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Degradation of widespread pharmaceuticals by activated sludge: Kinetic study, toxicity assessment, and comparison with adsorption processes

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ABSTRACT

The growing presence of pharmaceutical compounds in aqueous systems leads to the search for new efficient and eco-friendly solutions to this problem. Although pharmaceutical products are widely found in the aqueous environments, there is limited understanding of their ecological effects. To augment the removal of pharmaceuticals, a bench reactor was inoculated with activated sludge and fed with a synthetic medium. Two pharmaceuticals of widespread usage, ibuprofen (IBU) and paracetamol (PARA) in the range 0.4–1 mg L^{-1} were used. The uptake values increased from 0.192 to 0.660 mg g^{-1} for IBU and from 0.104 to 0.341 mg g^{-1} for PARA. The removal efficiency reached values from 99.1–99.5 % and is independent of the initial IBU concentration. For PARA the removal percentage ranged from 93.3–98.8 decreasing with the increase on the initial concentration. The removal mechanism is well described by pseudo-first order and pseudo-second order kinetic models for all concentrations tested. At same time, batch assays were performed in order to assess the toxicity of both pharmaceuticals into the activated sludge using quantitative image analysis (QIA). For IBU experiments, QIA studies showed that this compound favors the growth of aggregated biomass rather than filamentous bacteria. A comparison between this biological technology and adsorption by a commercial porous ceramic material and Pinus bark was also performed. The results showed that the biological technology allowed better results (99.5 % against 19.4 and 9.3 %, respectively for the ceramic material and Pinus bark) for IBU. The results obtained for PARA showed comparable results for the biological technology and for adsorption by the ceramic material. The activated sludge system presented here appears to be a promising treatment for pharmaceutical contaminated effluents and the biomass present in the activated sludge seems do not be negatively affected by the presence of high concentrations of both pharmaceuticals.

1. Introduction

The extensive use of personal care products and pharmaceuticals for human consumption and veterinary usage led to the detection of these products in wastewater effluents and aqueous systems as rivers, surface waters and others. This fact has increased the awareness of the potential threat that pharmaceuticals pose on the aquatic environment [1]. Studies on the occurrence of pharmaceuticals show that the widely used pharmaceuticals ibuprofen (IBU) and paracetamol (PARA) are present in relevant concentrations in the aqueous environment [2–5]. One of the additional problems associated with the release of these compounds to the water streams is that there are no legally regulated maximum permitted concentrations of pharmaceuticals in the environment. Only a few pharmaceuticals, more specifically four substances (macrolide antibiotics, diclofenac, 17α-ethynylestradiol, and 17β-estradiol) were included in the recent watch list of the European Commission [6] for wide monitoring. Biological technologies have been suggested by several authors to remove these compounds from aqueous systems including anaerobic digestion [7], membrane bioreactors [8,9], phytotreatment [10], biodegradation by pure cultures [5,11,12], and activated sludge [13,14]. Physicochemical technologies as activated carbon adsorption [15,16], pine bark and almond shell [17], and clays adsorbents [18,19], ozonation and ozone/activated carbon coupling [20], and chemical oxidation [21] have also been considered. Several disadvantages are associated with physicochemical methods as the generation of secondary pollutants and the high operational costs. The biological methods present less disadvantages: biodegradation of pharmaceuticals is being considered as an environmentally friendly option with low-cost operational requirements and the products of degradation are innocuous end products such as CO_2 and H_2O [22].

The mechanisms involved in the biological removal are mainly adsorption, biotransformation, and degradation [23]. The application

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of kinetic models is a worthy tool to study the mechanism that controls the biological process. Several authors applied pseudo-first order and pseudo-second order kinetics models for fitting the experimental biotransformation removal data [24,25]. Originally, these models were applied to physical adsorption processes. However, more recently studies have been reported that removal of trace amounts of organic pollutants using activated sludge can be described by a pseudo-first order reaction, due to the relatively low substrate concentration compared to the biomass concentrations [24] or by pseudo-second order kinetics [25].

The ecotoxicology of pharmaceuticals was studied by several authors [26–30]. However, these studies were main focused on the effects on soil organisms and marine flora and fauna and there is a lack of information about the effect of pharmaceuticals on microorganisms namely on activated sludge. Indeed, the effect of these compounds on microorganisms is an important study that should be taken into account when biological processes are used to remove these compounds from aqueous systems. The results of these studies will allow to conclude about the ecotoxicology of the compounds and to establish the range of concentrations where the biomass can be used.

Quantitative image analysis (QIA) has been employed for aggregated and filamentous bacteria contents determination, biomass structure and physiology [31]. Accordingly to these authors, image processing and analysis methodologies are able to provide valuable information about activated sludge systems, and can be recognized as valid monitoring tools. This technology allows to detect bulking phenomena and to prevent sedimentation problems.

The aims of the present study were: (i) to evaluate the potential of a bioreactor inoculated with activated sludge, for removing pharmaceuticals from wastewater and compare the behavior of this bioreactor with physicochemical methods (adsorption by a porous ceramic material and pinus bark); (ii) to determine the rate kinetic parameters of pseudo-first and pseudo-second order models for the removal of pharmaceutical compounds onto activated sludge biomass; and (iii) to analyze the effects of IBU and PARA on the biomass morphology using quantitative image analysis (QIA). Usually the removal of paracetamol using microorganisms is performed using pure cultures [12] and the use of conventional activated sludge wastewater treatment is considered inadequate for the effective removal of many emerging trace organic contaminants [32]. This research aims to show that the use of activated sludge (microbial consortium) presents several advantages over the use of pure cultures to remove paracetamol and ibuprofen. It is imperative to stimulate water reuse, and the complete removal of emerging contaminants, as pharmaceuticals, from wastewater will contribute to this aim. The use of QIA to analyze the effects of the pharmaceuticals on the biomass is also a novelty of this study. This team believes that the successful execution of these objectives will contribute to fill the knowledge gap that exists in the field of the removal of pharmaceuticals by activated sludge.

