Science of the Total Environment 610–611 (2018) 1147–1153

Contents lists available at ScienceDirect

Science of the Total Environment

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

# The role of sorption processes in the removal of pharmaceuticals by fungal treatment of wastewater

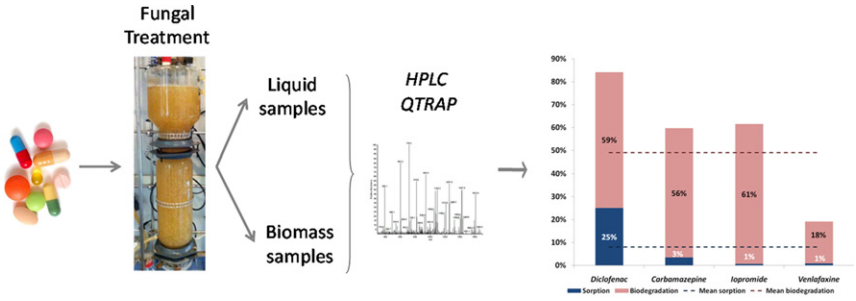
D. Lucas [a], F. Castellet-Rovira [b], M. Villagrasa [a], M. Badia-Fabregat [b], D. Barceló [a,c], T. Vicent [b], G. Caminal [d], M. Sarrà [b], S. Rodríguez-Mozaz [a,*]

[a] Catalan Institute for Water Research (ICRA), H2O Building, Scientific and Technological Park of the University of Girona, 17003 Girona, Spain
[b] Departament d'Enginyeria Química, Biològica i Ambiental, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Catalonia, Spain
[c] Water and Soil Quality Research Group, Department of Environmental Chemistry (IDAEA-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain
[d] Institut de Química Avançada de Catalunya (IQAC) CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

## HIGHLIGHTS

- Analytical methodology for PhACS in fungal biomass was developed.
- Pharmaceuticals sorbed in the biomass in degradation experiments was measured.
- Between 3 and 13% of PhACs elimination can be attributed to sorption processes.
- Accumulation of PhACs in fungi is similar to that in sludge of conventional treatments.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 14 April 2017
Received in revised form 11 August 2017
Accepted 12 August 2017
Available online 30 August 2017

Editor: D. Barcelo

*Keywords:*
Wastewater treatment
Trametes versicolor
Emerging contaminants
Water quality
Chromatography
Sludge

## ABSTRACT

The contribution of the sorption processes in the elimination of pharmaceuticals (PhACs) during the fungal treatment of wastewater has been evaluated in this work. The sorption of four PhACs (carbamazepine, diclofenac, iopromide and venlafaxine) by 6 different fungi was first evaluated in batch experiments. Concentrations of PhACs in both liquid and solid (biomass) matrices from the fungal treatment were measured. Contribution of the sorption to the total removal of pollutants ranged between 3% and 13% in relation to the initial amount. The sorption of 47 PhACs in fungi was also evaluated in a fungal treatment performed in 26 days in a continuous bioreactor treating wastewater from a veterinary hospital. PhACs levels measured in the fungal biomass were similar to those detected in conventional wastewater treatment (WWTP) sludge. This may suggest the necessity of manage fungal biomass as waste in the same manner that the WWTP sludge is managed.

© 2017 Elsevier B.V. All rights reserved.

## 1. Introduction

The concern about pharmaceutical contamination of the environment has increased over the last years. Several studies have shown that conventional technologies used in wastewater treatment plants (WWTPs) are not effective enough to degrade pharmaceutically active

* Corresponding author.
*E-mail address:* srodriguez@icra.cat (S. Rodríguez-Mozaz).

http://dx.doi.org/10.1016/j.scitotenv.2017.08.118
0048-9697/© 2017 Elsevier B.V. All rights reserved.

compounds (PhACs) because they are not designed to remove such compounds particularly (Verlicchi et al., 2012; Frédéric and Yves, 2014). As a consequence, new wastewater treatment technologies have been investigated and tested in order to provide higher removal efficiency of these compounds. Among them, fungal treatment of wastewaters has been pointed out as a promising technology due to the unspecific enzymatic system (in the case of lignolytic fungi), which is able to degrade a wide range of xenobiotics even at very low concentrations (Pointing, 2001; Guillén et al., 2005; Asgher et al., 2008). Many studies have been performed focusing on the fungal treatment of PhACs (Tran et al., 2010; Feijoo et al., 2011; Rodríguez-Rodríguez et al., 2012b; Yang et al., 2013; Cruz-Morató et al., 2014; Gros et al., 2014; Badia-Fabregat et al., 2015a, 2015b) showing quite good PhACs removal values specially when using the white rot fungi *Trametes versicolor*. However, there are still many questions to be answered regarding the fungal treatment, e.g., the role of the sorption processes in contrast to biodegradation processes in the elimination of the pollutants. Sorption in this article gathers both absorption (entry of pollutants inside the biomass) and adsorption (adhesion of pollutants to the biomass surface). Sorption processes in fungal treatment have been studied for contaminants such as textile dyes (Wang and Yu, 1998; Blanquez et al., 2004; Bayramoğlu and Arıca, 2007), personal care products (Badia-Fabregat et al., 2012) and some specific PhACs (Marco-Urrea et al., 2009; Marco-Urrea et al., 2010; Tran et al., 2010). However, in these studies, sorption evaluation was done indirectly, i.e., setting up a control flask with thermal inactivated fungus, and measuring the pollutants concentration in the water at the beginning and at the end of the experiment; the difference in concentration is then attributed to sorption processes assuming that biodegradation processes are not taking place. In contrast, no study in the literature so far has investigated the sorption of organic micropollutants in fungal biomass by means of direct measurement of these compounds in the solid phase.

The main objective of the present work was to evaluate the role of the sorption processes in the removal of PhACs during fungal treatment. Direct measurement of contaminants in fungal biomass were performed considering two different types of experiments; namely batch experiments with different fungi performed with spiked synthetic water and experiments in a continuous bioreactor with the fungus *T.versicolor* for the treatment of veterinary hospital wastewater (VHW).

## 2. Materials and methods

### 2.1. Chemicals and reagents

All PhACs standards and isotopically labelled compounds, used as internal standards, were of high purity grade (>90%). Compounds were purchased from Sigma–Aldrich (Steinheim, Germany), US Pharmacopeia USP (MD, USA), European Pharmacopeia EP (Strasbourg, France), Toronto Research Chemicals TRC (Ontario, Canada) and CDN isotopes (Quebec, Canada). Quantification parameters and internal standards used can be found in table S1.

Individual stock standard, isotopically labelled internal standard and surrogate solutions were prepared on a weight basis in methanol (at a concentration of 1000 mg $L^{-1}$), except ofloxacin and ciprofloxacin, which were dissolved in methanol adding 100 μL of NaOH1 M, and cefalexin, which was solved in HPLC grade water, as described in the literature (Ibáñez et al., 2009; Kantiani et al., 2009), since these substances are barely soluble or insoluble in pure methanol. After preparation, standards were stored at −20 °C.

