

MECHANISMS INVOLVED IN THE REMOVAL OF EMERGING CONTAMINANTS BY A COMBINED UASB-MBR SYSTEM

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ABSTRACT

Generally, a conventional treatment is enough to remove suspended solids and organic matter in urban wastewater treatment plants, but unfortunately, many emerging contaminants (ECs) remain in water. The aim of this research is to study the removal of several ECs from wastewaters using a combined system (Upflow Anaerobic Sludge Blanket-UASB followed by a Membrane Bioreactor-MBR) at two organic loading rates, studying the mechanisms involved in their removal. The combined system consisted on a UASB (25 L) followed by a MBR (20 L) (hollow fiber; 0.4 μm ; 0.2 m^2). The system was fed with synthetic wastewater doped with ECs (pharmaceutical products, personal care products and industrial) at an inlet concentration of 10 $\mu\text{g}\cdot\text{L}^{-1}$ and operated at two organic loading rates, low 0.09–0.15 $\text{kg DQO}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ (L1) and high 0.60–0.80 $\text{kg DQO}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ (L2). Aqueous samples were extracted by Solid Phase Extraction (SPE) and were analysed by GC-MS. ECs were extracted from sludge by Accelerated Solvent Extraction (ASE). With the anaerobic-aerobic combination of processes that takes place in the UASB-MBR system, almost complete removal from water was reached for methylparaben, ethylparaben, propylparaben, estrone, butylparaben, 4-octylphenol, ibuprofen and triclosan at both organic loading rates. Carbamazepine and diclofenac were the most recalcitrant compounds, whose removal was enhanced at L2. Almost complete removal was reached for 17-a-ethinylestradiol, 17-b-estradiol, 4-t-octylphenol and bisphenol A at L2, decreasing slightly the efficiencies at L1. The first stage (UASB) of the system was analyzed Anaerobic biomass was especially efficient in the removal of parabens, 4-octylphenol, 17-b-estradiol and triclosan, reaching biotransformation percentages of 80–99.7%. Biotransformation percentages were: 53–95% for bisphenol A, ibuprofen, 4-t-octylphenol, 17-a-ethinylestradiol and estrone; 38–49% for carbamazepine and 15–57% for diclofenac. Hormones, alkylphenols, bisphenol A and triclosan were found in the anaerobic sludge (fraction: 0.3–2.4%): in addition, the second stage (MBR) was analyzed Aerobic biomass also contributed to ECs removal, reaching biotransformation percentages of 18–41% for carbamazepine and 48–71% for diclofenac. Estrone, 17-a-ethinylestradiol and alkylphenols were detected in the aerobic sludge.

Keywords: UASB, emerging contaminants, membrane bioreactor, biotransformation, sorption, reuse.

1 INTRODUCTION

Generally, a conventional treatment is enough to remove suspended solids, pathogens and organic matter in urban wastewater treatment plants (WWTP), but unfortunately, many emerging contaminants (ECs) remain in water because these plants are not designed for this purpose.

ECs reach the aquatic environment and can cause adverse effects on human's health as well as on the environment through their bioaccumulation in living beings and/or sediments. *Directive 2000/60/EU* [1] provided 33 substances (4-t-octylphenol among others) and groups of priority substances list in the water field as well as prevention and pollution control strategies. *Directive 2013/39/EU* [2] updated the list to 45 substances and included an observation list for compounds whose effects are not yet known. The first list included



hormones 17- α -ethinylestradiol and 17- β -estradiol, diclofenac and macrolide antibiotics among others [3].

