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Prospects on coupling UV/H_2O_2 with activated sludge or a fungal treatment for the removal of pharmaceutically active compounds in real hospital wastewater

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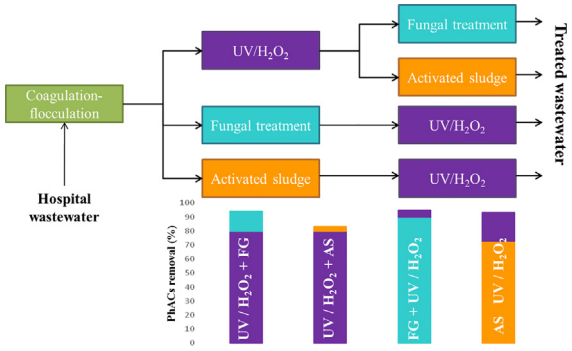
HIGHLIGHTS

- Fungal treatment was the most efficient as first treatment.
- Removal efficiency was higher than 90% for most of the treatment combinations.
- Deconjugation was evidenced after both biological treatments.
- Carbamazepine and its TPs highly removed (90%) by UV/H_2O_2 alone.
- UV/H_2O_2 more convenient as a polishing than pre-treatment step.

GRAPHICAL ABSTRACT



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ABSTRACT

Conventional active sludge (AS) process at municipal centralized wastewater treatment facilities may exhibit little pharmaceuticals (PhACs) removal efficiencies when treating hospital wastewater (HWW). Therefore, a dedicated efficient wastewater treatment at the source point is recommended. In this sense, advanced oxidation processes (AOPs) and fungal treatment (FG) have evidenced promising results in degrading PhACs. The coupling of the AOP based on UV/H_2O_2 treatment with biological treatment (AS or FG) treating a real non-sterile HWW, was evaluated in this work. In addition, a coagulation-flocculation pretreatment was applied to improve the efficiency of all approaches. Twenty-two PhACs were detected in raw HWW, which were effectively removed (93–95%) with the combination of any of the biological treatment followed by UV/H_2O_2 treatment. Similar removal results (94%) were obtained when placing UV/H_2O_2 treatment before FG, while a lower removal (83%) was obtained in the combination of UV/H_2O_2 followed by AS. However, the latest was the only treatment combination that achieved a decrease in the toxicity of water.

Abbreviations: HWW, hospital wastewater; AOP, advanced oxidation process; CAS, conventional activated sludge; WRF, white-rot fungi; PhAC, pharmaceutically active compound; CBZ, carbamazepine; AS, activated sludge; TPs, transformation products; TU, toxicity unit; bld, below limit of detection; blq, below limit of quantification.

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UV/H_2O_2
Decentralized treatment

Moreover, deconjugation of conjugated PhACs has been suggested for ofloxacin and lorazepam after AS treatment, and for ketoprofen after fungal treatment. Monitoring of carbamazepine and its transformation products along the treatment allowed to identify the same carbamazepine degradation pathway in UV/H_2O_2 and AS treatments, unlike fungal treatment, which followed another degradation route.

1. Introduction

Hospital wastewater (HWW) contains a complex mixture of hazardous chemicals and harmful microbes, which can pose a threat to the environment and public health. Although the contribution of hospital facilities to the total volume uploaded in the municipal WWTP usually range between 0.2 and 2% (Carraro et al., 2016), a specific directive or guideline for the management of hospital wastewater effluents in Europe is missing, and national legal regulations quite rarely define how to manage and treat HWW before its disposal (Rodriguez-Mozaz et al., 2017). Therefore, hospital effluents are usually discharged in the municipal sewer system without any previous pretreatment. The common practice of co-treating hospitals and urban wastewaters jointly at a municipal WWTP (centralized treatment) is considered as an inadequate solution for the removal of compounds such as some pharmaceuticals (PhACs), because highly polluted effluents can be detrimental for their removal by biological treatment (Verlicchi et al., 2015; Pauwels and Verstraete, 2006; Badia-Fabregat et al., 2015; Joss et al., 2006). Therefore, the use of alternative wastewater treatments at the source point (decentralized treatment) has been highly recommended (Verlicchi et al., 2015; Pauwels and Verstraete, 2006; Joss et al., 2006; Cruz-Morató et al., 2014; Verlicchi et al., 2010) and extensive research has been conducted in the development of appropriate decentralized treatments for hospital effluents (Verlicchi et al., 2015). However, full-scale dedicated treatment of hospitals effluents has only been implemented in a limited number of places (Rodriguez-Mozaz et al., 2017). In the case of psychiatric hospitals, on-site wastewater treatment can be particularly recommended since the effluents contain remarkable loads of psychiatric drugs as well as their metabolites and transformation products (Herrmann et al., 2015; Yuan et al., 2013a). These type of pharmaceuticals are more recalcitrant than most of the PhACs in both conventional WWTP and in the natural environment (Baena-Nogueras et al., 2017; Mir-Tutusaus et al., 2017; Verlicchi et al., 2012; Calisto and Esteves, 2009). They have also been targeted as contaminants to be prioritized by several authors (Ashton et al., 2004) as well as by the Global Water Research Coalition (2008). In addition, the use of antidepressants has significantly increased in most OECD countries in the last years, as a reflection of the prevalence of mental illness, increase in health coverage, new treatment opportunities and population ageing (Organization for Economic Cooperation and Development, 2015). An increase in the worldwide consumption of this type of PhACs class can thus be foreseen in the next years.

Wastewater treatment plants are among the main point sources of pharmaceutical release into the environment. Therefore, the improvement of WWTP capabilities is of high importance (Verlicchi et al., 2015; Collado et al., 2013; Fatta-Kassinos et al., 2011) and physical, chemical and biological processes have been tested with varying degrees of success. Conventional activated sludge (CAS) process is the standard practice in conventional WWTPs, which usually does not achieve high removal efficiencies of recalcitrant micropollutants (Verlicchi et al., 2015). Three main removal pathways are usually accounted in activated sludge: microbial processes (biodegradation, either metabolic or co-metabolic), sorption onto sludge flocs and volatilization (mainly during aeration). However, volatilization can be considered negligible for most PhACs (Joss et al., 2006). Some analgesics and anti-inflammatories are well removed by CAS but other drug families such as psychiatric drugs and antibiotics are more resistant to bacterial degradation (Yuan et al., 2013a). Other biological processes such as fungal treatment have also proved to remove PhACs from wastewater streams. Particularly white-rot fungi (WRF) have succeeded on degrading a wide range of pollutants thanks to its unspecific intracellular and extracellular enzymatic systems (Rodarte-Morales et al., 2011; Nguyen et al., 2013; Cruz-Morató et al., 2013). Fungal operations perform reasonably well in terms of PhACs removal but some compounds still remain in the effluents (Cruz-Morató et al., 2014). Advanced Oxidation Processes (AOPs) are being largely studied in regards to PhACs degradation with promising results, and an increasing number of articles has been published in recent years (Klavarioti et al., 2009). When the UV light is absorbed by H_2O_2, •OH radicals are formed by the photolysis of the –O–O– peroxidic bond. Hydroxyl radicals can react with organic pollutants in different ways: by transferring electrons, by oxidising organic compounds, by adding hydroxyl groups, by abstracting a hydrogen atom or by initiating a radical (Kim et al., 2009a).

