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Additional Information

Removal of pharmaceutical compounds commonly-found in wastewater through a hybrid biological and adsorption process

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Abstract

Nowadays, alternative options to conventional wastewater treatment should be studied due to rising concerns emerged by the presence of pharmaceuticals compounds (PhCs) in the aquatic environment. In this work, a combined system including biological treatment by activated sludge plus adsorption with activated carbon is proposed to remove three selected drugs (acetaminophen (ACT), caffeine (CAF) and ibuprofen (IBU)) in a concentration of 2 $mg \cdot L^{-1}$ of each one. For it three sequencing batch reactors (SBR) were operated. SBR-B treated a synthetic wastewater (SWW) without target drugs and SBR-PhC and SBR-PhC+AC operated with SWW doped with the three drugs, adding into SBR-PhC+AC 1.5 $g \cdot L^{-1}$ of a mesoporous granular activated carbon. Results showed that the hybrid system SBR-activated carbon produced an effluent free of PhCs, which in addition had higher quality than that achieved in a conventional activated sludge treatment in terms of lower COD, turbidity and SMP concentrations. On the other hand, five possible routes of removal for target drugs during the

biological treatment were studied. Hydrolysis, oxidation and volatilization pathways were negligible after 6 h of reaction time. Adsorption route only was significant for ACT, which was adsorbed completely after 5 h of reaction, while only 1.9% of CAF and 5.6% of IBU were adsorbed. IBU was the least biodegradable compound.

Keywords: Emerging pollutants; Pharmaceutical compounds; Sequencing bath reactor; Activated carbon

1. INTRODUCTION

The first synthetic therapeutic compounds appeared in the first decades of the 20th century due to medical science advances. From this time, a rapid development of these substances was performed as a result of growing demand, both to improve human health and to prevent or treat diseases in animals [1], resulting in thousands of different active compounds. In this way, European Public Assessment Reports (EPARs), published in October 2019 by European Medicines Agency, reported an amount of 1584 authorised medicines in Europe. Additionally, the amount of consumed pharmaceuticals compounds (PhCs) has increased over the years. According to estimations of QuintilesIMS [2], the use of PhCs will be increased by 24% between 2015 and 2020, achieving a globally amount of 4500 billion doses.

A portion of these substances are excreted after their consumption, both in their original form or in their active metabolites, ending up in the sewage and, consequently, in the wastewaters treatment plants (WWTPs) [3]. Furthermore, as reported in many studies [4–6], the main entrances of PhCs on the aquatic environments are through the treated effluents in WWTPs, due to incomplete removals by the conventional techniques, which are designed to eliminate

organic matter and nutrients, as phosphorous and nitrogen compounds. Although PhCs in these effluents are found in very low concentrations ($ng \cdot L^{-1}$ or $\mu g \cdot L^{-1}$), it should be highlighted that these active compounds are designed to be bioactive at low concentrations and for having an effect on living organisms.

Nowadays, a greater attention is paid to PhCs occurrence in the aquatic environment due to their potential toxic effect on the aquatic species [7–9], which could affect to human health. This concern is reflected in the European Union legislation, which has included diclofenac into the list of substances to be monitored [10]. Thus, alternative treatments to the conventional activated sludge process or tertiary treatments should be performed to remove completely PhCs in WWTPs, since more restrictive conditions for water quality can be expected in the future.

Occurrence of PhCs substances is achieved in surface waters [11,12], seawater [13,14] and sediments [15,16]. Analysing 50 samples of different aquatic samples collected in 20 countries around the world, it was observed that diclofenac and ibuprofen were present in 90% of cases [9]. Another drugs commonly found in Mediterranean sea were the analgesics, antibiotics and stimulants like acetaminophen (paracetamol), sulfamethoxazole or caffeine [14,17].

In this work a synthetic wastewater (SWW) containing three of the PhCs commonly found in different aquatic environments, was treated combining an activated sludge process and adsorption with activated carbon. The selected PhCs were a nonsteroidal anti-inflammatory drug (ibuprofen) and an analgesic (acetaminophen), which are over-the-counter drugs employed both from human and veterinary medicine. The other one was the most used stimulating compound, caffeine, present in medicines, food and drinks. A deep study of the different removal routes, which took place during the biological wastewater treatment to

eliminate these PhCs, was carried out. This study included biodegradation, adsorption on active sludge flocs, oxidation, hydrolysis and volatilization. It was performed through several batch experiments. Additionally, an alternative treatment to the conventional active sludge system, which included adsorption with activated carbon, was performed. For it, three sequencing batch reactors (SBRs) were operated during 35 days: the first SBR worked with a SWW without PhCs, as a control reactor, the second and third reactors treated the same SWW that also contained the three target drugs, adding activated carbon into the third SBR to enhance the PhCs removal efficiency.

2. MATERIALS AND METHODS

2.1. Synthetic wastewater and Target pharmaceuticals

Synthetic wastewater was prepared with peptone and meat extract, in equal amount (225 mg per liter of SWW), as nitrogen and organic matter sources, and K_2HPO_4 as phosphorus source (28 mg per liter of SWW). Concentration of these reagents were calculated to achieve a food to microorganisms (F/M) of 0.23 g COD·g $MLSS^{-1}$·d^{-1}. Despites the recommendation for COD:N:P relationship in aerobic systems is 100:5:1 [18,19], due to peptone and meat extract composition, the final rate was 100:12:1, achieving a final COD of 575 mg·L^{-1}. All the reagents were diluted with tap water, which provided other necessary minority compounds.

In Table 1 some physicochemical characteristics of the three PhCs studied in this work are shown [20]. All of these reagents had purities higher than 99.8% (Sigma-Aldrich; Germany). Standard solutions of 10,000 mg·L^{-1} were prepared from these substances, dosing the needed volume into SWW up to a final concentration of 2 mg·L^{-1}. Caffeine (CAF) and

acetaminophen (ACT) were diluted with osmotized water. Ibuprofen (IBU) was diluted with methanol due to its low water solubility.

Table 1. Physicochemical properties of target pharmaceuticals.