Several of these substances have been recently detected in wastewaters from the region of Alicante and Murcia. Cartagena et al. [4] reported concentrations of ibuprofen, carbamazepine, diclofenac, triclosan, 4-octylphenol, 4-t-octylphenol and bisphenol (among others) in the influent of the secondary treatment of WWTP Rincón de León (Alicante, Spain) from $0.06 \mu\text{g}\cdot\text{L}^{-1}$ to $59.50 \mu\text{g}\cdot\text{L}^{-1}$. Fernández-López et al. [5] reported the presence of the pharmaceutical compounds carbamazepine and diclofenac in influent samples from several WWTP located in Murcia in the ranges $0.56\text{--}26.52 \mu\text{g}\cdot\text{L}^{-1}$ and $0.39\text{--}5.78 \mu\text{g}\cdot\text{L}^{-1}$, respectively. It dares saying that many of these substances can produce endocrine disruption, such as the plasticizer bisphenol A, alkylphenols 4-t-octylphenol and 4-octylphenol, natural hormones estrone and 17-b-estradiol and synthetic hormone 17- α -ethinylestradiol.

Parabens and triclosan are also detected in wastewaters. These compounds inhibit the growth of microorganisms including bacteria and fungi, thus they are commonly used as preservatives cosmetics and toiletries. Methyl and propylparaben belong to category 1 of the “*Endocrine Disrupter Priority List for wildlife and human health*” [6] due to their endocrine disruptor potential.

Membrane bioreactor (MBR) and Upflow Anaerobic Sludge Blanket (UASB) are technologies that can remove ECs from wastewaters. Their effectiveness depends on the nature of the micropollutants and the operating conditions (temperature, organic loading rate, hydraulic retention time, sludge retention time, upflow velocity).

The mechanisms involved in the removal of ECs in these systems are mainly biotransformation and sorption onto sludge, and to a lesser extent, volatilization and photolysis. Biotransformation involves a series of catabolic processes either transforming the original compounds into metabolites, or completely mineralizing the original parent compound to carbon dioxide and water. Depending on the value of the biodegradation constant (k_{bio}), ECs can be classified according to Joss et al. [7] into: very good biodegradability ($k_{\text{bio}} > 10 \text{ L}\cdot\text{g SS}^{-1}\cdot\text{d}^{-1}$), good biodegradability ($0.1 < k_{\text{bio}} < 10 \text{ L}\cdot\text{g SS}^{-1}\cdot\text{d}^{-1}$), and no substantial removal by biodegradation ($k_{\text{bio}} < 0.1 \text{ L}\cdot\text{g SS}^{-1}\cdot\text{d}^{-1}$).

Sorption can occur through absorption processes due to hydrophobic interactions of the aliphatic and aromatic groups of a contaminant with the lipid fraction of suspended solids and bacterial cells. In addition, sorption can occur through adsorption processes due to the electrostatic interaction between the negative surface of microorganisms and positively charged groups of a compound. The absorption mechanism is related to the compound hydrophobicity, related to the partition coefficient octanol-water (K_{ow}) and expressed as $\log K_{\text{ow}}$ for neutral compounds. Connell [8] classified contaminants with $\log K_{\text{ow}} \geq 2$ as lipophilic compounds and compounds with $\log K_{\text{ow}} \leq 2$ as hydrophilic compounds. This parameter allows to predict the potentiality of the substances to be incorporated to the biomass by their easiness to be adsorbed or not to organic matter among other phenomena. The adsorption mechanisms are related to the partition coefficient K_d . Compounds with $\log K_d > 2.7$ possess high adsorption potential whereas compounds with $\log K_d < 2.7$ possess low adsorption potential. Volatilization takes place for compounds with high Henry constants.

The aim of this research was to study the removal of several ECs (methylparaben, ethylparaben, propylparaben, butylparaben, triclosan, ibuprofen, carbamazepine, diclofenac, estrone, 17- α -ethinylestradiol, 17- β -estradiol, bisphenol A, 4-t-octylphenol and 4-octylphenol) from wastewaters using a combined system UASB-MBR at two organic loading rates, studying the mechanisms involved in their removal.



2 MATERIAL AND METHODS

2.1 Experimental set up

The combined system consisted on a UASB (25 L) followed by an aerobic MBR with an external submerged configuration: an aerobic tank (12 L) and a membrane tank (8 L). The UASB was inoculated with 8 L of granular anaerobic fluidized sludge from a beer company. The following parameters were kept constant: flowrate was $0.67 \text{ L}\cdot\text{h}^{-1}$; temperature was $28.3\text{--}31.3^\circ\text{C}$; hydraulic retention time (HRT) was 37 h; sludge retention time (SRT) was > 90 days. pH was $6.97\text{--}7.68$ in the anaerobic reactor. UASB was operated at two organic loading rates (OLR): low $0.09\text{--}0.15 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ (L1) and high $0.60\text{--}0.80 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ (L2).