2. Materials and methods

2.1. Selection, main characteristics and preparation of pharmaceuticals

The pharmaceuticals chosen for this study are high consumption drugs. Paracetamol is one of the most commonly used oral analgesics and antipyretics and ibuprofen is an anti-inflammatory of widespread usage. Both substances are extensively used as non-prescription medicine, with an annual consumption of hundreds of tons around the world [33]. Both pharmaceutical products were of the highest purity commercially available. IBU was purchased from Acros Organics and PARA was purchased from Sigma-Aldrich. The main characteristics of both pharmaceuticals are presented in Table SM-1 (Supplementary material). A stock solution of each pharmaceutical was prepared from powdered substances in distilled water at a concentration of 20 mg L^{-1} and then stored at 4 °C. This stock solution was then diluted to the working concentrations.

2.2. Experimental unit and procedure

2.2.1. Removal and kinetics assays

Experiments with different ibuprofen (IBU) and paracetamol (PARA) concentrations (0.4, 0.5, 0.6, 0.8, and 1.0 mg L^{-1}) were conducted in a 4 L lab-scale batch reactor operated at room temperature during approximately three days. Although the concentrations found in the environment are in the ng/L–μg/L range, higher concentrations are found in wastewater originated from hospitals or elderly houses [34]. In what concerns to Portugal, ibuprofen consumption has been increasing during the last decade, moving from the 15th to 8th position in the sales ranking [35]. Considering the increase on the consumption of non-prescribed drugs, as ibuprofen and paracetamol, and taking into account the presence of higher concentrations on specific locations the authors decided to test the reactor for concentrations in the range 0.4 − 1 mg/L. The system was inoculated with activated sludge from a domestic wastewater treatment plant where an initial mixed liquor suspended solids (MLSS) concentration of 3 g L^{-1} was used. A synthetic medium was fed to the system in the beginning of each experiment and contained (per liter): 2.55 g $C_2H_3O_2Na.3H_2O$, 0.34 g $C_3H_5NaO_2$, 0.59 g NH_4Cl, 0.95 g $MgSO_4{\cdot}7H_2O$, 0.44 g $CaCl_2{\cdot}2H_2O$, 0.03 g EDTA, and 3.16 mL of a trace metals solution. The trace metals solution was well described by Smolders et al. [36]. Compressed air was used to ensure aerobic conditions and the pH was maintained around 7 by a pH controller using two-way controller pumps dosing 0.3 M HCl or 0.3 M NaOH when the pH was above/below the set point. The agitation was kept constant at 150 rpm. Samples were taken at different time intervals, centrifuged (13,400 rpm for 10 min) and the aqueous phase was stored at 4 °C. Prior to analysis, the liquid samples were thawed and homogenized by vortexing. The IBU and PARA concentrations were determined using a UHPLC system. All measurements were conducted in duplicate. The results presented are an average of both results. The relative standard deviation and relative error of the experimental measurements were < 1 % and 3 %, respectively.

2.2.2. Adsorption assays

For comparison reasons the same 4 L reactor used for the biological treatment was used for adsorption assays. These assays were performed using porous ceramic material (diameter 8 − 16 mm and 274 kg m^{-3} of density) and pinus bark (diameter 1 − 5 mm and 1377 kg m^{-3} of density) as adsorbents, for an initial concentration of IBU and PARA of 1 mg L^{-1} (4 L of pharmaceutical compound solution, 30 *g* of adsorbent, 150 rpm, at room temperature). Samples were taken at different time intervals, centrifuged (13,400 rpm for 10 min) and the aqueous phase was stored at 4 °C. Prior to analysis, the liquid samples were thawed and homogenized by vortexing. The IBU and PARA concentrations were determined using a UHPLC system. All measurements were conducted in duplicate. The results presented are an average of both results. The relative standard deviation and relative error of the experimental measurements were < 1 % and 3 %, respectively.

2.2.3. Ecotoxicity assessment

To analyze morphological changes and ecotoxicology of IBU and PARA, four identical batch reactors (300 mL working volume) were used during 24 h, at room temperature and agitated at 150 rpm. The reactors were inoculated with activated sludge from a domestic wastewater treatment plant (MLSS = 3 g L^{-1}) and the initial concentrations of IBU/PARA were 1, 10, and 20 mg L^{-1}. A control batch, without IBU/PARA, was also analyzed. The synthetic medium used was previously presented in Section 2.2.1. Aggregated and filamentous biomass contents and structure were assessed by images acquired through bright-field microscopy from MLSS samples obtained in each experiment. Samples were taken from the batch reactors for visualization and image acquisition in five periods of time using an Olympus BX51 microscope

(Olympus, Tokyo, Japan) coupled with an Olympus DP71 camera (Olympus, Tokyo, Japan). A recalibrated micropipette with a sectioned tip at the end, with a large enough diameter to allow larger aggregates to flow, was used to deposit 10 µL of the sample on to the slide, and covered with a 20 mm × 20 mm cover slip. Three slides per sample were used taking 50 images per slide resulting in 150 images. Samples were examined at 100x total magnification and acquired at 1360 × 1024 pixels in 8-bit format. Images were acquired in the upper, middle and bottom of the slide to improve the representativeness of the microbial community. The QIA results presented are an average of the 150 images acquired.

