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UASB reactor

High rate algal pond

Settler

MICROPOLLUTANTS IN SEWAGE TREATED BY ANAEROBIC – AEROBIC SYSTEM			
FAMILY	**UASB REMOVAL**	**HRAP REMOVAL**	**GLOBAL EFFICIENCY**
Hormones	-25% to 84 %	7% to 55%	89% to 95%
Pharmaceuticals	10% to 22%	17% to 54%	39% to 71%
Xenoestrogens	1.5% to 17%	41% to 53%	43% to 70%

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Can high rate algal ponds be used as post-treatment of UASB reactors to remove micropollutants?

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Abstract

The present study evaluated the removal capacity of a UASB-HRAP treatment, combining anaerobic treatment with microalgae-based, aerobic treatment, for eleven micropollutants present in raw sewage, including pharmaceuticals, estrogens and xenoestrogens. The UASB reactor and the HRAP were operated at a hydraulic retention time (HRT) of 7 hours and 8 days, respectively. Samples were collected periodically from the influent and UASB reactor and HRAP effluents. All the target compounds were found in raw sewage, with an occurrence ranging from 70 to 100%. Removal in the UASB reactor was generally incomplete, ranging from no removal (-25.12% for the hormone EE2-ethinylestradiol) to 84.91% (E2 - estradiol), but the overall performance of the UASB+HRAP system was highly efficient for most of the compounds, with removal rates ranging from 64.8% (ibuprofen) to 95% (estrone). Gemfibrozil and bisphenol A were the only exceptions, with overall removal rates of 39% and 43%, respectively. Hormones were the compounds with the highest removal index in the system.

Keywords:

Endocrine disruptors; High Rate Algal Ponds; Pharmaceuticals; Sewage; UASB reactors.

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1 – Introduction

The ubiquity of organic micropollutants in all kind of environmental matrices has become a well-known problem for the scientific community during the last decades, and has derived in a increasing scientific and social concern worldwide (Schwarzenbach et al., 2006; Shao et al., 2019). This is directly associated with all the uncertainties regarding human chronic exposure to these compounds, as their effects at both ecological and human level are not yet fully understood. (Eggen et al., 2014; Noman et al., 2019). It is notorious, though, that these compounds are generating a series of imbalances in non-target organisms causing undesired effects, such as the decrease of entire populations (e.g the case of vultures in Pakistan after diclofenac intake, (Virani et al. 2004)), or endocrine disruption (Hoga et al., 2018).

Pharmaceuticals (PhACs) and endocrine disruptors (ED) are the organic micropollutants most frequently addressed in studies relating environmental occurrence and the derived ecotoxicity (Kuster et al. 2010; Luo, et al. 2014). Both ED and PhACs reach the environment via excretion, partly in their active form and partly as metabolites, and are also often disposed inappropriately in sanitary appliances or in garbage, especially in developing countries (Quadra et al., 2019). It has been widely demonstrated in several research studies that conventional sewage treatment, based on activated sludge (CAS), is usually inefficient removing the vast majority of organic micropollutants, including EDs and PhACs (Luo et al., 2014; Queiroz et al., 2012). For instance, many of the most commonly used PhACs, such as the anti-inflammatory diclofenac, the antiepileptic carbamazepine or the antibiotic sulfamethoxazole are not completely removed in conventional sewage treatment plants (STP) (García-Galán et al., 2011; Kostich et al., 2014).

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The use of upflow anaerobic sludge blanket (UASB) reactors is an alternative to consider, which is already consolidated in developing countries such as Brazil, Colombia, India and Africa (Chernicharo et al., 2018). Indeed, it has permitted that sewage treatment reached populations in places with a low surface availability to install conventional treatment plants, or to cover the needs of small populations. UASB technology essentially allows the removal of suspended or dissolved carbonaceous organic matter from sewage water. However, a post-treatment of the UASB reactor effluent is usually required to improve the effluent quality. Furthermore, regarding organic micropollutants removal, UASB reactors have proved to be inadequate, obtaining low and even negative removals (with higher concentrations in the effluent than in the influent) (Brandt et al., 2013; Stasinakis et al., 2013). These same authors have concluded that most PhACs and EDs are only considerably eliminated under aerobic conditions. Even for those compounds with high adsorption coefficients to sludge (k_d), such as the hormone EE2 or the analgesic paracetamol, their deprotonation under anaerobic conditions (due to the operational pH of the UASB) leads to their repulsion from the sludge blanket (also negatively charged), remaining in the liquid phase (Suárez et al., 2008). In addition, the operating conditions of the reactor (low HRT), the physical-chemical characteristics of the pollutant, environmental conditions, sludge concentration and metabolite formation directly influence the removal of micropollutants in UASB reactors (Alvarino et al., 2014; Gonzalez-Gil et al., 2016).

Taking all this into consideration, the need to treat UASB effluent is clear. Microalgae-based treatments, specifically in open systems such as high rate algal ponds (HRAP), have demonstrated to be highly adequate to treat secondary effluents (de Godos et al., 2016; Mulla et al., 2019). Microalgae-based treatments are gaining a renewed popularity due to their high efficiency removing nutrients and organic matter in a much more sustainable way than conventional treatments. These systems can operate at low operation and maintenance (O&M)

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costs, as they do not require external aeration due to photosynthesis, or any chemical input (García et al., 2006; Muñoz and Guieysse, 2006). Microalgae biomass grows fixating CO_2 and assimilating the nutrients (mostly nitrogen (N) and phosphorus (P)) present in the raw sewage. Through photosynthesis, microalgae generate the oxygen needed by heterotrophic bacteria to aerobically degrade the organic contaminants present in the water. HRAPs are efficient removing both organic matter and nutrients and also a wide variety of micropollutants by photodegradation, bioadsorption and biodegradation (Matamoros et al., 2015; García-Galán et al. 2018, 2020). Indeed, biodegradation is an important route of removal in HRAPs since the large presence of microalgae and heterotrophic bacteria can remove these compounds intra and extracellularly, by means of adsorption on the cell wall (Xiong et al., 2018). The removal efficiency of these ponds for different PhACs has been only recently studied by different authors, obtaining good results for most of the target analytes (de Godos et al., 2012; Hom-Diaz et al., 2017b; García-Galán et al., 2020), but there is still a lack of knowledge regarding the different mechanisms governing these systems (Young et al., 2017). Likewise, the removal of PhACs during UASB treatment, which operates at much lower HRT, needs to be further investigated. Regarding the combination of both systems, to the authors best knowledge, anaerobic-aerobic treatment using UASB and HRAPs has been only barely studied.