The MBR was inoculated with sludge from a wastewater treatment plant. The filtration unit consisted on a hollow fiber membrane (Micronet Porous Fibers) with a filtration area of 0.2 m^2 and a porous size of $0.4 \mu\text{m}$. The volatile suspended solids (VSS) remained from 0.5 to $1 \text{ g}\cdot\text{L}^{-1}$. Flowrate was $0.67 \text{ L}\cdot\text{h}^{-1}$ (with recirculation: $1.1 \text{ L}\cdot\text{h}^{-1}$); temperature was $20.5\text{--}32.3^\circ\text{C}$; SRT was 90 days; flux (J) was $5.35 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$. pH was $6.30\text{--}7.68$ in the aerobic reactor. To remove reversible membrane fouling, the MBR worked with filtration-backwash cycles (10 minutes/30 seconds). A constant air flow was pumped into the MBR in order to diminish membrane fouling, maintain the suspended biomass and provide oxygen to microorganisms.

The UASB-MBR system (Fig. 1) was automatically controlled by means of a Programmable Logic Controller. It was fed with synthetic wastewater doped with a mixture of micropollutants at an inlet concentration of $10 \mu\text{g}\cdot\text{L}^{-1}$. Personal care products (methylparaben, ethylparaben, propylparaben, butylparaben and triclosan), pharmaceutical products (carbamazepine, diclofenac and ibuprofen), hormones (estrone, 17- α -ethinylestradiol and 17- β -estradiol), plasticizer (bisphenol A) and surfactants (4- t -octylphenol and 4-octylphenol) were selected based on their highly frequent usage and ubiquitous presence in wastewaters.

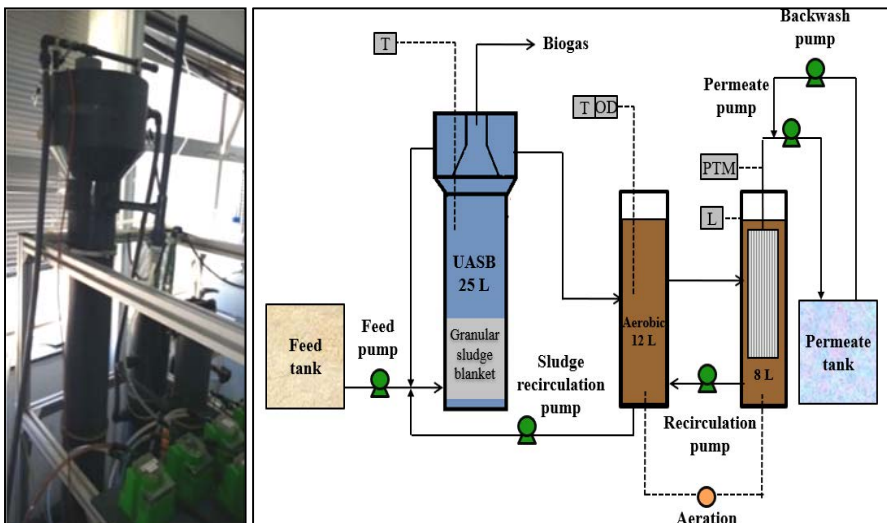


Figure 1: UASB-MBR system. (a) Picture; (b) Schematic diagram. (Source: own elaboration.)

2.2 Analytical items and methods

The analytical standards of each compound were purchased from Sigma Aldrich. Stock solutions were prepared in ethyl acetate or methanol depending on the compounds and stored at -20°C . Standard solutions at different concentrations were prepared. Ethyl acetate HPLC grade and methanol HPLC grade were purchased from Sigma Aldrich. Milli-Q water was generated from a Milli-Q Synthesis A10 System from Millipore. Nitrogen for drying (purity 99,995%) was purchased from Air Liquide (Madrid, Spain). The derivatization reagents BSTFA:TCM (99:1) and pyridine were purchased from Sigma Aldrich.

