Impact of UASB reactors operation mode on the removal of estrone and 17α-ethinylestradiol from wastewaters

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Abstract

This work aims to compare the performance of the continuous operation (CO) and intermittent operation (IO) of Upflow Anaerobic Sludge Blanket (UASB) reactors for the removal of estrone (E1) and 17α-ethinylestradiol (EE2) from wastewaters. Results suggest that the IO contribute to the improvement of the overall removal of estrogens (above 95% for E1 and EE2) when compared to CO (49% for E1 and 39% for EE2). For both CO and IO, biodegradation was the main removal mechanism for E1, while for EE2, adsorption to sludge was the major removal pathway. Moreover, a higher biodegradation of estrogens was obtained with the IO compared to CO (69.4% *vs.* 43.3% for E1 and 21.8% *vs.* 8.0% for EE2). The favourable effect of IO can be justified by effluent recirculation during the feedless period which promotes the adaptation of microbial biomass to estrogens' biodegradation.

Keywords: Estrogens; removal mechanisms; biodegradation; adsorption; intermittent UASB reactor.

1. Introduction

The emergent consumption of so-called endocrine-disrupting chemicals (EDCs), both in human and veterinary medicine, has triggered a remarkable increase in the occurrence of these pollutants in the environment. The main concern is the high persistence of these drugs, and the main mechanism for the removal of these micropollutants from water is the adsorption onto sludge (Johnson and Sumpter, 2001). Thus, wastewater treatment plants (WWTPs) are not effective barriers to their actual removal, reaching the environment mainly due to sludge application in agriculture (Pessoa et al., 2014). The occurrence of EDCs in the environment can lead to several harmful effects on human health and aquatic living organisms (Sanfilippo et al., 2010). The estrogens, estrone (E1), 17β-estradiol (E2) and synthetic 17α-ethynylestradiol (EE2), are the EDCs recognised as the major contributors to estrogenic activities (Pessoa et al., 2014). Among estrogens, E1 and EE2 are the ones that occur most frequently and at higher concentrations (up to 242 and 124 ng L^{-1}, respectively) in WWTPs treated effluents and sludge samples (ranging from 8 to 887 ng g^{-1} and from 1.2 to 139 ng g^{-1}, respectively) (Martín et al., 2015; Pessoa et al., 2014).

Biological processes are usually the most cost-effective on the removal of organic contaminants from wastewaters. The removal of estrogens in aerobic systems is well documented (Bernardelli et al., 2015). Previous batch experiments conducted under aerobic conditions have shown that approximately 94% of spiked E1 was removed from the liquid phase (15% by adsorption and 79% by biodegradation), after 4 h of contact and using the initial estrogen concentration of 1 mg L^{-1} (Hashimoto and Murakami, 2009). In the case of EE2, the authors observed that this compound was more resistant to removal and after 4 h of contact with sludge approximately 75% of spiked concentration was removed from the liquid phase (~25% by adsorption and ~50% by biodegradation) (Hashimoto and Murakami, 2009).

Despite high estrogen removals reported in the literature by aerobic processes, by adsorption and/or biodegradation, these processes often generate persistent metabolites or produce conjugated compounds which may also suffer deconjugation in the environment, recovering their initial toxicity (Khunjar et al., 2011). Some studies described that the substrate present in the raw influent competitively inhibits the degradation of estrogens (Joss et al., 2004). Nevertheless, estrogens are present in WWTPs at low concentrations, usually in the range of ng L^{-1} to µg L^{-1} (Fleming et al., 2016; Pessoa et al., 2014). Consequently, the insufficient amount of these compounds cannot be used as a carbon source to support the growth of the microorganisms, being mostly removed by co-metabolism instead of primary metabolism (Li et al., 2020).

In the last decades, important developments were achieved in anaerobic treatment systems owing to the advantages that these processes present comparatively to conventional aerobic systems. Although intensive research efforts have been undertaken to better understand the estrogens' removal from wastewaters under anaerobic conditions, there is contradictory evidence in the literature. Some authors have observed estrogens biodegradation efficiencies above 95% (Zeng et al., 2009), while others have not detected biological degradation, even after long incubation periods (Alvarino et al., 2016; Arias et al., 2018; Vassalle et al., 2020). Moreover, some researchers showed that in anaerobic systems some substrates are easily metabolized whilst others, such as estrogens, are not immediately metabolized and may be adsorbed onto the microbial aggregates (Xu et al., 2014; Zeng et al., 2009).