The present work aims to evaluate the removal capacity of a treatment system consisting of a UASB reactor (anaerobic treatment) followed by a HRAP (aerobic treatment) for eleven micropollutants: four estrogens, five PhACs and two xenoestrogens.

2 - Material and Methods

2.1. Experimental set-up and operation

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The experimental set-up consisted of a UASB reactor followed by two HRAPs, used as post treatment of the UASB effluent, and a settler to separate the microalgal biomass from the liquid phase (see graphical abstract)). The system received real raw sewage from a nearby sewage treatment plant (STP) located in Belo Horizonte (Brazil) designed for a flow rate of 4.5 $m^3 s^{-1}$.

The UASB reactor was made of fiberglass and had a working volume of 343 L (operational height of 4 m). It operated at a flow rate of 49 L h^{-1} under a HRT of 7 hours and sludge retention time (SRT) of 20 days. The HRAPs were also made of fiberglass, had an operational volume of 205 L each (1.70 m length x 0.3 m depth; 0.68 m^2 surface). The ponds operated at a flow rate of 25.5 L day^{-1} each, corresponding to a HRT of 8 days. The algal biomass produced was harvested in a 30 L volume settler of PVC that operated at a 14 hour HRT. The system was operated continuously for 4 months to reach a steady-state prior to the monitoring campaign.

2.2 Analytical methods

2.2.1. Chemicals and reagents

Eleven different target compounds, including PhACs and EDs, were selected considering their frequency of detection in similar matrices (raw and treated sewage) and their potential ecotoxicity effects. Five PhACs, namely ibuprofen, diclofenac, naproxen, paracetamol and gemfibrozil; four estrogens, namely estrone (E1), 17β-estradiol (E2), 17α-ethinynestradiol (EE2) and estriol (E3), and two xenoestrogens, nonylphenol and bisphenol A, were studied.

2.2.2. *Sample collection and preparation*

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Grab samples of raw sewage, UASB and HRAP effluents samples (1 L) were collected twice per week during 5 weeks between 10:00 am and 11:00 am (n=10 for each point). The sampling was done during the dry season (in May and June – autumn and winter in Brazil). These samples were used both for analysis of the target micropollutants (200 mL) and for physical-chemical analysis. During the sampling campaign, average air temperature was 21.5 ºC, and no precipitation events were registered (Brasil National Meteorology Institute, INMET, http://www.inmet.gov.br).

2.2.3. Sewage quality analyses

Water quality parameters such as pH, temperature, dissolved oxygen (DO), total and volatile suspended solids (TSS and VSS), total nitrogen (TN), ammonium ($N\text{-}NH_4^+$) and chemical oxygen demand (COD) were evaluated daily. pH, DO and temperature were measured using Hach® probes (HQ30D) (Hach, Colorado, US). $N\text{-}NH_4^+$ was analysed by ionic chromatography (IC), using a 940 professional IC Vario instrument (Metrohm®, Herisau, Switzerland). TN was analyzed by a TOC-L analyzer (Shimadzu®, Kioto, Japan). COD was analysed by a Hach® kit for high range. Microalgal biomass was also characterized in terms of volatile solids (VS), carbohydrates, Total Kjeldahl Nitrogen (TKN) and proteins. All of the analyses were done in the laboratory following the Standard Methods (APHA, 2012). pH, temperature and DO, were measured on-site. For qualitative evaluation of microalgae populations, mixed liquor samples were regularly examined under an optic microscope (Olympus BX-50) using a 40x magnification lens and a digital camera (Olympus DP70).

2.2.4 GC-MS analysis of the target PhACs and EDs

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Samples were analyzed by gas chromatography coupled to mass spectrometry (GC-MS), adapting the methodology by Queiroz et al. (2012). Previously, 200 ml of both influent and effluent samples were filtered through glass fiber filters (0.7µm Macherey-Nagel®, GF-3). Then, before the solid phase extraction (SPE), pH was adjusted to 2.0 ± 0.5 with HCl, and the antiquelating agent Ethylenediaminetetraacetic acid (EDTA) was added (50 mg). SPE procedure was adapted from USEPA Method 1694 (Sanson et al., 2014). Briefly, two different cartridges were used, Strata SAX® for ionic compounds (500 mg) and Strata X® for non-polar and aromatic compounds (500 mg), (Phenomenex, California, EUA). SPE was carried out in parallel for each specific cartridge, using 100 mL of each aliquot sample per cartridge. After extraction, Strata SAX cartridges were eluted with 10 mL of ethyl acetate and the Strata X cartridges were rinsed with 10 mL of ultrapure water and then eluted with 10 mL of methanol and 6 mL of a mixture methanol and acetone (1:1). The extracts were collected and evaporated under N_2 flow until they reached 1 mL volume. The extracts were then transferred to vials, completely dried under N_2 flow and frozen until analysis.

Before GC-MS analysis, derivatization of the samples was performed, adding BSTFA (trifluoroacetamide) + 1% TCMS (trimethylchlorosilane). GC/MS analyses were carried out on a QP2010 plus instrument (Shimadzu®). Further information on the methodology is given in supplementary material.