Liquid samples, feed (200 mL), UASB effluent (500 mL) and MBR permeate (500 mL), were collected in a 0.5 L sterilized plastic bottles (Tecnoquim S.L.). Samples were vacuum filtered through a $1.2\ \mu\text{m}$ glass fiber filter (Millipore) and stored at -20°C until their analysis. Mixed liquor samples from UASB and MBR were also vacuum filtered. Sludge samples were wrapped in aluminium paper and stored at -20°C . Sludge samples were lyophilised in a FreeZone 2.5 Liter Benchtop Freeze Dryer (Labconco) at -40°C and with 0.044 bar vacuum. Then, lyophilized sludge samples (0.5–1 g) were extracted by accelerated solvent extraction (ASE) in an ASE-100 equipment (Dionex) equipped with 11 mL stainless extraction cell following the method described by Radjenovic et al. [9].

Prior to extraction, liquid samples were acidified with H_2SO_4 (96%, w/w). The analysis of micropollutants from liquid samples and the reconstituted extracts obtained from solid samples consisted on solid phase extraction (SPE) and gas chromatography followed by quantitative determination of mass spectrometry with electron ionization (GC-MS). SPE was executed in a Dionex Auto Trace 280 (Thermo Scientific) equipment using Oasis HLB 6 cc/200 mg cartridges purchased from Sigma Aldrich. The analytical method used for SPE was adapted from Gómez et al. [10]. Firstly, the cartridges were preconditioned with 5 mL of ethyl acetate ($4\ \text{mL}\cdot\text{min}^{-1}$), 5 mL of methanol ($4\ \text{mL}\cdot\text{min}^{-1}$), and 5 mL of Milli-Q water ($4\ \text{mL}\cdot\text{min}^{-1}$). The samples were enriched through the SPE cartridges ($10\ \text{mL}\cdot\text{min}^{-1}$). Then, the cartridges were rinsed with 6 mL of Milli-Q water ($20\ \text{mL}\cdot\text{min}^{-1}$) and dried by nitrogen current for 30 min. The compounds were eluted with 4 mL of ethyl acetate ($4\ \text{mL}\cdot\text{min}^{-1}$) and 4 mL ethyl acetate: methanol 1:1 ($4\ \text{mL}\cdot\text{min}^{-1}$). The eluates were evaporated to dryness under a nitrogen stream. Then, the extracted residues were dissolved with 100 μL of carbamazepine-d10 solution ($500\ \mu\text{g}\cdot\text{L}^{-1}$ in methanol) and they were transferred into 1.5 mL vials, and further evaporated to dryness with nitrogen stream. The dry residues in the vials were derivatized by addition of 50 μL of BSTFA: TMCS (99:1) and 50 μL of pyridine in a thermoblock at 60°C for 30 min (adapted from Hai et al. [11]). The derivatives were cooled to room temperature and analysed by GC-MS.

GC-MS quantification was conducted using an Agilent 7890 gas chromatograph connected to a mass spectrometer Agilent 5975C. An Agilent HP-5MS (5% Phenyl 95% dimethylpolysiloxane) capillary column (30 m x 0.25 mm ID, $\text{df}=0.25\ \mu\text{m}$) was used. The flow rate of the carrier gas (helium) was maintained constant at $1.3\ \text{mL}\cdot\text{min}^{-1}$. The GC column temperature was programmed from 105°C (initial equilibrium time 1 min) to 200°C via a ramp of $17^{\circ}\text{C}\cdot\text{min}^{-1}$ and maintained 1 min, 200 – 220°C via a ramp of $2^{\circ}\text{C}\cdot\text{min}^{-1}$, 220 – 290°C via a ramp of $5^{\circ}\text{C}\cdot\text{min}^{-1}$ and maintained 1 min. The injector port and the interface temperature were maintained at 250°C and 280°C , respectively. Sample injection (1 μL) was in spitless mode.