Upflow anaerobic sludge blanket (UASB) reactors are considered as very competitive anaerobic systems due to the production of high-quality effluents, lower energy requirements, minimization of sludge production, and production of higher methane content in biogas (Alvarino et al., 2016, 2014). The intermittent operation (IO) of UASB reactors has been recommended to enhance the performance of the treatment process for the biodegradation of

complex substrates (Nadais et al., 2005). The advantage effect of IO is attributable to the improved ability to develop microbial consortia well adapted to the biodegradation of a wide variety of substrates (Couras et al., 2014). IO consists of a periodical interruption of the reactor feed, in an alternating sequence of feed and feedless periods. During feeding periods simple substrates are readily degraded whilst complex substrates are adsorbed onto the biomass aggregates. During feedless periods, the microbial population is deprived of simple substrates, forcing the metabolism of complex substrates that are retained in the sludge.

Some studies are available on the treatment of wastewaters contaminated with estrogens using UASB reactors. However, a combination of a UASB reactor as pre-treatment followed by aerobic treatment was the most widespread combination applied (Alvarino et al., 2019, 2016; Moya-Llamas et al., 2018; Vassalle et al., 2020) and a detailed study restricted to the UASB reactor has been poorly documented. Furthermore, E1 and EE2 are hydrophobic substances easily adsorbed to the sludge (Ying et al., 2002) and most surveys indicated the removal of estrogens only in the liquid samples, neglecting the amount of compound adsorbed in the sludge during the treatment process (Moya-Llamas et al., 2018; Vassalle et al., 2020), and, consequently, a complete mass balance is seldom accomplished.

To the best of the authors' knowledge, there are no research studies dealing with the IO of UASB reactors for the treatment of wastewaters contaminated with estrogens. The aim of the present study was to compare the performance of the IO and continuous operation (CO) of UASB reactors on the removal of the estrogens E1 and EE2 from wastewaters, operated under the same initial conditions (initial sludge, feed organic loads and temperature). The overall removal efficiency of estrogens was evaluated for both systems considering the main removal mechanisms, biodegradation and adsorption, and the mass balances of estrogens in the liquid (LP) and solid phases (SP) were determined.

2. Materials and Methods

2.1. Chemicals and solutions preparation

E1 (99%) and EE2 (98%) were provided by Sigma-Aldrich. HPLC grade methanol, acetone and acetonitrile, were from Fischer Chemical, Carlo Erba and VWR (Prolabo), respectively. Ultrapure water was obtained from a Milli-Q Millipore system (Milli-Q plus 185). Individual standard stock solutions of estrogens were prepared by dissolving the compounds in acetonitrile at a concentration of 1,000 mg L^{-1}, sonicated for 60 min and stored at 4 °C in the dark until their use.

2.2. Seed sludge and synthetic wastewater

Samples of anaerobically digested sludge used in this work contained a mixture of primary and waste activated sludge, collected from a WWTP treating 39,278 m^3 wastewater per day and located in Aveiro, Portugal. The sludge collected was washed 3 times with tap water (Ren et al., 2007), prior to use. Between each wash, sludge was allowed to settle for one day and the supernatant was removed.

A synthetic wastewater stock solution was prepared according to Hashimoto and Murakami (2009) with minor modifications, containing peptone (6 g L^{-1}), meat extract (4 g L^{-1}), urea (1 g L^{-1}), NaCl (0.3 g L^{-1}), KH_2PO_4 (1 g L^{-1}), KCl (0.14 g L^{-1}), $CaCl_2$ (0.14 g L^{-1}), and $MgSO_4 \cdot 7H_2O$ (0.1 g L^{-1}). Immediately after preparation, the solution was stored up to one week in the dark at 4 °C. The average total chemical oxygen demand (COD) of the synthetic wastewater stock solution was 226 ± 20 mg L^{-1}.

2.3. Experimental set-up and operation

Four lab-scale UASB reactors were operated during 21 days with an upflow velocity (ν_{up}) of 0.03 m h^{-1}. Two UASB reactors were used for the removal experiments with E1 (R1 and R2) and

the other two with EE2 (R3 and R4). For each EDC studied, one UASB reactor was operated in the IO and the other in the CO (Table 1 and supplementary material Table S1). UASB reactors were operated with a hydraulic retention time (HRT) of 30 h (considering the feeding period for the IO) (Table 1). A schematic diagram of the UASB reactor used in this work is shown in Figure 1. The UASB reactors with a working volume of 6 L were inoculated with approximately 4 L of flocculent sludge and topped with three-phase separators (Couras et al., 2014). The IO reactors were operated for 6 cycles (I to VI), each with 12 hours of feeding period, followed by 72 hours of feedless. The feedless period consisted of the recirculation of the treated effluent collected during the feeding period, while the CO reactors were fed continuously during all the assay (supplementary material, Table S1).

Table 1 Operating conditions of UASB reactors.