Pharmaceutical active compound	Category class	Formula	Molecular weight (g/mol)	Water solubility ($mg \cdot L^{-1}$)	pKa	Log K_{ow} *
Acetaminophen	analgesic	$C_8H_9NO_2$	151.17	14,000	9.40	0.52
Caffeine	stimulating	$C_8H_{10}N_4O_2$	194.19	22,000	2.30	0.07
Ibuprofen	anti-inflammatory	$C_{13}H_{18}O_2$	206.29	10-49	4.91	1.16

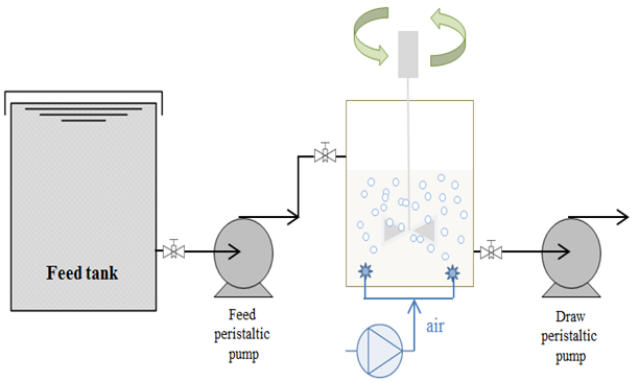
(*) The log $\overline{K_{OW}}$ values at pH = 7.0 were calculated by ACD/LogD version 12.0.

2.2. Activated carbon

The activated carbon used in this work was MG1050 from ChiemiVall, which was a granular bituminous carbon activated by thermal process. This carbon has a basic character (pH of the aqueous extract between 8 and 10). This is a mesoporous carbon (average pore diameter = 30 Å), which particle size range between 2.38-0.59 mm. To remove fine particles, the material was sieved at 0.6 mm before use.

2.3. Sequencing batch reactors (SBR)

Three identical SBRs were operated during 35 days at 21.6 ± 2.2ºC. Components of each SBR and common operating conditions of three reactors are presented in Figure 1.



Operating parameters		
V_R	6	L
HRT	24	h
F/M ratio	0.23	kg COD·kg MLSS^{-1}·d^{-1}
MLSS	≈ 2.5	g·L^{-1}
Number of cycles	3	cycles/d
Cycle characteristics		
Filling +Aerobic reaction	6	h
Sedimentation	1.5	h
Draw	25	min
Idle	5	min

Figure 1. SBRs characteristics and configuration.

Each SBR consists of a cylindrical Plexiglas tank (30 × 20 cm in height and diameter) with two valves to carry out the feeding and extraction by means of two peristaltic pumps (Dinko Instruments). During reaction phase, which included feed filling, homogeneous conditions were achieved by a mechanical stirrer (Heidolph), which operated at 200 rpm. In this phase, an air compressor (Eheim) supplied the necessary oxygen to achieve aerobic conditions (≈ 2.5 mg $O_2 \cdot L^{-1}$) through two air diffusers located at the bottom of the reactor.

The initial activated sludge was collected from a municipal wastewater treatment plant from Valencia (Spain). Periodical sludge withdrawals were carried out during the experimental procedure, to maintain 2.5 g·L^{-1} of mixed liquor suspended solids (MLSS) concentrations in the reactors.

The three SBRs were fed with the same SWW in the first 4 days, to acclimate the biomass to the new conditions. From 5th day on, several parameters were varied in each reactor. The first reactor, referenced as SBR-B, worked as a control system and was fed with SWW until the end of the experiment. The second one, named SBR-PhC, treated SWW in which the three target pharmaceuticals were added in concentrations of 2 mg·L^{-1} each one (SWW+PhC). The third reactor, referenced as SBR-PhC+AC, was fed with SWW+PhC and, additionally, 1.5

$g \cdot L^{-1}$ of activated carbon (AC) was added into the reactor. The amounts of AC removed by sludge withdrawals were replaced with fresh carbon to maintain this concentration.

In order to follow the evolution of treated water, pH, conductivity, turbidity, soluble COD and suspended solids (SS_{ef}) of the effluents were measured three times a week. In addition, once a week soluble total nitrogen (N_T), ammonia nitrogen (NH_4-N), nitrates (NO_3-N), total phosphorous (P_T) and phosphates (PO_4-P) were controlled. Finally, influent and effluent streams of SBR-PhC and SBR-PhC+AC were analyzed three times (on days 7, 21 and 35) to measure the PhC concentrations.

Regarding the mixed liquors of SBRs, MLSS concentration was measured three times a week and mixed liquor volatile suspended solids (MLVSS) concentration once a week. Activated carbon was separated previously by sieving before to MLSS and MLVSS measurement in SBR-PhC+AC samples. For this aim, ML was filtrated using a metallic filter of 0.5 mm, to ensure that all the AC was removed. In SBRs the stirring was smooth and no friction areas inside the reactor were observed. Thus, no fractionation of activated carbon particles was detected. From this information, the average sludge production (ΔX) was calculated according to Eq.(1) and Eq.(2):

$$X_{i-j} = \frac{\left(MLSS_j - MLSS_i\right) \cdot V_R}{j - i} + \left[SS_{ef} \cdot Q_{ef}\right]_i^j \qquad \text{Eq.(1)}$$

$$\Delta X = \sum X_{i-j} \qquad \text{Eq.(2)}$$

where SS_{ef} is the suspended solids concentration ($mg \cdot L^{-1}$) and Q_{ef} is the flow rate ($L \cdot d^{-1}$) of the effluent.

Eq.(1) allowed to calculate the sludge production between two days in which sludge withdrawal was not performed (i and j), taking into account the biomass growth (first term)

and the biomass lost through the treated effluent (second term). Additionally, the sludge retention time (SRT) can be calculated using Eq.(3).

$$SRT = \frac{\overline{MLSS} \cdot V_R}{\Delta X} \qquad \text{Eq.(3)}$$

where $\overline{MLSS}$ was the average of MLSS concentrations during the experimental time.