Working standard solutions, containing all compounds, were also prepared in methanol/water (10:90, *v*/v). Separate mixtures of isotopically labelled internal standards, used for internal standard calibration, and surrogates, were prepared in methanol and further dilutions were also prepared in a methanol/water (10:90, *v*/v) mixture (Gros et al., 2012).

Fiberglass GF/A filters (1 μm) and nylon membrane filters (0.45 μm) were purchased from Whatman (London, UK) and Millex Millipore (Barcelona, Spain) respectively. Glucose, ammonium tartrate dibasic and malt extract were purchased from Sigma Aldrich (Barcelona, Spain).

### 2.2. Experimental design

The monitoring of sorption processes was performed in two different fungal treatment experiments: i) Batch experiments with 6 different fungi performed with synthetic medium spiked with selected PhACs ii) Fungal bioreactor experiment performed with VHW.

#### 2.2.1. Batch experiments with fungi and spiked synthetic medium

Six different species of fungi were used in this study, three white-rot fungi (WRF): *Trametes versicolor* (ATCC #42530 strain), *Irpex lacteus* (AX1 strain) and *Ganoderma lucidum* (FP-58537-Sp strain); and three litter decomposing fungi (LDF): *Stropharia rugosoannulata* (FBCC 475 strain), *Gymnopilus luteofolius* (FBCC 466 strain) and *Agrocybe erebia* (FBCC 476 strain). Experimental details are summarized below and further information can be found in the work by Castellet-Rovira et al. (2017).

All these fungi were subcultured on 2% malt extract agar petri plates (pH 4.5) at 25 °C. Pellet production was achieved for all the fungi, and it was done following the same procedure as previously described Font et al. (2003).

The experiments were performed in 250 mL Erlenmeyer flasks, an amount of mycelial pellets (a mean of 0.5 ± 0.1 g in dry weight) was added in 100 mL of defined medium, consisting of 8 g $L^{-1}$ of glucose, 3.3 g $L^{-1}$ of ammonium tartrate, 1.168 g $L^{-1}$ of 2,2-dimethylsuccinate buffer and 1 and 10 mL of a micro and macronutrient solution from Kirk medium (Kirk et al., 1978). The inoculums were always the same mass of pellets but measured as wet weight and later translate to dry weight by the ratio wet weight/dry weight measured in each set of experiments and each fungi. The medium was prepared in ultrapure water, so initial COD was related to the glucose concentration, this is about 8500 mg $O_2$ $L^{-1}$, but this is mainly reduced up to the end of the experiment because the glucose is uptake by fungi and no glucose is detected at day 6. Four selected PhACs were added to the defined medium reaching a final individual concentration between 47 and 184 μg $L^{-1}$. The water was spiked at slightly higher concentrations than those commonly found in wastewater treatment plants. However, concentrations were low enough to avoid any possible toxic effect of the PhACs on the fungi (Castellet-Rovira et al., 2017). The pollutants present in the stock solution included diclofenac (anti-inflammatory), carbamazepine (anticonvulsant), venlafaxine (antidepressant) and iopromide (media contrast agent). PhAcs were selected based on its ubiquity in hospital wastewater effluents and low biodegradability exhibited in previous fungal treatments performed with *T. versicolor* (Cruz-Morató et al., 2014; Ferrando-Climent et al., 2015). After fungus inoculation and PhACs addition, flasks were incubated under orbital shaking (135 rpm) at 25 °C for six days in dark conditions to prevent a possible photodegradation on compounds.

Apart from the experimental treatment (*Exp*) above described, heat-killed (*KC*) control, and abiotic control were performed. Heat-killed controls consisted on autoclaved cultures, which were set-up under identical conditions to those of the *Exp* cultures. These experiments allowed us to evaluate sorption differences between *Exp* and *KC* samples. Abiotic control cultures were performed in the same manner as the *Exp* culture but without fungus. These experiments were intended to account the degradation of the contaminants due to physico-chemical processes. Each treatment was done in triplicate for each fungus.

Water samples and fungal biomass samples were taken, after 15 min and 6 days of PhACs addition in the cultures. Fungal biodegradation is a quite slow process, so 15 min are considered enough time to ensure homogeneity in the concentration of the flask and too short to detect a

significant adsorption process. On the contrary, according our experience, 5 days is the time that takes to uptake the added glucose and to decrease the degradation rate. So we decided to take the sample after 6 days of treatment to ensure the accomplishment of both objectives: to uptake glucose and to measure degradation yield. Biomass samples were taken at the end of the experiment since in the original experiment (Castellet-Rovira et al., 2017) the analysis of the sorption kinetics was not considered. In both cases, the whole content of the flasks was sacrificed at each sampling time. All samples were filtered through 1 μm fiberglass in order to separate the fungal biomass from water fraction. Liquid samples were kept frozen whereas solid samples were first freeze dried and then kept frozen until their analysis.

*2.2.2. Continuous bioreactor with T.versicolor and VHW*

Two air-pulsed glass bioreactors (1.5 L) (Blánquez et al., 2006) were set up in parallel, one inoculated with pellets of *T.versicolor* and the other non-inoculated as a control, for 26 days (Badia-Fabregat et al., 2015b). *T.versicolor* was chosen among the other fungi considered in this article because of its great yield in terms of PhACs removal (Castellet-Rovira et al., 2017) and to take advantage of the expertise of the group in performing experiments with this fungus. The applicability of fungal treatment technology has already been studied for *T.versicolor*, for treating wastewater containing pharmaceuticals in non-sterile conditions (Cruz-Morató et al., 2013; Cruz-Morató et al., 2014; Badia-Fabregat et al., 2012; Badia-Fabregat et al., 2015a, 2015b). In the inoculated bioreactor, fungal pellets were maintained fluidized through the air pulses. Wastewater was obtained from a veterinary hospital located in the Universitat Autònoma de Barcelona campus (Bellatera, Barcelona, Spain) (Table S2) the same day that each bioreactor was set up and also once a week during continuous bioreactors operation; this veterinary hospital receives 15,455 visitors per year: 12,435 dogs, 859 cats, 300 horses and 1861 exotic animals. The bioreactors were filled with 1.5 L of VHW, temperature was set up at 25 °C, and pH kept constant at 4.5 ± 0.5 by adding HCl 1 M or NaOH 1 M. Fungal pellets were kept inside the bioreactor by means of a mesh. *T. versicolor* was initially added at 3.7 g dry cell weight (DCW) $L^{-1}$. However, every 2–5 days, approximately 1/3 of the biomass was replaced by fresh one as determined by Blánquez et al. (2006). Hydraulic retention time (HRT) was 3.3 days. Glucose and ammonia tartrate were added at rates of 343–1040 and 0.77–433 mg g $DCW^{-1}$ $day^{-1}$, respectively, according to the nutritional requirements (Badia-Fabregat et al., 2015b), and in pulses of 0.6 min $h^{-1}$ from a concentrated stock. VHW in the feed storage tank was replaced every 2–3 days by fresh one stored at 4 °C. A more detailed description of the experimental set-up can be found elsewhere Badia-Fabregat et al. (2015b).

