

# Evaluation of two fungal strains for the degradation of pharmaceutical and personal care products (PPCPs)

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Pharmaceuticals and Personal Care Products (PPCPs) refers to any compound used for personal health or cosmetic reasons. After their consumption, these products are released into the environment at low doses and are hardly degraded in wastewater treatment plant (WWTPs). A potential alternative to attain effective degradation may be based on the use of white-rot fungi (WRF), a group of microorganisms capable of degrading recalcitrant compounds, thanks to the enzymes that they secrete. The objective of this work was to evaluate the potential of elimination of seven PPCPs by two fungal strains, an anamorph of *Bjerkandera* sp. R1 and *Phanerochaete chrysosporium*. The results reported total elimination of Diclofenac (DCF), Ibuprofen (IBP), Naproxen (NPX), Carbamazepine (CBZ) and the fragrances (ADBI, HHCB and AHTN) by the two fungal strains after 14 days of incubation in cultures with pellets; whereas the tranquilizer Diazepam (DZP) was partially removed in percentages from 27% up to 54%. In the case of cultures with immobilized fungi, the anti-inflammatories were the only compounds totally removed; meanwhile CBZ, DZP and the fragrances were partially removed demonstrating a partial removal of 44%.

## 1. Introduction

Pharmaceutical and Personal Care Products (PPCPs) are a wide group of chemicals compounds used for human and veterinary medicine, but also added as fragrances in cosmetic, perfumes and household products (Ternes and Joss, 2006). After its administration the molecules are absorbed, distributed, metabolized and finally excreted into the environment across the urine and feces (Ikehata *et al.*, 2006). Several studies have been carried out regarding the degradation of these compounds in Sewage Treatment Plants (STPs) demonstrating significant concentrations in the effluents (Joss *et al.*, 2006). Membrane filtration and activated carbon proved remarkable removal efficiencies for IBP and NPX; however the elimination is limited for CBZ, DCF and fragrances (Reif *et al.*, 2008; Ternes and Joss, 2006). By means of physicochemical processes such as coagulation-flocculation and flotation, removal rates between 45% and 60% were achieved for DZP, DCF and the fragrances; whereas CBZ, IBP and NPX were the most persistent (<35%)

(Carballa *et al.*, 2005). In a nitrifying-denitrifying process the results showed that CBZ and DZP were degraded less than 10% (Suárez *et al.*, 2005). However, compounds such as CBZ could be oxidized using Advanced Oxidation Processes (AOPs), though this technology is still rather expensive (Ternes *et al.*, 2003). Recently, the ability of four white-rot fungi (WRF) was studied for the elimination of pharmaceutical compounds, showing that IBP could be degraded by all the strains, including *P. chrysosporium*, meanwhile CBZ showed a recalcitrant behavior, only *T. versicolor* attained significant degradation (Marco-Urrea *et al.*, 2009). This capacity is related to the secretion of oxidative enzymes such as Lignin Peroxidase, Manganese Peroxidase, Versatile Peroxidase and Laccase (López *et al.*, 2004; Eibes *et al.*, 2005; Feijoo *et al.*, 2008). The aim of this work is to evaluate the ability of two fungal strains, an anamorph of *Bjerkandera* sp. R1 and *P. chrysosporium*, to eliminate a wide range of PPCPs belonging to different therapeutic groups: anti-inflammatories (DCF, IBP and NPX), anti-epileptics (CBZ), tranquilizers (DZP) and fragrances (ADBI, HHCB and AHTN) in cultures with pellets and immobilized fungi.

## **2. Materials and methods**

### **2.1 PPCPs and chemicals**

The PPCPs used in this work were: Diclofenac (DCF), Ibuprofen (IBP), Naproxen (NPX), Carbamazepine (CBZ) all from Sigma-Aldrich, grade pure; Diazepam (DZP) from Roche farma, grade pure; Celestolide (ADBI), Galaxolide (HHCB) and Tonalide (AHTN) from LGC-Promochem, 98% pure. The chemicals used for the extraction were: Acetone, Ethyl acetate, n-hexane, Acetonitrile all from J.T. Baker (99.5% pure) and Methanol from J.T. Baker (HPLC grade 99.8%).

### **2.2 Microorganisms**

Two fungal strains, an anamorph of *Bjerkandera* sp R1 and *Phanerochaete chrysosporium* BKM-F-1767 (ATCC 24725) were cultured in plates with agar (15 g/L), glucose (10 g/L) and malt extract (3.5 g/L) and incubated at 30°C for 7 days. Then, 3 plugs of agar with active fungus were transferred to a Fernbach containing 100 mL of modified Kirk culture medium (Tien and Kirk, 1988) and incubated at 30°C for 7 days until the experiment start.

### **2.3 Culture conditions**

The experiments were carried out in Erlenmeyer flasks (250 mL) containing 90 mL of modified Kirk culture medium and then were inoculated with 9 mL of homogenized mycelium from the Fernbach. For the immobilized fungi, the same procedure was carried out, adding polyurethane foam cubes (0.015 g/mL) to each flask before the inoculation. The flasks were agitated at 150 rpm at 30°C during 3 days until the pellets were formed and the fungi were immobilized, then the PPCPs solution was added (1 mg/L of each compound). The experiment was developed by triplicate together with abiotic controls to verify any possible adsorption or evaporation of the compounds. The flasks were incubated at 30°C at 150 rpm and the samples were taken after 2 hours, 4, 7 and 14 days of the experiment.