EI mass spectra were obtained at 70 eV electron energy and monitored from m/z 40 to 500. The ion source and quadrupole analyser temperatures were fixed at 250°C and 280°C ,



respectively. Quantitative analysis was carried out using selected ion monitoring mode (SIM).

3 RESULTS AND CONCLUSIONS

To characterize the removal of ECs in a combined system UASB-MBR, the contribution of each technology and the mechanisms involved in their removal were studied.

3.1 Removal of micropollutants in UASB-MBR system

The removal percentages of the selected contaminants in the aqueous fraction of each process (UASB, MBR and UASB-MBR) were calculated using eqn (1):

$$\%_{removal,i} = \left(1 - \frac{C_{i,effluent}}{C_{i,influent}}\right) \times 100, \tag{1}$$

where $C_{i,influent}$ and $C_{i,effluent}$ are the concentrations in the influent and effluent of UASB, MBR or UASB-MBR ($\mu\text{g}\cdot\text{L}^{-1}$).

The removal percentages at both OLR in UASB and in UASB-MBR are shown in Fig. 2.

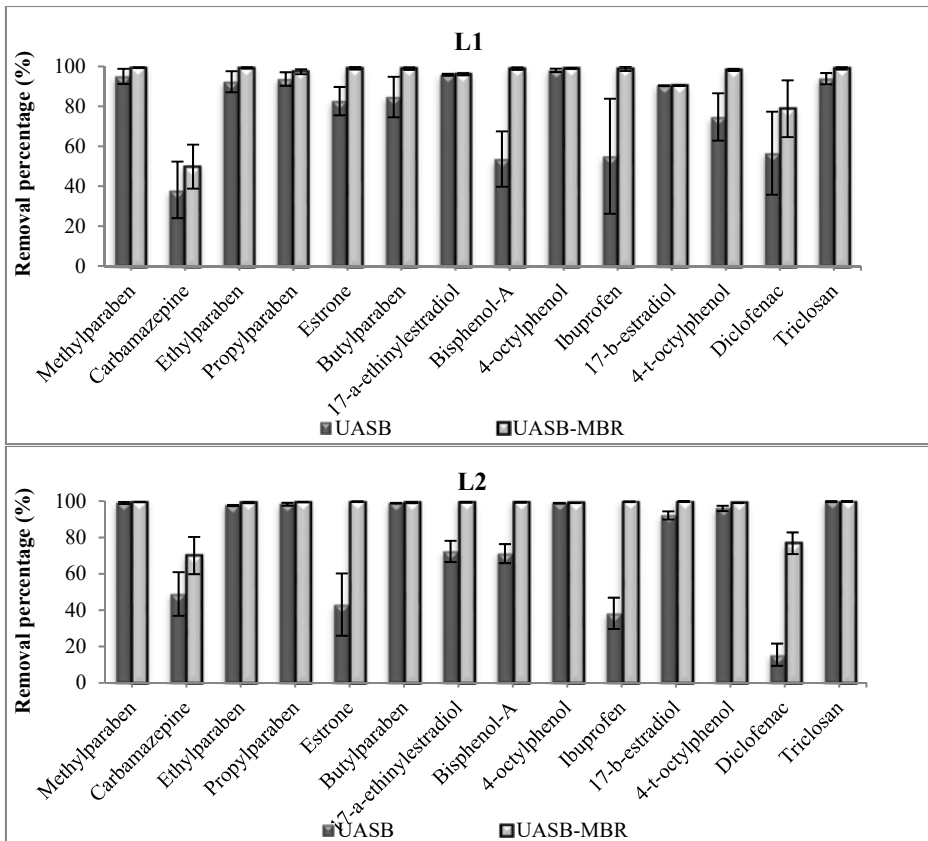


Figure 2: Removal percentages from the aqueous fraction at both OLR in UASB and in UASB-MBR.

