Behavior of micropollutants in polishing units that combine sorption and biodegradation mechanisms to improve the quality of activated sludge effluent

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Abstract

The current study evaluated the removal of six micro pollutants (Estrone (E1); 17β-estradiol (E2); 17α-ethynylestradiol (EE2); Ibuprofen (IBP), Diclofenac (DCF) and Paracetamol (PCT)) from the final effluent of an activated sludge domestic sewage treatment plant using polishing filters. Four polishing filters were assembled as columns and filled with a mixture of sand and vermiculite, sand and charcoal, sand and granulated activated carbon (9:1 by volume) and sand only. The column filters were placed near the outlet of a full-scale activated sludge treatment plant and were fed with a treated effluent containing from 4.71 ng/L to 28.93 ng/L of the target compounds at a hydraulic loading rate (HLR) of 50 $m^3.m^{-2}.d^{-1}$. Samples were collected periodically from the influent (biologically treated sewage) and effluent of the four columns and analysed for estrogens, anti-inflammatories and analgesic compounds. Liquid samples were submitted to a solid phase extraction (SPE) and analysed by gas chromatography coupled with mass spectrometry after their derivatization. Among the compounds found, Diclofenac was distinguished by the high ocurrence of detection in the samples (85%) and higher mean concentration (~17ng.L^{-1}). High removal efficiency (>90%) of the estrogens was observed in the polishing systems studied, whilst for the other targets, the removal efficiency varied from 10% to 30%. Observe that the compounds concentrations were low, maybe this fact is because the rainfall in the collect period.

Keywords

Activated sludge, Endocrine disrupters, Pharmaceuticals, Polish Unit and Post treatment.

1 – Introduction

In last decades the presence of micro pollutants in the aquatic environment has become an environmental concern (Luo et al. 2014), since the large development of industries and cities has resulted in the production and discharge of chemical compounds into the water streams (Bolong et al. 2009). Micro pollutants are segregated in six major categories:

pharmaceuticals, personal care products, steroid hormones, surfactants, industrial chemicals and pesticides (Luo et al. 2014). Within these categories there are numerous subclasses that make up a range of existing micro pollutants. Among them it is possible to highlight the appetite regulators, anticonvulsant, antibiotics, β-blockers, anti-inflammatories, fragrances, sunscreens, repellents, estrogens, xenoestrogens, plastic constituents, non-ionic surfactants and insecticides (Benfenati et al. 2003). In this article it is analysed the behavior of hormones (estrogens) and pharmaceuticals (anti-inflammatory) in filtration units for the post-treatment of activated sludge effluent. These contaminants have been found in concentration ranging from $ng.L^{-1}$ to $\mu g.L^{-1}$ in domestic and industrial effluents (Durhan et al. 2006), surface water and groundwater (Fernandez et al. 2007), in sediments and even in drinking water (Petrović et al. 2003). There are many inputs of these compounds into the environment, but the discharge of sanitary effluents (raw and treated) are known to be one of the most significant pathways (Tan et al. 2007).

The occurrence of some micro pollutants in trace concentrations in the aquatic environment has been frequently associated with increased incidence of cancer (Fent et al. 2006), endocrine disruption (caused by endocrine disrupters compounds – EDC) and resistance of microorganisms to antibiotics (Pruden et al. 2006). Studies conducted in the 1990s in Denmark showed that with prolonged exposure to estrogens, adult males had a decline in fertility due to deterioration in the quality of their semen (Carlsen et al. 1992). Another study confirmed the appearance of cancer in the reproductive system of daughters of women who used diethylstilbestrol (a non-steroidal estrogen) in pregnancy (Nakada et al. 2006). A classic example involving contamination by xenoestrogens occurred in the USA (Birkett and Lester, 2003). In this case researchers realized the decrease of crocodiles in the lake Apopka in the 1980s.

Environmental sinks for the micro pollutants are: volatilization, photo and chemical degradation, biological degradation and sorption (adsorption or absorption) (Cirja et al. 2008). Within a sewage treatment plant (STP), numerous units use these mechanisms to promote the treatment of raw sewage. Thus, a small amount of micro pollutants is occasionally removed in these units, although there is no general rule for the pathways (Luo et al. 2014). This is because the mechanisms responsible for the removal of this type of compounds depends on their physicochemical properties, the wastewater matrix, and the STP design and operational parameters (HLR, sludge age, temperature and pH) (Liu et al. 2015). According to the above pathways, (Cirja et al. 2008; Luo et al. 2014) showed the potential for the micro pollutants removal by using filters with activated carbon – AC (granular or powdered), advanced oxidative processes (AOP), membrane bioreactors and filters that provide the growth of a biofilm adhered to the packing media (bioactive). However, AC, AOP and membrane bioreactors are relatively expensive and have a more complex operation (Mahamuni and Adewuyi 2010), which makes them difficult to implement in small STP (Mulder et al. 2015). On the other hand, units that have a bioactive packing media provide satisfactory biological removal of the micro pollutants at low implantation and operation costs (Margot et al. 2015; Paredes et al. 2016; Zorita et al. 2009)

Despite the promising performance of the bioactive filters for micro pollutants removal, efforts to make a cost-effective technology are still needed since better efficiency on micro pollutant removal should be sought using low cost materials. To overcome the technology development challenges, it is considered here the use of novel packing media designed for the conjugation of both adsorption and biological degradation mechanisms, by using bioactive beds (sand) and low-cost adsorbent media. This hypothesis was based on the premise that a packing media that provides adhered growth and at the same time sorption of the micro

93 pollutants may increase the bioavailability of the target compounds in the biofilm and,
94 consequently, their biodegradation rates.