Other parameters, related to the biomass, were analysed to assess the influence of PhCs on the activated sludge. These parameters were the soluble microbial products (SMP; measured once a week) and six microbial hydrolytic enzymatic activities (MHEA; analysed in the same three days in which PhC concentration were tested). Additionally, PhCs adsorbed on activated sludge of SBR-PhC were analysed at the end of the experiment after a previous extraction by organic solvents.

2.3. Batch experiments

The removal of pollutants in the activated sludge process are carried out through several pathways: hydrolysis (H), oxidation (O), volatilization (V), adsorption (A) and biodegradation (B) [21,22]. In this work, four batch experiments were carried out to evaluate the five pathways of PhCs removal during a reaction cycle corresponding to SBR-PhC operation. These tests were performed in four beakers of 1 L, at 22.1 ± 0.4°C, stirring (in the four batch experiments) and aerating (in three of the batch experiments) the mixture for 6 h, to study in each test the removal routes presented in Table 2.

Table 2. Batch experiments.

Test	Removal routes	ML (L)	SWW+PhC (L)	Aeration (mg $O_2 \cdot L^{-1}$)	$HgCl_2$
I	B + A + O + H + V	0.67	0.33	2 – 3.5	0
II	A + O + H + V	0.67	0.33	2 – 3.5	30 mg·g $MLSS^{-1}$
III	O + H + V	0	1	2 – 3.5	5 mg·L^{-1}
IV	H + V	0	1	0	5 mg·L^{-1}

As it is shown in this table, Test I and II were performed with the necessary amounts of mixed liquor (acclimated to the drugs presence) and SWW+PhC to achieve the same operational conditions of SBR-PhC. Additionally, in Test II 30 mg·g $MLSS^{-1}$ of mercury chloride ($HgCl_2$, 99.5%, Sigma–Aldrich) were added to achieve the activated sludge inhibition [23]. Test III and IV were carried out with 1 L of SWW+PhC, with and without aeration, respectively. In both tests, 5 mg·L^{-1} of $HgCl_2$ were dosed to inhibit the eventual microbial activity.

In each test, PhCs concentrations were measured at the initial time and every hour to study its removal percentage. In addition, these results allowed to obtain the kinetics of biodegradation routes, evaluated through the equations of zero-order [Eq.(4)], first-order [Eq.(5)], and second-order [Eq.(6)].

$$C_t = C_0 - k_0 \cdot t \qquad \text{Eq.(4)}$$

$$C_t = C_0 \cdot e^{-k_1 \cdot t} \quad \rightarrow \quad \ln C_t = \ln C_0 - k_1 \cdot t \qquad \text{Eq.(5)}$$

$$C_t = \frac{C_0}{1 + C_0 \cdot k_2 \cdot t} \quad \rightarrow \quad \frac{1}{C_t} = \frac{1}{C_0} + k_2 \cdot t \qquad \text{Eq.(6)}$$

where C_0 and C_t are the concentration measured at initial conditions and at time t; k_0, k_1, and k_2 are kinetic constants.

2.4. Analysis

pH and conductivity were measured with GLP 21+ and GLP 31+ equipment (both from Crison), respectively. Turbidity was analysed using a turbidimeter D-122 from Dinko. COD, N_T, P_T and the others ions were analysed in Spectroquant NOVA 30, using reagent kits from Merck. MLSS and MLVSS were measured according to APHA, 2005 [24].

2.4.1. PhC characterisation

Concentration of caffeine, acetaminophen and ibuprofen were measured by a HPLC from Japan Spectroscopy Corporation (JASCO). All samples were filtered with 0.22 μm pore size syringe filter (Labbox) before analysis. Separation of different compounds was carried out by a Kinetex C18 (1.7 μm; 50 mm x 2.1 mm) column from Phenomenex. Caffeine and acetaminophen were analysed by a MD-2018 Photodiode Array detector using the same chromatographic method. Ibuprofen was measured with FP-4020 HPLC Fluorescence detector. The quantification limit for the three PhCs was 20 ppb. Table 3 shows the main conditions of the two established chromatographic methods.

Table 3. Chromatographic methods conditions.

Detector	Retention time (min)	Maximum absorption	Flux ($mL \cdot min^{-1}$)	Mobile phase*
MD-2018	ACT; 3.7	230 nm	0.8	0-15; 85% A + 15% B
	CAF; 12.1	270 nm		
FP-4020	IBU; 11.4	-	0.7	0-1 min; 70% A + 30% C 1-9 min; linearly increase up to 60% C 9-14 min; 40% A + 60% C 14-15 min; linearly decrease up to 30% C

(*) A=1% acetic acid in water; B=1% acetic acid in methanol; C=1% acetic acid in acetonitrile (Panreac).

The effluent samples of SBR-PhC and SBR-PhC+AC were subjected to a concentration procedure to ensure that the concentrations of ACT, CAF and IBU were higher that HPLC quantification limit. This procedure was performed by a solid phase extraction (SPE), following the methodology described by Vona A. et al. [25], to achieve a sample concentrated 500 times from the initial one. The other samples (SWW+PhC and samples of batch experiments) were measured directly.

2.4.2. Biomass characterisation

At the end of the reaction time, 30 mL of ML were centrifuged at 12000 x g. The liquid phase was filtered at 0.45 μm to analyse soluble microbial products (SMP). The solid phase was resuspended with Tris–HCl buffer (same volume of removed liquid) to perform hydrolytic enzymatic activities (MHEA) measurements.

SMPs were evaluated from proteins (BCA method [26]) and carbohydrates (anthrone method [27]) concentrations, since these compounds are the main elements of these substances [28]. Samples were measured in triplicate.

Several methods were applied to obtain MHEA concentrations. Since carbohydrates (25-50%) and proteins (40-60%) are the main compounds of the wastewater organic fraction [29], it can be considered that phosphatases, glucosidases and proteases are the most relevant hydrolytic activities, which are related to the active biomass [30]. Acid an alkaline phosphatase, α-D-Glucosidase and protease concentrations were analysed according to Goel et al. [31] method. MHEA measurements were normalized according to MLVSS concentration.

