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Removal of herbicides in a biopurification system is not negatively affected by oxytetracycline or fungally pretreated oxytetracycline

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HIGHLIGHTS

- Oxytetracycline (OTC) successfully pretreated in a bioreactor containing *Trametes versicolor*.
- Fast elimination of OTC was achieved in the bioreactor, but no detoxification.
- Pretreated OTC was disposed in a biomixture capable to remove two herbicides.
- OTC or OTC-residues did not inhibit herbicide removal in the biomixture.
- Detoxification achieved in the biomixture even in the co-application of herbicides.

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ABSTRACT

The disposal of agricultural antibiotic-containing wastewater in biopurification systems (BPS) employed in the treatment of pesticides, may negatively affect the removal capacity of these devices. This work aimed to employ a fungal pretreatment of oxytetracycline (OTC)-rich wastewater, before its disposal in a BPS used for the treatment of two pesticides. The fungal treatment at reactor scale (stirred tank reactor, 3L) with biomass of *Trametes versicolor* efficiently removed 100 mg L^{-1} OTC in only 60 h. However, ecotoxicity tests on seed germination with *Lactuca sativa* revealed that antibiotic elimination did not correlate with a decrease in toxicity. After the pretreatment, treated OTC was discarded in biomixtures used for the elimination of the herbicides ametryn and terbutryn. The co-application of treated or untreated OTC did not inhibit the removal of the herbicides; moreover, in both cases their removal seemed to be slightly enhanced in the presence of OTC or its residues, with respect to antibiotic-free biomixtures. Estimated half-lives ranged from 28.4 to 34.8 d for ametryn, and 34.0–51.0 d for terbutryn. In addition, the biomixture was also able to remove OTC in the presence of the herbicides, with an estimated half-life of 38 d. Remarkably, the toxicity of the wastewater containing OTC or treated OTC was mostly eliminated after its disposal in the biomixture. Overall results suggest that, given the high efficiency of the biomixture, the fungal pretreatment of OTC-containing wastewater is not mandatory before its disposal in the BPS.

1. Introduction

Formulated antibiotics are used in agriculture as prophylactic agents or for the treatment of bacterial diseases in a wide variety of crops (McManus et al., 2002). The number of antibiotics used in plant agriculture is modest; only oxytetracycline (OTC) and streptomycin are registered by the United States Environment Protection Agency, and in Latin American countries other compounds such as gentamicin, oxolinic acid and validamicin are also permitted. Every year in Costa Rica an average of more than 5000 kg of OTC active ingredients are imported as formulated products for agricultural use (Ramírez et al., 2012); in many crops this antibiotic is applied at concentrations of 150 mg L^{-1} in volumes of 500–1000 gallons of water per hectare, thus resulting in the spread of large amounts of active compound in every application (Vidaver, 2002). Other ways by which OTC accesses environmental

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compartments include its administration to livestock and subsequent excretion, aquaculture practices and the disposal of wastewaters from OTC production. Once in the environment, OTC shows low mobility which translates into high adsorption to soil, where reported half-lives are usually over 30 d (Lewis et al., 2016; Li et al., 2008).

Antibiotic use in agriculture results in the production of wastewaters from spraying leftovers. Inadequate disposal of such wastewaters can produce groundwater and soil contamination. Given its mode of action, persistent antibiotic residues in the environment can alter microbial processes within biogeochemical cycles, as deleterious effects of antibiotics have been described on processes such as nitrification, and iron or sulfate reduction (Toth et al., 2011). Also, the presence of tetracycline antibiotics has revealed negative effects on biodegradation processes including activated sludge (Prado et al., 2009), anammox plants (Shi et al., 2011) and composting (Stone et al., 2009). In addition, concern has been raised about the role of environmental exposure to these therapeutic agents, on the development of resistance to antibiotics by human and veterinary pathogens (Witte, 1998; McManus et al., 2002).

Biopurification systems (BPS), used in agricultural fields for the detoxification of pesticides, are potentially useful for the disposal of antibiotic-containing wastewaters. BPS represent a bioremediation approach, which bases its action on a biomixture that hosts a rich degrading microbiota. The biomixture is composed of three materials: a lignocellulosic substrate, employed to biostimulate lignolytic fungi, widely described as capable to degrade organic pollutants (Yang et al., 2013); soil, pre-exposed to the target pesticides, which provides an adapted degrading-microbial community (Sniegowski et al., 2012); and a humic-rich component to enhance the retention of the pesticides (Karanasios et al., 2012). However, due to the mode of action of BPS, highly dependent on microbial activity, antibiotics could hinder their performance. In this respect, a pretreatment of the antibiotic-containing wastewater before final disposal in the biomixture could prevent subsequent adverse effects on BPS performance.