However, total mineralization of organic pollutants in both biological and AOP treatment of wastewater is highly unlikely (Cavalcante et al., 2015) and transformation products (TPs) of these contaminants are usually formed. These TPs are typically more biodegradable than the corresponding parent compounds but, in some cases, they can be even more toxic.

Biological processes can be coupled to other treatment technologies, like AOP: On the one hand, biological treatment such as CAS and fungal processes can enhance PhAC removal with AOP as pretreatment in order to increase contaminant biodegradability (Changotra et al., 2019); on the other hand, AOP as a post-treatment could not only improve overall pollutant removal efficiency but also reduce treatment economic cost and ecological footprint (Oller et al., 2011). Specifically in the case of wastewater with high amounts of PhACs, like HWW, the combination of bio-oxidation and AOP has been recently proven to be economically feasible (Changotra et al., 2020; Martínez et al., 2018). Some studies have discussed the coupling of selected AOPs with some biological processes, mainly CAS-based and using the AOP as a polishing step (Giannakis et al., 2015; Sirtori et al., 2009; Vidal et al., 2004; Hörsch et al., 2003; Ahmed et al., 2017). The hypothesis in this work is that the coupling of advanced oxidation with biologically based technologies may improve PhAC removal, compared to individual treatments, especially in a complex matrix like HWW. However, depending of the wastewater nature, AOP has reported both to increase the biodegradability of contaminants (Changotra et al., 2019) and to decrease it, and also to enhance the effluent toxicity due to the formation of toxic by-products (Plahuta et al., 2014; Cuerda-Correa et al., 2020). Therefore, more thorough and wider-spectrum studies need to be performed to fully understand the interactions between the systems and to provide feasible combinations from which to choose. Therefore, the main objective of this study was the evaluation of coupling an AOP process, in this work the well-known UV/H_2O_2 treatment, with activated sludge or a fungal treatment treating real non-sterile HWW. The removal of a broad set of PhACs was evaluated in each treatment separately, and in several combinations of them. Moreover, a detailed evaluation of carbamazepine, considered as a model compound, and of some of its TPs was performed.

2. Materials and methods

2.1. Reagents and hospital wastewater

All the PhACs and the corresponding isotopically labelled standards used in the analysis were of high purity grade (>90%) and they were

purchased from Sigma–Aldrich (Steinheim, Germany), US Pharmacopeia USP (MD, USA), Europea Pharmacopeia EP (Strasbourg, France), Toronto Research Chemicals TRC (Ontario, Canada) and CDN isotopes (Quebec, Canada). Individual as well as isotopically labelled standard solutions were prepared according to Gros et al. (2012). Malt extract was acquired from Scharlau (Barcelona, Spain) and glucose, ammonium chloride and other chemicals were purchased from Sigma-Aldrich (Barcelona, Spain). All other chemicals used were of analytical grade. In the UV/H_2O_2 experiments titanium (IV) oxysulfate reagent (1.9–2.1% from Sigma-Aldrich), H_2O_2 (30% w/v 100 vol. stabilized PRS from Panreac) and sodium thiosulfate (Panreac) were used.

The HWW was collected directly from the sewer manifold of Sant Joan de Déu Hospital (Barcelona, Catalonia) in the NE of Spain. Fresh samples were pretreated with a coagulation-flocculation process as described previously (Mir-Tutusaus et al., 2016). The pretreatment used 43 mg L^{-1} of coagulant Hyfloc AC50 and 4.8 mg L^{-1} of flocculant Himoloc DR3000, both kindly provided by Derypol, S.A. (Barcelona, Spain). Characterization of the wastewater samples in terms of PhAC concentrations are summarized in Table 1.

2.2. HWW treatments

Fig. 1 summarizes the experiments carried out on the coupling of AOP and the biological treatments.

2.2.1. Fungal treatment

Trametes versicolor (ATCC#42530) was maintained on 2% malt agar slants at 25 °C until use. Subcultures were routinely made. A mycelial suspension of *T. versicolor* was obtained as in Borràs et al. and pellets were obtained as previously described (Borràs et al., 2008; Blánquez et al., 2006). An air-fluidized bed bioreactor was operated as a batch for 7 d. Fluidized conditions in the reactor was maintained by using 1 s air pulse every 4 s, resulting in an aeration rate of 0.8 $Lmin^{-1}$. Nutrients for maintenance, namely, glucose and NH_4Cl, were added with a molar C/N ratio of 7.5 at *T. versicolor* consumption rate (1200 mg glucosegDCW$^{-1}\cdot d^{-1}$). Temperature was maintained at 25 °C and pH was controlled at 4.5 by HCl 1 M or NaOH 1 M addition.

2.2.2. Activated sludge treatment

All the biodegradation tests with activated sludge were performed using 1 L lab-scale Applikon stirred tank reactor coupled with a proportional-integral-derivative (PID) controller for pH, oxygen and temperature. Bioreactors were operated as batch for 24 h and each experiment was conducted in duplicate. The biomass originated from Celrà WWTP (Catalonia, Spain, 20,000 equivalent inhabitants, 2100 m^3d^{-1}), with a hydraulic retention time of 48 h and a sludge retention time of 20–22 d (Collado et al., 2014). The experiments started at the latest 24 h after withdrawing the biomass from the full scale WWTP. Biomass concentration during the experiments was 3 gTSS L^{-1} and aerobic conditions (>2.5 mg O_2 L^{-1}) were achieved with a continuous air supply. pH was controlled at 7.5 and the temperature maintained at 25 °C. All these parameters were selected based on the optimum conditions needed for this treatment (Rubirola et al., 2014). Organic solution (sodium acetate, propionate and yeast extract), phosphate buffer, trace and inorganic solution were added as described elsewhere (Collado et al., 2013).

2.2.3. UV/H_2O_2 treatment

Photo-oxidation was carried out in a UV Laboratory Reactor System from UV-Consulting Peschl® which consists of an immersion-type photo-reactor with a working volume of approximately 550 mL. The UV lamp used was a 15 W Heraeus Noblelight TNN 15/32 low-pressure mercury vapor lamp emitting at 254 nm. The photo-reactor was covered with aluminum foil to minimize loss of UV light and avoid any reflections, and magnetically stirred. Potassium ferrioxalate actionometry (Hatchard and Parker, 1956) was used to characterize

Table 1
Levels of selected PhACs (ng L^{-1}) in the initial wastewater and after each treatment. Carbamazepine is discussed separately.