2.3 Methodology validation and data statistical analysis

Recovery rates (R%), method limit of detection (LOD) and limit of quantification (LOQ) for all the target compounds are given in Table S1 of the Supplementary Materials. LODs and LOQs were calculated as the minimum detectable amount of analyte with a signal-to-noise ratio of 3 and 10, respectively, in the different samples analyzed. LOQs in the raw sewage ranged from 0.5 ng L^{-1} for estrone to 5.9 ng L^{-1} for naproxen. For the recovery study,

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100 mL of each type of sample were fortified to a final concentration of 20 ng mL^{-1} of all the target analytes and were submitted to SPE, following the methodology explained in section 2.2.4. Analyses were performed in triplicate. The recovery percentage was calculated according to the equation 1:

$$R(\%) = \frac{C_D + C_B}{C_T} \cdot 100 \quad (1)$$

where C_T corresponds to the theoretical added concentration, C_D is the actual concentration determined in the fortified sample and C_B is the concentration determined in the non-fortified sample (background concentration of the real sample). The R% values obtained were >50% in most cases.

The Mann-Whitney U-Statistical test was used for independent samples to confirm the statistical difference between influent and effluent samples. Statistica 10.0® software was used, using a significance level for all tests of 95%.

3 - RESULTS AND DISCUSSION

3.1 Sewage quality parameters

The physical-chemical properties of the raw sewage, UASB effluent and final effluent from the HRAP are summarised in Table 1.

Table 1. Physical-chemical characterization of the different water samples evaluated: Raw sewage, UASB effluent ($UASB_{eff}$) and final effluent of the treatment system ($HRAP_{eff}$).

Parameters For n = 10	Sample point					
	Raw Sewage		$UASB_{eff}$		$HRAP_{eff}$	
	Mean ± SD	Min / Max	Mean ± SD	Min / Max	Mean ± SD	Min / Max
TSS (mg L^{-1})	366.58 ±134.62	15.12 / 443.46	64.38 ± 41.36	1.03 / 283.94	149.27 ± 92.82	18.32 / 503.94

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VSS (mg L^{-1})	254.31 ±213.32	92.31 / 389.32	40.38 ± 41.19	0.31 / 219.92	106.32 ± 77.17	16.92 / 410.14
COD (mgO_2 L^{-1})	518.64 ±123.21	102.80 / 598.43	232.69 ±109.21	111.14 / 482.24	146.08 ±65.40	98.15 / 321.43
pH	7.4 ± 0.2	7.0 / 8.0	7.1 ± 0.4	6.8 / 8.3	8.4 ± 0.7	7.1 / 10.2
DO (mg L^{-1})	1.04 ± 0.55	0.05 / 2.33	0.34 ± 0.12	0.13 / 1.36	9.04 ± 2.95	3.45 / 14.45
Temperature (ºC)	24.5 ± 2.5	20.6 / 29.4	23.5 ± 2.6	19.8 / 29.1	21.8 ± 3.3	16.3 / 30.1
TN (mg L^{-1})	34.43 ± 9.31	26.21 / 53.12	54.33 ± 5.32	42.21 / 63.95	24.31 ± 11.02	14.13 / 48.04
N-NH_4^+ (mg L^{-1})	25.21 ± 8.13/	7.10 / 32.25	34.21 ± 13.43	14.92 / 54.75	14.31 ± 9.13	10.32 / 55.54
VS* (g L^{-1})	-	-	-	-	1.01 ± 2.55	0.20 /3.25
TKN* (mg L^{-1})*	-	-	-	-	101.37 ± 65.16	29.71 / 235.77
Carbohydrates* (mg L^{-1})	-	-	-	-	102.98 ± 86.43	13.89 / 318.82
Proteins* (mg L^{-1})	-	-	-	-	633.56 ± 407.25	185.68 / 1,473.56

TS – Total Solids; VS – Volatile Solids; COD – Chemical oxygen demand ; DO – Dissolved Oxygen; TN – Total Nitrogen; N-NH_4^+ - Ammonium - * measured only in HRAP biomass

Average COD values obtained were 232 mg L^{-1} in the UASB reactor effluent and 146 mg L^{-1} in the HRAP effluent, with average removals of 55% and 72% in the UASB reactor and UASB+HRAP, respectively. In general, COD removals between 55 to 65% have been reported for UASB reactors and between 65 and 80% for UASB followed by polishing ponds systems (Von Sperling 2007) or HRAPs (Villar-Navarro et al., 2018).

In the UASB reactor, no nitrogen removal was observed, but organic nitrogen was mineralized. Under anaerobic conditions, the decomposition of the organic matter by anaerobic microorganisms leads to the hydrolysis of proteins and urea and the consequent increase of NH_4^+-N (Metcalf & Eddy et al., 2003). Mean concentrations found in the raw sewage were 25 mg N-NH_4 L^{-1}, increasing to 34 mg N-$NH_4 \cdot L^{-1}$in the UASB effluent. In the HRAPs, an average removal of 44% for NH_4^+-N was observed, with final effluent

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concentrations of 14 mg N-NH_4 L^{-1}. The reduction of NH_4^+ in the ponds was due to nitrification and volatilization. Regarding TN, an overall removal of 30% for was observed.

The observed TSS and VSS concentrations increased from the UASB reactor to the HRAP by 130% and 165% respectively. However, the total removal for TSS and VSS was about 59% and 58%, respectively. These results are consistent with the typical values of domestic effluents (Metcalf & Eddy, 2003), UASB reactor effluents (Chernicharo 2007) and HRAPs effluents as UASB post treatment (Santiago et al. 2017). UASB reactors operating at HRT of 15 hours followed by an HRAP working at HRT of 4- 6 days showed overall removals of 60% for NH_4^+-N, and an average increase of 130% for TSS and 165% for VSS due to microalgae growth (Santiago et al., 2017; Villar-Navarro et al., 2018).

