include hematological changes in fish, endocrine disrupting activity and in general high toxicity for mammals and aquatic life [6–9]. As a result, CFN has been banned for use in agricultural activities in the US and the EU, but it is still widely employed in developing countries, where unregulated application may translate in constant incorporation to the environment, as shown by its detection in soil, water sources and fresh vegetables along the tropic [10,11].

An approach to reduce the contamination of water sources with pesticides is the use of biopurificaction systems (BPS), also known as biobeds. These systems were first developed in Sweden and have been used in Europe since the nineties. The active core of the BPS is the biomixture, composed of soil, a humified material (peat or compost) and a lignocellulosic matrix waste [12]. Biomixtures employ the degrading capacity of microorganisms to minimize, in a simple and low cost way, the contamination by pesticide-containing wastewaters from agricultural activities [1,3].

The composition of biomixtures depends on the availability of local agroindustrial wastes, particularly lignocellulosic wastes, and design should be adapted to every region. The incorporation

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http://dx.doi.org/10.1016/j.procbio.2014.10.006
1359-5113/

of a lignocellulosic substrate in the composition of biomixtures is intended to favor the growth and activity of ligninolytic fungi, widely known for their ability to degrade diverse organic contaminants. This ecological group produces lignin-modifying enzymes (LMEs) including laccases and peroxidases of highly unspecific capability to oxidize a broad range of organic compounds [13]. Moreover, these fungi employ the intracellular enzymatic complex cytochrome P450 to widen their degrading spectrum [14,15]. Overall, the list of organic pollutants known to be degraded by ligninolytic fungi or their LMEs includes pesticides [16–20], polycyclic aromatic hydrocarbons (PAH) [21,22], polychlorinated biphenyls (PCB) [23,24], textile dyes [25,26], brominated flame retardants [27,28] and pharmaceuticals and personal care products [29,30], among others. In this context, the evaluation of the pesticide-removal capacity of ligninolytic fungi on lignocellulosic substrates which can be potentially added to biomixtures, becomes a priority task in the design of BPS for tropical lands.

The aim of the work is to describe the performance of rice husk (RH) as a lignocellulosic substrate in the degradation of CFN by the white-rot fungus *Trametes versicolor*, to be used as a component in BPS biomixtures. *T. versicolor* is a ligninolytic fungus of high performance in the elimination of organic pollutants from diverse matrices [29–31]. Comprehensive evaluation of the substrate includes elimination of the parental compound, detection and monitoring of transformation products, mineralization of CFN and monitoring of toxicological changes of the matrix during the degradation process.

2. Materials and methods

2.1. Chemicals and reagents

Formulated CFN (Furadan®48SC, 48% w/v) was acquired from a local store. Analytical standards CFN (2,2-dimethyl-2,3-dihydro-1-benzofuran-7-ylmethylcarbamate, >99% purity), 3-hydroxycarbofuran (99.5%) and 3-ketocarbofuran (99.5%) were obtained from Chemservice (West Chester, Pennsylvania, USA). Radio-labeled CFN (^{14}C-CFN; [Ring-U-^{14}C]-Carbofuran; 2.89×10^9 Bq g^{-1}; radiochemical purity 100%; chemical purity 99.59%) was obtained from Izotop (Institute of Isotopes Co., Budapest, Hungary). Carbendazim-d_3 (surrogate standard, 99.0%) and carbofuran-d_4 (internal standard, 99.5%) were purchased from Dr Ehrenstorfer (Augsburg, Germany).

Acetonitrile (ACN) and methanol of HPLC (high performance liquid chromatography) grade, formic acid (purity 98–100%) and glacial acetic acid (purity ≥ 99.7%) were obtained from Merck (Darmstadt, Germany). Water was purified with a Direct-Q UV3 (resistivity 18.2 MΩ cm) on a water purification system (Millipore, Bedford, MA). Magnesium sulfate anhydrous, sodium chloride, and sodium acetate anhydrous analytical grade were purchased from J.T. Baker (Phillipsburg, NJ). Bondesil-PSA (primary secondary amine, 40 μm) was obtained from Varian (Palo Alto, CA); silica-C18 were acquired from Chromabond (Duran, Germany). Potassium hydroxide analytical grade was purchased from Merck (Darmstadt, Germany). Ultima Gold cocktail for Liquid Scintillation Counting was purchased from Perkin Elmer (Waltham, Massachusetts, USA).