Finally, both fungal biomass from the inoculated bioreactor (inoculated biomass) and biomass from the non-inoculated control bioreactor (control biomass), were collected through a filtration system at the end of the experiment (26 days) and then were frozen and kept at −20 °C until analysis. Samples were taken at the end of the experiment because no stationary state was achieved because different nutrient additions were being tested during the process to determine optimal conditions for fungal survival. It was at the end of the experiment when the conditions were fixed and no removal of biomass was done; therefore it was the period that can be considered as stationary (Badia-Fabregat et al., 2015b)

*2.3. Sample pre-treatment*

For the analysis of micropollutants in the fungal biomass samples, an extraction methodology was previously adapted and implemented. Three extraction methodologies, initially developed for the analysis of several pollutants (biocides and pharmaceuticals) in solid samples, were selected from the literature to be tested (Capone et al., 1996; Jelić et al., 2009; Rubilar et al., 2011). A fourth methodology was also tested following the scheme of Capone et al. (1996) but using a different extraction solvent (MeOH-$Na_2$EDTA). Methods description is summarized in table S3. The latest extraction methodology exhibited the best recovery values for the selected PhACs (Fig. S1) and was thus used in the present work for the analysis of micropollutants in biomass samples from both experiments, batch experiment with different fungi and continuous bioreactor with *T.versicolor* and VHW.

In Capone et al. (1996) adapted methodology, the biomass was freeze dried and then homogenized using a Robot Coupe Blixer food processor (Robot Coupe USA, Jackson, MS) and a mortar. For each sample, 1 g of biomass was used; then 4 mL of MeOH-$Na_2$EDTA (50:1.5 *v*/v) was added and vortexed for 30 s. Later on, samples were sonicated for 3 min and centrifuged at 1500 rpm for 5 min at 5 °C. The supernatant was kept together with the pellet, and the procedure was repeated twice more using 3 mL of MeOH-$Na_2$EDTA each time. The total resulting supernatant was centrifuged at 3200 rpm for 20 min, decanted, filtered with nylon membrane filters, evaporated under nitrogen stream using a Reacti-Therm 18,824 system (Thermo Scientific) and reconstituted with 1 mL of methanol-water (10:90 *v*/v). Lastly, 10 μL of internal standard mix at 10 ng $\mu L^{-1}$ were added in the extracts for internal standard calibration. $Na_2$EDTA were added since it has been observed that it considerably improves extraction efficiency of antibiotics as well as other pharmaceuticals (Yang et al., 2005; Hernández et al., 2007). This is attributed to the fact that these compounds can potentially bind residual metals present in the sample matrix and glassware, resulting in low extraction recoveries. By adding $Na_2$EDTA, soluble metals are bound to the chelating agent, increasing the extraction efficiency of PhACs (Gros et al., 2012).

*2.4. PhACs quantification*

Quantification of the PhACs in both experiments was performed according Gros et al. (2012) procedure, with an ultraperformance liquid chromatography system (Waters Corp. Mildford, MA, USA), using an Acquity HSS T3 column (50 × 2.1 mm i.d. 1.7 μm particle size) for the compounds analyzed under positive electrospray ionization (PI) and an Acquity BEH C18 column (50 × 2.1 mm i.d., 1.7 μm particle size) for the ones analyzed under negative electrospray ionization (NI), both from Waters Corporation. The UPLC instrument was coupled to 5500 QqLit, triple quadrupole linear ion trap mass spectrometer (5500 QTRAP, Applied Biosystems, Foster City, CA, USA) with a Turbo V ion spray source. Two MRM transitions per compound were recorded by using the Scheduled MRM™ algorithm, and the data were acquired and processed using the Analyst 2.1 software.

Analytical quality parameters of the analytical methodologies applied can be found in table S1 but briefly: the limit of detection (LOD) achieved range from 0.001 to 0.08 μg $L^{-1}$ for the water samples and from 0.001 to 0.1 ng $g^{-1}$ for the biomass samples. The recoveries ranged from 26 to 117% in the water samples and from 25 to 132% for the biomass samples.

*2.5. Sorption and biodegradation calculation*

In order to determine the role of biodegradation and sorption processes in the elimination of PhACs in the batch experiments with fungi, the following calculations were performed: Total elimination for each PhAC was first calculated as Eq. (1):

$$E = (C_{wi} - C_{wf}) \cdot V \quad (1)$$

where E is total elimination (ng), $C_{wi}$ and $C_{wf}$ the concentration (ng $mL^{-1}$) of each PhAC measured in the water samples (Table S4) at the beginning and at the end of the experiment respectively, and V is the volume of the water in each flask (mL). PhACs sorbed for each compound can be expressed as Eq. (2)

$$A = C_{bf} \cdot B_f \quad (2)$$

where A is the amount of PhAC sorbed (ng), $C_{bf}$ is the concentration (ng $g^{-1}$) of each PhAC measured in the biomass at the end of the experiment, and $B_f$ the amount of biomass (g) at the end of the experiment. Finally, the biodegradation for each PhAC were calculated as it can be seen in Eq. (3):

$$Bd = E - A \quad (3)$$

where Bd is the quantity of PhAC (ng) eliminated by biodegradation, E is the total amount of PhAC eliminated (ng), and A is the quantity of PhAC (ng) eliminated by sorption processes.

Solid–water partition coefficients ($K_d$), (Ternes et al., 2004) which defines the distribution of a compound between water and solid phase, were calculated from PhACs concentrations in water and biomass samples obtained at the end of the experiment in the continuous bioreactor. $K_d$ takes into account both absorption and adsorption and is used to evaluate the overall sorption in solids exposed to different concentration of pollutants in the liquid phase. This coefficient takes into account both absorption and adsorption and is used when evaluating the sorption in solids exposed to different concentration of pollutants in the liquid phase. It was used with the biomass of the continuous bioreactor at the end of the experiment in order to have a picture of the sorption of contaminants at the end of the experiment;

$$K_d = \frac{C_{sorbed}}{C_{soluble}}$$

where $C_{sorbed}$ is the sorbed PhACs concentration onto biomass (μg $Kg^{-1}$) and $C_{soluble}$ the dissolved concentration of the compound (μg $L^{-1}$).

### 2.6. Statistical analysis

Values were compared using Student's *t*-test, in which $p < 0.05$ was considered significant (IBM SPSS Statistics 21.0 software; IBM, Chicago, IL, USA).