Family	Pharmaceutical	Initial concentration (ng L^{-1})		Final concentration (ng L^{-1})																											
				UV/H_2O_2			FG			AS			UV/H_2O_2 + FG			UV/H_2O_2 + AS			FG + UV/H_2O_2			AS + UV/H_2O_2		
Analgesics and anti-inflammatories	Acetaminophen	27,569	± 954	bld			3	± 0	3	± 0	bld			3	± 0	6	± 1	bld								
	Diclofenac	1448	± 398	bld			bld		1045	± 35	bld			bld		bld										
	Ibuprofen	26,939	± 3506	7128	± 595		113	± 4	bld		bld			bld		60	± 5	bld								
	Ketoprofen	3169	± 1205	581	± 132		3450	± 506	2342	± 390	3778	± 554		1052	± 175	886	± 201	892	± 203							
	Naproxen	6883	± 799	bld			1578	± 60	bld		bld			bld		995	± 214	bld								
Antibiotics	Ciprofloxacin	6738	± 846	2921	± 289		bld		1713	± 499	bld			1247	± 363	bld		bld								
	Ofloxacin	2052	± 111	2	± 0		757	± 1	3887	± 95	bld			5319	± 130	2	± 0	1212	± 38							
Antihypertensives	Valsartan	414	± 118	60	± 4		bld		328	± 40	bld			232	± 28	79	± 5	132	± 9							
β-blockers	Atenolol	1370	± 574	93	± 39		136	± 2	11	± 1	797	± 14		694	± 55	97	± 40	46	± 19							
Diuretics	Furosemide	2188	± 601	bld			bld		1053	± 27	bld			bld		bld		bld								
	Hydrochlorothiazide	1670	± 411	bld			bld		1565	± 100	bld			178	± 11	bld		47	± 1							
H1 and H2 antagonists	Ranitidine	1970	± 509	bld			bld		2439	± 20	bld			bld		bld		bld								
Lipid regulators	Atorvastatin	77	± 12	bld			bld		41	± 0	bld			bld		bld		bld								
	Gemfibrozil	13,955	± 1051	7655			bld		6259		bld			4718		bld		2773								
Psychiatric drugs	Carbamazepine	4118	± 314	2067	± 52		1897	± 48	2952	± 75	790	± 20		1897	± 48	1545	± 39	892	± 23							
	Citalopram	898	± 107	53	± 1		264	± 0	663	± 7	196	± 0		253	± 3	224	± 5	123	± 2							
	Lorazepam	538	± 178	154	± 13		543	± 27	901	± 53	275	± 14		1214	± 71	bld		612	± 50							
	Trazodone	225	± 31	bld			bld		96	± 13	bld			bld				bld								
	Venlafaxine	5766	± 295	1295	± 16		2504	± 66	4202	± 30	435	± 11		1056	± 8	1800	± 22	462	± 6							
Total		107,987	± 12,019	22,009	± 1141		11,246	± 714	29,499	± 1386	6270	± 614		17,862	± 893	5694	± 532	7190	± 352							
Removal (%)				80	± 13		90	± 6	73	± 19	94	± 5		83	± 9	95	± 4	93	± 2							

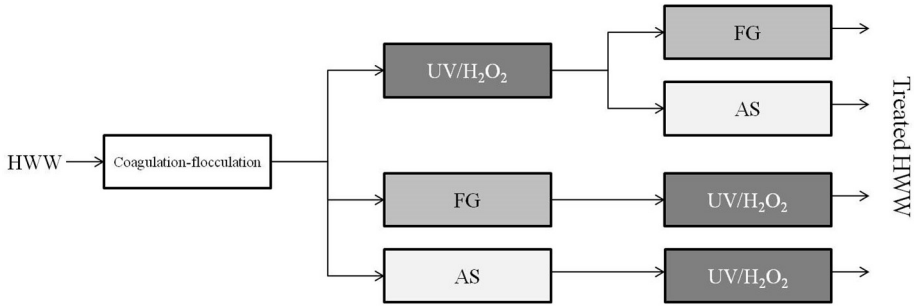

Fig. 1. Diagram of the coupled treatments. FG represents the fungal treatment; AOP, the UV/H_2O_2 treatment; AS, the activated sludge process.

the intensity of the light, resulting in an irradiance of 0.049 W cm^{-2} (Benito et al., 2017). The experiments were carried out with 500 mL of wastewater, 15 mgL^{-1} of H_2O_2 and a reaction time of 10 min that corresponds to a UV dose of 29.4 J cm^{-2}. Sodium thiosulfate was added to interrupt the oxidation reaction (with stoichiometric excess of 20%). The presence of H_2O_2 was analyzed by a spectrophotometric method using titanium (IV) oxysulfate (Shahbazi et al., 2014) to check that if it was completely quenched by the added sodium thiosulfate.

2.3. Analysis of pharmaceutically active compounds

Samples collected from the experiments were filtered through 0.45 μm PVDF filters (Millipore, Barcelona, Spain) and kept in PET containers at −20 °C until PhAC analysis. Sample pretreatment was carried out using the methodology described elsewhere (Gros et al., 2012). Briefly, samples were filtered through 1 μm glass fibber followed by 0.45 μm PVDF membrane filters (Millipore; Billerica, MA, USA) and an appropriate volume of Na_2EDTA was added to obtain a final concentration of 0.1% (w/w). Oasis HLB cartridges (60 mg, 3 mL) (Waters Corp.; Mildford, MA, USA) were conditioned with 5 mL of methanol followed by 5 mL of HPLC-grade water. After conditioning step, 25 mL and 50 mL for raw and treated wastewater respectively were percolated through cartridges, rinsed with 6 mL of HPLC grade water and further dried with air for 5 min to remove remaining water. Elution was performed with 10 mL of pure methanol. 5 mL of the eluate was evaporated under gentle nitrogen stream and reconstituted in 1 mL of methanol-water (10: 90, v/v). Finally, 10 μL of 10 $ngμL^{-1}$ internal standard mix was added in all samples.

Analysis was performed by using an Ultra-Performance Liquid Chromatography system (Waters Corp. Mildford, MA, USA) coupled to a quadrupole-linear hybrid ion trap mass spectrometer 5500 QTRAP (Applied Biosystems, Foster City, CA, USA) equipped with a Turbo V ion spray source. Chromatographic separation was carried out by using an Acquity HSS T3 column (50 mm × 2.1 mm i.d, 1.8 μm particle size; Waters Corp. Mildford, MA, USA) for positive ionization (PI) mode and an Acquity BEH C18 column (50 mm × 2.1 mm i.d. 1.7 μm particle size; Waters Corp. Mildford, MA, USA) for negative ionization (NI) mode. All transitions were recorded by using Scheduled MRM™ algorithm monitoring two SRM transitions for each compound; the first one for quantification and the second one for confirmation of the compounds. Concentrations were calculated by internal calibration and processed by using Analyst 1.5.1 software.

2.4. Toxicity analysis

To determine the ecotoxicological effects on treated samples, decay percentages on bacterial bioluminescence were measured by using a Microtox™ Model 500 Toxicity Analyzer (Strategic Diagnostics Inc. Newark, DE, US). For this purpose, samples were adjusted at pH 7 and centrifuged in glass vials to remove any suspended solids. Then, samples were put in contact with the bacterium *Vibrio fischeri*. Bioluminescence was recorded after 15 min exposure and compared with that of blank controls. Toxicity values are expressed in Toxicity Units (TUs). The possible presence of trace sodium thiosulfate in the analysis was tested in a previous work and had no toxic effect on luminescent bacteria at the added concentration (Jaén-Gil et al., 2019).

2.5. Statistical analysis

For removal calculations, values below limit of detection (bld) and below limit of quantification (blq) were considered to have a concentration half of the limit of detection and half of the limit of quantification, respectively (EPA, 2000). Summary statistics were performed with R: A language and environment for statistical computing (R Core Team, 2015).

3. Results and discussion

General physicochemical parameters of the HWW studied were in the same range than previously sampled HWW from the same sewer manifold and from other hospitals (Mir-Tutusaus et al., 2017; Mir-Tutusaus et al., 2016). The use of a coagulation-flocculation pretreatment can be highlighted as necessary, as several studies have reported low removal efficiencies while operating UV/H_2O_2 and fungal reactors with raw wastewater, due to high suspended solids and microorganisms concentration, respectively (Verlicchi et al., 2015; Mir-Tutusaus et al., 2016). The coagulation-flocculation pretreatment reduced the absorbance at 650 nm to zero, the COD from 174 to 87 mg O_2 L^{-1} and total suspended solids from 108 to 16 mg L^{-1}.

