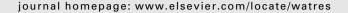


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Biodegradation of the X-ray contrast agent iopromide and the fluoroquinolone antibiotic ofloxacin by the white rot fungus *Trametes* versicolor in hospital wastewaters and identification of degradation products



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ABSTRACT

This paper describes the degradation of the X-ray contrast agent iopromide (IOP) and the antibiotic ofloxacin (OFLOX) by the white-rot-fungus $Trametes\ versicolor$. Batch studies in synthetic medium revealed that between 60 and 80% of IOP and OFLOX were removed when spiked at approximately 12 mg L⁻¹ and 10 mg L⁻¹, respectively. A significant number of transformation products (TPs) were identified for both pharmaceuticals, confirming their degradation. IOP TPs were attributed to two principal reactions: (i) sequential deiodination of the aromatic ring and (ii) N-dealkylation of the amide at the hydroxylated side chain of the molecule. On the other hand, OFLOX transformation products were attributed mainly to the oxidation, hydroxylation and cleavage of the piperazine ring.

Experiments in 10 L-bioreactor with fungal biomass fluidized by air pulses operated in batch achieved high percentage of degradation of IOP and OFLOX when load with sterile (87% IOP, 98.5% OFLOX) and unsterile (65.4% IOP, 99% OFLOX) hospital wastewater (HWW) at their real concentration (μ g L⁻¹ level). Some of the most relevant IOP and OFLOX TPs identified in synthetic medium were also detected in bioreactor samples. Acute toxicity

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tests indicated a reduction of the toxicity in the final culture broth from both experiments in synthetic medium and in batch bioreactor.

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1. Introduction

Large amounts of different pharmaceuticals, belonging to several therapeutic groups, are used worldwide and their sales have been continuously increasing in the last decade (Kummerer, 2001; Verlicchi et al., 2012). Wastewaters are the primary route of entry of pharmaceuticals in the environment and hospitals are considered important sources and significant contributors of pharmaceutical residues in influent municipal wastewater treatment plants (WWTP) (Hawkshead, 2008; Nagarnaik et al., 2012). Despite their specific nature, in many countries, hospital effluents are discharged into public sewer networks and collected to WWTP where they are cotreated with urban wastewater (Verlicchi et al., 2012). Several scientists have questioned this common practice of co-treating hospital and urban wastewaters (Kovalova et al., 2012; Pauwels and Verstraete, 2006), and have recommended a pre-treatment of hospital effluents before being discharged into public wastewaters. Treatment of the wastewater at the source has advantages of avoiding dilution due to mixing with the urban sewage and avoiding losses into the environment due to sewer leakage and combined sewer overflows (Kovalova et al., 2012). In the case of hospital wastewater (HWW), specific concerns are to avoid spread of multiresistant or pathogenic bacteria, viruses and parasite eggs as well as to avoid input of large quantities of pharmaceuticals, diagnostic agents and disinfectants (Kovalova et al., 2012).

Some studies published have reported on the efficiency of several advanced wastewater treatment technologies to remove pharmaceutical residues from HWW (Beier et al., 2011, 2012, 2010; Kovalova et al., 2012; Nielsen et al., 2013). Even though these techniques proved to be very efficient in the removal of recalcitrant pharmaceuticals one of their main limitations may be the formation of undesirable and sometimes toxic by-products (Gomez-Ramos et al., 2011; Trovo et al., 2011).

The ability of white-rot fungi (WRF) to oxidize a large number of organic contaminants from liquid medium has been widely proven (Pointing, 2001). This high degradation capability is attributed to the non-specific nature of their ligninolytic enzymes, which include high redox potential peroxidases and laccases (Asgher et al., 2008; Cerniglia, 1997; Harms et al., 2011). In addition, WRF have the potential to metabolize xenobiotics intracellularly by means of the cytochrome P450 system in a similar way to mammals (Cerniglia, 1997; Doddapaneni and Yadav, 2004). Another advantage of WRF is that they do not require preconditioning to particular pollutants because a number of their ligninolytic isoenzymes are produced constitutively and others after induction by nutrient deprivation (Harms et al., 2011; Munoz et al., 1997).

The high unspecificity of WRF in the removal of organic pollutants from environmental matrices makes these organisms an interesting option to be taken into account for remediation processes. Among the WRF, Trametes versicolor has the advantage to produce pellets when it grows in submerged cultures. In addition it has been proved to be a powerful decontaminant of different types of pollutants such as dyes, UV-filters, chlorobenzenes, polybrominated flame retardants and pharmaceuticals (Badia-Fabregat et al., 2012; Blanquez et al., 2004; Cruz-Morató C., 2012; Marco-Urrea et al., 2009; Rodriguez-Rodriguez et al., 2012). However, studies applying T. versicolor in lab-scale bioreactors are still quite scarce (Blanquez et al., 2008; Cruz-Morato et al., 2013; Jelic et al., 2012; Libra et al., 2003; Yang et al., 2013) and there are few of them working in non-sterile conditions (Blanquez et al., 2008; Cruz-Morato et al., 2013; Libra et al., 2003; Yang et al., 2013). This is the first study showing degradation of pharmaceuticals by WRF in batch bioreactors loaded with real hospital wastewater and at their realistic concentration.

Here, we evaluate the capability of the WRF Trametes versicolor to degrade the X-ray contrast agent iopromide (IOP) and the fluoroquinolone antibiotic ofloxacin (OFLOX) by two different approaches: (i) experiments performed in Erlenmeyer flasks containing defined liquid medium spiked with the target compounds at high concentrations, in order to identify possible transformation products (TPs) and (ii) experiments in a pilot scale bioreactor, operating in batch mode and load with real unspiked hospital wastewater under sterile and non-sterile conditions. IOP and OFLOX were selected because they are ubiquitous pharmaceuticals in hospital wastewaters (Brown et al., 2006; Chang et al., 2010; Perez and Barcelo, 2007), they were detected in the HWW used in the present study (approximately 190 $\mu g L^{-1}$ for IOP and 24 $\mu g L^{-1}$ for OFLOX) and show moderate to low removal efficiency under conventional wastewater treatment processes (Joss et al., 2005; Perez and Barcelo, 2007). TPs formed during the time-course experiments were analyzed and identified. Acute toxicity was tested in order to evaluate the toxicity of the treated aqueous medium.

2. Materials and methods

2.1. Fungus and chemicals

T. versicolor (ATCC#42530) was from the American Type Culture Collection and was maintained by subculturing on 2% malt extract agar slants (pH 4.5) at 25 °C. Subcultures were routinely prepared every 30 days. Pellet production was done as previously described (Blanquez et al., 2004).

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