95 Thus, this study, unlike others published in the literature, has focused on evaluating the
96 removal of 3 estrogens, 2 anti-inflamatories and 1 analgesic in polishing units that aggregate
97 sand for the most part, with inexpensive materials such as vermiculite or charcoal.

98 **2 - Material and Methods**

99 **2.1. Experimental apparatus features and operation**

100 The experimental apparatus is represented in the schematic drawing of Figure 1. The system
101 was composed of a centrifugal pump (Schneider®, BCR-2001), a plastic pre-filter (number 2
102 gravel and 2 mm stainless steel mesh), a reservoir (1,000 L in polyvinyl chloride), a peristaltic
103 pump with independent channels (Provitec®) and four Plexiglas columns (polishing units, as
104 detailed Figure 1). The experimental apparatus received the treated effluent from a
105 conventional activated sludge STP. The STP is designed for a flow rate of 4.5 $m^3.s^{-1}$ and is
106 located in Belo Horizonte, Brazil (coordinates 19º53'42" S and 43º52'42"W, 800 m of
107 altitude).

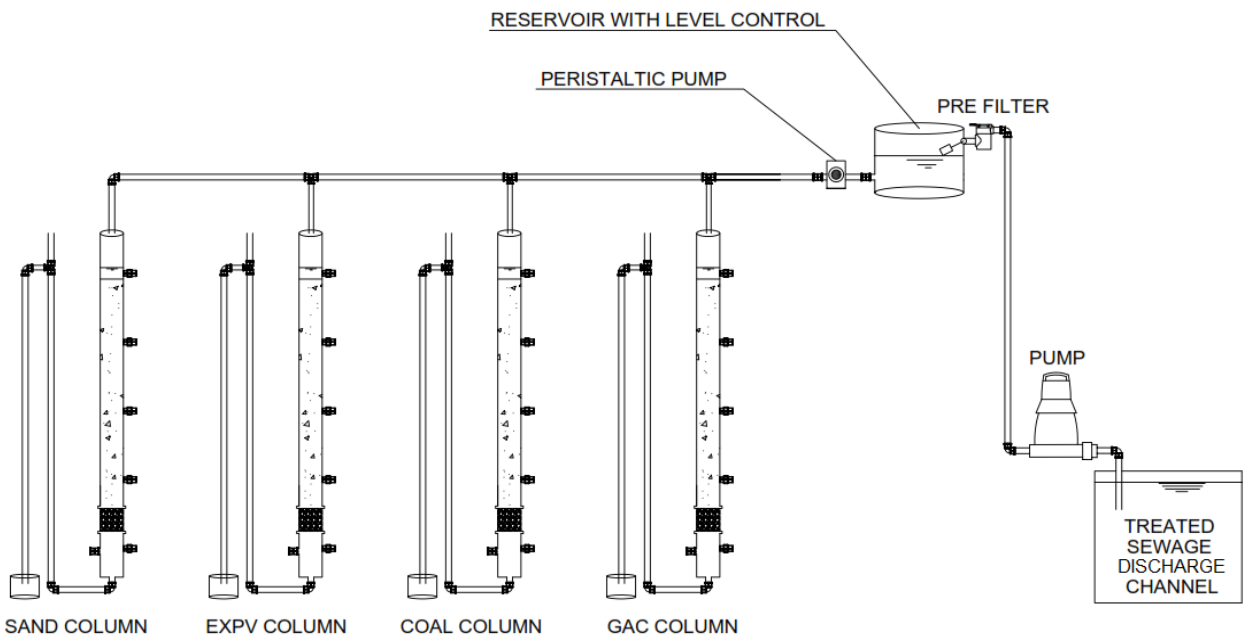

Figure 1. The experimental apparatus

Each Plexiglas column (height = 1.5 m, internal diameter = 0.096 m) was packed with different filter media (30 cm high) and all of which were placed on a layer (30 cm high) of a mixture of number 1 and number 2 gravels. The filter media were chosen to provide the column gains in terms of specific surface area and, hence, a greater attachment of the target contaminants, which could enhance their bioavailability and biodegradability.

One column was packed with only sand (control column); the second one was packed with a mixture of sand and expanded vermiculite (ExpV) in the proportion of 9:1 on a volumetric basis. This proportion was chosen through adsorption tests performed on small scale columns. The same proportion was used for the third and fourth columns, which had as packing media sand with non-activated charcoal (Coal) or sand and granular activated carbon (GAC), respectively. Henceforth, the packing media will be referred only by the fractions that distinguish the four used materials (Sand, ExpV, Coal, and GAC).

The columns operated continuously for two months at an HLR of 50 $m^3.m^{-2}.d^{-1}$, corresponding to a flow rate 0.33 $m^3.d^{-1}$ for each column, respectively. For the growth and stabilization of the biofilm within the columns, the system operated for 15 days uninterruptedly before sampling began. From the 15th day, the system was sampled systematically for 30 days with collections made on average 2 times per week. The time of 15 days was established by monitoring the apparent biofilm formed on the walls of the polishing columns. Moritz et al. 2010, showed that 14 days are enough for the growth and ripening of bacteria such as Pseudomonas sp. in biofilter. These bacteria according to Liang et al. 2012 are linked to the degradation of emerging contaminants, especially estrogens. Furthermore, the columns biofilm stabilization can be confirmed by the little variation of the COD measured (view in complementary material) in the system sampling period during the system operated period of 30 days.

2.2 Analytical methods

The target compounds have been defined based on the literature and considering the following attributes: occurrence in similar matrices (treated domestic effluents) (Bila and Dezotti 2007; Brandt et al. 2013; Ghiselli and Jardim 2007; Luo et al. 2014), effects on health, (Radjenović et al. 2009; Siegrist et al. 2005) concerns about regulation and the technical feasibility of the analytical method used. Under these conditions, six compounds including pharmaceuticals and EDC were chosen: Estrone (E1); 17β-estradiol (E2); 17α-ethynylestradiol (EE2); Ibuprofen (IBP), Diclofenac (DCF) and Paracetamol (PCT).