On one hand, as presented in Fig. 1, the UV/H_2O_2 was studied as a step for both removing micropollutants and incrementing the biodegradability of the effluent before the subsequent fungal or AS treatments (Oller et al., 2011). On the other hand, fungal and AS treatments were evaluated as a first step for reducing the pharmaceutical load prior to the UV/H_2O_2 stage, which would be considered as a polishing step. Only 23 PhACs (out of the total 77 analyzed; Table S1) were detected before the treatments, and only those detected at concentrations at least 10 times their limit of quantification are discussed in the following sections. Table 1 shows the concentrations of the 23 compounds, before and after single and coupled treatments. The analgesics and anti-inflammatories compounds were contributing the most to total PhACs concentration in raw wastewater, a common trend in urban and hospital wastewaters (Verlicchi et al., 2015; Verlicchi et al., 2010; Frédéric and Yves, 2014). However, a high concentration of psychiatric drugs can be highlighted, most likely due to a large psychiatric ward located within the hospital premises. For example, the levels of carbamazepine and lorazepam (4118 and 538 ng L^{-1}, respectively) were considerably higher than the levels found previously in urban wastewater (1200 ng L^{-1} and n.d., respectively) (Verlicchi et al., 2012). The same could be observed with citalopram and venlafaxine, measured at a much higher

concentration (898 and 5766 ng L^{-1}, respectively), than the concentrations observed in urban wastewater (5 and 287–371 ng L^{-1}, respectively) (Yuan et al., 2013a; Mackuľak et al., 2015).

3.1. Performance of individual treatments

Fungal and AS operations were carried out in parallel, with the same initial wastewater, and their removal efficiencies were compared. The single fungal step removed 90% of total PhACs concentration, 66% excluding analgesics and anti-inflammatories, which are commonly degraded in WWTPs (and accounted for roughly half of influent wastewater PhACs load). The fungus completely removed acetaminophen, diclofenac, ibuprofen, ciprofloxacin, furosemide, hydrochlorothiazide, ranitidine, atorvastatin, gemfibrozil, 2-hydroxycarbamazepine (2-hydroxyCBZ) and trazodone (Table 1). High removal was achieved for analgesics and anti-inflammatories (94%) as well as for antibiotics (91%) and around 42% for psychiatric drugs, in accordance with previous studies (Cruz-Morató et al., 2014; Mir-Tutusaus et al., 2017; Cruz-Morató et al., 2013; Lucas et al., 2016).

In the case of the AS treatment, 73% of the initial pharmaceutical load was eliminated, 92% disregarding the analgesics and anti-inflammatories. AS completely removed the analgesics and anti-inflammatories acetaminophen, ibuprofen and naproxen (Table 1), in line with the literature regarding these compounds and CAS (Verlicchi et al., 2012). AS also removed nearly 75% of the antibiotic ciprofloxacin, probably by adsorption to the biomass as it occurs in full-scale WWTPs (Verlicchi et al., 2012). Gemfibrozil is usually reported as recalcitrant in CAS (Verlicchi et al., 2010). In contrast it was partly removed in the present CAS experiment. In general, the AS treatment exhibited lower removal efficiencies than fungal treatment for most of the compounds analyzed, and in agreement with removal efficiencies usually reported in WWTPs (Verlicchi et al., 2012; Wang et al., 2014). Fungal operation was thus more efficient than reference AS operation for HWW treatment and it could be regarded, in some cases, as a standalone treatment of such PhAC-polluted streams.

UV/H_2O_2 removed 80% of the total PhACs load while the fungal treatment and the AS removed 90% and 73% respectively. The removal of psychiatric drugs in the UV/H_2O_2 treatment was 69% while in the FG and AS treatments was 55 and 24% respectively. Contrarily, the removal of antibiotics with UV/H_2O_2 was 67%, while with the biological treatments the removal efficiencies were 91% in FG and 36% in the AS. The removal of analgesics and anti-inflammatories with UV/H_2O_2 was similar than with the biological treatments.

3.2. Performance of the coupled treatments

3.2.1. Biological treatments coupled with UV/H_2O_2

The combinations FG + UV/H_2O_2 and AS+UV/H_2O_2 are shown in Fig. 2A and B, respectively. Placing the UV/H_2O_2 after the biotreatment aimed at degrading the remaining PhACs and the corresponding TPs produced by the first-placed biological processes. Psychiatric drugs were the most recalcitrant family in this combination of treatments, although carbamazepine, citalopram and venlafaxine were > 80% removed in the best-case scenario. Further discussion on CBZ and its TPs can be found in Section 3.4.

In the FG + UV/H_2O_2 combination (Fig. 2A), the UV/H_2O_2 removed only partially the residual PhACs content after the fungal treatment. Exceptions were CBZ TPs, produced by the biotransformation and deconjugation of carbamazepine, and ofloxacin, whose removal was improved by the UV/H_2O_2 treatment. Placing the UV/H_2O_2 after the AS (Fig. 2B), instead, improved significantly the overall PhACs removal, even though the removal efficiency of AS was lower than the one obtained by FG. AS can indeed decrease the COD, improving the effectiveness of the subsequent UV/H_2O_2: since OH radicals produced in UV/H_2O_2 treatment have non-selective reactivity to organic materials, their effectiveness in PhAC degradation is lower when treating matrices with high COD or TSS content (Kim et al., 2009b). Overall, placing the UV/H_2O_2 after the biological treatment could effectively increase the total PhACs removal, compared to the single treatment, up to 95%.

3.2.2. UV/H_2O_2 coupled with biological treatments

Overall removal values of UV/H_2O_2 coupled with the biological treatments, as post treatments, can be found in Table 1.

Fig. 3 presents the cumulative removal efficiencies of UV/H_2O_2 coupled with the biological treatments. The UV/H_2O_2 step alone completely removed the analgesics and anti-inflammatories acetaminophen, diclofenac and naproxen, the antibiotic ofloxacin, furosemide, hydrochlorothiazide, ranitidine, atorvastatin, and the psychiatric drug trazodone. It also removed by more than 80% valsartan, atenolol, 10,11-epoxyCBZ (by far the major contributor to the psychiatric drugs family), 2-hydroxyCBZ and citalopram. Carbamazepine and gemfibrozil were poorly degraded by UV/H_2O_2 in accordance with previous studies (Wols et al., 2013). It is noteworthy to remember that HWW was pretreated with coagulation-flocculation to lower its COD and TSS content, which could have allowed the good observed removals with UV/H_2O_2.

Placing the UV/H_2O_2 step before a biological treatment generally aims at increasing the biodegradability of biorecalcitrant compounds (Oller et al., 2011). This was confirmed in this work with both AOP + biologic treatment couplings. Regarding the first coupling (UV/H_2O_2 + FG treatment, Fig. 3A), although each single treatment was already able to remove more than 70% of the initial pharmaceutical load, this value raised up to 95% when the two treatments were coupled. In the case of the other coupling (UV/H_2O_2 + AS, Fig. 3B) a similar trend was observed: i.e. UV/H_2O_2 eliminated 74% of ibuprofen and the following biological treatment increased its removal up to 100%. Likewise, in the case of gemfibrozil an increase of the removal efficiency from 45% to 60% was observed, while the removal of any other compound was considerably affected. In fact, coupling the two treatments only increased the overall removal from 80% (UV/H_2O_2) to 83% (UV/H_2O_2 + AS). However, AS might obtain benefits from a previous pretreatment with UV/H_2O_2: for example to degrade part of the antibiotics, with some of them known to be toxic to the bacterial community in AS (Collado et al., 2013).