2.3. Analytical details

2.3.1. Pharmaceuticals analysis by UHPLC

Chromatographic analysis was performed using a Shimadzu Corporation apparatus (Tokyo, Japan) consisting of a UHPLC equipment (Nexera) with one multi-channel pump (LC-30AD), an autosampler (SIL-30AC), an oven (CTO-20AC), a diode array detector (M-20A) and a system controller (CBM-20A) with proper software (LabSolutions). For ibuprofen, a Kinetex5 u EVO C18 column (150×4.6 mm i.d.) supplied by Phenomenex, Inc. (CA, USA) was used. The mobile phase was sodium phosphate (20 mM; pH 2.4) (pump A) and acetonitrile (pump B). Starting mobile phase composition was 80 % A, decreased to 30 % A in 10 min and remains in this percentage for 5 min. The flow rate was 1.5 mL min^{-1}. The sample was monitored by the diode array detector from 190 to 400 nm, and chromatograms were extracted at 225 nm. Column oven was set at 40 °C and the volume of injection was 10 µL. For solid phase extraction (SPE), the sorbent cartridges (Strata SDB-L Styrene-Divinylbenzene Polymer, 100 mg/1 mL) were used. The cartridges were conditioned with acetonitrile and equilibrated with water. The sample was loaded and washed with methanol/water (30:70), dried 10 min under full vacuum and eluted with acetonitrile. For paracetamol, a Kinetex2.6 u EVO C18 column (150×4.6 mm i.d.) supplied by Phenomenex, Inc. (CA, USA) was used. The mobile phase was 0.1 % phosphoric acid in water (pump A) and 0.1 % phosphoric acid in acetonitrile (pump B). Starting mobile phase composition was 95 % A, decreased to 5 % A in 9 min and increased again to 95 % (9.01 min) and remains in this percentage for 3 min. The flow rate was 1.8 mL/min. The sample was monitored by the diode array detector from 190 to 400 nm, and chromatograms were extracted at 215 nm. Column oven was set at 50 °C and the volume of injection was 5 µL. For solid phase extraction (SPE), the sorbent cartridges (Strata SDB-L Styrene-Divinylbenzene Polymer, 100 mg/1 mL) were used. The cartridges were conditioned with methanol and equilibrated with water. The sample was loaded and washed with methanol/water (5:95), dried 10 min under full vacuum and eluted with methanol.

2.3.2. Quantitative image analysis (QIA)

2.3.2.1. Image processing and analysis in bright-field images. Bright field program was developed in Matlab 7.3 (The Mathworks, Natick, USA) for the recognition of aggregates and filamentous bacteria in grayscale images. A more detailed description of the image processing methodology can be found in Mesquita et al. [31]. Fig. 1 shows an illustrative bright-field image with corresponding aggregates and filaments binary images obtained from the QIA procedure.

2.3.2.2. Morphological parameters determination. Aggregates were classified according to their size in: small aggregates (Deq < 25 µm); intermediate aggregates (25 < Deq < 250 µm); and large aggregates (Deq > 250 µm), where Deq represents the equivalent diameter. For each studied class, the aggregates area percentage was calculated. The aggregates total area per volume (TA/Vol) and filaments total length per volume (TL/Vol) were also determined according to Mesquita et al. [37].

3. Results and discussion

3.1. Ibuprofen and Paracetamol removal performance by activated sludge and comparison with adsorption processes

In recent years, several reports about pharmaceuticals detected in a variety of environmental samples, as river water, seawater, and wastewater were published [35,38,39] proving that much scientific attention is being focused in the occurrence of pharmaceuticals in the environment. The present study shows that a reactor inoculated with activated sludge is able to remove IBU in percentages around 99 % whereas PARA was removed between 93.3 and 98.8 %. The pharmaceuticals were aerobically degraded by the biomass or sorbed to the solids, with IBU being faster removed than PARA (Fig. 2). Ibuprofen was removed within 50 h to undetectable concentrations and paracetamol was removed to acceptable levels in approximately 73 − 77 h. For IBU, the largest amount of this pharmaceutical was removed in 6 − 10 h. This quick removal may indicate not only biodegradation but also external sorption sites on the solids for IBU removal from water [40]. The removal of PARA was slower, for the concentration of 0.4 mg/L the largest amount of PARA was removed on the first 29 h and for the highest concentration, 1 mg/L, the time to reached equilibrium was more than 73 h. The uptake rates and removal percentages are presented in Table 1. The uptake varied from 0.192 mg g^{-1} to 0.660 mg g^{-1} for IBU and from 0.104 mg g^{-1} to 0.341 mg/g for PARA. As expected the uptake increase with the increase on the pharmaceutical concentration. Different authors suggested that this behavior could be justified by the fact that surface saturation was dependent on the initial pharmaceutical concentration [41]. As the concentration increase, the number of moles of IBU/PARA also increased for the same amount of available sites on the biomass. The removal percentage is similar for all the initial concentrations tested for IBU. The removal percentage around 99 % for IBU suggests that this activated sludge system could be used to remove higher concentrations of IBU than those tested in this assays. In fact, the biomass seems to be far from the maximum removal capacity. For PARA, the removal percentage decrease with the increase on the initial PARA concentration. This behavior can be explained easily: higher concentrations reduce the average distance between the