Considering the current Brazilian and Minas Gereais (local where this study was performed) legislation on urban wastewater (Conama Directive 430/2011 and Copam directive 01/2008), maximum effluent discharge concentrations for COD, TSS and N-NH_4^+ are set to 180 mg L^{-1}, 150 mg L^{-1} and 20 mg L^{-1} respectively (Morais and Santos, 2019). The studied system successfully meets all the required limits, parameters, as final concentrations for COD, TSS and N-NH_4^+ of 146 mg L^{-1}, 106 mg L^{-1} and 24 mg L^{-1} were obtained, respectively. On the other hand, considering the most restrictive European urban wastewater Directive (Council Directive 91/271/EEC), with COD, TSS and TN discharge limits in effluents set to 125 mg L^{-1}, 35 mg L^{-1} and 15 mg L^{-1} respectively, the quality of our final effluent would not be compliant. Nevertheless, it is important to note that for a complete analysis of the domestic effluent treatment capacity in natural systems (such as this one), an assessment of at least one year of data would be required to verify the seasonality effect (Von Sperling, 2007).

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The microalgae biomass harvested had a VS concentration of 1.01 g L^{-1}, and 101.4 mg TKN L^{-1}, 102.9 mg L^{-1} of carbohydrates and 635 mg L^{-1} of proteins. Carbohydrates and proteins corresponded to 10% and 62% of the biomass wet weight, respectively. Regarding the different microalgae species present in the HRAPs, *Chlorella vulgaris* was predominant, together with *Scenedesmus* sp., *Westella botryoides* and different species of diatoms.

3.2 Occurrence of micropollutants in raw sewage

The concentrations of the targeted micropollutants in the raw sewage are shown in Figure 1. The frequency of detection was indicated on top of the box whiskers. Values ranged from 4 ng L^{-1} (E3) to 445 ng L^{-1} (naproxen).

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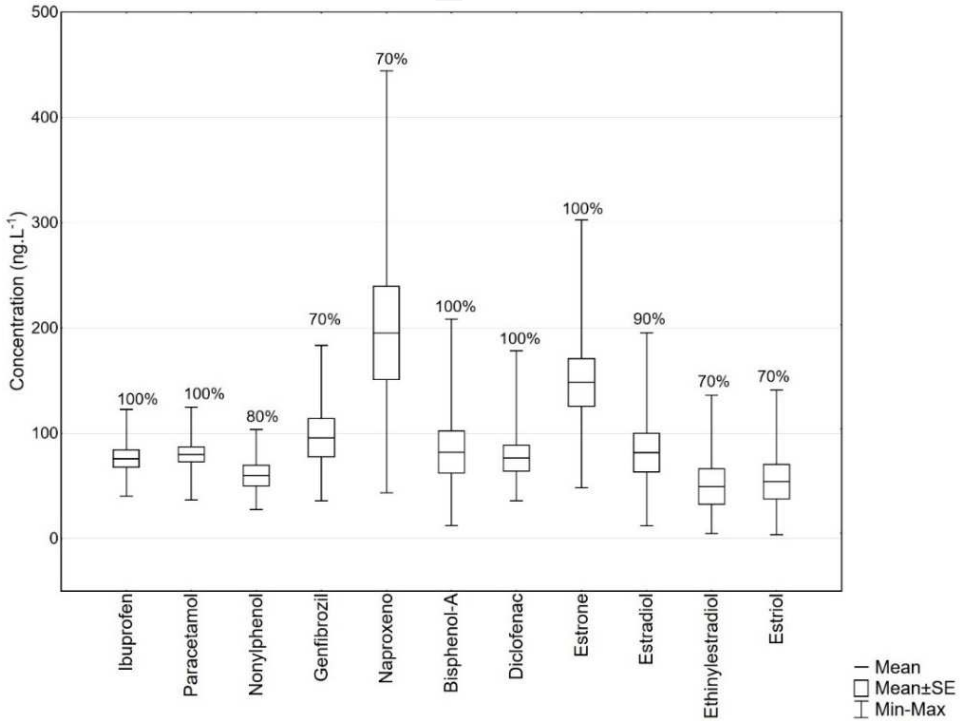

Figure 1. Concentrations of the target micropollutants in the raw sewage. Frequencies of detection (%) are shown on top of the box-plots.

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In order to simplify the discussion of the results, the target micropollutants were divided into family groups, PhACs (ibuprofen, naproxen, diclofenac, paracetamol and gemfibrozil) estrogens (E1, E2, EE2 and E3), and xenoestrogens (nonylphenol and bisphenol A).

For PhACs, naproxen and gemfibrozil were those detected at the highest concentrations (195.14 and 95.57 ng L^{-1}, respectively). Concentrations for the anti-inflammatories ibuprofen and diclofenac were much lower than those generally found in sewage water (75.82 ng L^{-1} and 76.38 ng L^{-1}, respectively) (Américo et al., 2012). For gemfibrozil, the values found are within the reported in the literature in different countries (i.e. France, USA and China) (Luo et al., 2014).

For hormones, E1 and E2 were detected at the highest concentrations (148.42 ng L^{-1} and 81.5 ng L^{-1}, respectively). Similar levels have been reported in raw sewage from Brazil (Américo et al., 2012b; Froehner et al., 2011). EE2 and E3 were detected at 49.29 ng L^{-1} and 54.05 ng L-1, respectively. When compared to concentrations from different countries, EE2 concentrations are usually lower, ranging from 1 to 3 ng L^{-1} (Luo, et al. 2014; Nie et al. 2012; Zorita et al. 2009). This difference could be explained by a different consumption pattern the study sites. Regarding the natural estrogens E1, E2 and E3, values found in the present study are within those reported in the literature (Luo, et al. 2014) However, when compared to each other, higher concentrations of E3 than E2 and E1 are usually found in the literature, since the human excretion rate of E3 is far larger than that of E2 or E1 (Liu et al., 2015). But it should be noticed that both E3 and E2 can be dissociated to E1, causing a mass increase of the latter compound (Fan et al., 2011).

EE2 is a hormone formed from E1 by ethinylation, which makes it more stable within the organisms, thus able to exert its contraceptive function. During its metabolism in the organism, it is conjugated with glucuronic and sulfuric acids and so they are excreted and

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274 released into sewage in its less active form. However, under anaerobic conditions, these
275 metabolites can revert back to their active form, deconjugating and being transformed back
276 into E1 (Benfenati et al., 2003; Shore et al., 1993). This may also be observed within the
277 pipelines that lead the sewage to the STP (Brandt et al., 2013).