Though promising, some reports have expressed concern about the application of AOPs for micropollutants removal because the TPs generated in these oxidation processes may have higher toxicity than the corresponding parent compounds (Yang et al., 2017). However, in some cases a reduction in ecotoxicity to larval zebrafish could be observed in the effluent of several advanced wastewater treatment systems, suggesting a lack of negative short-term biological effects (Angeles et al., 2020). The toxicity values evaluated in raw wastewater and after the treatments performed in this study are presented in Fig. 4. As it can be observed, UV/H_2O_2 diminished the toxicity of flocculated HWW by almost one TU. In contrast, the UV/H_2O_2 did increase the effluent toxicity when placed after any biological treatment. This behavior could be attributed to the complexity of the matrix: the degradation of some compounds can lead to less toxic effluents (Cavalcante et al., 2015; Bilińska et al., 2016; Díaz-Garduño et al., 2016) while degradation of others might not affect the toxicity (Dantas et al., 2008; González et al., 2007) and other compounds might create more toxic intermediates (Calza et al., 2006; Méndez-Arriaga et al., 2008; Le et al., 2017). Therefore, toxicity increase/decrease after UV/H_2O_2 might be regarded as matrix-specific, as a function of the pharmaceutical load and category. Despite it has not been measured in this work, we cannot discard that the overall mineralization could have a direct impact on the toxicity (Perisic et al., 2016; Sági et al., 2018). The FG treatment consistently increased the toxicity of the effluent, although previous studies with analogous wastewater showed opposite results (Cruz-Morató et al., 2014; Mir-Tutusaus et al., 2017; Cruz-Morató et al., 2013). It was likely that some fungal products interfered with *Vibrio fischeri* survival. Contrarily, AS was the only treatment that constantly reduced the acute toxicity of

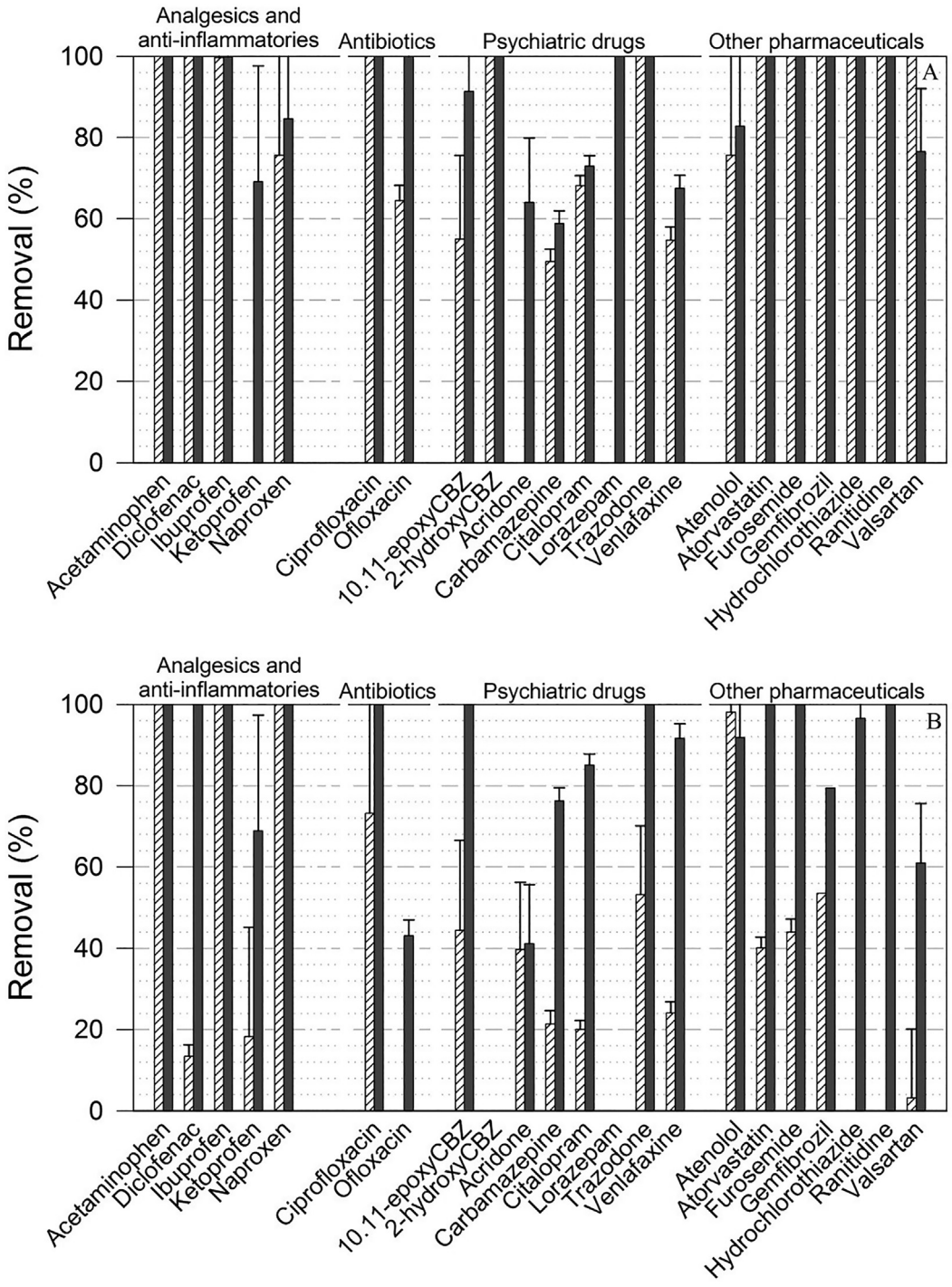

Fig. 2. Cumulative removal percentages of PhACs with FG + UV/H_2O_2 (A) and AS + UV/H_2O_2 (B) couplings. Dashed bars represent the removal of the fungal (A) and activated sludge (B) treatments; grey bars the overall removal of the corresponding biological step followed by UV/H_2O_2 process.

the effluent, whether it was placed before or after the UV/H_2O_2. In all cases, it is important to highlight that there are many non-analyzed compounds that can be contributing to sample toxicity.