Reactor	Operation mode	Compound	Spiked concentration [a] ($\mu g\ L^{-1}$)	Average spiked mass per cycle [b] (μg)	Flow ($L\ h^{-1}$)	HRT [a,c] (h)	v_{up} [d] ($m\ h^{-1}$)	SRT [e] (d)	COD feed [a] ($g\ COD\ L^{-1}$)	OLR [a,f] ($g\ COD\ L^{-1}\ d^{-1}$)	pH feed	Temperature (°C)	Feed period per cycle (d)	Feedless period per cycle (d)	Total period of operation (d)	Number of cycles
R1	Continuous	E1	1 000	16800	0.2	30	0.03	92.5	1.4 ± 0.2	1.1 ± 0.2	7.4 ± 0.5	25.8 ± 0.6	3.5	0	21	-
R2	Intermittent	E1	7 000	16800	0.2	30	0.03	92.3	9.8 ± 1.6	7.8 ± 1.3	6.5 ± 0.1	25.6 ± 0.7	0.5	3	21	VI
R3	Continuous	EE2	500	8400	0.2	30	0.03	278.9	0.87 ± 0.08	0.70 ± 0.07	7.1 ± 0.8	22.2 ± 0.4	3.5	0	21	-
R4	Intermittent	EE2	3 500	8400	0.2	30	0.03	82.0	6.1 ± 0.6	4.9 ± 0.5	6.5 ± 0.1	19.7 ± 1.3	0.5	3	21	VI

[a] Operating conditions in the feed period.

[b] Average spiked mass = total fed mass during one cycle times the number of feeding days.

[c] HRT, a hydraulic retention time.

[d] v_{up}, upflow velocity.

[e] SRT, solids retention time.

[f] OLR, organic loading rate.

For the CO experiments, the estrogens were spiked to the influent at initial concentrations of 1 000 μg L^{-1} and 500 μg L^{-1}, for E1 and EE2, respectively. For the IO experiments, the estrogen concentration in the feed was seven times higher than the feed concentration applied for the CO reactors, so that in each cycle (total period of 84 h) the total mass of estrogen and the COD feed concentration admitted to the reactor were identical (Table 1). The feed consisted of diluted synthetic wastewater (2%, *v/v*) (Hashimoto and Murakami, 2009) in distilled water, spiked with the target estrogen (E1 or EE2).

To obtain the anaerobic conditions, the sludge placed inside of each reactor was purged with nitrogen for 15 min and capped. The desired temperature (between 20 °C to 25 °C) (Alvarino et al., 2019, 2016, 2014; Buntner et al., 2013; Vassalle et al., 2020) was maintained by the recirculation of water through the external jacket of the reactor connected to a thermostatic bath. In the top layer of each UASB reactor, a layer of fibreglass and aluminium foil was used to prevent heat loss and exposure to sunlight.

During the whole experiment, samples of biogas, influent and effluent were collected from the UASB reactors at the beginning and at the end of each cycle. For the IO reactors, the effluent was also collected at the end of the feeding period. To determine the amount of estrogens adsorbed onto sludge solid sample, the initial estrogens mass in the collected sludge was quantified. The estrogens' concentration adsorbed onto solid sludge phase at the end of the experiment was also determined. Due to the considerable volume of sludge needed for the E1 or EE2 analysis (about 600 mL) and in order to preserve the quantity of microorganisms inside of the UASB reactors, estrogens concentration adsorbed onto the sludge was not determined along the time, but only at the end of the experiment.