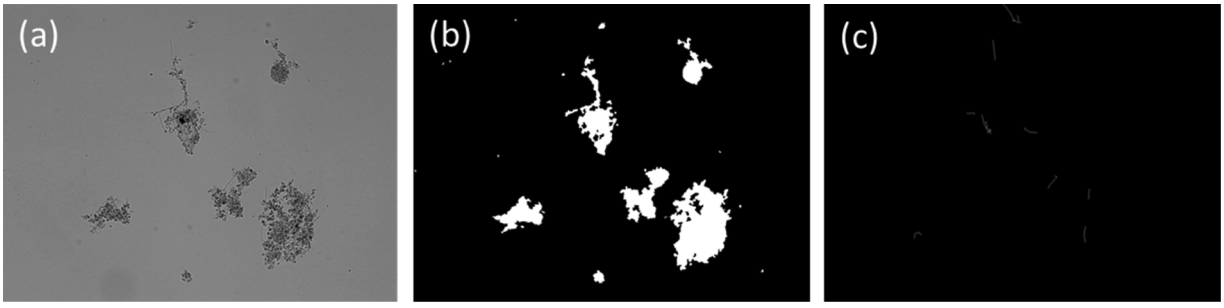

Fig. 1. (a) original grayscale image, (b) aggregates binary image, (c) filaments binary image.

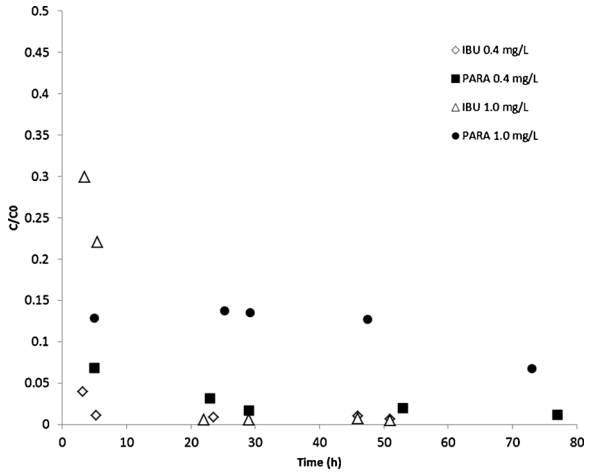

Fig. 2. Ratio between residual and initial pharmaceutical concentration (C/C0) as a function of contact time, for the initial concentrations of 0.4 and 1 mg L^{-1} (Data not shown for t_0).

Table 1
Values of removal percentage (%) and q_e (mg g^{-1}) calculated for different initial concentration of IBU and PARA using a reactor inoculated with activated sludge.

Ci(mg L^{-1})	% Removal		Uptake	
	IBU	PARA	IBU	PARA
0.4	99.33	98.84	0.192	0.104
0.5	99.06	97.86	0.228	0.170
0.6	99.49	95.83	0.312	0.168
0.8	99.45	94.75	0.365	0.325
1.0	99.47	93.25	0.660	0.341

adsorbing species and this affect the charge distribution of all the species involved and alter the capacity of the species to migrate to the biomass surface, resulting in reduced adsorption [42]. For this compound, the lower removal percentage obtained was 93.3 % (Ci = 1 mg L^{-1}) in 73 h. This result indicates that, as for IBU, the system could be used to treat higher concentration of PARA, however the time required to reach the equilibrium will be higher what could be a disadvantage. The difference on the removal percentage and uptake of IBU and PARA indicates that the microbial populations in the inoculum show a different affinity towards the degradation of the selected pharmaceuticals.

The mechanisms involved in the removal of pharmaceuticals by microorganisms include biodegradation, bio-sorption, volatilization and hydrolysis [43]. Ibuprofen and paracetamol are usually resistant to hydrolysis, because they are designed for oral intake [44]. Ibuprofen is not significantly volatilized in the activated sludge [14] and is known for its easy biodegradability [34] suggesting that the main mechanisms involved in the removal of IBU are biodegradation and bio-sorption. Paracetamol is also mainly degraded by microorganisms [22] or by sorption to solids and biodegradation when an activated sludge system is used. Indeed, both mechanisms, biodegradation and sorption to the solids, were responsible for IBU/PARA removal on activated sludge, resulting in a large IBU/PARA removal capacity of about 66 % by weight and 34 % by weight, respectively for the highest concentration of IBU and PARA. Several authors tested the use of biomass, activated sludge or other biological systems, to remove IBU and PARA. Carballa et al. [45] obtained removal percentages of IBU around 40 % using an anaerobic activated sludge system; Nakada et al. [46] achieved 90 % of IBU removal during activated sludge treatment; and Langenhoff et al. [34] obtained the complete removal of IBU, in 4 days, using activated sludge from a pilot plant treating hospital wastewater. The aerobic degradation of IBU by an indigenous bacterial community was tested by Fortunato et al. [47]. These authors found removal percentages around 99 % (33 h, 100 mg L^{-1} initial concentration), proving the ability of the indigenous communities to improve the treatment of wastewaters containing IBU. Biodegradation and bio-sorption of both, IBU and PARA, was analyzed by Yu et al. [43] using immobilized cell process. The removal of approximately 100 % PARA was achieved via bio-sorption or sorption within 8 d and more than 25 % PARA removed by biodegradation within 2 d. For IBU, 77 % was removed via biodegradation in 4 d and 28 % via bio-sorption in 14 d. Zhang et al. [12] studied the removal of PARA by pure bacterial cultures and their microbial consortium and found that the consortium of three microbial strains recovered from the same culture was shown to be necessary for complete PARA degradation and mineralization, suggesting a possible complementary interaction among the various isolates. The system used in the present study allows high efficiency in a shorter period.