3.3. Lin/deconjugation of compounds

The increase in the concentration of some PhACs during biological wastewater treatment has been reported in the literature and it is usually attributed to desorption from solids and also to conjugation/deconjugation phenomena of PhACs metabolites Conjugation is a mechanism used by several organisms to detoxify xenobiotics as part of the Phase II metabolism and it involves the covalent addition of a molecule to a compound (Badia-Fabregat et al., 2015; Sanchez and Kauffman, 2010). In the human liver, for example, the conjugation leads to the formation of water-soluble compounds that can be excreted through urine, being glucuronidation the most common conjugation pathway in the biotransformation of xenobiotics (Sanchez and Kauffman, 2010). Therefore, while both PhACs TPs and conjugated forms are commonly present in wastewater, the analytical methods are mostly targeting only parent compounds and non-conjugated

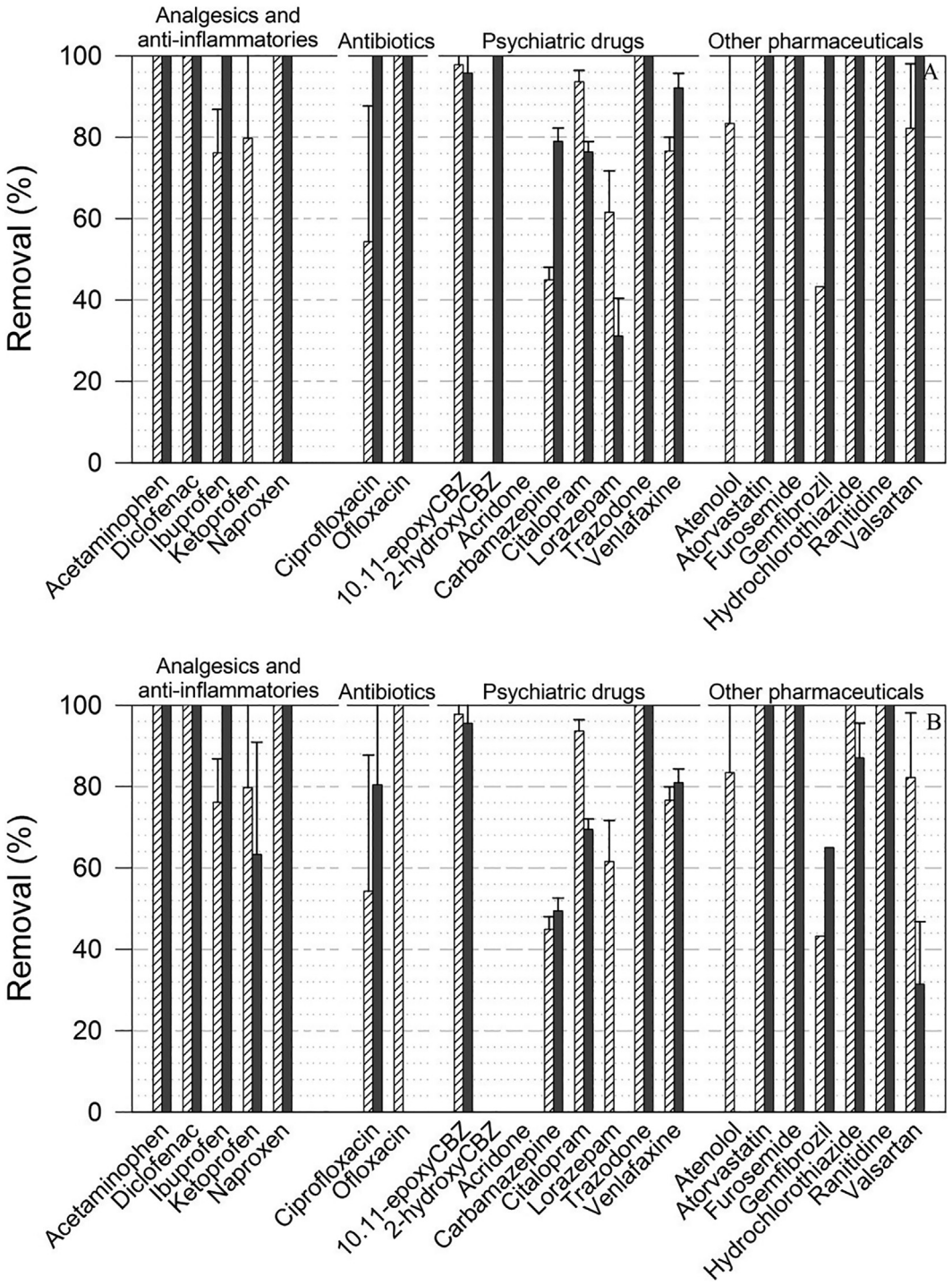

Fig. 3. Cumulative removal percentages of PhACs with UV/H_2O_2 + FG (A) and UV/H_2O_2 + AS (B) couplings. Dashed bars represent the removal of the UV/H_2O_2 treatment; grey bars, the removal of the coupled fungal (A) and activated sludge (B) treatments.

molecules. Deconjugation in activated sludge and fungal treatments has been described elsewhere, but in general it can occur in biological systems where specific enzymes exist that break the covalent bond in conjugated compounds (Badia-Fabregat et al., 2015; Jelic et al., 2015). It probably occurred in the present study as it can be inferred by the increase of the concentration of some compounds (discussed after Table 1). Specifically, atenolol (Yuan et al., 2013b), citalopram (Dalgaard and Larsen, 1999), ketoprofen, naproxen, ibuprofen, diclofenac, gemfibrozil, ofloxacin, carbamazepine, 2-hydroxyCBZ, 10,11-epoxyCBZ, acridone and lorazepam (Jelic et al., 2015) can be excreted to some extent as conjugates.

Without taking into account CBZ and its TPs (discussed in detail in Section 3.4), the compounds detected at higher-than-initial concentration were treatment-specific: concentration of ofloxacin and lorazepam increased only after the AS treatment, while ketoprofen only after the fungal treatment. This fact could mean that the two treatments had distinct deconjugation capacities. In an attempt to shed light into the subject, ketoprofen can be considered as an example: it is well removed by

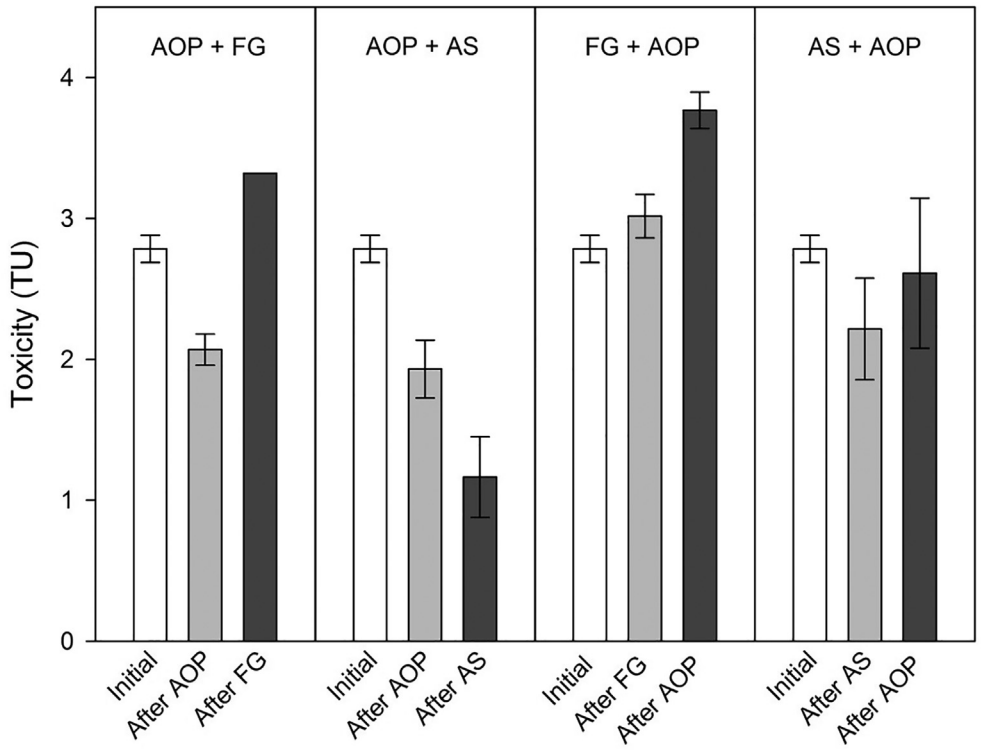

Fig. 4. Toxicity values of the raw HWW (white bars), after the first treatment (grey bars) and at the end of the coupled treatments (black bars).

fungal treatments in spiked defined matrices (Mir-Tutusaus et al., 2016) but an increase in its concentration has been observed with real matrices (Badia-Fabregat et al., 2015; Mir-Tutusaus et al., 2017). Similarly, ketoprofen concentration rose in the fungal treatments both before and after the UV/H_2O_2, proving that conjugated forms of ketoprofen were present in HWW and that UV/H_2O_2 did not deconjugate –nor remove– them. Nevertheless, deconjugation of PhACs could not be confirmed in this study as no conjugated compounds were quantified. Thus, the concentration of the whole set of PhACs, their TPs, metabolites and conjugates was potentially undervalued to an unknown degree.