## 3. Results and discussion

### 3.1. Batch experiments with fungi and spiked synthetic medium

In general terms, results obtained from the abiotic cultures reveal no PhACs degradation attributed to physico-chemical processes such as volatilization, thermal degradation and sorption in the bioreactor (Castellet-Rovira et al., 2017). Therefore, PhACs elimination observed in *Exp* cultures can only be associated to biodegradation and/or sorption processes. The role of sorption was thus calculated (as explained in section 2.5) based on the results obtained from the measurements of PhACs both, in liquid and fungal phase after 6 days of treatment (Table S4). The sorption contribution in the PhACs elimination ranged from 3% to 13% (Fig. S2). The contribution of the sorption processes to overall removal was different depending on the fungus considered; many factors are actually involved in the sorption process and specific interactions between PhACs and the surface components of each fungus can occur. The fungi can be ordered from most to least efficient as follows: S.*rugosoannulata* *G.luteofolius*, *T.versicolor*, *G.lucidum*, *A.erebia* and *I.lacteus*; *S.rugosoannulata* was the most effective fungus in terms of elimination of the 4 selected PhACs (75%), but also in terms of biodegradation; 72% biodegradation + 3% sorption (Fig. S2). On the contrary, *I.lacteus* exhibited the lowest elimination value, 44% (37% biodegradation + 7% sorption) (Fig. S2). Comparing biodegradation and sorption values it can be highlighted that, in the experiments with *S.rugosoannulata* the sorption processes has less relevance to the total elimination observed; only 4% of total elimination can be attributed to sorption processes while 96% of the elimination is due to biodegradation. In contrast; the relevance of the sorption processes achieves the highest values in the case of *G.lucidum*. For this fungus, 26% of total elimination can be attributed to sorption processes and 74% can be attributed to biodegradation.

Behaviour of each compound in the six different fungal experiments was also evaluated (Figs. 1 and 2). Diclofenac was the compound with the highest sorption in the fungal biomass. Up to 9153.2 ng $g^{-1}$ (33% of initial amount) of this compound were retained in *A.erebia* biomass, being the average retained concentration of diclofenac, considering all fungi, 5213.2 ng $g^{-1}$ (25% of initial amount). These high diclofenac sorption values are in agreement with those exhibited in previous fungal 80% (Marco-Urrea et al., 2010) and conventional activated sludge (CAS) treatments (Radjenović et al., 2009). The great sorption of diclofenac in the present experiment might be due to its high partition coefficient (log Pat pH 4.5 = 3.6) indicating its hydrophobic character (Mannhold et al., 2009). In contrast, iopromide and venlafaxine present the lowest concentrations in the fungal biomass (212.5 and 202.5 ng $g^{-1}$), c.a. 1% of the initial amount for each compound (Fig. 2), which is in agreement with the low sorption shown by these compounds in sludge, reported in studies performed with CAS (Batt et al., 2006; Joss et al., 2008). The high hydrophilicity of iopromide (log P at pH 4.5 = −0.44) and venlafaxine (log P at pH 4.5 = −0.69) may explain the low concentrations found for these compounds in the fungal biomass.

Sorption values obtained in the *Exp* treatments were compared with those sorption values of *KC* treatments, and the feasibility of launching *KC* experiments to calculate indirectly the sorption contribution in the total removal of PhACs was also evaluated. *KC* treatments are very common as control treatment in biodegradation experiments; they are frequently used to evaluate the contribution of the sorption processes in the elimination of the pollutants. Sorption of the active biomass is thus considered to be the same as the sorption of the inactive (killed control) biomass. Therefore, the difference in elimination in both treatments is attributed indirectly to biodegradation processes. However this approach might sometimes lead to some inaccuracies, since the structure of biomass, and therefore their sorption capacities, may change according to the inactivation mechanism. It is indeed well known that, e.g., some fungal cells exposed to heat treatments alter the physicochemical properties of their surfaces leading to a greater, equivalent or less bioadsorptive capacities than that of living cells depending on the pollutant (Arıca et al., 2003; Bayramoğlu and Arıca, 2007). In addition, active transport in living cells may play an important role in the sorption processes, which is inactive in the killed control culture. The biodegradation values obtained measuring both types of matrices (liquid and solid) from the *Exp* culture (direct method), were compared (Table 1) with the biodegradation values calculated using the KC culture to distinguish between biodegradation and sorption (indirect method) (Castellet-Rovira et al., 2017). For most of the compounds, degradation percentages calculated using both strategies were similar (Table 1). However, for some compounds the differences were quite remarkable, like in the case of the degradation of diclofenac by *I.lacteus* (42% degradation calculated through the direct method and 71% with the indirect method), or the degradation of venlafaxine by *G.luteofolius* (15% of degradation calculated with the direct method and no degradation measured with the indirect method). These differences detected can be attributed, on one hand, to the different sorption values between the active and the inactive biomass. On the other hand, biodegradation processes of absorbed compounds can occur in the active biomass due to intracellular enzymes, whereas no degradation occurs in inactivated biomass, thus affecting the distribution of the contaminants in the two compartments.

### 3.2. Continuous bioreactor with T.versicolor and VHW

28 out of 47 compounds analyzed, were detected in the biomass sampled at the end of the experiment performed in the continuous bioreactor (Table S5). As it can be seen in Fig. 3 the sorption of the