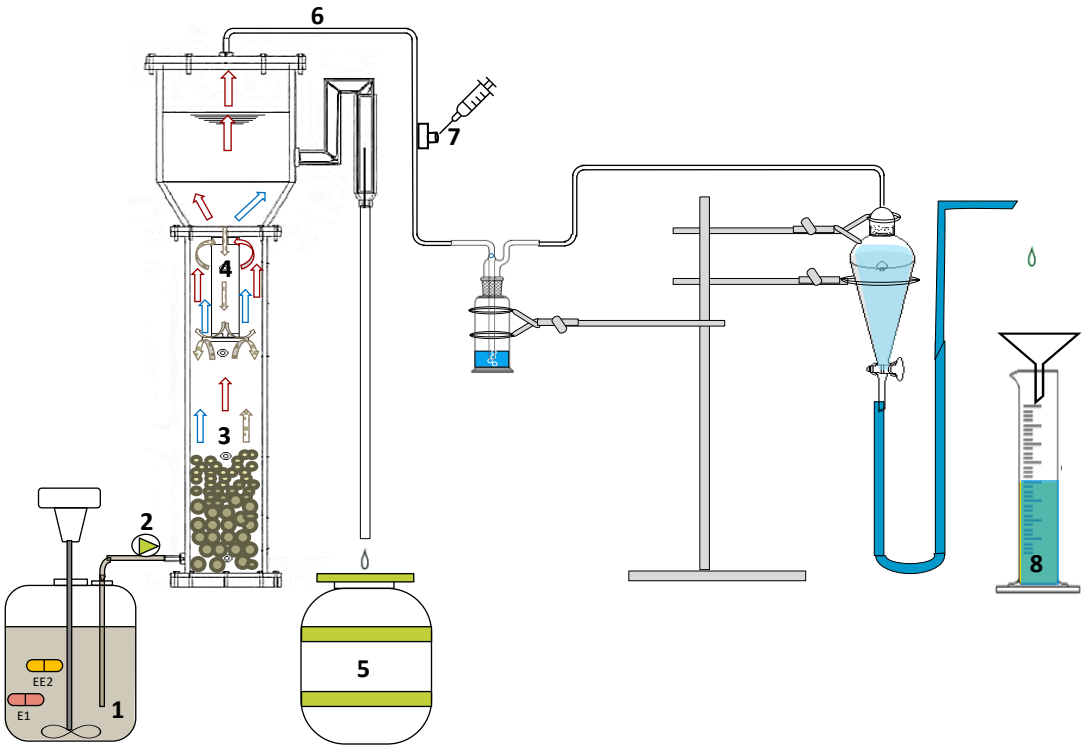

Figure 1 Schematic diagram of UASB reactors. Legend: (1) feed tank; (2) feed pump; (3) UASB reactor; (4) gas-liquid-solid separator; (5) treated effluent tank; (6) biogas outlet; (7) biogas sampling septum; (8) water displacement method for biogas measuring.

Biogas production was measured by the water displacement method and its composition was monitored using an SRI® 8610 C gas chromatograph equipped with a Haysep® Q (2.5 m × 2.1 mm) column and a Thermal Conductivity Detector (T = 100 °C). Injection temperature was 61 °C and Helium was used as carrier gas (Flow = 10 mL min^{-1}). The sludge samples concentration of total suspended solids (TSS), volatile suspended solids (VSS), and COD were determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The pH was measured with a Consort C861.

The percentage of COD removal (COD_R) was determined according to equation 1 (Couras et al., 2014):

$$COD_R\ (\%) = \frac{COD_{T,feed} - COD_{s,effluent}}{COD_{T,feed}} \times 100 \quad (1)$$

Where $COD_{T,feed}$ is the total COD of the feed (g COD L^{-1}) and $COD_{s,effluent}$ is the soluble COD of treated effluent (g COD L^{-1}).

The methanization percentage was calculated using equation 2 (Couras et al., 2014):

$$\text{Methanization}\ (\%) = \frac{\text{COD-CH}_4}{\text{COD}_R} \times 100 \quad (2)$$

Where $COD\text{-}CH_4$ is the mass of COD converted to methane (g).

2.4. Estrogens analysis

Quantification of E1 and EE2 in the LP of the influent and treated effluent, as well as in LP and SP of the sludge, was based on the methods described in Louros et al. (2019) with minor modifications. Briefly, immediately after collection the LP samples were filtered with a 0.2 µm PVDF filter, stored at 4 °C and analysed by high-performance liquid chromatography with a fluorescence detector (HPLC-FLD) within 24 h. For the sludge samples, the aliquots sampled were immediately separated by centrifugation (4000 rpm, 10 min), yielding the LP and SP. The supernatant LP sample was immediately filtered, stored at 4 °C and analysed by HPLC-FLD within 24 h. The sludge SP was freeze-dried, grounded, and then successively extracted with 18 and 9 mL of methanol, followed by 9 mL of acetone per g dry sample. In each extraction step, the samples were vigorously vortexed (Velp Scientifica) during 1 min and subjected to ultrasonic liquid extraction (ULE) using an Ultrasonic Cleaner USC-T ultrasonic bath (VWR) for 1 h. After ULE, the three solvent fractions were combined, filtered, and analysed by HPLC-FLD.

2.5. Estrogens mass balance and kinetics

A mass balance of E1 and EE2 was performed to assess the amount adsorbed and biodegraded for the CO and IO of UASB reactors and were quantified using equation 3 (Alvarino et al., 2014):

$$F_{biod} = F_{inf} - (F_{eff} + F_{sor}) \quad (3)$$

where F_{inf}, F_{efl}, F_{sor} and F_{biod} correspond to the mass flows (in µg) of the influent, effluent (analysed both in a dissolved fraction of LP), and adsorbed onto sludge, respectively. Thus, the amount of biodegraded E1 and EE2 (F_{biod}) can be determined.