The biodegradation of IBU and PARA was well studied by several authors [13,22,48,49]. Accordingly to Zwiener et al. [48], IBU is transformed in ibuprofen carboxylic acid (CBX-IBU) and hydroxylated ibuprofen (oxic conditions), and only in CBX IBU (anoxic conditions). These authors concluded that only 10 % of ibuprofen removed could be attributed to its transformation into these metabolites. Other authors detected two isomers of hydroxy-ibuprofen (2-OH IBU and 1-OH IBU) as intermediates in the mineralization of ibuprofen by microorganisms and concluded that both intermediates were also degraded or disappeared quickly from the bioreactor [49]. Paracetamol is mainly degraded to 4-aminophenol and hydroquinone [12]. As reported by other authors some intermediates (not identified) appeared during the UHPLC analysis after some hours of experiment. The presence of intermediates proves that PARA and IBU are being degraded. These intermediates disappeared during the experiment meaning that they were also degraded by the activated sludge biomass.

The removal of IBU and PARA was also tested using adsorbents aiming to compare the behavior of biological methods and physico-chemical methods. A porous ceramic material, mainly constituted by expanded clay material, and pinus bark were selected as adsorbents. Porous ceramic materials were already used in the treatment of wastewater contaminated with IBU [50,51] and Pinus bark was used for the removal of steroids [17] and bisphenol A [52]. The removal percentages obtained for the removal by expanded clays are 19.4 % and 97.5 %, respectively for IBU and PARA as reported in Table 2. The difference between the removal percentages for both pharmaceuticals seems to be related with the size of the molecule. Ibuprofen has a molecular weight of 206.3 g mol^{-1} and paracetamol has a molecular weight of 151.2 g mol^{-1}. The molecules of IBU are too large for the porous of ceramic material and the entrapment of this compound is more difficult. The removal percentage of 19.4 % obtained is related with the adsorption of IBU by adsorption sites on the material surface. Behera et al. [53] used different clays (kaolinite, montmorillonite, goethite) to remove IBU and obtained removal percentages between 4–12% (Ci = 60 mg L^{-1}). On the other hand, Dordio et al. [50] used a matrix of light expanded clay aggregates (LECA) planted with *Typha* spp. to remove IBU and obtained removal percentages of 96 % proving that the lower removal percentages obtained when clays materials are used could be incremented by combination with biological treatment.

Pinus bark was also used as adsorbent and obtained 9.3 % and 20.5

Table 2
Values of removal percentage (%) and q_e (mg g^{-1}) using ceramic material and Pinus bark adsorbents, for an initial concentration of 1 mg L^{-1} of IBU and PARA pharmaceuticals.

Adsorbent	% Removal		Uptake	
	IBU	PARA	IBU	PARA
Ceramic Material	19.42	97.47	0.067	0.217
Pinus Bark	9.28	20.47	0.024	0.067

% removal percentages, respectively for IBU and PARA. The results obtained for Pinus bark as adsorbent are worse than the obtained using ceramic material as adsorbent. This can be related to the porous structure of both materials. Expanded clay materials are well known for its mesoporous structure [54], which allows better results.

From the analysis of Tables 1 and 2, it is possible to conclude that the use of a biological method (activated sludge) presents better results than the obtained using a physicochemical method (ceramic material and Pinus bark as adsorbents), for IBU. For PARA, the removal obtained for the biological method is comparable to the obtained using ceramic material as adsorbent. However, the results are worst for Pinus bark.

As the use of biological methods presents several advantages over the use of adsorption, as already explained in introduction section, the use of activated sludge is also an attractive method from the economical point of view. Although the adsorbent materials tested, pinus bark and ceramic material are relatively inexpensive, they have a higher cost than activated sludge biomass. These findings reinforce our opinion that this system is very promising in the treatment of water contaminated with high concentration of pharmaceuticals.

3.2. Ibuprofen and paracetamol removal kinetics

Kinetic modeling must be taken into account to develop suitable mathematical models for predicting the performance of treatment systems. Aiming to study the process of removal of the selected pharmaceuticals, two simplified kinetic models, namely pseudo-first order and pseudo-second order, were used to study the mechanism that controls the biological process. The nonlinear forms of both equations [55] are respectively expressed as:

$$q = q_e\,(1 - e^{-k_1 t}) \tag{1}$$

$$q = \frac{k_2 q_e^2\, t}{1 + k_2 q_e\, t} \tag{2}$$

The value of q_e represents the amount (uptake) adsorbed onto adsorbent at equilibrium (mg g^{-1}), k_1 and k_2 are rate sorption constants, and t is time. The values of k_1 and k_2, and predicted q_e uptakes are presented in Tables 3 and 4. For IBU, the values of k_1 and k_2 decrease as the initial concentration increase (except for 0.8 mg L^{-1}), which means that the removal becomes slower as the concentration increases. For PARA, the values of k_1 and k_2 do not follow any trend.