## 2.4 Extraction and determination of PPCPs

After incubation, 100 mL of Acetonitrile were added for the extraction; the flasks were sealed with teflon and then were agitated for 2 hours at 180 rpm in a shaker (Ika Labortechnik, HS 501 Digital, Germany). Then 10 mL were taken from every flask and were centrifuged in glass tubes for 15 minutes at 7000 rpm. A sample was taken out and the soluble content of the seven PPCPs was determined after solid-phase extraction (SPE) using 30 mg OASIS HLB cartridges (Waters closet, Milford, USA) previously conditioned with ethyl acetate, methanol and distilled water (pH 2.5). After the samples passed through the cartridges, 10 mL of distilled water (pH 2) was added to avoid any possible interference. The cartridges were then dried with a stream of nitrogen for 45 min and eluted with 3 mL of ethyl acetate. This extract was divided into two fractions: one of them for the determination of DCF, IBP and NPX, adding 200  $\mu$ L of MTBSTFA (N-Methyl-N-(tert-butyl)dimethylsilyl)trifluoroacetamide) for derivatization and then the sample was placed in a GC oven at 60°C for 1 hour. On the other hand, the remaining fraction was used for the determination of CBZ, DZP, ADBI, HHCB and AHTN, where an aliquote of 0.8 mL was taken from the extract, then 0.7 mL of ethyl acetate were added and 10  $\mu$ L of PCB 30 (99.5%), so the sample was ready for the GC-MS analysis (Rodríguez *et al.*, 2003; Reif *et al.*, 2008).

## 3. Results

### 3.1 Elimination of PPCPs by pellets of two fungal strains

Figure 1 shows the elimination of the DCF, IBP, NPX, CBZ, DZP, ADBI, HHCB and AHTN after 14 days of incubation in presence of pellets of an anamorph of *Bjerkandera* sp. R1 and *P. chrysosporium*.

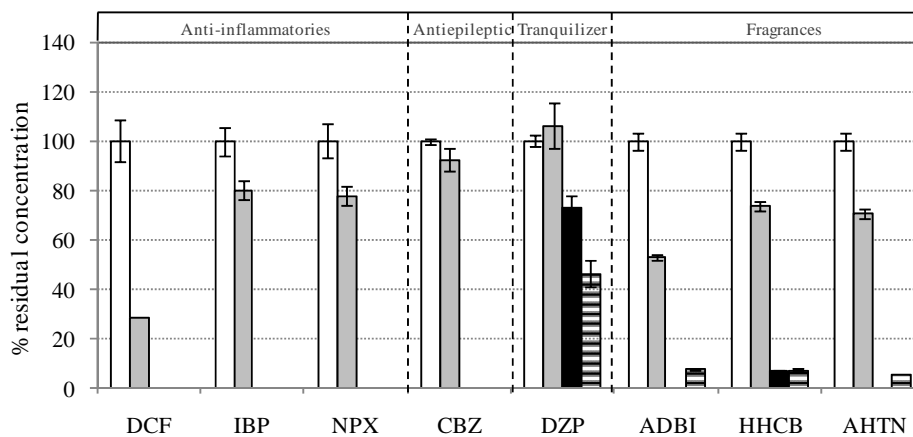


Figure 1. Residual concentration of PPCPs after 14 days of treatment with pellets from fungi. Symbols:  $\square$  Control (day 0);  $\blacksquare$  Control (day 14);  $\blacksquare$  Anamorph of *Bjerkandera* sp. R1 (day 14);  $\blacksquare$  *P. chrysosporium* (day 14).

The results show that the anti-inflammatories (DCF, IBP, and NPX) were totally removed even at day 4<sup>th</sup> (Data not shown). The residual percentage of these compounds in the abiotic controls was of 80% except in the case of DCF, which showed a decrease of its initial concentration (> 70%). In the case of recalcitrant compounds such as CBZ and DZP, a total elimination of the former was achieved by the two strains; whereas, the latter was partially degraded by the anamorph of *Bjerkandera* sp. R1 and *P. chrysosporium* (73% and 46% of residual percentage, respectively). After evaluated the concentration ADBI, HHCB and AHTN, the results show that these compounds were eliminated after 4 days of incubation (Data not shown), while the initial concentration of abiotic controls suffered a slight decline (47%, 26% and 29%, respectively).

### 3.2. Elimination of PPCPs by two fungal strains immobilized in polyurethane foam

Previous assays were conducted with and without extraction using acetonitrile, to prove that this solvent is able to extract the compounds that might be adsorbed on polyurethane foam (Data not shown). In this work, after 2 weeks of incubation in flasks with immobilized fungi, the elimination of DCF, IBP, NPX, CBZ, DZP, ADBI, HHCB and AHTN was evaluated (Figure 2). The initial concentration in the abiotic controls was maintained, except in the case of NPX and CBZ (~50% of residual percentage).