Previous studies have reported that biodegradation constant (k_{biol}) could be determined considering the pseudo-first-order kinetics (Alvarino et al., 2014) reported in equation 4:

$$F_{biod} = k_{biol} \cdot X_{VSS} \cdot C_{eff} \cdot V \quad (4)$$

Where k_{biol} is the first-order-rate constant (L g_{vss}^{-1} d^{-1}), X_{VSS} is the volatile suspended solids concentration (g_{vss} L^{-1}) inside the reactor, C_{eff} is the final compound concentration of treated effluent in the LP (µg L^{-1}), and V is the reactor volume (L).

3. Results and Discussion

3.1. Reactors performance

The main characteristics determined for anaerobically digested sludge used as inoculum for UASB reactors are shown in the supplementary material (Table S2). In the experiments with E1, the average pH values of treated effluent were 5.6 ± 0.6 and 7.4 ± 0.6, for the CO and IO,

respectively. For the EE2 assays, the average pH values of treated effluent were 7.2 ± 0.4 and 6.7 ± 0.9, for the CO and IO, respectively. The VSS obtained in treated effluent were below 0.15 g SSV L^{-1} in the assays with E1, while in the experiments with EE2 VSS values were below 0.18 g SSV L^{-1}.

The biogas composition from experiments with E1 and EE2 was analysed. In E1 experiments, the average concentration of methane in the biogas was 81% and 72% for the CO and IO, respectively. In EE2 experiments, the methane content was 64% and 96% for the CO and IO, respectively. Under IO conditions, a higher average biogas flow rate was achieved compared to CO (0.16 L d^{-1} *vs.* 0.08 L d^{-1} for E1 and 0.16 L d^{-1} *vs.* 0.13 L d^{-1}). These results confirmed the high quality of the biogas produced, in agreement with the findings attained by other authors, with values ranging 65 % - 80 % for the treatment of wastewaters in UASB reactors (Alvarino et al., 2016, 2014; Arias et al., 2018; Buntner et al., 2013; Moya-Llamas et al., 2018). However, higher values of average biogas production (between 46 to 100 L d^{-1}) have been reported, which can be justified by the substrate used (dairy wastewater), the higher UASB reactor volume (>120 L), the lower HRT (between 12 h-13 h) and the higher time of operation (150-292 days) (Alvarino et al., 2016; Buntner et al., 2013). Instead, Moya-Llamas et al. (2018) obtained much lower results for this parameter (below 5 L d^{-1}) using synthetic wastewater as substrate and higher HRT (37 h).

For the IO, higher COD removal efficiencies were achieved (ranging 26.8%-78.5% for E1 and 40.4%-71.7% for EE2) that for the CO (ranging 18.3%-76.4% for E1 and 34.5%-61.3% for EE2) (Figure 2). Using CO, the methanization of COD ranged 2.0%-11.8% and 7.2%-22.9% in the experiments with E1 and EE2, respectively. When using IO, the methanization of COD ranged 2.5%-22.7% and 5.2%-37.8% in experiments with E1 and EE2, respectively. Alvarino et al. (2016) pointed out higher COD removal values (above 95%) under anaerobic conditions treating dairy wastewater. Other authors have reported COD removal values ranging 40% and 86% when treating synthetic wastewaters and raw sewage (Moya-Llamas et al., 2018; Vassalle et al., 2020).

Higher COD methanization values have also been pointed in the literature (45%-74%) when treating dairy wastewater (Arias et al., 2018; Buntner et al., 2013), most probably as the result of a highly adapted microbial population present in the sludge.