Finally, an external laboratory (Iproma Castellón, Spain) performed the extraction of PhCs adsorbed onto activated sludge of SBR-PhC. Each drug was quantified by HPLC analysis. For that, all the sludge of this reactor was dried in an oven at 30ºC until total dryness, in the last operational day. The extraction process was carried out by a solid-liquid extraction through the following methodology: 1) 2 mL of Mili-Q water and 1 g of dried sludge were shaken for 0.5 min in a 15 mL flask; 2) 8 mL of acetonitrile were added, shaking for 0.5 min; 3) 3 g of magnesium sulphate and 0.75 g of sodium acetate trihydrate were added, mixing the sample for 1 min; 4) This sample was centrifuged 12 min at 3900 rpm; 5) Finally, 1 mL of the liquid phase was treated until total dryness, adding then 1 mL of methanol, which was analyzed by HPLC. The quantification limits were 2 $\mu g \cdot kg^{-1}$ for ACT and CAF and 5 $\mu g \cdot kg^{-1}$ for IBU.

2.4.3. Statistical analysis

One-way ANOVA analysis (confidence level of 95 %) with Statgraphics Centurion XVII was performed to assess the effects of PhCs and carbon addition on the system. For it, two parameters were calculated: F-ratio and p-value. F-ratio provided information about the differences in the mean of the variances between several groups and the mean of the variances within the groups, so that the higher F-ratio, the greater the difference. In addition, a p-value less than 0.05 indicated that this difference showed a statistical significance and not a random result.

3. RESULTS AND DISCUSSION

3.1. Batch experiments

Oxidation, hydrolysis and volatilization pathways were negligible for the three PhCs, since a removal percentage for each drug lower than 1.0% was measured in Tests III and IV (data not

shown). These results agree with earlier studies [32,33], where the same behaviour was reported. Conversely, 100% of the three PhCs concentrations were removed in Test I (concentrations were below the quantification limit of HPLC for the three drugs), after 6 h of reaction time. On the other hand, Test II results varied with the analysed PhC. Figure 2 shows the evolution of normalized concentrations (C/C_0) of each PhC in Test I (left) and Test II (right), throughout the 6 h of experiments.

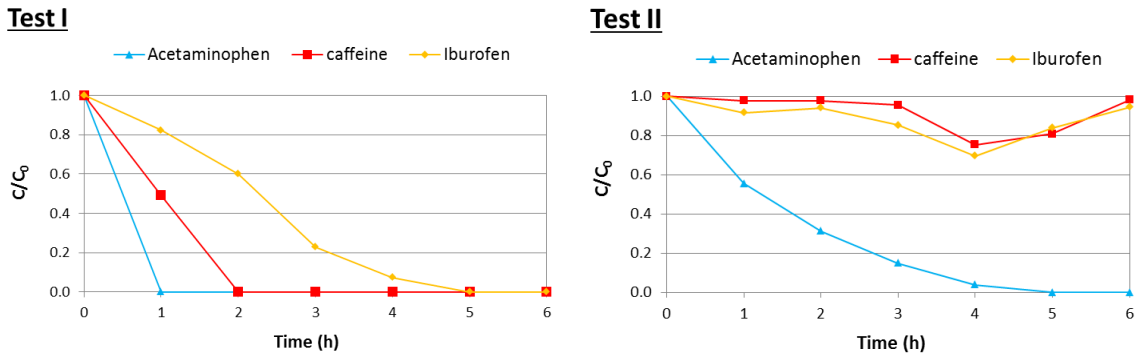


Figure 2. Evolution of normalized PhC concentrations during Test I and Test II.

Test II illustrates the elimination of drugs by adsorption as the only possible route of PhCs removal, since biomass was inhibited (unlike Test I) and O, H and V routes are negligible. In this Test, ACT was adsorbed on activated sludge after 6 h of reaction time, achieving from the 5th hour a concentration below of HPLC quantification limit. However, insignificant adsorption of CAF (1.9%) and IBU (5.6%) was observed at the end of the test. It's important to mention that the removal efficiencies obtained in both drugs from sampling times 4 and 5 hours of could be due to the eventual adsorption of CAF and IBU on the SWW components. These PhC were desorbed at the end of the experiment, probably due to the weakness of the bonds. Similar behavior (unexpected adsorption in some samples taken during batch tests) were also reported by Li and Zhang [32], Fan et al. [34] and Peng et al. [33]. The lower adsorption of CAF and IBU are related by the physicochemical characteristics of drugs and

activated sludge. Some researchers reported a positive correlation between the octanol–water partition coefficients values (log Kow) and the adsorption removal efficiencies for several PhCs [35,34]. Thus, considering only this parameter, the PhCs sequence that promotes the adsorption process should be IBU > ACT > CAF (according Table 1), which does not agree with the experimental results. This is because pKa of substances must also be taken into account. It is known that under neutral pH (typical value in the biological reactor of WWTPs), the activated sludge is negatively charged. Under this condition an electrostatic attraction between the sludge and ACT (pKa > 7) and an electronic repulsion between the sludge and the negatively charged IBU and CAF (pKa < 7) occur. This phenomenon explains the small IBU and CAF concentrations adsorbed on the sludge.

Test I, which include B and A pathways (since O, H and V were negligible as mentioned above), shows that ACT was the fastest drug removed by this route, followed by CAF and IBU. This behaviour has been reported in other works, in which the biological wastewater treatment showed higher removal efficiency for ACT than for CAF [36], and better results for CAF than for IBU [37]. Regarding Figure 2, it can be concluded that IBU was the least biodegradable compound. It can also be seen that ACT was removed faster than CAF, but it should be taken into account that both B and A routes contributed to ACT elimination, while only B route was responsible for CAF removal. Thus, it cannot be possible to know which of these two substances was the most biodegradable.