2.2.1. Sample preparation and collection

To evaluate the occurrence of the target compounds after the STP as well as the level of removal of these compounds in the polishing system, liquid grab-samples (1 L each) of the effluent from the STP (influent of the polishing columns) and the effluent of each column

were taken between 8:00 am and 10:00 am in 10 different days for each operational period (30 days), making a total of 50 samples throughout the entire experiment. The rainfall in sampling period were presented in supplementary materials. These samples were used both for analysis of the target micro pollutants and for physical-chemical analysis as described below.

All target micro pollutants were concentrated using solid phase extraction (SPE) and analyzed by gas chromatography coupled to mass spectrometry (GC–MS) after a derivatization procedure. Aliquots of 200 mL were taken from the grab samples and filtered in glass fiber filters with nominal retention capacity of 0.7 µm (Macherey-Nagel®, GF-3). Filtration and SPE were performed immediately after collection, with no storage of samples. SPE procedure was adapted from USEPA Method 1694 as described elsewhere (Sanson et al. 2014). The remaining sample aliquots were used for analyzes of chemical oxygen demand (COD), suspended solids, pH, temperature and dissolved oxygen (DO). These analyzes were carried out in accordance with Standard Methods (APHA, 2012).

2.2.2. Solid phase extraction - SPE

For the SPE two specific cartridges, Strata SAX® (500 mg) and Strata X® (500 mg), both of Phenomenex brand, were used to clean samples, eliminate interfering compounds, and concentrate the target compounds. Filtered aliquot samples had the pH adjusted to 2.0 ± 0.5 with HCl and then received 50 mg of Ethylenediaminetetraacetic acid (EDTA). After that, the sample rested for two hours at ambient temperature. SPE was carried out in parallel for each specific cartridge, using 100 mL of each aliquot sample per cartridge. The cartridges used were preconditioned with 10 mL of methanol, 10 mL of ultrapure water and 6 mL of ultrapure water acidified with HCl (pH 2.0) in this order. Castro 2017 shows in detail the assembly used for SPE. For the recovery of the target compounds, Strata SAX cartridges were eluted with 10

mL of ethyl acetate and the Strata X cartridges (previously cleaned as 10 mL of ultrapure water) were eluted with 10 mL of methanol and 6 mL of a mixture methanol and acetone (1:1). The extracts were collected and evaporated with nitrogen gas flow until they reached 1 mL volume. The evaporated extracts were transferred to vials and the contents inside the vials were completely dried with gaseous nitrogen flux and frozen for further analysis by GC/MS.

2.2.3 Gas chromatography coupled with mass spectrometry

For GC/MS analyses, derivatization was done by the addition of BSTFA (trifluoroacetamide) + 1% TCMS (trimethylchlorosilane) into glass vials. GC/MS analyses were performed using a GCMS-QP2010 plus equipment (Shimadzu®) and the injection was done in splitless mode. Injection time and temperature were set in 0.5 min and 280 ºC. The carrier gas was helium with a linear velocity of 45 $cm.s^{-1}$ and a pressure of approximately 90.7 kPa. The Rtx-5MS column (30 m × 0.25 mm × 0.25 mm) was used. The initial oven temperature was set as 50 ºC by 1 min and then increased for 100 ºC in a gradient of $25ºC.min^{-1}$. In sequence, the temperature was increased to 300ºC by 5 min in a gradient of $15ºC.min^{-1}$. The gas flowrate through the column was 1.54 $mL.min^{-1}$. Temperature for the MS ionization source was set at 250 °C (interface = 280 °C) and the mass analyzer voltage was set at 1.3 kV with a total time analysis of 21.33 min. The methodology employed was duly validated and some validation parameters are depicted in Table 1.

192 Table 1: Validation parameters for the method used to analyse the selected micro pollutants.

Compound	Linearity (R^2)	LOD ($ng.L^{-1}$)[a]	LOQ ($ng.L^{-1}$)[a]	Recovery Index (%)
Estrone (E1)	0.9903	2.66	8.88	34.3
17β-estradiol (E2)	0.9977	3.68	12.28	36.4
17α-ethynylestradiol	0.9968	6.06	20.19	32.5
Ibuprofen (IBP)	0.9893	1.36	4.53	79.8
Diclofenac (DCF)	0.9576	3.58	11.94	60.0
Paracetamol (PCT)	0.9917	1.03	3.44	60.7

193 . [a]LOD and LOQ refer to the method limits of detection and quantification, respectively, and were calculated
194 considering the signal-to-noise ratio method (S / R = 3 for LD and S / R = 10 for LQ), a concentration factor of
195 250, and the estimated recovery index.

196 **2.3 Data statistical analysis**

197 In order to perform the statistical analysis, Statistica 10.0® software was used. To verify the
198 hypothesis of removal of macro and micro pollutants in a particular polishing column, the
199 Mann-Whitney U-Statistical test was used for independent samples. In order to compare the
200 performance between the polishing columns and to verify the effect of the different packing
201 media on the macro and micro pollutants removal, the Wilcoxon test was used for paired or
202 dependent samples. The significance level of all tests was 95%.

203 **3 - RESULTS AND DISCUSSION**

204 **3.1 Physical-chemical characteristics of influent and effluent from the polishing system**

205 Table 2 shows the physical chemical data of the effluents and influent (INF) – activated sludge final
206 effluent - of the polishing columns (Sand, ExpV, Coal, and GAC). The activated sludge effluent
207 exhibited physical-chemical characteristics within the typical patterns for this type of matrix. For pH,
208 DO and temperature, it was observed that during the experimental period there was no great variation

($\alpha = 0,05$), which means that these parameters are not responsible for variations of dependent variables such as micropollutants. Likewise, the parameters COD and suspend solids did not change significantly according to the statistical methods used, implying that the differences observed in the columns performance cannot be imputed to these variables.