The comparison between the experimental results and those predicted by both models is shown in Fig. 3(a–d). The analysis of Fig. 3 and the correlation coefficients (R^2) (values not shown) suggest that both models, pseudo-first order and pseudo-second order models, fits well the removal of IBU and PARA by the reactor inoculated with activated sludge. Accordingly to Garcia-Rodrigues et al. [64] a good fit to the pseudo-first order model should be related to the effect of biodegradation processes, generally described as first order reactions. The assumptions of both models are different: pseudo-first order models assumes that the reaction rate is limited by one mechanism, one class of sorbing sites and all of them of time dependent type; pseudo-second order model assumes that the adsorption process is controlled by surface reaction, with chemisorption involving valence forces, through sharing or exchange of electrons between bacteria and pharmaceutical compounds [56]. Results indicate that a mixed of both removal processes are involved in the removal of IBU/PARA by the activated sludge. The calculated q_e uptakes agree with the experimental data, especially for the lower concentrations and for both models. Other important parameter, named approaching equilibrium factor (R_w) (Eq. 3), was proposed by Ofomaja [57]. These authors studied the relationship between pseudo-second order parameters and the removal performance by the analysis of the characteristic kinetic curve. The equilibrium factor is expressed as:

$$R_W = \frac{1}{1 + k_2\, q_e\, t_{ref}} \tag{3}$$

where t_{ref} is the longest operation time (based on kinetic experiments). Values of $R_w = 1$ represent a linear type kinetic curve which means that the process is not approaching equilibrium. Values of R_w between 0.1–1 indicate a kinetic curve slightly curved, approaching equilibrium; between 0.01-0.1 represent a kinetic curve largely curved, well approaching equilibrium; and values lower than 0.01 indicate a kinetic

Table 3
Rate kinetic parameters of pseudo-first and pseudo-second order models for the removal of ibuprofen at different initial concentrations, onto activated sludge biomass.

C_i (mg L^{-1})	q_e (mg g^{-1})			k_1 (h^{-1})	k_2 (g mg^{-1} h^{-1})	R_W
	Experimental	predicted (1 st order)	predicted (2nd order)			
0.4	0.192	0.192	0.193	1.063	53.605	0.0019
0.5	0.228	0.228	0.232	0.606	4.785	0.0174
0.6	0.312	0.311	0.321	0.387	2.725	0.0218
0.8	0.365	0.363	0.373	0.447	3.255	0.0157
1.0	0.660	0.657	0.698	0.320	0.798	0.0340

Table 4
Rate kinetic parameters of pseudo-first and pseudo-second order models for the removal of paracetamol at different initial concentrations, onto activated sludge biomass.

C_i (mg L^{-1})	q_e (mg g^{-1})			k_1 (h^{-1})	k_2 (g mg^{-1} h^{-1})	R_W
	Experimental	predicted (1 st order)	predicted (2nd order)			
0.4	0.104	0.104	0.104	0.601	30.771	0.0426
0.5	0.170	0.170	0.170	0.647	37.008	0.0021
0.6	0.168	0.168	0.167	0.387	54.337	0.0014
0.8	0.325	0.321	0.321	1.401	124.317	0.0003
1.0	0.341	0.323	0.325	0.863	24.322	0.0017

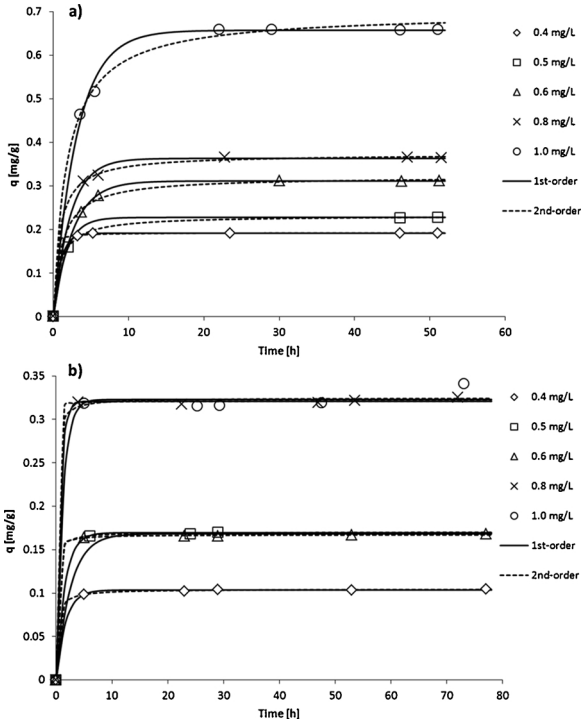

Fig. 3. Pseudo-first order and pseudo-second order kinetics plots for removal of a) IBU and b) PARA by activated sludge.

curve pseudo-rectangular, drastically approaching equilibrium. In this study, the values for R_w shown in Tables 3 and 4 were found to range between 0.001 and 0.03, confirming the good performance of the system used.

Garcia-Rodrigues et al. [Garcia-Rodrigues et al., 2015] tested the biodegradation of several pharmaceuticals including IBU and PARA using two aquatic plants (Lemna sp. and Spirogyra sp.) and reported that the best fit was obtained using pseudo-first order model with values of k_1 of 0.132 day^{-1} and 0.479 day^{-1}, respectively using Spirogyra sp. and Lemna sp., for PARA, 0.099 day^{-1} and 0.109 day^{-1}, respectively using Spirogyra sp. and Lemna sp., for IBU. Santaeufémia et al. [58] studied the removal of IBU using microalga Phaeodactylum tricornutum and found that the pseudo-second order kinetic model was the most suitable for the description of the ibuprofen sorption using this microalga with values of k_2 of 2.92 g mg^{-1} h^{-1} and 2.61 g mg^{-1} h^{-1}, respectively for living and dead forms of the microalga. In the present work, the values obtained for the kinetic constants are higher which indicates a faster removal. These results contribute to highlight the good performance of the reactor.