Previous reports have evaluated the effect of white-rot fungi in order to degrade residues of tetracycline antibiotics. The exposure of high OTC concentrations to crude or purified enzyme extracts from the fungi *Trametes versicolor* and *Phanerochaeta chysosporium* have resulted in antibiotic removals greater than 85% in periods ranging from minutes to one day (Wen et al., 2009, 2010; Suda et al., 2012; Becker et al., 2016). In order to decrease the possible inhibitory effect of OTC on the biomixture, this work aimed to apply a fungal pretreatment to OTC-containing wastewater, using *T. versicolor* pellets in a stirred-tank bioreactor. Then, in order to evaluate the effect of residual OTC on pesticide removal, the treated wastewater from the reactor was discarded into a biomixture used for the degradation of herbicides. Pesticide removal was compared to a system simultaneously exposed to untreated OTC-containing wastewater, and ecotoxicological assays were performed to estimate the environmental suitability of the processes.

2. Materials and methods

2.1. Chemicals and reagents

Commercial formulations of OTC (Terramicina Agrícola®, 5% w/w), ametryn (Agromart®, 50% w/v), and terbutryn (Terbutrex®, 50% w/v) were purchased from a local market. Analytical standards ametryn (*N*2-ethyl-*N*4-isopropyl-6-methylthio-1,3,5-triazine-2,4-diamine; 98.0%) and terbutryn (*N*2-*tert*-butyl-*N*4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine; 98.1%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and Chem Service Inc. (West Chester, Pennsylvania, USA), respectively. OTC standard ((4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,6,10,11,12a-hexahydroxy-6-methyl-,12-dioxo-1,4,4a,5,5a,6,12,12a-octahydrotetracene -2-carboxamide) was obtained from Sigma (St. Louis, Missouri, USA). Carbofuran-d_3 (surrogate standard, 99.5%) and linuron-d_6 (internal standard, 98.5%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Solvents and extraction chemicals are listed in Ruiz-Hidalgo et al. (2014) and Jiménez-Gamboa et al. (2018).

2.2. Fungal strain, culture medium and biomixture

T. versicolor (ATCC 42530) was maintained by subculturing on 2% malt extract agar slants (pH 4.5) at 25 °C. Subcultures were routinely made every 30 d. A mycelial suspension was prepared as described elsewhere (Font Segura et al., 1993) using Sabouraud broth as the culture medium. Pellets of *T. versicolor* were produced by inoculating 1 mL of the mycelial suspension in an Erlenmeyer flask (1 L) containing 250 mL of Sabouraud broth and kept under continuous shaking (130 rpm), in the dark at 25 °C for 7 d. The reactor assays were performed using a chemically defined medium (CDM, composition per liter: 8 g glucose, 1.0 g NH_4Cl, 4 g casein hydrolysate, 0.2 g $CaCl_2$, 0.2 g $MgSO_4$, final pH of 4.5) (Murillo-Zamora et al., 2017). A biomixture containing coconut fiber, compost and soil at a volumetric composition of 45:13:42 (Chin-Pampillo et al., 2015) was employed for the simultaneous treatment of OTC-residues and herbicides.

2.3. Experimental procedures

2.3.1. Removal of OTC in a stirred tank bioreactor with T. versicolor

A 7 L glass stirred tank bioreactor (Applikon Z611000720) equipped with a pH controller (AppliSens Z001032551) was employed. The reactor was aseptically filled with 3 L filtered (0.45 μm) CDM (used as synthetic wastewater) supplemented with 100 mg L^{-1} OTC (commercial formulation) and inoculated with an amount of *T. versicolor* pellets equivalent to 80 g L^{-1} (measured as wet weight). Set point of pH was adjusted at 4.5 ± 0.3 and controlled by the addition of H_2SO_4 2 M or NaOH 2 M. Agitation with a Rushton impeller was set at 350 rpm; aeration was supplied at 3 L min^{-1} and temperature was maintained at 25 °C. The bioreactor was operated in the dark in batch mode for 8 d. Samples were aseptically withdrawn from the reactor for OTC quantification (every 12 h, 10 mL) and seed germination tests (at times 0, 96 and 160 h; 20 mL); at time 96 h, 400 mL pellet-free samples were withdrawn to be used as "treated OTC" in the biomixture assays. A control reactor without fungal pellets was employed to determine abiotic losses of OTC.

2.3.2. Treatment of OTC and OTC residues in a biomixture during co-application of herbicides

The removal of the herbicides ametryn and terbutryn during co-application of OTC or treated OTC (from the bioreactor), was assayed in systems composed of 5.0 g of the biomixture placed in 50 mL polypropylene tubes. Each tube was spiked with the commercial formulation of ametryn (50 mg kg^{-1}) and terbutryn (50 mg kg^{-1}); then, 2.0 mL of the OTC treated sample (96 h) were added to every tube of the treated group, 2.0 mL of a 100 mg L^{-1} OTC solution were added to the untreated group, and 2.0 mL of CDM were added to the negative control (antibiotic-free) group. Agrochemical concentrations were selected based on estimated concentrations found in BPS, according to application indications of the formulations. Triplicate systems of each group were prepared per sampling point. All the tubes were incubated in the dark at (25 ± 1) °C and the remaining concentration of ametryn and