Due to the fast removals of ACT and CAF in Test I, biodegradation kinetic of both PhCs could not be calculated. The experimental results for IBU (in the first 4 h of reaction in which concentration was higher than 0) showed a better correlation with zero order kinetics, as it can be seen when comparing the R^2 of the three kinetic equations:

$$\text{Zero} - \text{order:} \qquad C_t = 0.577 + 0.136 \cdot t \qquad R^2 = 0.982$$

$$\text{First} - \text{order:} \quad \ln C_t = \ln(0.784) + 0.649 \cdot t \qquad R^2 = 0.900$$

$$\text{Second} - \text{order:} \quad \frac{1}{C_t} = \frac{1}{-0.433} + 5.065 \cdot t \qquad R^2 = 0.708$$

However, other researchers reported that IBU biodegradation with activated sludge was correlated with a first-order kinetic, after a test of 50 h in both cases. At the final of the experiment Min et al. [22] achieved a removal of 97.9%, while Peng et al. [33] reported 32.8%. In these works, it is unclear whether the sludge was previously adapted to PhCs presence, which could explain the wide difference between reported results.

3.2. Biological treatment

3.2.1. Effluent characteristics

Table 4 shows the average values for effluent parameters of each reactor and the one-way ANOVA analysis results (F-ratio and p-value) both calculated for the 35 experimental days, which were performed for all the parameters when the reactors are taken as a factor.

Table 4. Statistical results for effluent parameters of SBR-B, SBR-PhC and SBR-PhC+AC for 35 experimental days.

Effluent parameter	SBR-B	SBR-PhC	SBR-PhC+AC	F-ratio	p-value
pH	7.3 ± 0.3	7.3 ± 0.3	7.6 ± 0.2	5.92	0.0055
Conductivity ($mS \cdot cm^{-1}$)	1.20 ± 0.09	1.27 ± 0.09	1.27 ± 0.15	1.25	0.2978
Turbidity (NTU)	0.062 ± 0.022	0.179 ± 0.326	0.044 ± 0.015	3.96	0.0279
COD ($mg \cdot L^{-1}$)	35.3 ± 7.6	64.9 ± 17.5	37.3 ± 27.2	4.79	0.0224
N_T ($mg \cdot L^{-1}$)	46.4 ± 7.5	42.8 ± 5.2	40.7 ± 14.6	0.89	0.4284
$N\text{-}NH_4^+$ ($mg \cdot L^{-1}$)	≈ 0	≈ 0	7.5 ± 10.6	1.65	0.2274
$N\text{-}NO_3^-$ ($mg \cdot L^{-1}$)	39.3 ± 7.8	35.9 ± 5.8	24.6 ± 17.0	2.43	0.1240
P_T ($mg \cdot L^{-1}$)	8.2 ± 2.1	8.3 ± 1.3	6.2 ± 0.8	1.36	0.2843
$P\text{-}PO_4^{3-}$ ($mg \cdot L^{-1}$)	7.8 ± 2.6	7.5 ± 2.2	6.1 ± 1.4	0.90	0.4286

It can be observed a statistical significance for pH, turbidity and COD. Observing the Tukey diagrams for both pH and turbidity parameters presented in Figure 3, it can be seen that in

SBR-PhC+AC higher pH and lower turbidity than in the other two reactors were achieved during the 35 experimental days.

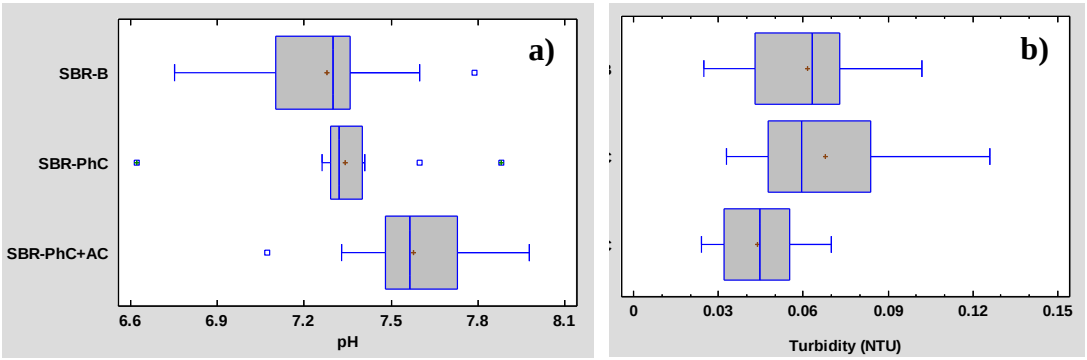


Figure 3. Tukey diagram for pH (figure a) and turbidity (figure b).

The higher pH in this reactor can be due because MG1050 has more affinity for acidic compounds, as a consequence of the alkaline character of this carbon. Regarding turbidity values, the adsorption capacity of colloidal particles on the carbons is known [38,39]. In this way, as expected, this parameter was lower in SBR-PhC+AC.

Concerning COD values, Figure 4 shows its evolution in each reactor during the experimental procedure. In this graph it can be seen that after drugs dosage the initial effluents COD values were similar in all the reactors (the average value of three reactors in 5^{th} day was 46.3 ± 7.6 $mg \cdot L^{-1}$).

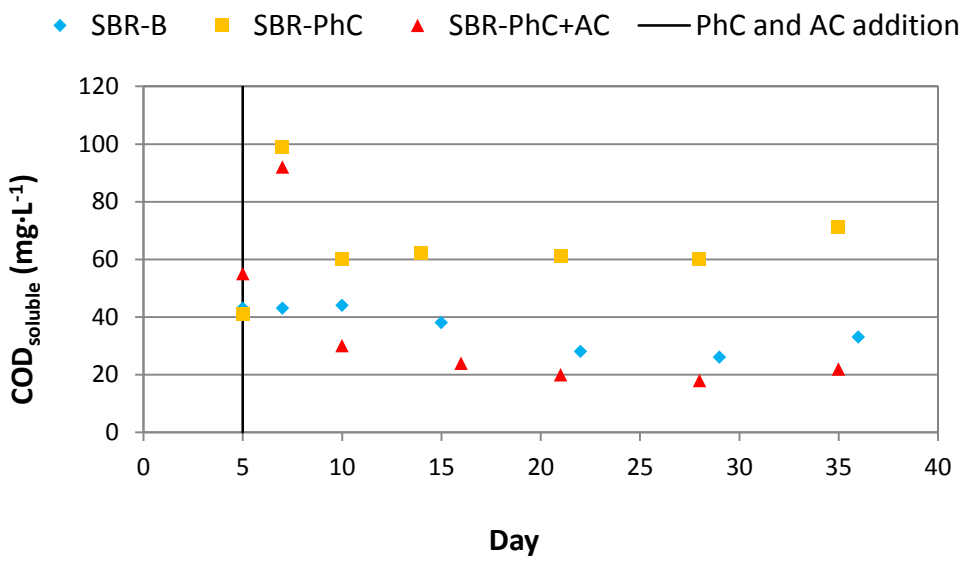


Figure 4. Effluent COD evolution of SBRs.
Vertical line indicates the day in which PhC and AC were added.