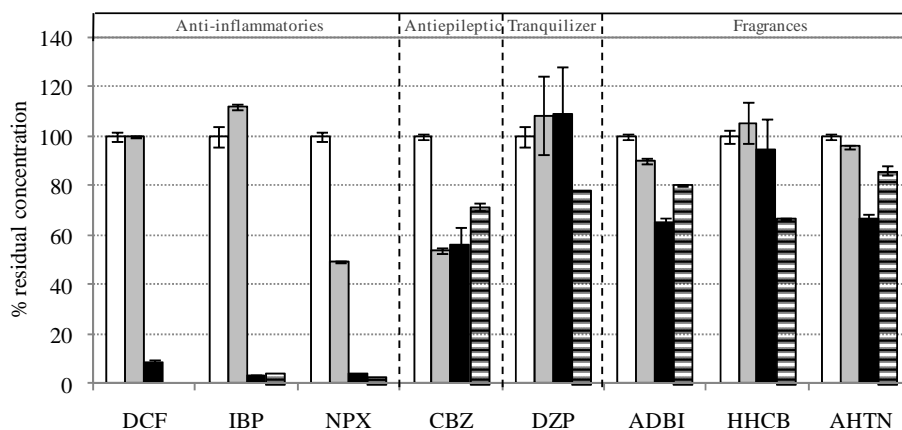


Figure 2. Residual concentration of PPCPs after 14 days of treatment with immobilized fungi in polyurethane foam. Symbols: □ Control (day 0); ■ Control (day 14); ■ Anamorph of *Bjerkandera* sp. R1 (day 14); ▨ *P. chrysosporium* (day 14).

In presence of the immobilized anamorph of *Bjerkandera* sp. R1 and *P. chrysosporium*, compounds such as DCF, IBP and NPX were totally removed, even at day 7 (Data not shown). These results are in agreement with those obtained in this work using pellets. The initial concentration of the abiotic controls was maintained at the end of the experiment, except in the case of NPX (49% residual concentration). After 14 days of incubation the antiepileptic CBZ was removed partially by immobilized anamorph of *Bjerkandera* sp. R1

and *P. chrysosporium* (44% and 28%, respectively), whereas the tranquilizer DZP was recovered totally in presence of the anamorph, and a slightly elimination (21%) was achieved in presence of *P. chrysosporium*. The three fragrances could not be eliminated by the immobilized fungi showing residual percentage of 65% - 95%, while the concentration in the abiotic control was maintained after 14 days (up to 90%) (Figure 2).

#### 4. Discussion

In this work, the elimination of seven PPCPs was evaluated using cultures with pellets and immobilized fungi. In the case of anti-inflammatories (DCF, IBP and NPX), previous studies using physicochemical and biological treatments demonstrated that IBP and NPX could be eliminated in a 55% and 70%, respectively, in WWTPs effluents (Carballa *et al.*, 2004). Using a membrane bioreactor (MBR), Reif *et al.* (2008) attained high levels of elimination for the same compounds, while for DCF only 10% was achieved. Using advanced oxidation process (AOPs) such as UV irradiation and ozonation, Gagnon *et al.* (2008) demonstrated removal efficiencies of 70%. The results obtained in this work was larger than the previous reported, since the three compounds were totally eliminated, even at day 4<sup>th</sup>, in both cultures. However, the initial concentration of DCF and NPX in the abiotic controls with pellets and immobilized fungi, respectively, showed a substantial decrease. Recalcitrant compounds such as CBZ and DZP, two of the most persistent pharmaceuticals in the environment, were partially degraded in this work. As it was mentioned, using pellets of both fungal strains, the antiepileptic was degraded totally after 14 days, whereas DZP was partially eliminated. In the case of immobilized fungi, the elimination rates were below 50% for CBZ and 20% for DZP. However, this results are greater than those reported in previous studies where degradation rates from 9% up to 35% for both compounds were achieved using an anaerobic digestion, nitrifying-denitrifying plants, membrane bioreactor and two physicochemical processes: coagulation-flocculation and flotation (Carballa *et al.*, 2005; Reif *et al.*, 2008; Suárez *et al.*, 2005; Joss *et al.*, 2006). Using WRF, Marco-Urrea *et al.* (2009) reported high degradation percentages between 47% to 58% for CBZ. In the case of the fragrances, these compounds are found in household detergents, perfumes and cosmetics (Peck *et al.*, 2006). In the case of fragrances, the treatment with pellets reported a total removal, however the initial concentration in the abiotic controls showed a slightly decrease. By contrast, in the case of immobilized fungi, abiotic controls retained their initial concentration and these compounds could not be eliminated. These compounds could be attached to the polyurethane foam, which could be attributed to their lowest solubilities in water and by their strong lipophilic character (Suárez *et al.* 2008). In this work the use of pellets from an anamorph of *Bjerkandera* sp. R1 and *P. chrysosporium* achieved higher rates of removal of seven compounds analyzed.

## 5. References

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