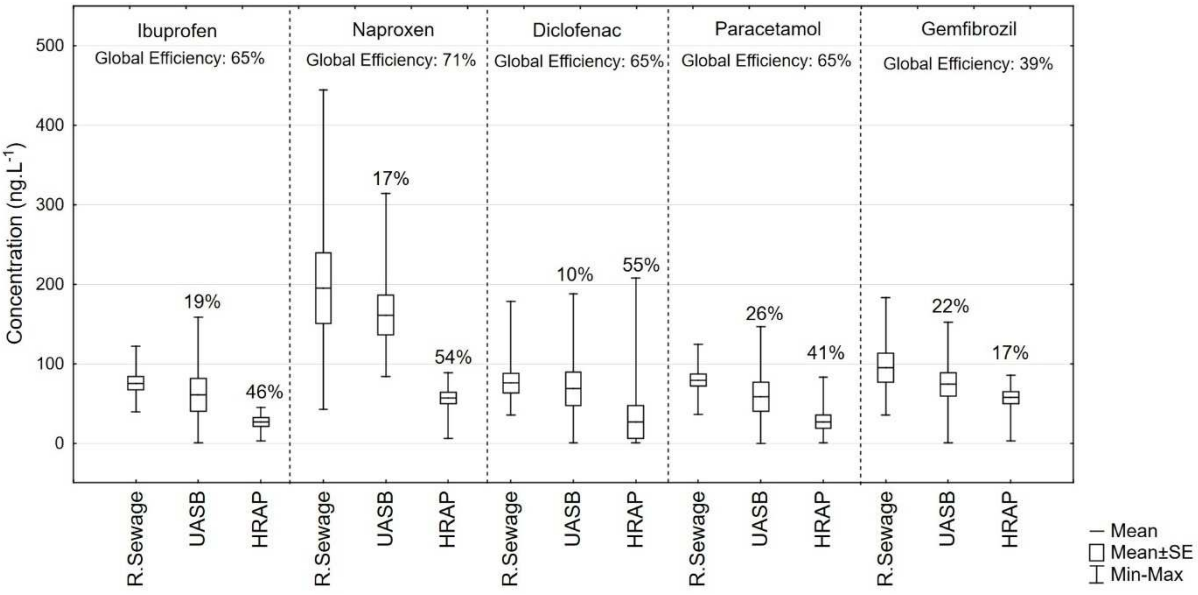
278 Regarding the xenoestrogens nonylphenol and bisphenol A, the average concentrations
279 found were 59.86 ng L^{-1} and 82.04 ng L^{-1} respectively, which are similar to those published in
280 previous studies (Brandt et al. 2013; Luo, Guo, Ngo, et al. 2014; Nie et al. 2012; Pothitou and
281 Voutsa 2008).

282 **3.3 Behavior and fate of micropollutants in the UASB + HRAP system**

283 **3.3.1. Pharmaceuticals**

284 Concentrations of the five PhACs studied in the effluents from the UASB reactor and
285 HRAP are illustrated in Figure 2.

286



287

288 **Figure 2.** Removal efficiency of pharmaceutical in UASB-HRAP system. The percentage placed on top of the
289 plots refers to the average removal observed for the corresponding compound in the corresponding system

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Removal rates obtained for the five PhACs in the anaerobic phase of the treatment were lower than 30%, which agrees with previous results (Brandt et al., 2013; de Graaff et al., 2011; Reyes-Contreras et al., 2011). PhACs have pK_a values between 4 and 5 and log K_{ow} between 3 and 4, (with the exception of paracetamol, pK_a = 9.8 and log K_{ow} = 0.46) (Castro, 2017; Castro et al., 2018). Therefore, sorption could be the main removal mechanism and the ionization of these PhACs may be the factor that causes these compounds to remain in the liquid phase. The low HRT and upflow velocity can cause a detachment of these compounds from the solid phase to the liquid phase. In addition, the repulsion of these compounds (deprotonated) from the sludge blanket (negatively charged) makes them mostly present in the liquid phase in the UASB. Paracetamol follows a different pattern, as it is highly hydrophilic with no tendency to adsorb onto the biosolids. Although it has a high tendency for biodegradation (K_{bio} = 80 $L \cdot gSS^{-1} \cdot d\text{-}^{1}$) (Joss et al., 2006), the HRT applied to the reactor studied was too low (7 hours) for an efficient biodegradation. Indeed, previous publications obtained removals higher than 90% for paracetamol in a UASB reactor operating at an HRT 30 times higher than that in the present study (de Graaff et al., 2011).

Gemfibrozil was only removed a 20% in the UASB reactor, despite its high tendency to adsorption. This could be explained in terms of its competition with humic substances present in the reactor for the active adsorption sites of the sludge blanket (Maeng et al., 2011).

In the HRAP, removals were greater than 60% for all the anti-inflammatories and analgesics studied (ibuprofen, paracetamol, diclofenac and naproxen). Diclofenac was removed in a 65%. Its low K_{bio} (less than 0,1 $L \cdot gSS^{-1} \cdot d^{-1}$) indicates that biodegradation can be neglected as removal pathway (De Laurentiis et al., 2014). On the other hand the high photosensitivity of this compound has been demonstrated in previous studies, and photodegradation is probably the main elimination route for this anti-inflammatory (García-

Galán et al., 2020; Xiong et al., 2018; Iovino et al. 2017; Zhang et al., 2008). Contrary to diclofenac, ibuprofen is highly biodegradable. A recent experiment performed at laboratory scale, using effluent water from a stabilization pond, demonstrated that, regardless the presence or absence of light, the concentration of ibuprofen remained the same when microalgae were not present (Larsen et al., 2019). On the contrary, when inoculating the system with a pure culture of *Scenedesmus obliquus*, the removal of ibuprofen reached almost a 90%.