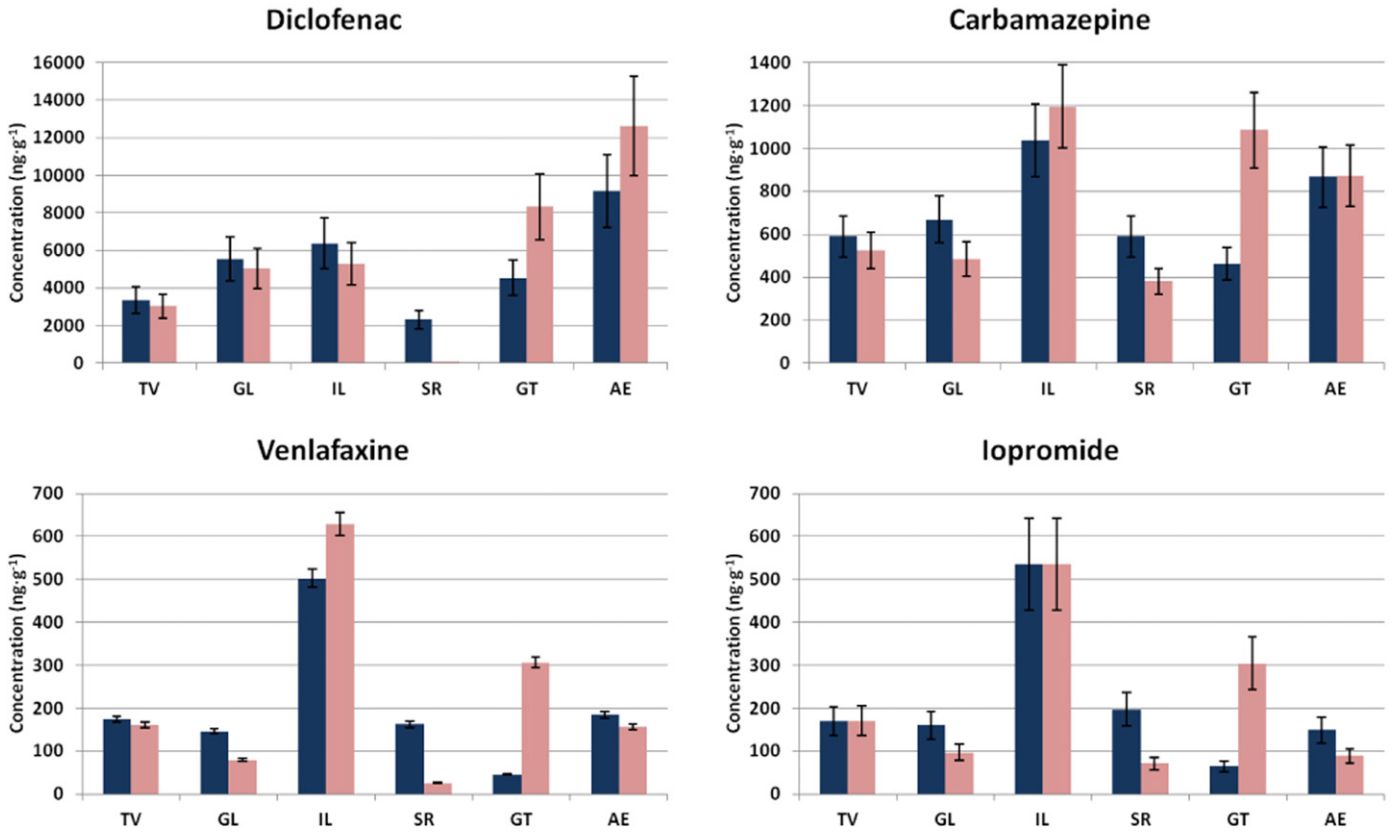



**Fig. 1.** Concentrations (ng $g^{-1}$) of each pollutant in the corresponding fungi at the end of the batch experiments performed with ultrapure spiked water; experimental (blue) and killed control (pink) cultures. TV: *Trametes versicolor*; GL: *Ganoderma lucidum*; IL: *Irpex lacteus*; SR: *Stropharia rugosoannulata*; GT: *Gymnopilus luteofolius*; AE: *Agrocybe erebia*. Error bars correspond to deviation of analytic results of the concentrations measured.

compounds is different between the biomass from the inoculated bioreactor (obtained in the experiment performed with inoculated fungi) and the biomass obtained in the experiment performed without any fungal inoculation (control bioreactor), which can be considered as mainly bacterial biomass with some fungal strains detected at very low concentrations (Badia-Fabregat et al., 2015b). Most of the compounds were better sorbed into the control biomass (23 out of 28 compounds), whereas for only 5 of them the sorption was higher in the inoculated biomass. However, in global terms, no big differences can be pointed out in the total PhACs concentration measured in both biomasses; 174.6 ± 5.0 ng $g^{-1}$ in the inoculated biomass and 204.9 ± 5.2 ng $g^{-1}$ in the control biomass. On the other hand, PhACs levels in sludge from conventional WWTPs reported in other studies (Table S5) were higher than levels encountered in the control biomass in our experiment, with the exception of propranolol, with similar concentrations in all the samples analyzed; 35.2 ng $g^{-1}$, 36.8 ng $g^{-1}$ and 32.3 ng $g^{-1}$ for inoculated biomass, control biomass and sludge from conventional WWTPs, respectively. However, comparison between levels of PhACs in the biomass from the different treatments without taking into account the levels in the water phase (which can vary enormously from one WWTP to the other) is not accurate since both are closely related. Also biomass concentration in the treatments is likely to vary between the treatments. Therefore, the sorption is compared in terms of $K_d$ coefficients (Ternes et al., 2004). Using this coefficient we assumed that the bioreactor has achieved a stationary state, which is the case in CAS, since the samples selected were taken, at the end of the experiment where, no removal of biomass was done. For example, $K_d$ values (Table S6) calculated for salycilic acid were similar in the inoculated biomass and in the biomass of the control bioreactor (33 and 14 respectively) and in the sludge of a CAS treatment (23) (Barron et al., 2009). Also for carbamazepine, $K_d$ values were quite similar: 48 in the inoculated biomass, 31 in the control biomass from the control bioreactor, 43 and 61,2 from CAS treatment (Barron et al., 2009; Radjenović et al., 2009). Considering these results, it could be suggested that there are no major differences in the sorption of pharmaceuticals in the

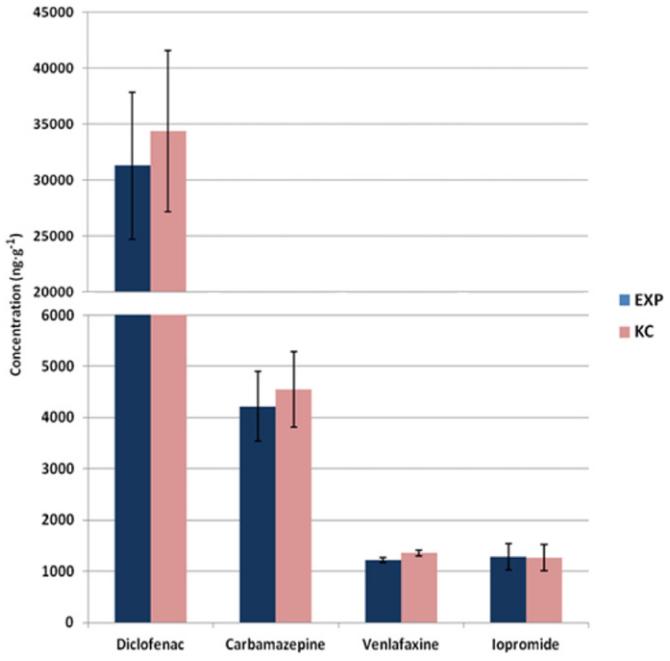



**Fig. 2.** Mean concentration of each PhACs detected in the fungal biomass at the end of the batch experiments: *EXP* culture and *KC* culture, both performed with spiked synthetic medium. Error bars correspond to deviation of analytic results of the concentrations measured.