Operating at both OLR, it was observed that the effectiveness in the removal of the studied ECs depended on the nature of the compounds. In UASB process, the removal percentages of parabens (methyl, ethyl, propyl and butylparaben), alkylphenols (4-t-octylphenol and 4-octylphenol), hormones (estrone, 17-a-ethinylestradiol and 17-b-estradiol) and triclosan were comprised from 74 to 99.7%. Bisphenol A was partially removed, with percentages from 53 to 71%. Carbamazepine, diclofenac and ibuprofen were the most recalcitrant compounds with removal rates from 15 to 56%.

At both OLR, methylparaben, ethylparaben, butylparaben, estrone, 4-octylphenol, ibuprofen and triclosan were efficiently removed from aqueous fraction by the combined treatment UASB-MBR, reaching removal percentages superior to 99%. Similar removal efficiencies were reached for bisphenol (98.2–99.3%). The removal of propylparaben was slightly enhanced with the increase on the OLR (removal percentages 97.3% at L1 and 99.5% at L2). Hormones 17-a-ethinylestradiol and 17-b-estradiol were partially removed at L1 (96% and 90%, respectively); removal percentages up to 99% were reached operating at L2. Carbamazepine was poorly removed at L1 (49.8%); the increase on OLR enhanced its removal till 70%. The removal efficiencies of diclofenac were 76.8–78.8%.

In summary, the removal efficiencies of the studied emerging contaminants in the combined system UASB-MBR increased with increase in OLR. UASB was the main contributor to the removal of ECs in the combined system. The subsequent treatment of the effluent in the MBR process increased the removal efficiencies.

3.2 Mass balance

The contributions for adsorption and biodegradation of the micropollutants at each process were obtained through a mass balance including measurements in the liquid and solid phase.

3.2.1 Mechanisms involved in the removal of ECs in the anaerobic process

The UASB was fed with a flowrate of $0.67 \text{ L} \cdot \text{h}^{-1}$ and a concentration of the studied pollutants of $10 \mu\text{g} \cdot \text{L}^{-1}$. Mass balances were applied to each compound in the anaerobic process and results have been graphically represented in Fig. 3, including the contribution of both, biological transformation and sorption. The residual fraction of each compound that leaves the system with the effluent has been also included in the plot.

In the UASB, the mass balance showed that biotransformation was the main mechanism involved in the removal of the studied ECs. In minor proportion, sorption onto sludge contributed to the removal of some ECs.

Regarding biotransformation, methylparaben, ethylparaben, propylparaben and butylparaben were highly biodegraded (coinciding with other authors as Li et al. [12] and Wijekoon et al. [13]) with removal percentages from 84 to 95% at L1 and 97-99% at L2. The antimicrobial activity of parabens increases according to the length of the chain [14], fact that explains the lesser removal percentage obtained for butylparaben (84%) by biotransformation operating at low OLR (L1) compared to methylparaben (95%). At high OLR, the UASB was more efficient and their removal were not significantly different.

According to Verlicchi et al. [15], hormones 17-b-estradiol and estrone ($k_{\text{bio}} > 10 \text{ L} \cdot \text{g} \text{ SS}^{-1} \cdot \text{d}^{-1}$) are compounds very biodegradable. In this paper 17-a-ethinylestradiol showed good biodegradability. It dares saying that estradiol is transformed into estrone during its degradation. The removal percentages by biotransformation reached for these compounds in the UASB were 82 to 95% at L1 and 42 to 92% at L2.

The anaerobic biomass was also so efficient in the biotransformation of 4-t-octylphenol and 4-octylphenol, with removal percentages of 73% and 96% at L1 and 95% and 98% at