Sapling point	Parameter (Mean ± SD / Min / Max)						
	SS ($mg.L^{-1}$)	**SS Mean Removal (%)**	**COD ($mg.L^{-1}$)**	**COD Mean Removal (%)**	**pH**	**DO ($mg.L^{-1}$)**	**Temperature (°C)**
INF	13±4 / 10 / 23	-	36±8 / 25 / 51	-	7.7±0.2 / 7.4 / 8.0	2.3±0.6 / 1.3 / 3.4	24.9±1.4 / 22.4 / 26.8
Sand	6±3 / 2 / 11	23	28±7 / 22 / 42	24	7.6±0.3 / 7.2 / 8.0	2.5±0.6 / 1.7 / 3.3	23.8±1.3 / 21.3 / 26.1
ExpV	6±2 / 1 / 8	20	30±7 / 22 / 43	20	7.6±0.2 / 7.3 / 7.9	2.6±1.4 / 1.4 / 6.2	23.5±1.3 / 21.3 / 25.8
Coal	5±3 / 1 / 9	44	24±7 / 17 / 41	35	7.6±0.2 / 7.3 / 8.0	2.0±0.6 / 1.5 / 3.5	23.1±1.3 / 21.3 / 24.8
GAC	4±2 / 1 / 7	43	26±7 / 19 / 40	28	7.7±0.2 / 7.5 / 8.0	3.0±0.6 / 2.3 / 3.9	23.1±0.9 / 21.9 / 24.2

SS – Suspend Solids; COD- Chemical Oxygen Demand; DO – Dissolved Oxygen

Table 2: Physical-chemical characteristics of influent (INF) and effluents of the polishing columns (Sand, ExpV, Coal, and GAC)

The values of SS and COD are in agreement with Brazilian effluent treated by activated sludge technology. Obseve that the values of SS found in this study were 13 ± 4 mg.L-1 (mean), 10 mg.L-1 (min) and 23 mg.L-1. (max), while those reported in the literature are from 20 mg.L-1 (min) to 40 mg.L-1 (max). The COD values found in this study were 36 ± 8 (mean), 25 mg.L-1 (min) and 51 mg.L-1 (max), while those reported in the literature were 10 mg.L-1 (min) at 40 mg.L-1 (max) (Oliveira and von Sperling 2002). It should be noted that the values closer to the lower levels, especially SS, may actually be related to rainfall dilution, but it is important to note that the value of precipitation in the operating period was not exorbitant (Cumulative rainfall (24h) - 7, 87 ± 14.68 mm) 0.00 (min) 63.00 (max)

3.2 Occurrence of the micro pollutants in the STP effluent

Figures 2 shows the changes in concentrations of the micro pollutants investigated in the STP effluent (column influent - INF). In the figure, the frequency of detection of each micro pollutant was also placed at the top of the Box-Whiskers.