**Table 1**
Average removal, biodegradation and sorption values plus minus standard deviation calculated directly (with PhACs concentration in both liquid and biomass) and indirectly (subtracting the degradation value measured in the liquid from the killed control culture from the degradation measured in the liquid from the experimental culture (Castellet-Rovira et al., 2017) from the batch experiments with fungi and spiked synthetic medium)

| | Total removal | | | | | |
|---|---|---|---|---|---|---|
| | *T.versicolor* | *G. lucidum* | *I. lacteus* | *S. rugosoannulata* | *G. luteofolius* | *A. erebia* |
| Carbamazepine | 58 ± 8% | 36 ± 7% | 62 ± 6% | 86 ± 7% | 55 ± 12% | 45 ± 10% |
| Diclofenac | 96 ± 24% | 98 ± 15% | 97 ± 14% | 79 ± 7% | 76 ± 13% | 77 ± 20% |
| Iopromide | 47 ± 7% | 31 ± 7% | 27 ± 12% | 94 ± 8% | 96 ± 25% | 55 ± 20% |
| Venlafaxine | 55 ± 8% | 30 ± 5% | 15 ± 4% | 12 ± 8% | 15 ± 3% | 10 ± 3% |

| | Biodegradation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *T.versicolor* | | *G. lucidum* | | *I. lacteus* | | *S. rugosoannulata* | | *G. luteofolius* | | *A. erebia* | |
| | Direct method | Indirect method | Direct method | Indirect method | Direct method | Indirect method | Direct method | Indirect method | Direct method | Indirect method | Direct method | Indirect method |
| Carbamazepine | 51 ± 7% | 50 ± 7% | 31 ± 6% | 23 ± 2% | 58 ± 5% | 57 ± 7% | 84 ± 7% | 77 ± 12% | 52 ± 11% | 45 ± 10% | 42 ± 9% | 38 ± 3% |
| Diclofenac | 64 ± 16% | 54 ± 15% | 58 ± 8% | 64 ± 6% | 65 ± 6% | 71 ± 9% | 68 ± 6% | 25 ± 5% | 59 ± 9% | 20 ± 2% | 43 ± 8% | 3 ± 0% |
| Iopromide | 46 ± 7% | 39 ± 3% | 30 ± 7% | 13 ± 1% | 25 ± 11% | 22 ± 3% | 93 ± 8% | 70 ± 13% | 96 ± 25% | 93 ± 36% | 55 ± 20% | 43 ± 4% |
| Venlafaxine | 53 ± 8% | 49 ± 9% | 29 ± 5% | 19 ± 1% | 14 ± 3% | 9 ± 1% | 11 ± 7% | 2 ± 0% | 15 ± 3% | 0 ± 0% | 9 ± 3% | 8% ± 1% |

| | Sorption | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *T.versicolor* | | *G. lucidum* | | *I. lacteus* | | *S. rugosoannulata* | | *G. luteofolius* | | *A. erebia* | |
| | Direct method | Indirect method | Direct method | Indirect method | Direct method | Indirect method | Direct method | Indirect method | Direct method | Indirect method | Direct method | Indirect method |
| Carbamazepine | 6 ± 1% | 8 ± 1% | 5 ± 1% | 13 ± 1% | 4 ± 0% | 5 ± 1% | 2 ± 0% | 9 ± 1% | 3 ± 1% | 10 ± 2% | 3 ± 1% | 7 ± 1% |
| Diclofenac | 32 ± 8% | 42 ± 12% | 40 ± 6% | 34 ± 3% | 31 ± 4% | 26 ± 3% | 10 ± 1% | 54 ± 11% | 17 ± 3% | 56 ± 6% | 33 ± 9% | 74 ± 10% |
| Iopromide | 1 ± 0% | 8 ± 1% | 1 ± 0% | 18 ± 1% | 2 ± 1% | 5 ± 1% | 1 ± 0% | 24 ± 4% | 0 ± 0% | 3 ± 1% | 0 ± 0% | 12 ± 1% |
| Venlafaxine | 2 ± 0% | 6 ± 1% | 1 ± 0% | 11 ± 1% | 1 ± 0% | 6 ± 1% | 1 ± 0% | 10 ± 1% | 0 ± 0% | 15 ± 3% | 1 ± 0% | 2% ± 0% |

inoculated biomass in comparison to sludge from CAS treatments ($p < 0.05$). Therefore, inoculated biomass after fungal treatment might be considered a potential waste (as it is the case of wastewater sludge in a conventional WWTP), and thus be treated accordingly before being released into the environment (Rodríguez Rodríguez, 2013). However, this is a preliminary experiment and further studies would be necessary to confirm these findings.

On the other hand, the appearance and removal of transformation products (TP) of PhACs has been addressed by some authors (García-Galán et al., 2011; Cruz-Morató et al. 2012; Jelić et al., 2012). According to studies performed in our research group (Llorca et al., 2016) ligninolytic fungi degradation rely on mechanisms occurring inside and outside the fungal cells, though Blanquez et al. (2004) evidenced that most of the biological transformation occurs at intracellular level. Therefore, the great majority of transformation products might be found in the fungal biomass. However, the occurrence of these TP in fungal biomass has not been reported so far and calls for further studies in order to reach a better understanding on the subject.

## 4. Conclusions

According to the results obtained in our studies, some ideas can be highlighted: i) It is worthwhile to measure target compounds in both, liquid and solid (biomass) phases in order to determine the role of the sorption and biodegradation mechanisms when the elimination of pollutants is evaluated, ii) Sorption processes accounted for a 7% of the PhACs elimination (mean value) in our batch experiments. However, the contribution of the sorption processes to overall removal is different