Similarly to diclofenac, paracetamol was removed a 65% in the HRAP. It is indeed a readily biodegradable compound (K_{bio} = 80 L $\cdot gSS^{-1} \cdot d^{-1}$) (Joss et al., 2006) and removals of up to 90% were obtained in microalgae-based systems (Zhou et al., 2014). Additionally, direct photolysis has also been reported as an important mechanism of paracetamol removal (De Laurentiis et al., 2014). Naproxen was removed a 70%, which is in accordance to previous results (Hom-Diaz et al. 2017; Matamoros et al. 2015). Naproxen removal in conventional STPs is mainly attributed to biodegradation (K_{bio} ~9 L$\cdot gSS^{-1}\cdot d^{-1}$) (Suarez et al., 2010), whereas sorption processes can be neglected due to its low K_{ow} (Kasprzyk-Hordern et al., 2009). Simultaneous removal of NH_4^+-N and naproxen could also take place, as a consequence of the co-metabolic biotransformations induced by autotrophic aerobic bacteria present in the HRAPs (Fernandez-Fontaina et al., 2012; Helbling et al., 2012; Yi et al., 2006). Some authors suggested that the enzyme ammonium monooxygenase (AMO) is the catalyst responsible for the micropollutants and NH_4^+-N co-metabolism (Yi et al., 2006).

For the antilipemic gemfibrozil, an average removal of 40% was obtained. The higher removal of this compound compared to that obtained in the UASB reactor may be related to a lower competition for adsorption sites with the humic substances and a greater availability of active sites in the microalgae biomass. However, the lower removal of this compound

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compared to the other PhACs studied could be due to the fact that the hydrophobicity of gemfibrozil decreases with an increasing pH, as recently demonstrated by Phan et al., (2018), who observed that the K_d of gemfibrozil was 2.1 when pH was 7, and so gemfibrozil migrated from the solid phase into the aqueous phase.

3.3.2. Estrogens

Concentrations of E1, E2, EE2 and E3 in the effluents from the UASB reactor and the HRAPs are shown in Figure 3. Removals < 50% were observed for E1 and E3 after the UASB treatment, which is in accordance with previous publications in anaerobic systems such as anaerobic membrane reactors and completed stirred tank reactors (Gonzalez-Gil et al., 2016; Ito et al., 2016). The low removal of E1 and E3 may be associated with the low HRT (7 hours) and the low sludge retention time (SRT) (20 days) applied in the UASB reactor. Previous studies have demonstrated that SRT between 10-30 days had nearly no effect on the biotransformation of PhACs and estrogens (Gonzalez-Gil et al., 2016). Nevertheless, E2 was removed a 85% in the UASB reactor, but this elimination was not associated to its biodegradation and full mineralization, but to its transformation to E1 under anaerobic conditions (Adeel et al., 2017). This could corroborate that the hormone E1, contrary to what is usually reported in the literature, occurs in greater quantities in the raw sewage than E2 due to this dissociation.

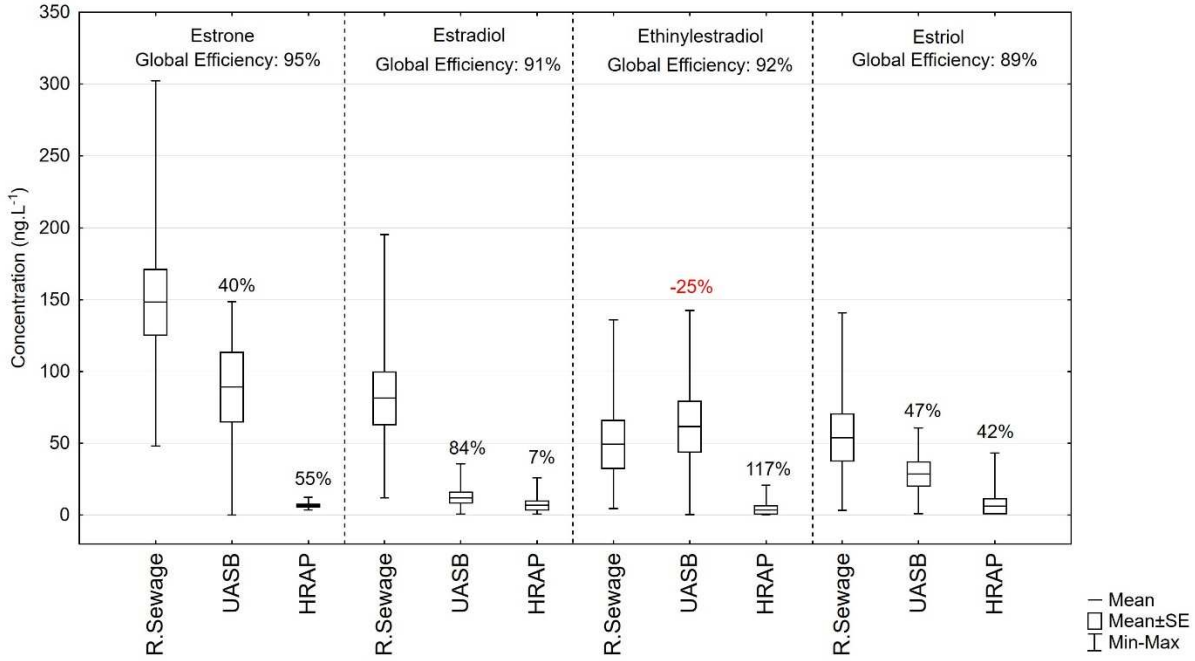

Figure 3. Concentrations of estrogens in UASB-HRAP system. The percentage placed on top of the plots refers to the average removal observed for the corresponding compound in the corresponding system**.**

Despite many studies have addressed the anaerobic degradation of hormones, the reactors evaluated usually operated at HRTs higher than 12 hours, directly affecting the degradation of the compounds. Indeed, two recent studies confirmed that HRT is critical in the removal of micropollutants from UASB reactors, as it is directly related to the contact time between the wastewater and the sludge inside the reactor (Queiroz et al. 2012; Alvarino et al. 2018). The short HRT of the present study may also account for the increased EE2 concentration in the UASB reactor effluent. It is important to point out that in most of the studies carried out only liquid samples, and not solid samples, are analyzed, so a complete mass balance is seldom achieved.