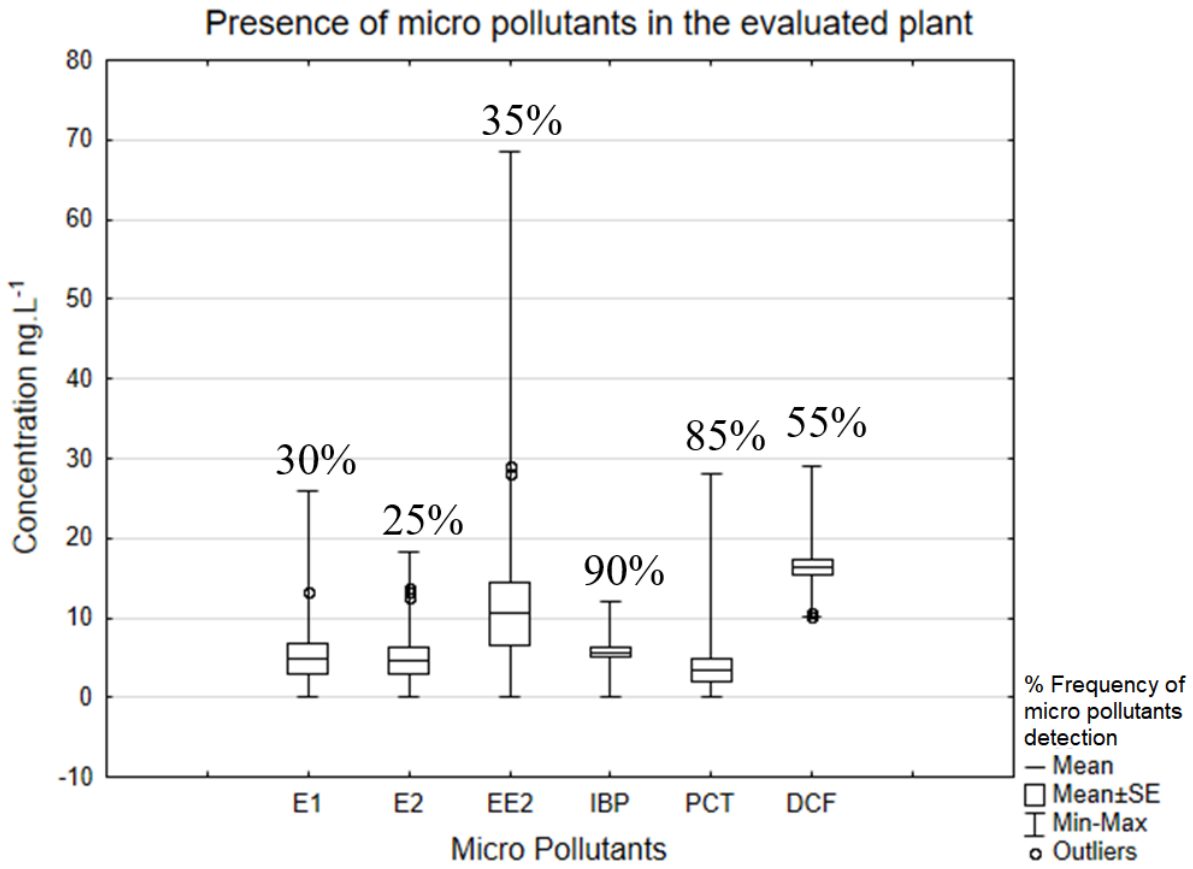

Figure 2. Concentrations of the target micropollutants in the activated sludge effluent

In a comparative analysis of the data presented in Figure 2 with the literature it is observed that for some micropollutants the concentration range reported by other researchers. For instance, Stumpf et al. (1999) observed similar concentration of ibuprofen (max. 12.06 $ng.L^{-1}$) in an effluent from an activated sludge treatment plant in Rio de Janeiro (Brazil). At the same research, the authors also investigated the compound diclofenac, and for this compound, the concentrations found by Stumpft et al. were higher than those found in this research. A reason

237 for this may rely on the dilution factor caused by rain throughout the sampling campaigns of
238 this research.

239 For a study carried out in treatment plants in Korea, Behera et al. (2011) investigated 20
240 micro pollutants in treated effluents and the results showed concentrations similar to those
241 found in this study for diclofenac (mean of 20 $ng.L^{-1}$) and estrone (max of 17$ng.L^{-1}$).
242 However, for ibuprofen the average concentrations found in Korean domestic treated effluents
243 were much higher (111 $ng.L^{-1}$) than that found in this study (40 $ng.L^{-1}$). These differences
244 might be explained by the pharmaceuticals consumption patterns of different countries, as
245 well as the climate differences since tropical countries, due to the higher water temperature,
246 may favour the biodegradation of some micropollutants.

247 For the hormones E1, E2 and EE2, Nie et al. (2012) found mean concentrations of 0.3.$ng.L^{-1}$,
248 < 0.8 $ng.L^{-1}$ and < 4.0 $ng.L^{-1}$, respectively, in domestic effluent from Chinese activated sludge
249 treatment plants. These values are similar to those observed in this study. The lower
250 concentration of these hormones in the activated sludge effluent may have occurred as a
251 consequence of the partial degradation of these estrogens throughout the treatment, since they
252 have relatively high K_{bio} values (Joss et al. 2006), which might lead to the production of by-
253 products (Cajthaml et al. 2009).

254 For the analgesic paracetamol, Stumpf et al (1999) reported concentrations in domestic
255 effluents treated by activated sludge in Brazil in the order of 5.89 $\mu g.L^{-1}$, which is much
256 higher than that found for this study of 5.05 $ng.L^{-1}$.

257 **3.3 Behavior of Estrogens in the polishing units**

258 The target estrogens (E1, E2 and EE2) have similar structural conformation. Basically they
259 consist of a phenolic ring, two cyclohexanes and one cyclopentane ring. Because of the high

octanol / water partition coefficient (log K_{ow}> 3.5), these estrogens are lipophilic and have a moderate tendency of absorption in the solid matrices. Indeed the log K_d are higher than 2.7 for the adsorption of such compounds onto secondary sludge from SWTP using activated sludge technology (Ternes et al. 1999), thus demonstrating a high affinity of such contaminants to this specific matrix. As mentioned, estrogens also have high biodegradability coefficients (K_{bio}> 10) resulting in a great potential for biodegradation in aerobic treatment units (Joss et al. 2006)

Figure 3 shows the concentrations of E1, E2 and EE2 in the effluents from the polishing columns studied (Sand, ExpV, Coal, and GAC). The percentage value placed on the plots refers to the average removal observed for the estrogens. Because of their similar molecular conformation and chemical properties, the same behavior was observed among the three estrogens in the polishing columns. The high level of estrogens removal in the polishing columns might be explained by its adsorption followed by biodegradation. According to Joss et al. (2006), compounds with K_{bio}> 10 tend to be removed above 90% by biological mechanisms, which was confirmed in this research. In addition, the three estrogenic substances exhibit logK_{ow}> 3.0 which gives them the characteristic of being lipophilic and having a moderate tendency to be absorbed in the solid matrices. Another important factor is logK_d> 2.7 for the three compounds, indicating a high tendency of the contaminant for the adsorption in the sludge of the STP. With these characteristics, the contaminant becomes bioavailable for biological degradation, which occurs due to the higher K_{bio} values (K_{bio}> 10). The adsorbent materials increases the retention of E2 and EE2 in the biofilter, thus increasing the chance for their degradation (Nakada et al. 2006).

From the results presented, it was noted that the columns were efficient in the removal of the three target estrogens independently of the filter media used for the treatment. Margot et al.

2015 reported results similar to those presented in this work regarding the behavior of estrogens in sand filters. The authors assigned the removal of these contaminants in sand filters to biodegradation and concluded that sorption in sand was not sufficient to promote the removal of estrogens from synthetic secondary effluents from anaerobic treatment and conventional activated sludge.

It is emphasized that no statistical difference was observed between the columns for the removal of estrogens. However, due to the composition of the filter media some materials (eg GAC) should retain more hydrophobic contaminants (estrogens) than others (eg vermiculite). A proposition that this difference has not been observed is given by the colonization of the material by the biofilm. That is, the growth of the biofilm on the filter material gives hydrophobicity to the supports and levels the level of estrogen removal in the columns, thus not showing the intrinsic differences between the materials.