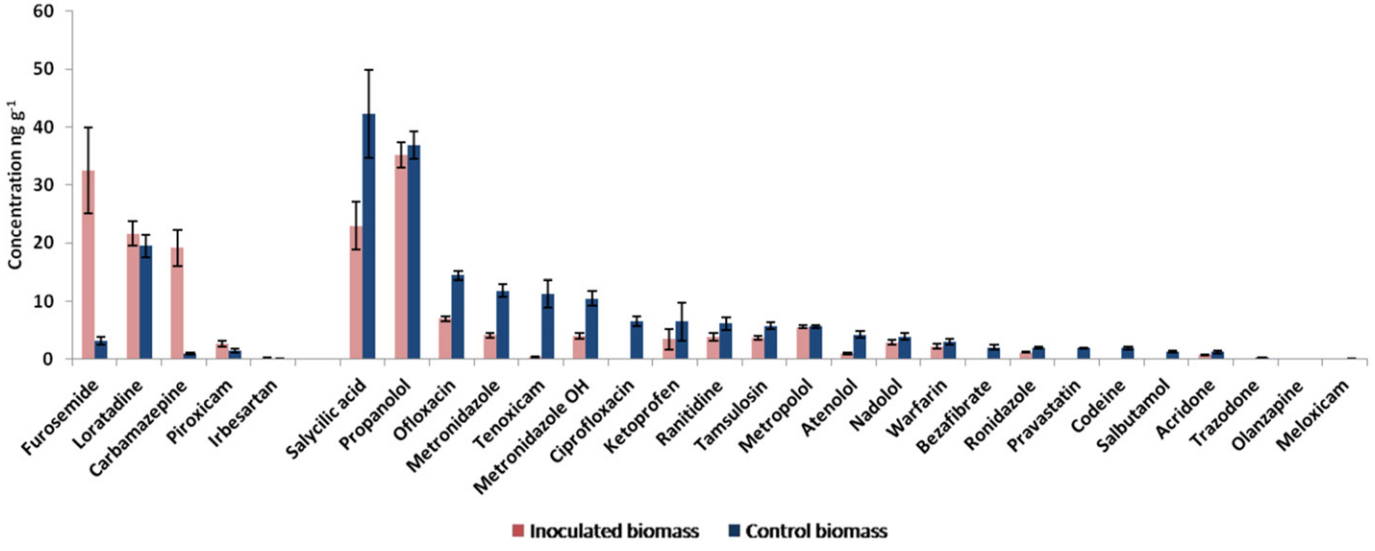



**Fig. 3.** Concentration of the PhACs detected in the biomass samples from the continuous bioreactor with *T.versicolor* and VHW at the end of the experiment. Error bars correspond to deviation of analytic results of the concentrations measured.

depending on the fungus considered, ranging from 4% in the case of *S.rugosoannulata* to 26% for *G.lucidum*. Iii) Sorption of PhACs on fungal biomass was similar to that observed into the sludge from conventional CAS treatments. Therefore, based on this preliminary study, fungal biomass should be managed in the same way as the sludge from CAS treatments.

## Acknowledgments

This work has been supported by the Spanish Ministry of Economy and Competitiveness (project CTM 2013-48545), co-financed by the European Union through the European Regional Development Fund (ERDF) and supported by the Generalitat de Catalunya (Consolidated Research Group: Catalan Institute for water Research 2014 SGR 291). The Department of Chemical, Biological and Environmental Engineering of the Universitat Autònoma de Barcelona (UAB) is member of the Xarxa de Referència en Biotecnologia de la Generalitat de Catalunya. Castellet-Rovira, F. acknowledges the predoctoral grant from UAB. D. Lucas acknowledges the predoctoral grant from the Spanish Ministry of Education, Culture and Sports (AP-2010-4926). S. Rodriguez-Mozaz acknowledges the Ramon y Cajal program (RYC-2014-16707).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.08.118.

## References

Arıca, M., Arpa, C., Kaya, B., Bektaş, S., Denizli, A., Genç, Ö., 2003. Comparative biosorption of mercuric ions from aquatic systems by immobilized live and heat-inactivated Trametes versicolor and Pleurotus sajur-caju. Bioresour. Technol. 89 (2), 145–154.

Asgher, M., Bhatti, H.N., Ashraf, M., Legge, R.L., 2008. Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. Biodegradation 19 (6), 771–777.

Badia-Fabregat, M., Rodríguez-Rodríguez, C.E., Gago-Ferrero, P., Olivares, A., Piña, B., Díaz-Cruz, M.S., Vicent, T., Barceló, D., Caminal, Glòria, 2012. Degradation of UV filters in sewage sludge and 4-MBC in liquid medium by the ligninolytic fungus Trametes versicolor. J. Environ. Manag. 104, 114–120.

Badia-Fabregat, M., Lucas, D., Gros, M., Rodríguez-Mozaz, S., Barceló, D., Caminal, G., Vicent, T., 2015a. Identification of some factors affecting pharmaceutical active compounds (PhACs) removal in real wastewater. Case study of fungal treatment of reverse osmosis concentrate. J. Hazard. Mater. 283, 663–671.

Badia-Fabregat, M., Lucas, D., Pereira, M.A., Alves, M., Pennanen, T., Fritze, H., Rodríguez-Mozaz, S., Barceló, D., Vicent, T., Caminal, G., 2015b. Continuous fungal treatment of non-sterile veterinary hospital effluent: pharmaceuticals removal and microbial community assessment. Appl. Microbiol. Biotechnol. 1–15.

Barron, L., Havel, J., Purcell, M., Szpak, M., Kelleher, B., Paull, B., 2009. Predicting sorption of pharmaceuticals and personal care products onto soil and digested sludge using artificial neural networks. Analyst 134 (4), 663–670.

Batt, A.L., Kim, S., Aga, D.S., 2006. Enhanced biodegradation of iopromide and trimethoprim in nitrifying activated sludge. Environ. Sci. Technol. 40 (23), 7367–7373.

Bayramoğlu, G., Arıca, M.Y., 2007. Biosorption of benzidine based textile dyes "direct blue 1 and direct red 128" using native and heat-treated biomass of Trametes versicolor. J. Hazard. Mater. 143 (1), 135–143.

Blanquez, P., Casas, N., Font, X., Gabarrell, X., Sarra, M., Caminal, G., Vicent, T., 2004. Mechanism of textile metal dye biotransformation by Trametes versicolor. Water Res. 38 (8), 2166–2172.

Blánquez, P., Sarrà, M., Vicent, M., 2006. Study of the cellular retention time and the partial biomass renovation in a fungal decolourisation continuous process. Water Res. 40 (8), 1650–1656.

Capone, D.G., Weston, D.P., Miller, V., Shoemaker, C., 1996. Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. Aquaculture 145 (1), 55–75.

Castellet-Rovira, F., Lucas, D., Rodríguez-Mozaz, S., Barceló, D., Sarrà, M., 2017. Novel fungal candidates for biodegradation of pharmaceuticals in wastewater treatment. J. Environ. Manag. Submitted.

Cruz-Morató, C., et al., 2012. Biodegradation of pharmaceuticals by fungi and metabolites identification. Emerging organic contaminants in sludges. Springer, pp. 165–213.

Cruz-Morató, C., Ferrando-Climent, L., Rodriguez-Mozaz, S., Barceló, D., Marco-Urrea, E., Vicent, T., Sarrà, M., 2013. Degradation of pharmaceuticals in non-sterile urban wastewater by Trametes versicolor in a fluidized bed bioreactor. Water Res. 47 (14), 5200–5210.

Cruz-Morató, C., Lucas, D., Llorca, M., Rodriguez-Mozaz, S., Gorga, M., Petrovic, M., Barceló, D., Vicent, T., Sarrà, M., Marco-Urrea, E., 2014. Hospital wastewater treatment by fungal bioreactor: removal efficiency for pharmaceuticals and endocrine disruptor compounds. Sci. Total Environ. 493, 365–376.

Feijoo, G., Moreira, M., Lema, J., 2011. Degradation of selectedpharmaceutical and personal care products (PPCPs) by white-rot fungi. World J. Microbiol. Biotechnol. 27, 1839–1846.