However, after two days of ACT, CAF and IBU presence, a lower organic matter removal performance was observed in SBR-PhC and SBR-PhC+AC, resulting in an increase of effluent COD in the 7th day up to 99.1 and 97.0 mg·L^{-1}, respectively. From 10th day on, the effluent COD of both reactors decreased reaching a stable value. The average value in the three reactors from this day until the end of the experiment were 33.8 ± 7.4, 62.8 ± 4.7 and 22.8 ± 4.6 mg·L^{-1} in the reactors B, PhC and PhC+CA, respectively. Thus, after the initial impact of PhCs on the two systems, a rapid biomass adaptation was observed. This adaptation was not complete in SBR-PhC since the initial organic removal yield was not recovered. On the contrary, the addition of MG1050, and the methodology followed to replace it on the reactor, resulted in an improvement of treatment, achieving lower COD concentrations than that obtained in SBR-B.

Finally, in Table 5 ACT, CAF and IBU concentrations both in feed solutions and in treated effluents of SBR-PhC and SBR-PhC+AC are presented.

Table 5. PhC concentrations.

Sample	Day	Feed concentrations ($mg \cdot L^{-1}$)		
		Acetaminophen	**Caffeine**	**Ibuprofen**
SBR-PhC	5	2.05	2.07	2.09
SBR-PhC+AC		2.02	2.09	2.06
Sample	**Day**	**Effluent concentrations ($\mu g \cdot L^{-1}$)**		
		Acetaminophen	**Caffeine**	**Ibuprofen**
SBR-PhC	7	n.d	0.33	5.14
	21	n.d	0.60	3.12
	35	n.d	0.67	2.26
SBR-PhC+AC	7	n.d	n.d	1.13
	21	n.d	n.d	n.d
	35	n.d	n.d	n.d

n.d: not detected

As it can be seen, ACT presented the best removal results in both reactors. This behaviour was expected according to batch experiments performed, in which ACT presented the fastest removal values due to the joint biodegradation and adsorption pathways, achieving values below the quantification limit. Regarding CAF and IBU concentrations in effluent of SBR-PhC, it can be observed that both drugs were detected on effluents samples, in which IBU removal was lower than that obtained for CAF. This behaviour also agreed with the batch experiments results, since in Test I CAF was removed faster than IBU. Regarding MG1050 effect, excepting IBU presence in the first analysis of effluent, it can be concluded that ACT and IBU that were not eliminated biologically were removed by carbon adsorption, achieving an effluent free of target drugs.

3.2.2. Biomass characteristics

3.2.2.1. Sludge production

PhCs presence affected the sludge production as it can be shown in Figure 5.

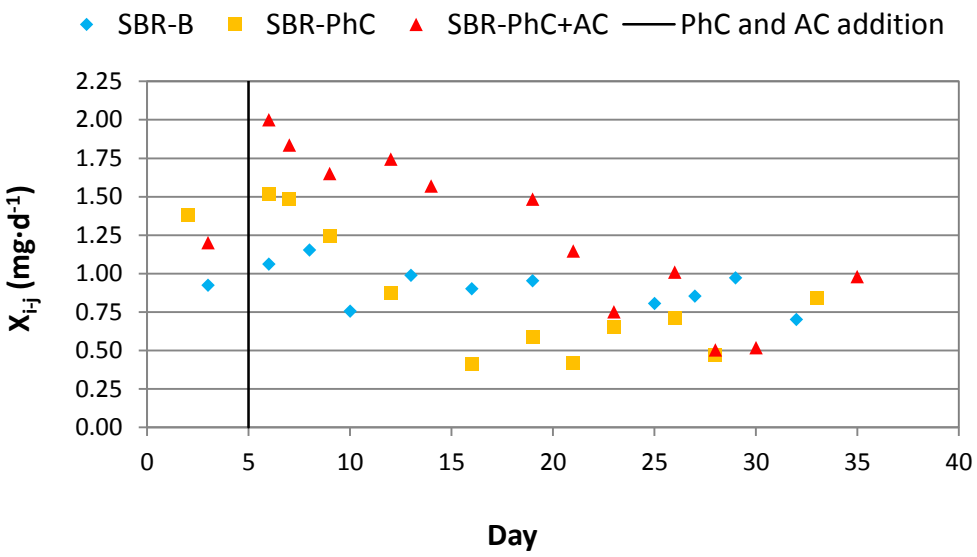


Figure 5. Evolution of sludge production of SBRs.
Vertical line indicates the day in which PhCs and AC were added.

Throughout the experiment, the evolution of X_{i-j} in SBR-B was stable, achieving a ΔX of 0.91 ± 0.13 mg·d^{-1}. However, PhCs dosage resulted in an initial increase of this parameter in SBR-PhC+AC, reaching 2.00 mg·d^{-1} in the 6th day. From this day on, sludge production decreased progressively until the last period (between 23 to 35 days), in which X_{i-j} reached more stable values, as it can be seen in Figure 5, achieving a ΔX value of 0.75 ± 0.24 mg·d^{-1} in the last 12 days. In SBR-PhC a progressive decrease of sludge growth was observed between the initial PhCs dosage until 16th day, due to partial biomass inhibition. From this day, ΔX reached a more stable value (0.67 ± 0.15 mg·d^{-1}). Thus, similar X_{i-j} values were achieved in the three reactors at the end of the experiment. This behaviour was reflected in the one-way ANOVA results, achieving a statistical significance when X_{i-j} of the three reactors were evaluated in the entire experiment (F-ratio = 3.51; p-value = 0.0419), meanwhile no significance was achieved between in the last 12 days (F-ratio = 0.77; p-value = 0.4900). The differences achieved on the sludge production drove to different sludge retention times in each reactor. Taking into account the last stable period of 12 days, SRT were 18.8 days in SBR-PhC and 18.4 days in

SBR-PhC+AC. Meanwhile, in SBR-B the sludge retention time was 13.1 days (calculated from the total of experiment).