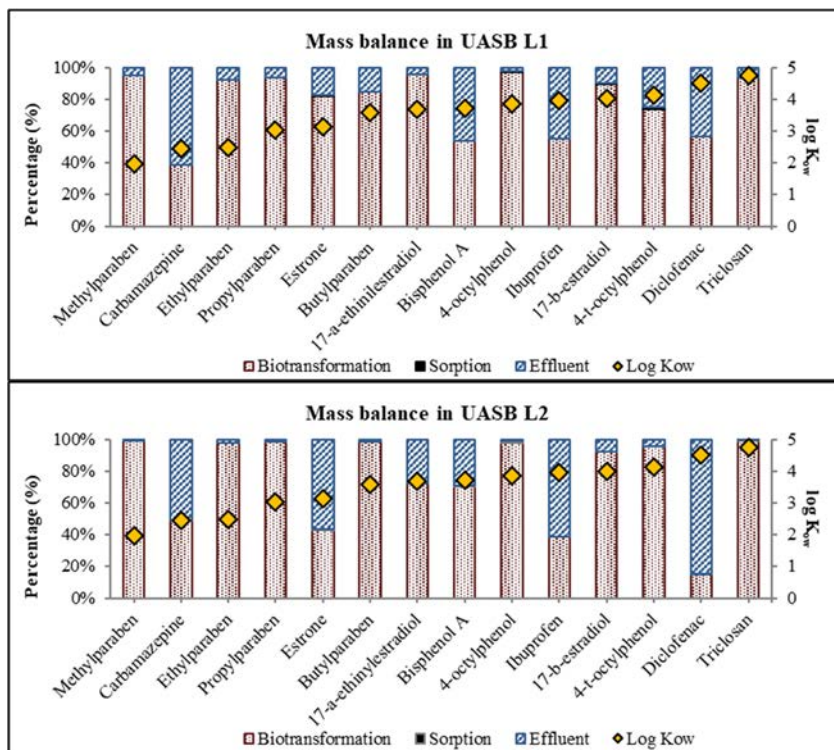


Figure 3: Micropollutants mass balance in UASB, contribution of biotransformation, sorption and release within the effluent at each OLR.

L2, respectively. Triclosan was highly biodegraded (removal percentages of 93% at L1 and 99% at L2, respectively).

Partial removal by biotransformation was observed for the plasticizer bisphenol A (with percentages of 53% at L1 and 70% at L2), and the pharmaceutical product ibuprofen (55% at L1 and 38% at L2). Alvarino et al. [16] also reported the low effectiveness of the anaerobic biomass to remove ibuprofen and Musson et al. [17] explained that this fact is probably due to the presence of substitutions in the para positions of its aromatic ring.

Carbamazepine and diclofenac showed to be very persistent during treatment in the UASB. The removal percentages by biotransformation were 38% and 49% for carbamazepine and 57% and 15% for diclofenac at L1 and L2, respectively. These compounds possess low biodegradation constants ($k_{bio} < 0.1 \text{ L} \cdot \text{g SS}^{-1} \cdot \text{d}^{-1}$ [15]). The presence of the heterocyclic group pyridine in the structure of carbamazepine hinders its degradation under anaerobic conditions [18].

Regarding sorption, hormones 17- β -estradiol, estrone and 17- α -ethinylestradiol were detected in the anaerobic sludge. The removal percentages by sorption varied from 0.4% (estrone and 17- α -ethinylestradiol) to 0.6% (17- β -estradiol) at L1, increasing with the increase of hydrophobicity ($\log K_{ow} = 3.13, 3.67$ and 4.01 , respectively) and adsorption potential ($\log K_d$ [15]: 2.4-2.8, 2.4-2.9 and 0.9-2.1, respectively). At L2, lesser concentrations of hormones were detected in the sludge, varying the removal percentages by sorption up to 0.2%.

Bisphenol A was also detected in the sludge, with removal percentages by sorption of 0.23% at L1 and 0.66% at L2. This compound is lipophilic ($\log K_{ow} = 3.72$).

The compounds with greater presence in the anaerobic sludge were 4-octylphenol, 4-t-octylphenol and triclosan (lipophilic compounds with low K_{ow} : 3.84, 4.12 and 4.76, respectively). Operating at L1, the removal percentages by sorption were 1.33%, 1.0% and 0.35%, respectively. At L2, lesser concentrations of these compounds were detected in the sludge, varying the removal percentages by sorption up to 0.79%, 0.53% and 0.22%, respectively.