In UASB reactors, the flow rate is applied from the bottom upwards and, therefore, the liquid upflow velocity can cause turbulence in the sludge blanket (Alvarino et al., 2014). Therefore, lower HRTs mean higher upflow rates applied and, consequently, a higher velocity within the UASB. The turbulence caused in the blanket increases the transfer of lipophilic

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372 compounds from the solid to the liquid phase. In addition, under the pH in the UASB (7.1),
373 compounds with high ionization coefficients (pK_a) as EE2 (pK_a = 10,5) tend to ionize (Brandt
374 et al., 2013). Negatively charged molecules are repelled by the negatively charged biomass
375 and, therefore, remain in the liquid phase (Schäfer et al., 2011).

376 No clear trend was observed for anaerobic hormone removal and indeed, most of the
377 previous studies on the removal of these compounds in anaerobic systems reported that
378 estrogens are not significantly removed (Brandt et al., 2013; Carballa et al., 2007; Shi et al.,
379 2010).

380 In contrast, the results after HRAP treatment showed removals between 88% and 95%
381 for all the hormones evaluated. Similar results were found in the literature for the same
382 compounds (Shi et al., 2010). Removal of micropollutants in HRAPs are related to
383 bioadsorption, biodegradation, photodegradation and volatilization (García-Galán et al.,
384 2020). Hormones have a moderate tendency to adsorb onto solid matrices, (log K_{ow} > 2.65),
385 but they are highly biodegradable ($K_{bio(EE2)}$ = 9L·gSS^{-1}·d^{-1}) (Joss et al., 2006). In microalgae-
386 based treatment systems, the microbiology community acts as a biosorbent (Gadd, 2009). The
387 cell wall of microalgae and bacteria contains polysaccharides and proteins that can provide
388 adsorption sites for the organic contaminants (Fomina and Gadd, 2014; Moritz et al., 2010).
389 In addition, microalgae may be actively involved in the biodegradation of the organic
390 contaminants, as their enzymes can metabolize these compounds (Wang et al., 2017).
391 Therefore, for the hormones evaluated in this work, sorption to the solid matrix and
392 biodegradation are probably the two main removal pathways. In particular for EE2, which
393 was the most stable hormone and had the lowest biodegradability coefficient, degradation
394 may also be associated also with the removal of NH_4^+-N (~ 60%, Table 2), as explained for
395 naproxen. Regarding photodegradation, half-lives of 10 days have been estimated for the

photolytic degradation of E2 and EE2 (Jurgens et al., 2002). These tests were done on a bench-scale system, using surface water, with daily and direct radiation, suggesting that irradiation could enhance the removal of these hormones. Nevertheless, biosorption to the biomass and biodegradation seem to be the main elimination routes in HRAPs (Hom-Diaz et al. 2015; Yongli Zhang et al. 2014).

3.3.3. Xenoestrogens

The concentrations and removals of nonylphenol and bisphenol A in raw sewage and effluents from the UASB reactor and HRAP are shown in Figure 4. The removals achieved for both compounds in the UASB were very low, and the statistical test confirmed that there was no significant removal in this treatment unit.

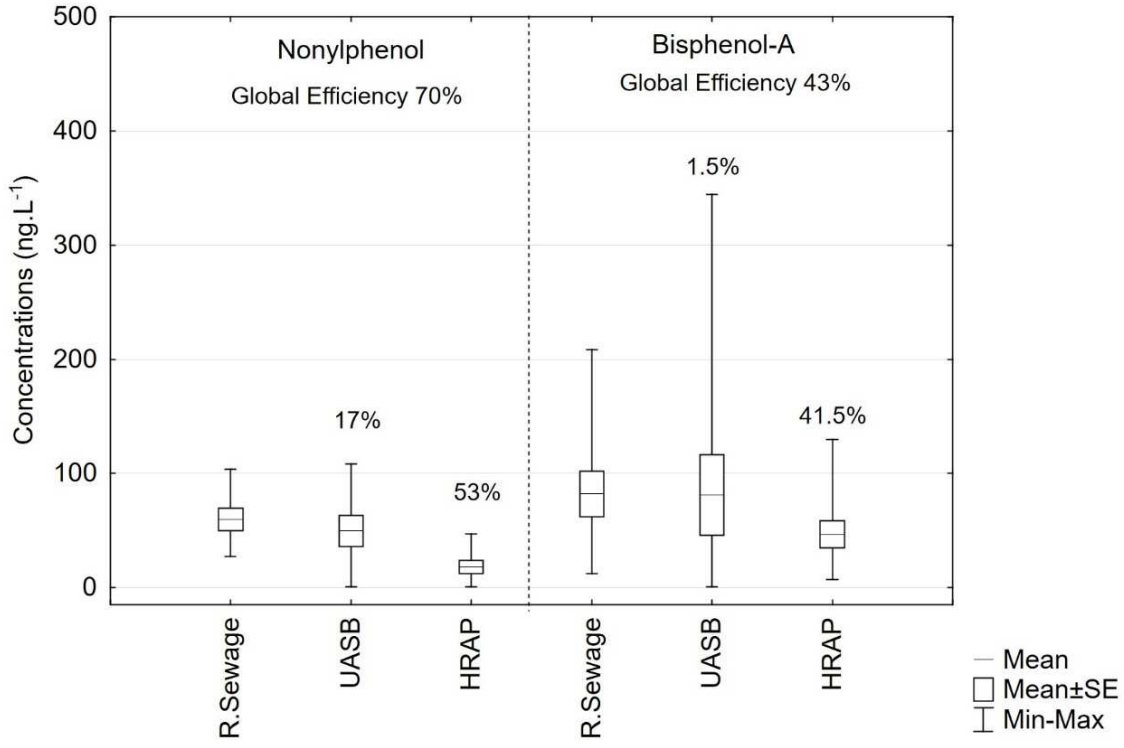

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Figure 4. . Concentrations of xenoestrogens in UASB-HRAP system. The percentage placed on top of the plots refers to the average removal observed for the corresponding compound in the corresponding system**.**

For nonylphenol, the mean removal was approximately 17%. Nonylphenol is a raw material for the production of non-ethoxylated nonylphenols (NEPO) (Virkutyte et al., 2010). The recalcitrance of nonylphenol in anaerobic sewage treatment systems is due to the degradation of the NEPOs present in the formulations of cleaning products (Aquino et al., 2013). Furthermore, nonylphenol has a slower degradation kinetics in anaerobic environments due to the alkyl chain and the aromatic ring in its molecular structure (Soares et al., 2008). Despite its pK_a >10, it can be deprotonated and repelled by the solids of the reactor (Brandt et al., 2013).