The great advantage of the biofilm is that it would allow the use of cheaper materials (eg sand) to do the same function as more expensive materials (eg coal). However, during the execution of the experiment the columns showed a great head loss, showing a fragility of the colonization in the filtering media, which is the sealing causing the head loss in the proposed unit.

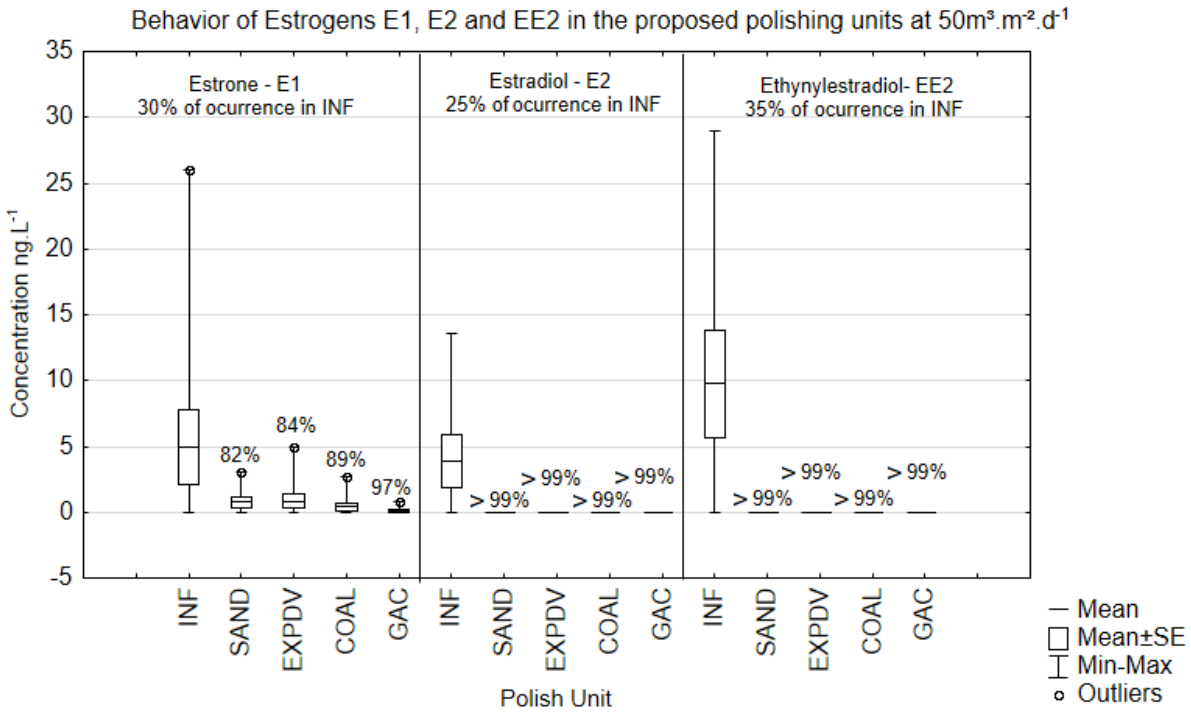

Figure 3. Removal efficiency of target estrogens in the polishing units

3.4 Behavior of analgesic and anti-inflamatory compounds in the polishing units

The anti-inflammatories targeted in this work have chemical structural conformation similar to each other, being composed by a connection of aromatic rings to an acid functional group. However, there are differences between the biodegradability and sorption properties of these compounds. For example, ibuprofen and diclofenac have a moderate to high tendency of sorption onto the solid matrices of a given treatment (log Dow <1.7 for actived sludge matrices to ph 7.65 (Petrovic et al. 2013)), whereas the analgesic paracetamol has a low sorption tendency as a consequence of its low hydrophobicity ($logK_{ow}$ = 0.46 < 2.5 and $logD_{ow}$ = 0.40 to pH 7.65). When the biodegradability aspect is analysed, diclofenac is at odds with its low K_{bio} (<0,1), which renders to this compound some recalcitrance towards biological treatment.

Figure 4 shows the concentrations for the pharmaceuticals ibuprofen, diclofenac and paracetamol in the activated sludge effluent (INF) and in the polishing columns effluents. During the experimental period the average removal of ibuprofen in the polishing systems ranged from 22% (SAND) to 31% (GAC). Statistical analyses (nonparametric Wilcoxon; α = 5%) showed that significant differences were observed among the polishing columns, indicating a better IBP removal in columns with sand and charcoal, either activated or *in natura*) when compared to the other columns. Finally, the statistical test (non-parametric Mann-Whitney U test; α = 5%) confirmed that the polishing systems were efficient in the removal of IBP, showing statistically significant differences between the influent and effluent concentrations.

Ibuprofen has a high $\log K_{ow}$ value (3.97) and a high K_{bio} value (35), indicating that the mechanisms that govern its removal in the polishing columns is likely to be both the sorption onto the filter medium followed by its biodegradation by biofilm microorganisms. Using sand filters as a polishing step in WWTP, (Nakada et al. 2007) reported levels of the anti-inflammatories removal similar to that observed in this research (30%). Moreover, peaks of 80% removal, as observed in this work, were pointed out by researchers who investigated IBP removal in sand filtration units with slow filtration rates (Erba et al. 2012).

Regarding the anti-inflammatory diclofenac, the statistical analysis (non-parametric t of Wilcoxon, α = 5%) did not indicate significant differences on the removal performances among the columns, thereby indicating the filter media was not a key factor in the process.