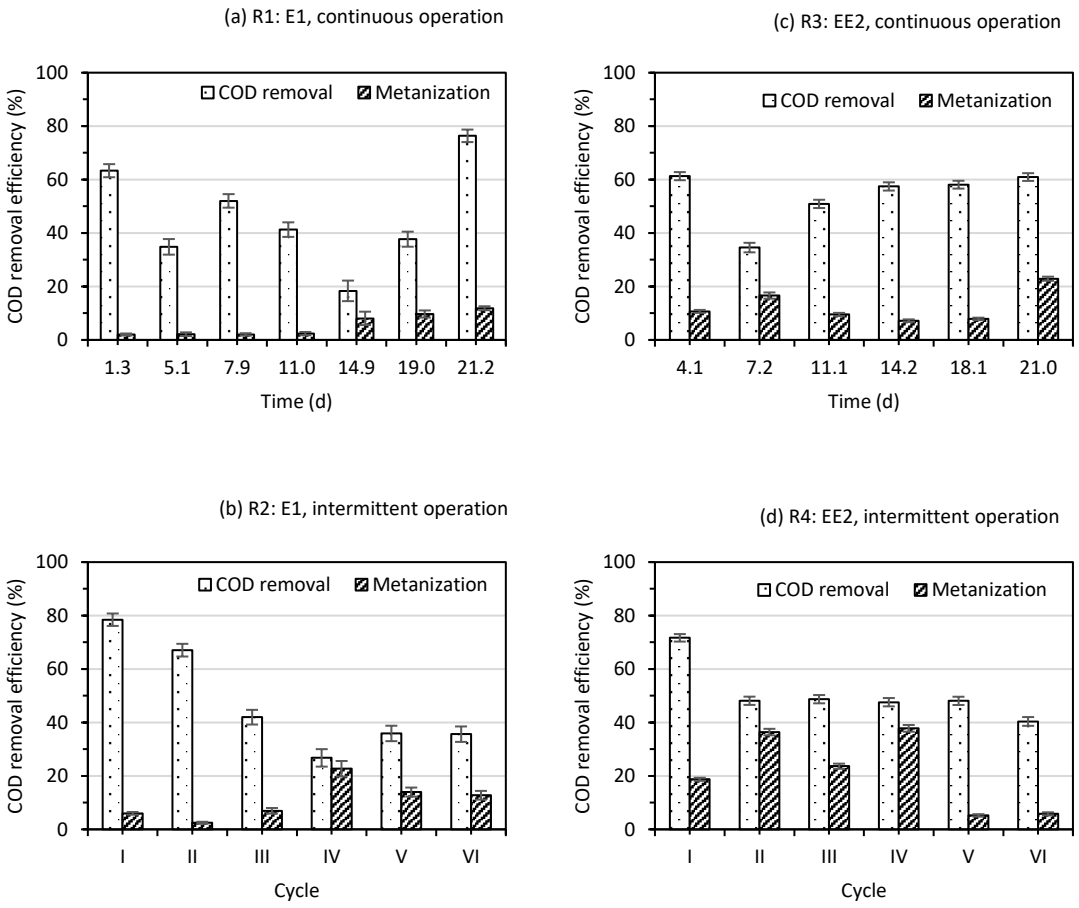

Figure 2 Results of COD removal and methanization in UASB reactor (a) R1: fed with synthetic wastewater spiked with E1 using CO; (b) R2: fed with synthetic wastewater spiked with E1 using IO; (c) R3: fed with synthetic wastewater spiked with EE2 using CO; (d) R4: fed with synthetic wastewater spiked with EE2 using IO. (Note that, for some of the experimental points, error bars are too small to be visible in the figure)

3.2. E1 and EE2 removal

For the IO, higher removal efficiencies of E1 and EE2 from the LP were obtained at the end (after feed and feedless periods) of all cycles analysed (above 90%) compared to CO (ranging 37% to 80% for E1 and 16% to 94% for EE2) at the same HRT of 30 h (Figure 3).

Under CO, an initial decrease of E1 removal from the LP of treated effluent was observed until 10 h, suggesting that E1 was removed mainly by an adsorption process, which decreases probably due to sludge saturation. After this period, an increase in the removal of E1 from the LP of the treated effluent was registered until approximately 20 h. This increase, maybe related to the increase in biodegradation, which will increase the number of binding sites available for adsorption. Following this time, the E1 removal remained constant, suggesting that an equilibrium between adsorption and biodegradation is reached. In the case of EE2, for CO, there was a significant decrease in the removal of this compound from LP of the treated effluent until approximately 10 h. This fact can be a result of sludge saturation, followed by a constant EE2 removal from LP, indicating an equilibrium between biodegradation and adsorption. The results obtained from the removal of E1 and EE2 in the liquid phase of treated effluent can be justified by the initially fast estrogen adsorption onto the surface of the sludge, which was faster and not followed by an immediate biodegradation, and consequently, a lower specific surface was available for the continuous estrogens adsorption.

In the IO, the clearly higher removal efficiencies of E1 and EE2 from LP obtained may have resulted from a better contact between the compounds and the biomass, which has provided a higher available surface for adsorption and an improved mass transfer from the LP to the SP, due to the recirculation of treated effluent. Moreover, the IO contributed to a quick and constant adsorption of estrogens in the SP of sludge throughout the six cycles, while for the CO their removal from the LP of treated effluent was significantly lower indicating a lower adsorption and biodegradation.

Similar estrogens removal efficiencies from LP have been pointed out by previous studies using the CO of UASB reactors. Vassalle et al. (2020) reported an E1 removal efficiency of 40% when treating raw sewage, using similar temperatures (23.5 °C) and solids retention time (SRT) of 20 days but a lower HRT (7 h). Arias et al. (2018) obtained EE2 removal rates in the range 20%-25% upon treating dairy wastewaters in a UASB reactor after more than 4 months of operation using similar temperatures (21 °C), lower HRT (20 h), and higher sludge concentration (30 g SST L^{-1}). Another survey conducted by Moya-Llamas et al. (2018) reported higher removal rates for E1 (ranging 40%-85%) and EE2 (ranging 70%-95%) after 172 days of operation of an UASB system for the treatment of wastewaters operated at higher temperatures (above 26 °C), SRT (90 days), and HRT (37 h).