2.2. Fungal strain and lignocellulosic substrate

The strain *T. versicolor* ATCC 42530 was obtained from the American Type Culture Collection, and maintained by subculturing every 30 d on dextrose potato agar slants (pH 4.5) at 25 °C. *T. versicolor* blended mycelial suspension was prepared according to a procedure by Font et al. [32], modified by the use of Sabouraud broth as culture medium. RH obtained from an agricultural input supplier from Tierra Blanca, Cartago, Costa Rica was employed as lignocellulosic substrate.

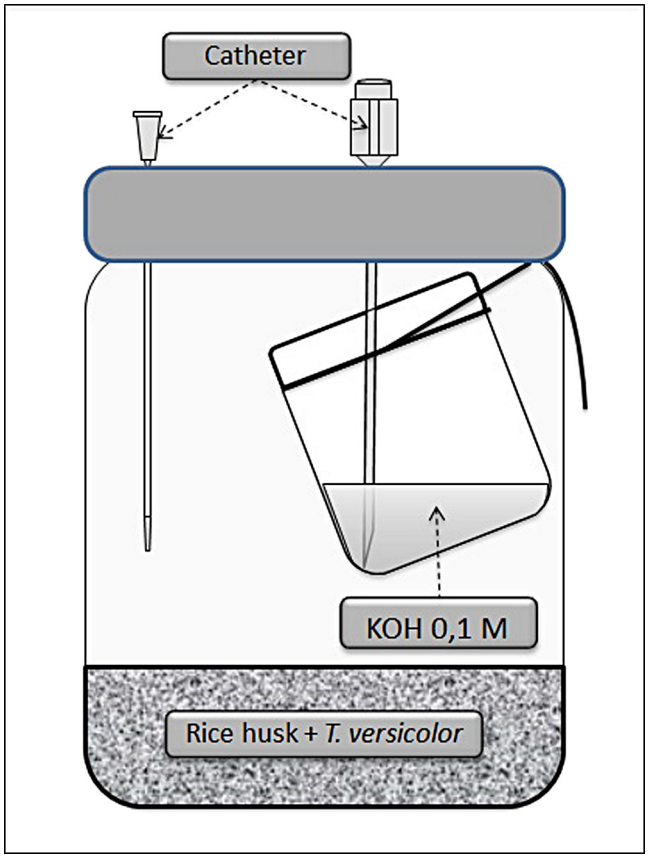

Fig. 1. Biometric system employed in the ^{14}C-CFN mineralization assays.

2.3. Experimental procedures

2.3.1. Mineralization assays

Biometric systems were constructed using 400 mL glass jars and 50 mL glass flasks suspended by copper wires inside the jars. A catheter was placed in the upper section of each jar and was used to remove and add the KOH solution (Fig. 1). The experiment was conducted in triplicate; 50 g RH were weighed into biometric flasks and spiked with commercial CFN, Furadan®48SC (~10 mg kg^{-1}, potential concentration within a biomixture) and ^{14}C-CFN (3000 dpm g^{-1}). $^{14}CO_2$ traps were prepared by adding KOH (10 mL, 0.1 M) into the 50 mL suspended flasks. The systems were incubated in the dark at (25 ± 1)°C during 63 d. The KOH in the flaks was withdrawn at 2, 6, 15, 22, 30, 38, 45, 52 and 64 d after treatment and replaced with the same amount of fresh KOH. Trapped $^{14}CO_2$ was analyzed in the KOH samples (Section 2.4.2).

2.3.2. Degradation assays

CFN degradation assays were performed in 12 cm × 3.5 cm plastic tubes containing 3.5 g dry RH and 11 mL distilled water. The tubes were then autoclaved (121 °C during 15 min) prior the inoculation with 1.5 mL of blended *T. versicolor* mycelium suspension. The microcosms were incubated in static conditions at 25 °C for 7 d and then spiked with Furadan®48SC to give a CFN final concentration of ~10 mg kg^{-1}. Temperature was kept constant at 25 °C until the end of the assay. Triplicate unitary microcosms were sacrificed at times 0, 8, 15, 34 and 64 d for the quantification of CFN and transformation products, and ecotoxicological tests. Uninoculated controls were included to estimate abiotic losses of