3.4. Fate of carbamazepine and transformation products

Monitoring of parent compounds is as important as monitoring of their TPs, both already present in the raw water and newly generated, in order to properly evaluate the real efficiency of a treatment. In this study CBZ has been taken as model compound and some of its TPs have been quantified. CBZ, in fact, is frequently found in the environment worldwide (Zhang and Geißen, 2010) and it is usually refractory to biodegradation with conventional wastewater treatments. For this reason it remains stable through the aquatic compartments (Zhang and Geißen, 2012), and several approaches have been tested to remove it. Different CBZ TPs may be formed based on the process/treatment. A summary of CBZ transformation pathways in humans, UV/H_2O_2, white-rot fungus and AS can be found in Fig. 5 and it embodies a compendium of different studies (Golan-Rozen et al., 2015; Mathieu et al., 2011; Pearce et al., 2009; Lekkerkerker-Teunissen et al., 2012; Kaiser et al., 2014; Kosjek et al., 2009; Jarrott, 1999; Lynn et al., 1978; Thorn et al., 2011; Jelic et al., 2012). Several TPs are depicted but only 2-hydroxyCBZ, 10,11-epoxyCBZ and acridone could be included in the present study. The raw HWW already contained human metabolites of the CBZ: the parent compound, CBZ, is primarily metabolized in the human liver generating 10,11-epoxyCBZ (CBZE) as main metabolite (Thorn et al., 2011). It is then further transformed to form acridine, acridone and the non-pharmaceutically active 10,11-dihydroxyCBZ (CBZD). A minor pathway is the transformation of CBZ to 2,3-epoxyCBZ to produce 2-hydroxyCBZ and 3-hydroxyCBZ. Human metabolites include also several glucuronides of CBZ, CBZE, CBZD, 2-hydroxyCBZ and 3-hydroxyCBZ, which were not analyzed in this study (Lynn et al., 1978).

The concentration of some of these TPs as well as CBZ in wastewater before and after each treatment can be found in Table 2. The initial load of CBZE in this study was 100-fold higher than 2-hydroxyCBZ (Table 2), because CBZE is the metabolite from the main route for CBZ transformation in humans and it is produced and excreted at much higher concentration than CBZ itself (Thorn et al., 2011). In fact, CBZE was the compound with the highest concentration in the raw HWW analyzed. In general, UV/H_2O_2 and AS seem to follow the same degradation pathway, namely, CBZE, acridine-9-carbaldehyde, acridine and acridone. White-rot fungal pathway resembles the human CBZE pathway (Jelic et al., 2012); and therefore, 2-hydroxyCBZ and 3-hydroxyCBZ could also be generated by WRF, as both humans and fungus have similar cytochrome P450 systems. In fact, fungi *C. elegans* and *U. ramanniana* were reported to produce such compounds when metabolizing CBZ (Kang et al., 2008).

The UV/H_2O_2 treatment removed 90% of the overall initial concentration of CBZ and TPs. It removed around 50% of CBZ, although UV/H_2O_2 has been reported to degrade up to a 70% of CBZ in some wastewaters (Giannakis et al., 2015). In contrast, 98% removal of the main metabolite, CBZE, was achieved whereas acridone concentration increased. Acridone is a byproduct of CBZE degradation although its increase does not account for the complete degradation of CBZE; AOPs are able to degrade the acridine-9-carbaldehyde, acridine and acridone (Kosjek et al., 2009), so acridone must have been degraded too.

The fungal treatment removed 54% of CBZ, being the single treatment with the highest CBZ reduction. This is in accordance with previous works, with achieved removal values of around 50% (Mir-Tutusaus et al., 2017). CBZE concentration decreased by 56% and acridone increased substantially, as expected when taking into account