DCF has pK_a and $\log K_{ow}$ values of 4.2 and 4.51, respectively, which gives it a high tendency to be sorbed onto solid matrices such as biofilms and filter media of the polishing columns. However, DCF occurs in the form of negative molecules under the polishing systems operating pH (7.0) as a consequence of its acidic nature ($pK_a < pH_{inf}$) value, and may suffer

repulsion by negatively charged biomass in the filter media (Suarez et al. 2010). These antagonistic factors might have been decisive in the removal performances observed during the experimental period. Possibly, the probability of repulsion by negative molecules was increased as a consequence of the higher amount of solids in the liquid matrices (Table 2). In addition, the K_{bio} value for DCF is less than $0.1 \cdot g.SS^{-1} \cdot d^{-1}$, which means a low biodegradability of this compound. Therefore it seems that DCF removal in the polishing columns was significantly affected by its sorption onto the solids rather than its biodegradation. The absence of significant biodegradation implies in saturation of the adsorption sites, which eventually would lead to the breakthrough of the column and require replacement of the filter media. Studies published in the literature report removal efficiencies up to 80% for DCF in sand filters with rapid rate of sand (Erba et al. 2012; Suarez et al. 2010). On the other hand, Phu 2016, found that the average DCF removal efficiency was 30%, similar to what have been found in this work.

Figure 4 shows that the average PCT removal in the polishing system varied from 10% to 29% throughout the experimental phases. The non-parametric Wilcoxon t test (α = 5%) applied for data in each operational period did not show statistically significant differences in the PCT concentration in samples of the distinct polishing columns effluents. Thus, statistically, no difference was found in the use of different filter media. The non-parametric Mann-Whitney U test (α = 5) also showed no significant difference, indicating that when operated at the hydraulic rates tested the polishing columns are inefficient to remove PCT from the activated sludge effluent.

PCT has a log K_{ow} value of 0.46 and hence has a low tendency to be sorbed in hydrophobic surfaces such as the solid matrices covered by biofilm. However, the high value of K_{bio} (80) indicates it is prone to biodegradation. On the other hand, Kruskal-Wallis statistical test did

not show a significant difference between PCT concentrations in the influent and effluent of the columns, and that the removal was negligible in all columns studied. This may be justified by the fact that the compound does not tend to be adsorbed and therefore does not become bioavailable for biodegradation.

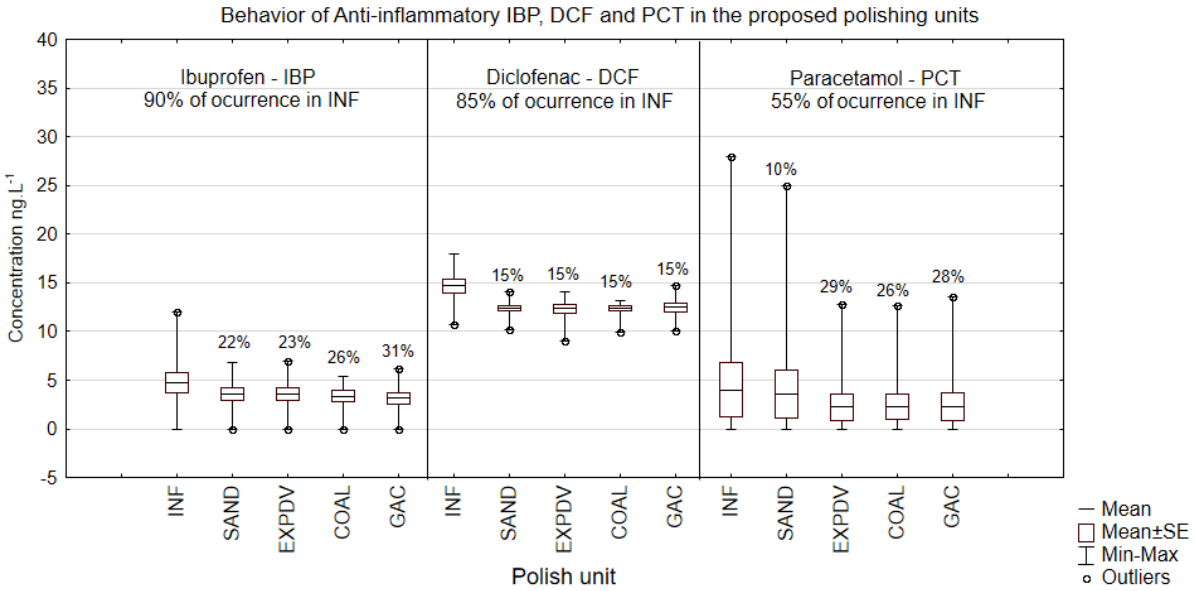

Figure 4. Removal efficiency of analgesic and anti-inflamatory compounds in the polishing units

4 - Conclusions

- All six micropollutants targeted in this study were found in the activated sludge effluent in concentration ranging from 4.71 ng/L to 28.93 ng/L. IBF was the most prevalent compound (detected in 90% of samples) followed by DCF and PCT which were detected in 85% and 55% of samples, respectively. The target estrogens were occasionally detected (frequency of detection of 25% for E2 and 35% for EE2); The polishing units were efficient to remove the estrogens with removal efficiencies varying from 82% to 97% for E1 and nearly 100% from E2 and EE2. The great removal of estrogens in the columns is related to their high biodegradability.

- Regarding the analgesic and anti-inflammatory compounds, the removal efficiencies were not high. For IBP the removal efficiency varied between 22% and 31%, whereas for DCF it was around 15% in all tested columns. Finally, for PCT there was no statistical difference between the columns influent and effluent concentrations, indicating the polishing units operating under described conditions were inefficient to remove this compound;

- The results gathered in this research refuted the hypothesis that the novel packing media would enhance biofilm growth which, in its turn, would lead to higher micro pollutants removal efficiencies. The fact that the column filled with just sand performed similar to those filled with charcoal and vermiculite indicates that biofilm grew well in all media tested and masked the sorption differences of the materials tested.