3.3. Ibuprofen and Paracetamol effects on the biomass

Microscopic examination of aerobic sludge has been useful for determining the physical nature of the biomass structure, and the type and abundance of aggregated and filamentous microorganisms. Coupled to microscopy survey and advances in digital imaging acquisition and computer processing capabilities allow for a fast and efficient classification and quantification of the biomass. Thus, image processing and analysis is, at present, a well-established technique for biological processes monitoring when combined to bright-field and/or fluorescent microscopy. Amongst other applications, quantitative image analysis (QIA) has been used to identify activated sludge disturbances [59], detect filamentous bulking problems [60], and predict the sludge settleability [61]. However, QIA studies for studying the effects of different concentrations of pharmaceuticals in aggregated and filamentous microorganisms are still lacking.

In this study, the automatic identification and quantification of aggregated and filamentous microorganisms was sought using QIA. The experiments were conducted through 24 h using different concentrations of IBU and PARA in order to investigate its effects on the biomass structure during a short period. The results here presented were normalized by the control experimental data without the addition of pharmaceuticals. Furthermore, the size distribution of aggregated biomass was also studied regarding the percentage of small (Deq < 25 μm) and intermediate (25 < Deq < 250 μm), since no large aggregates (Deq > 250 μm) were found in the samples.

During the experiment with 1, 10, and 20 mg L^{-1} of PARA, in spite of a slight variation, the aggregates area ratio (small and intermediate) (Fig. 4a, c) tended to the initial value, indicating that these experiments presented the same trend as the control. These results showed that no effects were found in the biomass structure during the experiments with different PARA concentrations. The study conducted by Lawrence et al. [62] showed that no significant impact was found in bacterial biomass that corroborates the findings of the present work.

Regarding the structure of the biomass during the experiment with 1 mg L^{-1} of IBU, it can be seen that the effect of aggregates fragmentation is extensively higher than with the same concentration of PARA. In this case, the small aggregates area percentage increased from 36 % to 53 % (Fig. 4b) and the intermediate aggregates percentage decreased from 62 % to 39 % (Fig. 4d). Considering the experiments using 10 and 20 mg L^{-1} of IBU, it was found that the breakage of intermediate to small aggregates was considerably greater when all the experimental data were compared. The small aggregates area percentage increased from 36 % at the beginning of both experiments to 68 % and 80 %, respectively (Fig. 4b). The intermediate aggregates area percentage decreased significantly from 62 % to 28 % when 10 mg L^{-1} of IBU was added and from 62 % to 17 % using 20 mg L^{-1} of IBU (Fig. 4d). It seems therefore clear that IBU presents substantial effect in bacterial biomass leading a deflocculating phenomenon with higher impact for 10 and 20 mg L^{-1}.

The aggregates total area (TA/Vol) and filaments total length (TL/Vol) contents were also studied as shown in Fig. 5(a–d). For all the concentrations tested with PARA, the TA/Vol parameter was quite similar (Fig. 5a). These results revealed that the same trend was achieved for all the experiments with the different concentrations of PARA and for the control. The TL/Vol presented a stable ratio for the experiment with 1 mg L^{-1} of PARA, indicating that the results for the lowest concentration were similar to the control results. Regarding the highest concentrations, it can be concluded that 20 mg L^{-1} of PARA slightly favored the growth of filamentous bacteria rather than aggregated biomass (Fig. 5c).

The behavior of TA/Vol ratio for 1, 10 and 20 mg L^{-1} of IBU revealed an increase for all the experiments (Fig. 5b). This could be explained by the lowest TA/Vol reached during the control experiment. The filamentous bacteria contents (TL/Vol) presented a decrease for all the concentrations studied (Fig. 5d). It seems therefore clear that IBU favors the growth of aggregated biomass rather than filamentous bacteria. Caracciolo et al. [63] reported that IBU was found to reduce the overall bacteria of biofilm communities. However, in the present work this effect was observed just for the filamentous bacteria community considering the decrease of TL/Vol throughout the experiments.

Although the acute ecotoxicity of ibuprofen and paracetamol is relatively limited, it definitely poses a risk on the ecosystems and more studies on effective removal of these pharmaceuticals are needed.

The fast IBU/PARA removal, large IBU/PARA removal capacity, and negligible ecotoxicological effects of both pharmaceuticals on the biomass, allow to classify the activated sludge as good material to remove IBU/PARA from wastewater contributing to an environmental friendly solution for the problems of pharmaceuticals. This system is very promising and can be tested for other pharmaceuticals.

4. Conclusions

This study indicates that the activated sludge biomass is efficient in removing IBU/PARA and is a good alternative for the removal of these compounds from aqueous solutions. The removal was almost total for IBU and is high for PARA, which indicates that the maximum removal capacity of the biomass is far from being reached and could be used from the treatment of higher concentrations. Kinetic data of pharmaceuticals followed both, the pseudo-first order and pseudo-second order kinetic models. The comparison between biological and adsorption technologies shown better results, in general, for the biological method tested. QIA studies shown that the biomass seems not be negatively affected by the presence of both compounds. In fact, this biomass showed to be very resistant to the xenobiotic effect of pharmaceuticals, which is a good indication that can be used for the treatment of other compounds.

Declaration of Competing interest

The authors have no competing interests.