3.2.2.2. Soluble microbial products

Figure 6 shows the evolution of SMP (as sum of proteins and carbohydrates) in each reactor.

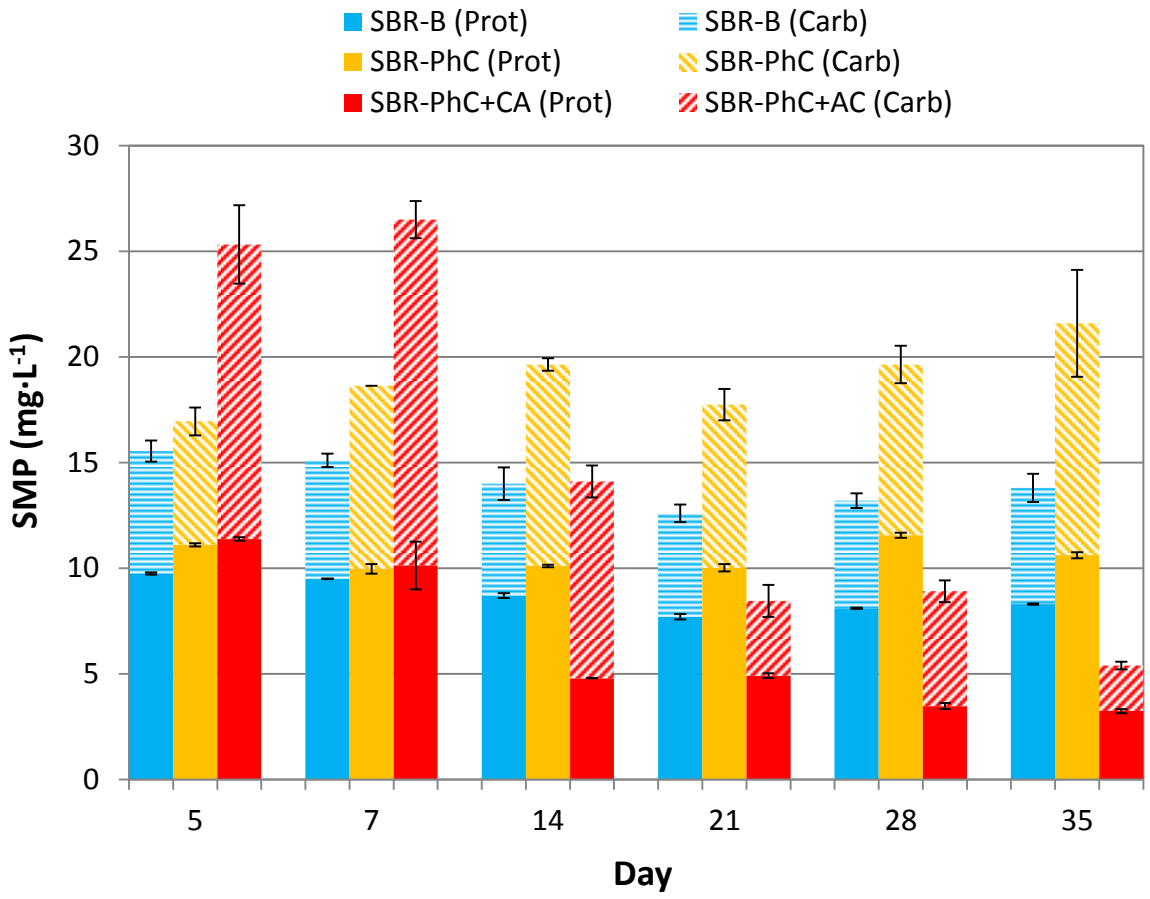


Figure 6. SMP concentration in SBR-PhC and SBR-PhC+AC. SMP are plotted as the sum of proteins (Prot) and carbohydrates (Carb) concentrations in each reactor.

It can be observed that, in general terms, the highest SMP concentrations were achieved in SBR-PhC, with an average value of 19.0 ± 1.6 $mg \cdot L^{-1}$. Thus, it can be concluded that PhCs increased this parameter by 26.3%, since the average value of SMP concentrations in SBR-B was 14.0 ± 1.1 $mg \cdot L^{-1}$. It should be commented that anomalous values in the first two samples of SBR-PhC+AC were observed, which were not related to PhCs dosage or AC

presence, since the sample analysed in 5th day was taken prior to addition. Taken into account SMP from 7th day , it can be observed that in SBR-PhC+AC the lowest values of SMP (9.2 ± 3.6 mg·L^{-1}) were measured, as expected, since activated carbon has the capacity to adsorb these substances [40]. However, no stable value was achieved. This behaviour can be related to the methodology followed to replace the activated carbon removed in the sludge withdrawals. In this way, on the days 12, 19, 26 and 33 (two days before SMP analysis), 0.17, 0.25, 0.26 and 0.50 g·L^{-1} of fresh activated carbon were added into the reactor, respectively, to maintain the target concentration of 1.5 g·L^{-1}. Thus, a direct correlation between the added fresh activated carbon amounts and the SMPs removal was observed.

3.2.2.3. Microbial hydrolytic enzymatic activities

Regarding MHEA analysis, in Figure 7 the evolution of acid an alkaline phosphatase, α-D-glucosidase and protease in each reactor is presented. In this figure, it can be seen that all the MHEA remained stable throughout the experimental period. However, both phosphatases and α-D-glucosidase concentrations increased in 7th day in SBR-PhC+AC. From this day on, a progressive decrease was observed. On the contrary, a little increase of protease activity was observed in the entire experiment. Several researchers reported a direct correlation between active biomass and both phosphatases and α-D-glucosidase activities and a negative correlation with protease [29,30]. Comparing biomass growth evolution (Figure 5) with MHEA values in SBR-PhC+AC it can be seen that these correlations were fulfilled. This behaviour was also observed in SBR-PhC, except for some anomalous values in acid phosphatase on day 21. This value was related with orthophosphate available for the microorganisms. In SBR-PhC the average value of P-PO_4^{-3} was 7.5 mg·L^{-1} (table 4), but in 21st day its concentration was 4.9 mg·L^{-1}. Thus, phosphatase activity of the microorganisms increased to generate orthophosphate available for their metabolic processes.