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Supplementary Material

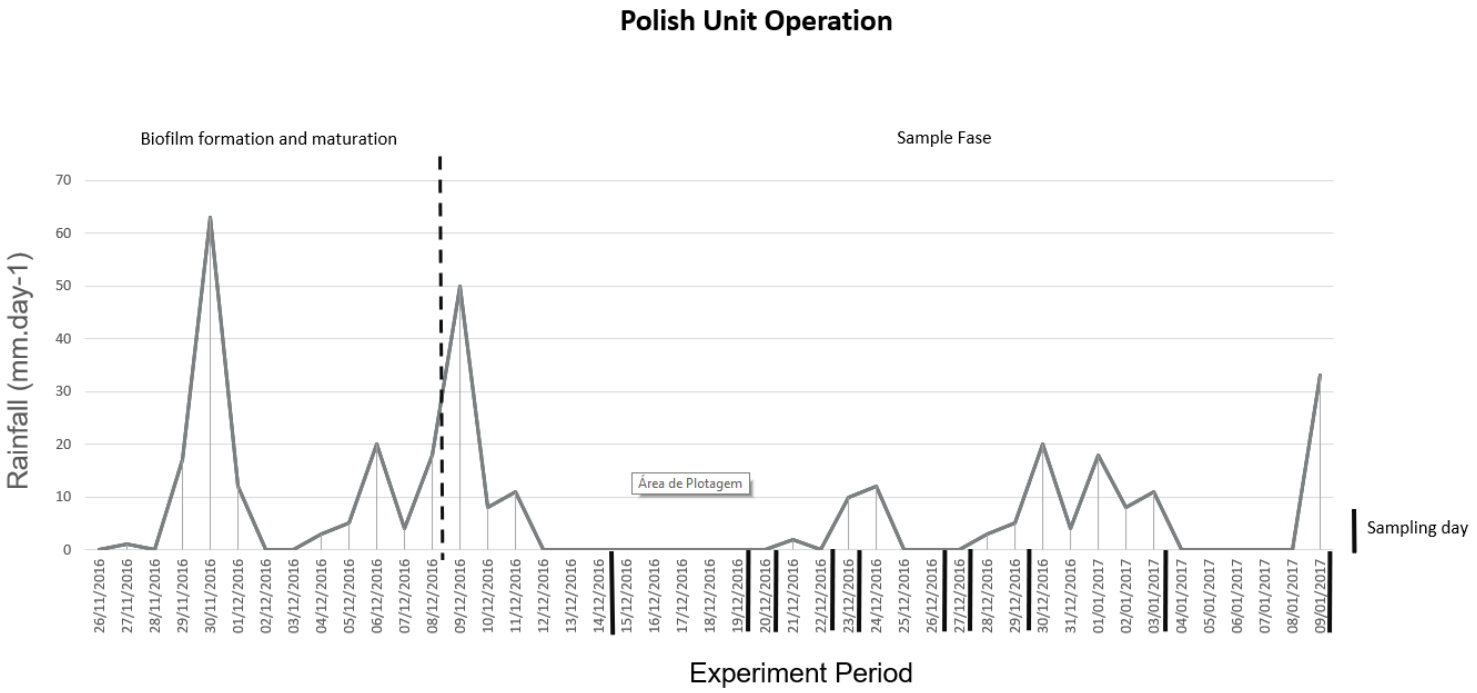

Figure 1. Rainfall in Polish Unit Operation

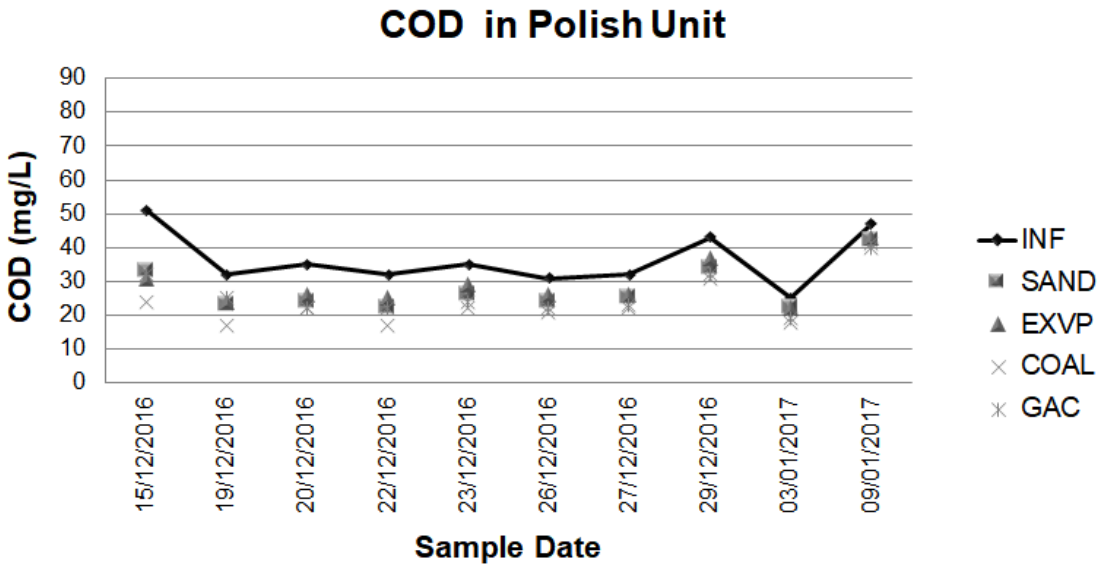

Figure 2. COD in Polish Unit Operation