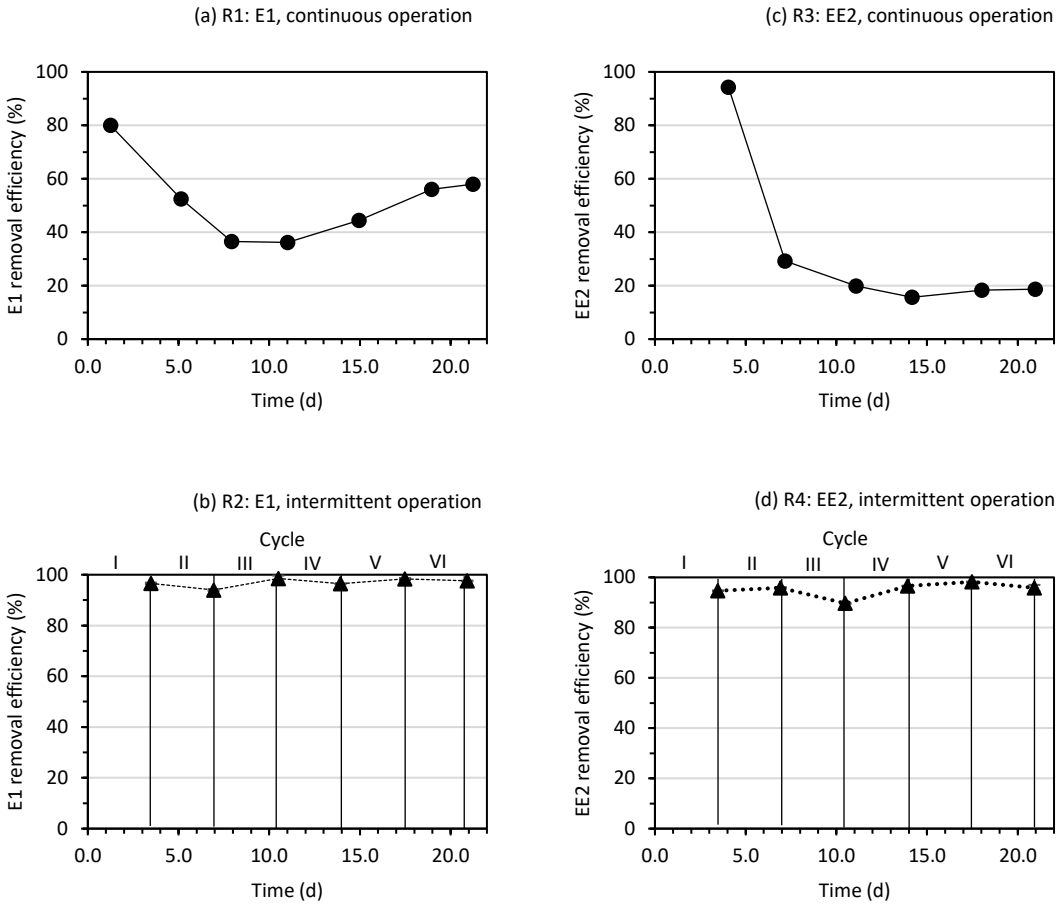

Figure 3 Results of E1 and EE2 removal from the LP in UASB reactor (a) R1: fed with synthetic wastewater spiked with E1 using CO; (b) R2: fed with synthetic wastewater spiked with E1 using IO; (c) R3: fed with synthetic wastewater spiked with EE2 using CO; (d) R4: fed with synthetic wastewater spiked with EE2 using IO. (Note that, for most of the experimental points, error bars are too small to be visible in the figure)

3.3. E1 and EE2 adsorption and biodegradation

The adsorption and biodegradation percentages for E1 and EE2 removal using the CO and IO modes were established through a mass balance, which includes measurement of estrogens in the LP of the influent and the treated effluent, as well as in SP and LP of the sludge (Figure 4). For the IO, only 4.1% of the spiked amount of E1 and 5.5% of the spiked amount of EE2 remained in the treated effluent, while for the CO, 51.0% of the spiked amount of E1 and 61.0% of the spiked amount of EE2 were detected in the treated effluent. Adsorption values of E1 and EE2

onto SP of sludge were 4.7 and 2.3 times higher using the IO compared to CO, respectively. These results indicate that IO may have contributed to improve the contact between estrogens and sludge.