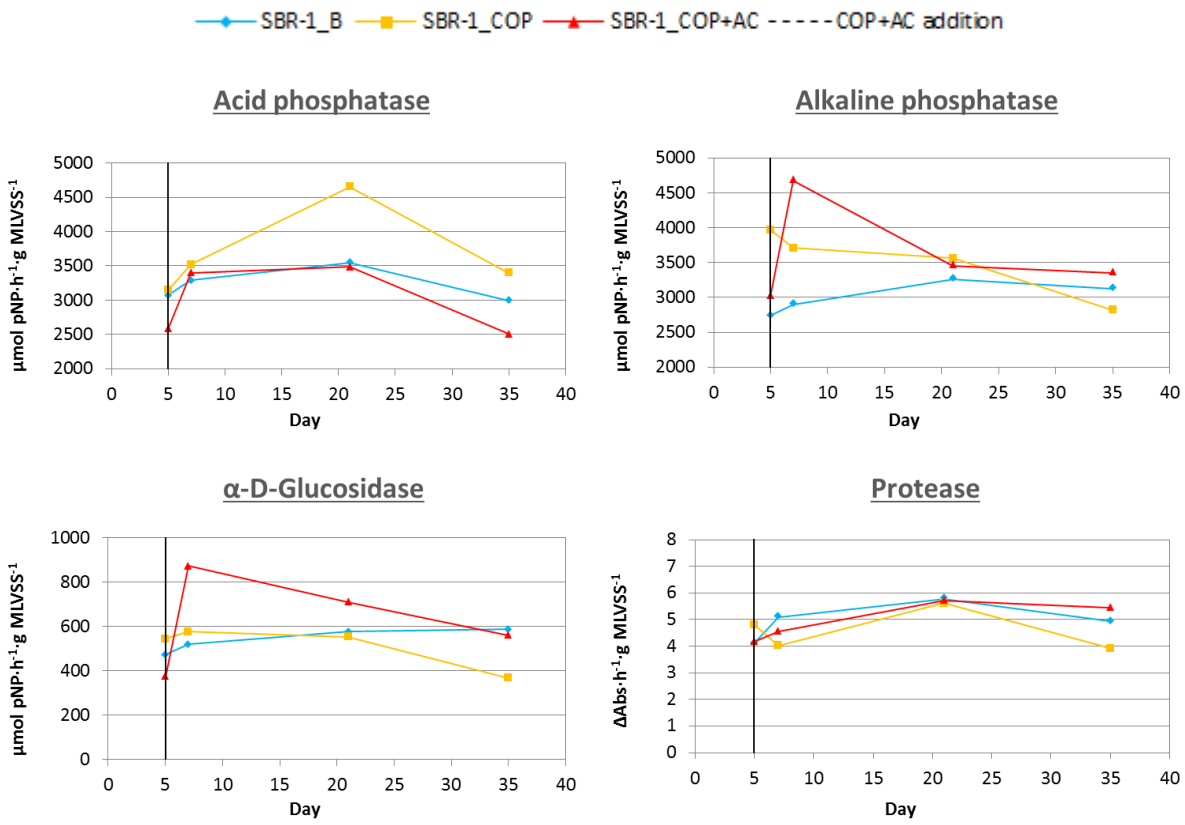


Figure 7. Evolution of four MHEA in SBRS.
Vertical line indicates the day in which PhCs and AC were added.

3.2.2.4. PhCs adsorbed on mixed liquor

Finally, PhCs adsorbed on activated sludge of SBR-PhC were analyzed. After 30 days of drugs dosage, ACT and CAF were lower than quantification limit (2 µg·kg^{-1}). These results agreed with those expected for CAF, because batch experiments showed a low capacity of the sludge to remove this drug by adsorption. However, it would be expected that a part of ACT had been adsorbed on sludge. This fact can be due to the high biodegradability of this compound. In this way there are two possible explanations: biodegradation is so fast that adsorption does not happen or adsorption was firstly carried out and there was a subsequent biodegradation on the sludge floc. Regarding IBU analysis, 25.2 µg of IBU for each kg of dried sludge was detected. Taken into account the sludge amount analysed, the sludge

withdrawals performed and the total IBU amount added into the reactor, only 0.0002% of this drug was adsorbed.

4. CONCLUSIONS

In this work the elimination of ACT, CAF and IBU, in a concentration of 2 $mg \cdot L^{-1}$, through five possible routes of removal during biological treatment with activated sludge system have been studied. Hydrolysis, oxidation and volatilization pathways were negligible after 6 h of reaction time. Adsorption route was only significant for ACT, which was adsorbed completely after 5 h of reaction, while only 1.9% of CAF and 5.6% of IBU were adsorbed. IBU was the least biodegradable compound.

On the other hand, a combined system that includes biological treatment by activated sludge plus adsorption with carbon was proposed to treat wastewaters which contain the three target drugs. The experiments carried out during 35 days showed that PhCs affected the organic matter removal performance increasing 46.2% effluent COD values, while combined system reduced it 32.5%. Additionally, PhCs presence also increased SMP concentrations by 26.3% and turbidity by 65.4%;meanwhile the combined system reduced both parameters comparing with the reactor working without PhCs. Regarding PhCs removal, combined system achieved an effluent free in target drugs, while small amounts of CAF and IBU were detected when biological treatment was carried out without activated carbon.

Finally, it can be concluded that the combined system proposed in this work generated an effluent free of PhCs, which in addition had higher quality that achieved in a conventional activated sludge treatment. In this way, this hybrid process is strongly recommended for

upgrading the WWTPs for PhCs removal, in view of the new regulations expected in the next future.

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