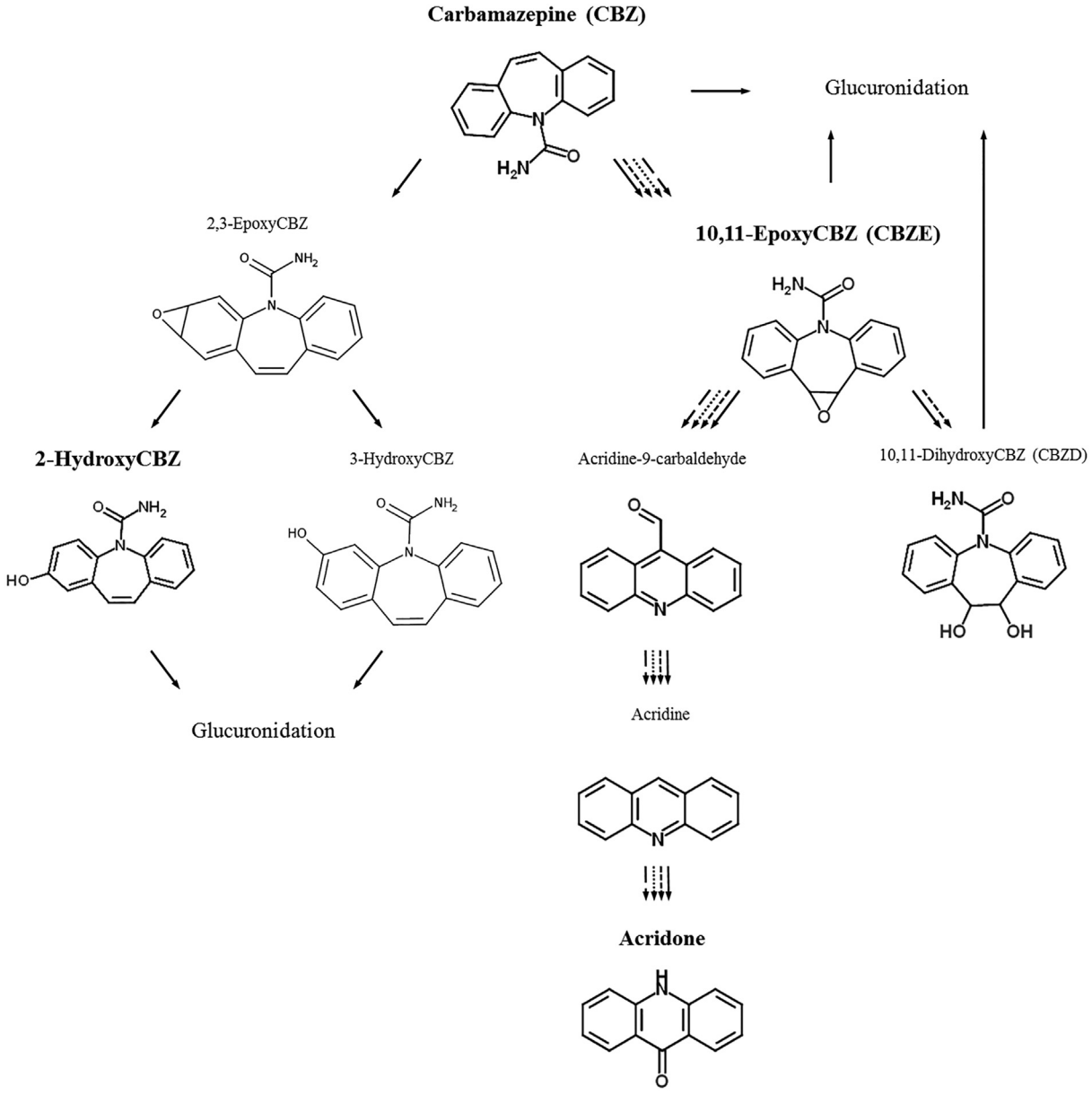

Fig. 5. Transformation pathways of CBZ: in humans (solid lines), white-rot fungi (dotted lines), AS (short-dashed lines) and UV/H_2O_2 (long-dashed lines). Analyzed compounds are presented in bold. Only TPs relevant to this study have been represented (Golan-Rozen et al., 2015; Mathieu et al., 2011; Pearce et al., 2009; Lekkerkerker-Teunissen et al., 2012; Kaiser et al., 2014; Kosjek et al., 2009; Jarrott, 1999; Lynn et al., 1978).

the fungal transformation pathway (Fig. 5) and in agreement with previous studies (Mir-Tutusaus et al., 2017). CBZE and 2-hydroxyCBZ might have been present as glucuronides in raw HWW and biological processes might have deconjugated such glucuronides, which would have in turn undervalued total removal capabilities of the treatment (Badia-Fabregat et al., 2015).

The AS process removed CBZ and CBZE less than the fungal treatment, but it did not accumulate acridone. CBZ is known to be very

Table 2
Levels of CBZ and CBZ TPs (ng L^{-1}) in the initial HWW and after each treatment.

Pharmaceutical	Initial concentration (ng L^{-1})	Final concentration (ng L^{-1})						
		UV/H_2O_2	FG	AS	UV/H_2O_2 + FG	UV/H_2O_2 + AS	FG + UV/H_2O_2	AS + UV/H_2O_2
Carbamazepine	4118	2067	1897	2952	790	1897	1545	892
10,11-epoxyCBZ	28,505	622	12,675	15,655	1195	1254	2442	bld
2-hydroxyCBZ	335	35	bld	5465	bld	1045	bld	605
Acridone	493	542	5710	257	1402	1544	153	251
Total	33,451	3266	20,282	24,329	3387	5740	4141	1747
Removal (%)		90	39	27	90	83	88	95

recalcitrant and poorly degraded in CAS, while acridone has been reported to be removed up to a 40% (Kosjek et al., 2009). Other unmeasured intermediates could have been accumulated during the treatment before reaching acridone. As it was commented for the fungal process, in fact, some compounds could have been present as glucuronides that the AS could deconjugate. This pathway was confirmed by the observed increase in 2-hydroxyCBZ (which is not a byproduct of CBZ in AS), supporting the hypothesis of deconjugation of already present 2-hydroxyCBZ glucuronides.

In terms of coupled treatments, an increase of 2-hydroxyCBZ was observed with both possibilities (UV/H_2O_2 + AS and AS + UV/H_2O_2). Contrarily, the increase in acridone produced by UV/H_2O_2 can be limited with AS + UV/H_2O_2, that resulted the most convincing strategy to remove CBZ and its TPs. This combination removed, in fact, around 80% of the initial CBZ, as if the removal percentages of UV/H_2O_2 and AS separately were additive. Removal of CBZ and acridone were lower than in other combinations, and 2-hydroxyCBZ increased. Nonetheless, considering that 10,11-epoxyCBZ was the most present compound and that it was completely removed, 94% overall removal of CBZ and its TPs could be achieved. In general, both combinations of the tested biotreatments with UV/H_2O_2 could eliminate most of initial CBZ. This is in accordance with other studies that used electrooxidation or UV/H_2O_2 combined with a membrane bioreactor, even though of course, a part of the biodegradation of the activated sludge the effect of the membrane filtration and related phenomena should be accounted for in that case (Monteoliva-García et al., 2020; Ouarda et al., 2018). In fact, the UV/H_2O_2 already removed 90% of the CBZ and its TPs even in real, flocculated HWW. The best coupling approach improved this removal only by an additional 5% (up to 95%, with AS+UV/H_2O_2). Nevertheless, if we take into account also the conjugates and/or other TPs, then placing UV/H_2O_2 after a biological treatment, as a polishing step, would be the optimal treatment train configuration: the biological treatment would deconjugate and remove part of the compounds and UV/H_2O_2 would degrade the remaining pollutants and byproducts.

4. Conclusions

In terms of overall PhACs removal, three out of the four considered treatment trains (namely UV/H_2O_2 + AS, UV/H_2O_2 + FG, FG + UV/H_2O_2 and AS+UV/H_2O_2), showed high removal values (93–95%), whereas UV/H_2O_2 + AS exhibited the lowest removal (83%). However, the latest, was the only treatment leading to a toxicity decrease.

Although placing UV/H_2O_2 prior to AS is regarded sometimes in the bibliography as beneficial due to the increase of compounds biodegradability (Bilińska et al., 2016), this study showed better results when UV/H_2O_2 was placed after AS. The performance of UV/H_2O_2 probably benefited from the decrease in terms of COD and suspended solids promoted by the AS, leading to overall higher removal values. This is in accordance with the bibliography, as AOPs (such as UV/H_2O_2) are mainly used as a polishing step after a biological treatment.

Concerning the coupling of UV/H_2O_2 with the fungal treatment, no significant difference in PhACs removal was observed regardless of the treatment order. Therefore, it might be advisable to place FG as a pretreatment so that UV/H_2O_2 can further degrade not only parent compounds, but also TPs and previously non-accessible conjugated compounds. Economically speaking, using UV/H_2O_2 as a polishing step is also preferable, as it reduces reagents and energy usage. However, an increase on water toxicity was observed in this configuration.

Concerning the removal and generation of transformation products along the treatment trains, the psychiatric drug CBZ and its TPs were highly removed (90%) by UV/H_2O_2 alone and the coupling with a biological treatment (AS + UV/H_2O_2) could improve it up to 95%.

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CRediT authorship contribution statement

Josep Anton Mir-Tutusaus: Investigation, fungal treatment Writing original draft. **Adrian Jaen-Gil:** Investigation and PhACs analysis. **Damià Barcelo**: Supervision and reviewing. **Gianluigi Buttiglieri:** Investigation, activated sludge treatments Rafael Gonzalez-Olmos: Investigation and H2O2/UV treatments **Sara Rodríguez-Mozaz:** Conceptualization, supervision analysis and Reviewing. **Gloria Caminal:** Conceptualization, Supervision and Reviewing. **Montserrat Sarrà:** Supervision and Reviewing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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