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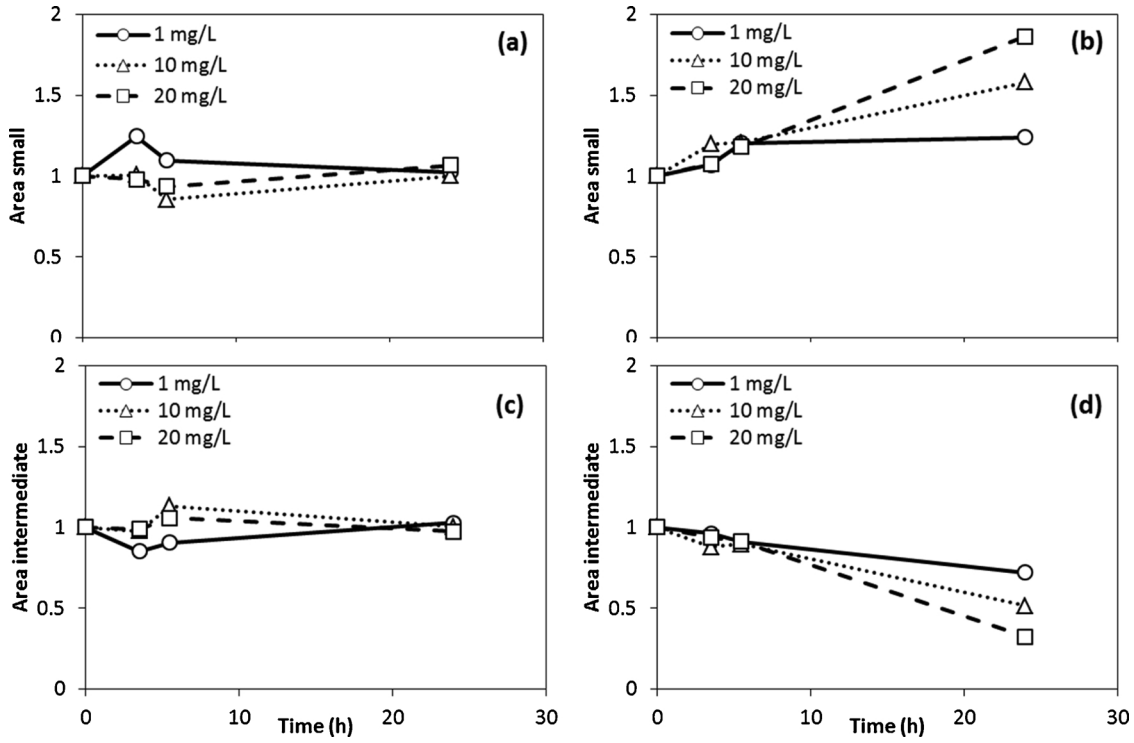

Fig. 4. Area of small and intermediate aggregates for the experiment with PARA (a, c) and with IBU (b, d). Experimental data were normalized by the control results.

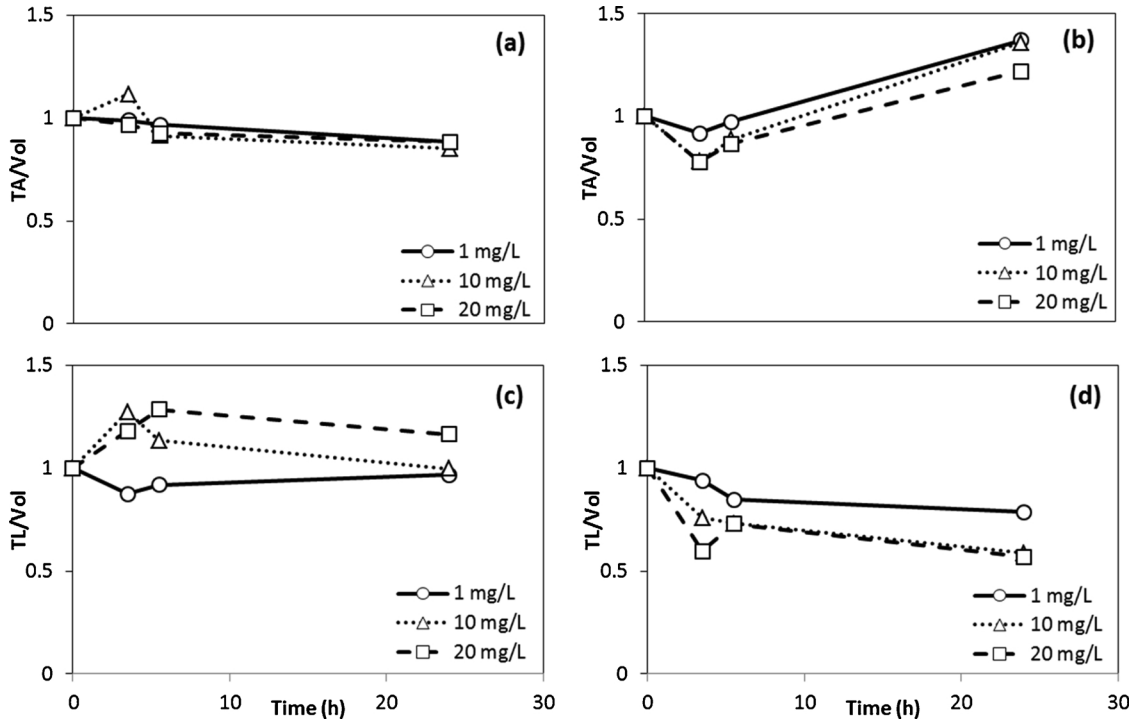

Fig. 5. TA/Vol and TL/Vol for the experiment with PARA (a, c) and for the experiment with IBU (b, d). Experimental data were normalized by the control results.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jwpe.2019.101061.

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