Of all the compounds evaluated in this study, bisphenol A was the most recalcitrant to anaerobic treatment. It should be noted, however, that this plasticizer is present in some of the components of the experimental treatment line of this study and thus can be transferred to the liquid phase. causing an increase in concentration. Bisphenol A has a moderate sorption capacity to solid matrices and, besides, the low HRT in UASB reactors led to a shorter contact time of the compound with the sludge blanket, reducing the possibility of sorption. Studies on the biodegradation of this compound in anaerobic environments have shown its recalcitrance under such conditions (Ronen and Abeliovich, 2000).

Regarding their removal in the HRAP, an average elimination of 70% for nonylphenol and 44% for bisphenol A was obtained. Removal of Nonylphenol is directly related to biodegradation, and previous studies have shown that it can be absorbed and adsorbed by *Chlorella sp*. (Gao et al., 2011). Photodegradation should not be neglected for this compound. For bisphenol A, Matamoros et al., (2016) obtained higher removals of 60-90% in HRAP. As aforementioned, the different results can be attributed to the concentration and transfer of bisphenol A from the different plastic materials associated to the HRAP. Oxidation of the

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hydroxyl radicals in the HRAP should also be neglected. with high DO in the HRAP that allows the oxidation

4 – Conclusions

The efficiency of an anaerobic-aerobic wastewater treatment system, consisting of an UASB reactor followed by an HRAP system, was evaluated, focusing in the removal of 11 selected organic micropollutants. This treatment strategy was efficient treating wastewater in terms of solids, organic matter and nutrients, and also in terms of organic micropollutants. The UASB reactor showed a limited removal for all the targeted compounds, and only the estrogen E2 was removed significantly (85%). The short operation HRTs of the UASB reactor seems to be the most feasible explanation for the low removals obtained. On the contrary, the HRAP system proved to be more efficient in removing estrogens (90%-95%), PhACs (64%-70%) with the only exception of gemfibrozil (39%), and to a lesser extent xenoestrogens, removals reached between 40% and 70%. Irradiance is key in these microalgae-based systems, as it can lead to both direct photodegradation and to an increased growth of microalgae and, in consequence, a higher bioadsorption and biodegradation.

Further research is required to optimize the different operational parameters of this dual system to eventually improve the removal of the studied compounds and also a broader range of contaminants. Different types of water and/or microalgae species should also be tested.

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Table 1. Physical-chemical characterization of the different water samples evaluated: Raw sewage, UASB effluent ($UASB_{eff}$) and final effluent of the treatment system ($HRAP_{eff}$).

Parameters For n = 10	Sample point					
	Raw Sewage		$UASB_{eff}$		$HRAP_{eff}$	
	Mean ± SD	Min / Max	Mean ± SD	Min / Max	Mean ± SD	Min / Max
TSS ($mg\ L^{-1}$)	366.58 ±134.62	15.12 / 443.46	64.38 ± 41.36	1.03 / 283.94	149.27 ± 92.82	18.32 / 503.94
VSS ($mg\ L^{-1}$)	254.31 ±213.32	92.31 / 389.32	40.38 ± 41.19	0.31 / 219.92	106.32 ± 77.17	16.92 / 410.14
COD ($mgO_2\ L^{-1}$)	518.64 ±123.21	102.80 / 598.43	232.69 ±109.21	111.14 / 482.24	146.08 ±65.40	98.15 / 321.43
pH	7.4 ± 0.2	7.0 / 8.0	7.1 ± 0.4	6.8 / 8.3	8.4 ± 0.7	7.1 / 10.2
DO ($mg\ L^{-1}$)	1.04 ± 0.55	0.05 / 2.33	0.34 ± 0.12	0.13 / 1.36	9.04 ± 2.95	3.45 / 14.45
Temperature (ºC)	24.5 ± 2.5	20.6 / 29.4	23.5 ± 2.6	19.8 / 29.1	21.8 ± 3.3	16.3 / 30.1
TN ($mg\ L^{-1}$)	34.43 ± 9.31	26.21 / 53.12	54.33 ± 5.32	42.21 / 63.95	24.31 ± 11.02	14.13 / 48.04
$N\text{-}NH_4^+$ ($mg\ L^{-1}$)	25.21 ± 8.13/	7.10 / 32.25	34.21 ± 13.43	14.92 / 54.75	14.31 ± 9.13	10.32 / 55.54
VS* ($g\ L^{-1}$)	-	-	-	-	1.01 ± 2.55	0.20 /3.25
TKN* ($mg\ L^{-1}$)*	-	-	-	-	101.37 ± 65.16	29.71 / 235.77
Carbohydrates* ($mg\ L^{-1}$)	-	-	-	-	102.98 ± 86.43	13.89 / 318.82
Proteins* ($mg\ L^{-1}$)	-	-	-	-	633.56 ± 407.25	185.68 / 1,473.56

TS – Total Solids; VS – Volatile Solids; COD – Chemical oxygen demand ; DO – Dissolved Oxygen; TN – Total Nitrogen; $N\text{-}NH_4^+$ - Ammonium - * measured only in HRAP biomass

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Highlights

- The fate of selected micropollutants during UASB + HRAP treatment has been evaluated
- UASB does not efficiently remove most of the target compounds.
- The UASB+HRAP system is highly efficient removing most of the target pollutants.
- This treatment has been tested for the first time, and successfully, for hormones.

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Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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