Ferrando-Climent, L., Cruz-Morató, C., Marco-Urrea, E., Vicent, T., Sarrà, M., Rodriguez-Mozaz, S., Barceló, D., 2015. Non conventional biological treatment based on Trametes versicolor for the elimination of recalcitrant anticancer drugs in hospital wastewater. Chemosphere 136, 9–19.

Font, X., Caminal, G., Gabarrell, X., Romero, S., Vicent, M.T., 2003. Black liquor detoxification by laccase of Trametes versicolor pellets. J. Chem. Technol. Biotechnol. 78 (5), 548–554.

Frédéric, O., Yves, P., 2014. Pharmaceuticals in hospital wastewater: their ecotoxicity and contribution to the environmental hazard of the effluent. Chemosphere 115, 31–39.

García-Galán, M.J., Rodríguez-Rodríguez, C.E., Vicent, T., Caminal, G., Díaz-Cruz, M.S., Barceló, D., 2011. Biodegradation of sulfamethazine by Trametes versicolor: removal from sewage sludge and identification of intermediate products by UPLC–QqTOF-MS. Sci. Total Environ. 409 (24), 5505–5512.

Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J. Chromatogr. A 1248, 104–121.

Gros, M., et al., 2014. 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. Water Res. 60, 228–241.

Guillén, F., Martínez, M.J., Gutiérrez, A., Del Rio, J., 2005. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. Int. Microbiol. 8 (195204) 187204Minami.

Hernández, F., Sancho, J.V., Ibáñez, M., Guerrero, C., 2007. Antibiotic residue determination in environmental waters by LC-MS. TrAC Trends Anal. Chem. 26 (6), 466–485.

Ibáñez, M., Guerrero, C., Sancho, J.V., Hernández, F., 2009. Screening of antibiotics in surface and wastewater samples by ultra-high-pressure liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry. J. Chromatogr. A 1216 (12), 2529–2539.

Jelić, A., Petrović, M., Barceló, D., 2009. Multi-residue method for trace level determination of pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid chromatography/quadrupole-linear ion trap mass spectrometry. Talanta 80 (1), 363–371.

Jelić, A., Cruz-Morató, C., Marco-Urrea, E., Sarrà, M., Perez, S., Vicent, T., Petrović, M., Barcelo, D., 2012. Degradation of carbamazepine by Trametes versicolor in an air pulsed fluidized bed bioreactor and identification of intermediates. Water Res. 46 (4), 955–964.

Joss, A., Siegrist, H., Ternes, T., 2008. Are we about to upgrade wastewater treatment for removing organic micropollutants? Water Sci. Technol. 57 (2), 251.

Kantiani, L., Farre, M., Sibum, M., Postigo, C., López de Alda, M., Barcelo, D., 2009. Fully automated analysis of β-lactams in bovine milk by online solid phase extraction-liquid chromatography-electrospray-tandem mass spectrometry. Anal. Chem. 81 (11), 4285–4295.

Kirk, T.K., Schultz, E., Connors, W., Lorenz, L., Zeikus, J., 1978. Influence of culture parameters on lignin metabolism byPhanerochaete chrysosporium. Arch. Microbiol. 117 (3), 277–285.

Llorca, M., Lucas, D., Ferrando-Climent, L., Badia-Fabregat, M., Cruz-Morató, C., Barceló, D., Rodríguez-Mozaz, S., 2016. Suspect screening of emerging pollutants and their major transformation products in wastewaters treated with fungi by liquid chromatography coupled to a high resolution mass spectrometry. J. Chromatogr. A 1439, 124–136.

Mannhold, R., Poda, G.I., Ostermann, C., Tetko, I.V., 2009. Calculation of molecular lipophilicity: state-of-the-art and comparison of log P methods on more than 96,000 compounds. J. Pharm. Sci. 98 (3), 861–893.

Marco-Urrea, et al., 2009. Ability of white-rot fungi to remove selected pharmaceuticals and identification of degradation products of ibuprofen by Trametes versicolor. Chemosphere 74 (6), 765–772.

Marco-Urrea, E., Pérez-Trujillo, M., Cruz-Morató, C., Caminal, G., Vicent, T., 2010. Degradation of the drug sodium diclofenac by Trametes versicolor pellets and identification of some intermediates by NMR. J. Hazard. Mater. 176 (1), 836–842.

Pointing, S., 2001. Feasibility of bioremediation by white-rot fungi. Appl. Microbiol. Biotechnol. 57 (1–2), 20–33.

Radjenović, J., Petrović, M., Barceló, D., 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res. 43 (3), 831–841.

Rodríguez Rodríguez, C.E., 2013. Degradation of pharmaceuticals in sewage sludge by Trametes versicolor. Doctoral Thesis. Universitat Autònoma de Barcelona, Barcelona.

Rodríguez-Rodríguez, C.E., Barón, E., Gago-Ferrero, P., Jelić, A., Llorca, M., Farré, M., Díaz-Cruz, M.S., Eljarrat, E., Petrović, M., Caminal, G., 2012. Removal of pharmaceuticals, polybrominated flame retardants and UV-filters from sludge by the fungus Trametes versicolor in bioslurry reactor. J. Hazard. Mater. 233, 235–243.

Rubilar, O., Tortella, G., Cea, M., Acevedo, F., Bustamante, M., Gianfreda, L., Diez, M., 2011. Bioremediation of a Chilean Andisol contaminated with pentachlorophenol (PCP) by solid substrate cultures of white-rot fungi. Biodegradation 22 (1), 31–41.

Ternes, T.A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., Joss, A., 2004. A rapid method to measure the solid–water distribution coefficient (K d) for pharmaceuticals and musk fragrances in sewage sludge. Water Res. 38 (19), 4075–4084.

Tran, N.H., Urase, T., Kusakabe, O., 2010. Biodegradation characteristics of pharmaceutical substances by whole fungal culture Trametes versicolor and its laccase. J. Water Environ. Technol. 8 (2), 125–140.

Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment—a review. Sci. Total Environ. 429, 123–155.

Wang, Y., Yu, J., 1998. Adsorption and degreadation of synthetic dyes on the mycelium of Trametes versicolor. Water Sci. Technol. 38 (4), 233–238.

Yang, S., Cha, J., Carlson, K., 2005. Simultaneous extraction and analysis of 11 tetracycline and sulfonamide antibiotics in influent and effluent domestic wastewater by solid-phase extraction and liquid chromatography-electrospray ionization tandem mass spectrometry. J. Chromatogr. A 1097 (1), 40–53.

Yang, S., Hai, F.I., Nghiem, L.D., Price, W.E., Roddick, F., Moreira, M.T., Magram SF, S.F., 2013. Understanding the factors controlling the removal of trace organic contaminants by white-rot fungi and their lignin modifying enzymes: a critical review. Bioresour. Technol. 141, 97–108.