3.2.2 Mechanisms involved in the removal of ECs in the aerobic MBR process

The effluent of the UASB system was fed to the MBR. Mass balances were applied to each compound and results have been graphically represented in Fig. 4, including the contribution of both, biological transformation and sorption. The residual fraction of each compound that leaves the system with the effluent has been also included in the plot. The studied compounds possess low Henry constant thus volatilization was considered negligible.

In the MBR, the mass balance showed that biotransformation was the main mechanisms responsible of the removal of the remaining compounds from the UASB effluent. Carbamazepine was resistant to the biological treatment with removal percentages by biotransformation of 18% at L1 and 41% at L2. The removal percentages of diclofenac by biotransformation were 48% at L1 and 71% at L2. Ibuprofen was highly removed by the aerobic biomass.

The removal of ECs by sorption was mostly negligible. Few compounds were detected in the aerobic sludge: estrone, 4-octylphenol, 4-t-octylphenol, bisphenol A and triclosan (compounds with high hydrophobicity).

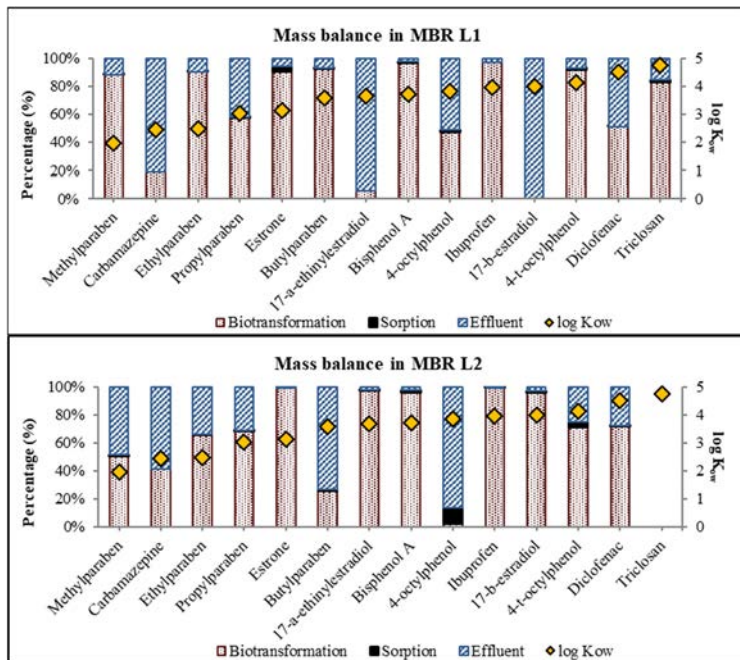


Figure 4: Micropollutants mass balance in MBR, contribution of biotransformation, sorption and release within the effluent at each OLR.

4 CONCLUSIONS

The effectiveness of the combined system UASB-MBR depended on the operating conditions and on the physico-chemical properties of the target compounds:

- Parabens (methyl, ethyl, propyl and butylparaben), hormones (estrone, 17- β -estradiol and 17- α -ethinylestradiol), alkylphenols (4-octylphenol and 4-t-octylphenol), the anti-inflammatory ibuprofen, the preservative triclosan and the plasticizer bisphenol A were efficiently removed from aqueous fraction by the combined treatment UASB-MBR, reaching removal percentages up to 99%, whereas carbamazepine and diclofenac were the most recalcitrant.

The removal of the studied ECs in the UASB-MBR system was enhanced with the increase in OLR.

Generally, the UASB was the main contributor to the removal of ECs in the combined system, except in the treatment with high organic loading rate where estrone, ibuprofen and diclofenac were little biodegraded by the anaerobic process. The subsequent treatment of the effluent in the MBR process increased the removal efficiencies.

The mass balance at each unit showed that biotransformation was the main mechanism involved in the removal of the studied ECs. Sorption onto sludge was mostly negligible; hydrophobic compounds such as hormones and alkylphenols were detected in the sludge.

The UASB-MBR system is a feasible alternative to remove emerging contaminants from wastewaters in combination with other technologies that allows to remove compounds resistant to the biological treatment.

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