The adsorption process is strongly dependent on the physicochemical properties of the compound, which can be characterized by the octanol/water partition coefficient (K_{ow}), specific sorption coefficient (K_d), and the molecular structure (Silva et al., 2012; Tran et al., 2018). Indeed, pollutants with higher log K_{ow} (above 4.0) often exhibit high sorption potential onto the particulate phase, while compounds with lower log K_{ow} (below 2.5) tend to have a low sorption potential (Caliman and Gavrilescu, 2009; Luo et al., 2014). Both E1 and EE2 are hydrophobic compounds with log K_{ow} values of 3.43 and 4.15, respectively, and consequently low water solubility values (13 mg L^{-1} and 4.8 mg L^{-1}, respectively) (Ying et al., 2002). Thus, these compounds tend to have a strong partition in the particulate phase and, consequently, exhibit a high sorption potential in the order EE2> E1 (Jones-Lepp and Stevens, 2007; Silva et al., 2012). Moreover, K_d, the ratio between the adsorbed and the dissolved concentrations of the compound at the equilibrium, is commonly used to quantify the affinity of a compound for a sorbent (Silva et al., 2012). Pollutants with log K_d values lower than 2 are negligible for adsorption, while for compounds with values higher than 4 adsorption to the sludge is a major removal mechanism (Clara et al., 2004). Data in the literature indicates log K_d values for E1 and EE2 between 2.4 and 2.8, indicating that adsorption is a relevant process in the removal of these compounds (Andersen et al., 2005; Clara et al., 2004; Ternes et al., 2004). Thus, the high estrogens adsorption values (5.7%-26.5% for E1 and 31.0%-72.7% for EE2) obtained in this study can be justified by the hydrophobic nature of these compounds, more evident in the case of EE2.

The mass balance for E1 and EE2 suggests that the IO caused an increase in E1 and EE2 biodegradation, with values of 69.4% and 21.8%, respectively. For the CO the biodegradation estimated values were 43.3% for E1 and 8.0% for EE2. The resistance of EE2 to biodegradation

using IO and CO can be linked to the ethynyl group in the 17-position, which blocks the potential formation of a ketone and stereotypically hinders access to the hydroxyl group in the 17-position. These features justify the higher persistence of EE2 when compared to E1 (Czajka and Londry, 2006).

Published information about estrogens removal mechanisms (involving biodegradation and adsorption) using UASB reactors is limited. Some studies have been undertaken using the UASB reactor followed by an aerobic process (Alvarino et al., 2016). Nevertheless, there is no data on the removal of estrogens using the IO of UASB reactors. Alvarino et al. (2016) investigated the behaviour of E1 and EE2 using the CO in a UASB reactor coupled to a hybrid aerobic membrane bioreactor (MBR). The authors reported that no biodegradation or adsorption was detected for both E1 and EE2. In another work carried out by Alvarino et al. (2014), the researchers found values similar to those reported in the present work for E1 adsorption onto sludge (~4%) and E1 biodegradation rate (~34%) using UASB units under CO for the treatment of dairy wastewater. In the experiments with EE2, the authors obtained a much lower adsorption value (~2%) and higher biodegradation rate (~47%) (Alvarino et al., 2014), as compared to the work hereby presented (31% of adsorption and 8% of biodegradation). The distinct biodegradation efficiencies observed can be attributed to different factors, such as the distinct HRT, the absence of nitrifying conditions, the COD concentration in the inlet, and the initial EE2 concentration. Moreover, in the work reported in the literature, the sludge used for the inoculum of the UASB reactor was granular sludge obtained from a full-scale reactor treating brewery wastewaters, whereas in our study, flocculent anaerobically sludge was used (Alvarino et al., 2014). Flocculent sludge exhibited much lower dimensions and, consequently, a higher specific surface is available for estrogens adsorption (Alvarino et al., 2016). Consequently, in our study, higher amounts of E1 and EE2 could adsorb onto the UASB reactor sludge.

Adsorption and biodegradation results obtained in our work may be related to the short time of operation of UASB reactors used (21 days). Operating times applied in the literature varied from several weeks to a few months (Alvarino et al., 2019, 2016, 2014; Arias et al., 2018; Moya-Llamas et al., 2018; Vassalle et al., 2020). A study carried out by Chan et al.(2018) have investigated the anaerobic co-digestion of a mixture of food waste and domestic wastewater using an UASB reactor operated during 10 days. Larcher and Yargeau (2013) investigated the biodegradation of EE2 by heterotrophic bacteria and observed that this compound can be efficiently removed after 48 h in the presence of *Rhodococcus rhodochrous*, a bacterial species commonly present in activated sludge. With the combination of bacterial cultures, the authors found about 43% of EE2 removal after 300 h. On the other hand, Yu et al. (2007) investigated the influence of the presence of bacteria (strains KC1-14) isolated from activated sludge on E1 degradation and remarked that E1 can be removed after 5 days. Thus, the operation time of UASB reactors proposed in this work can be considered suitable for comparison of the two operation modes.

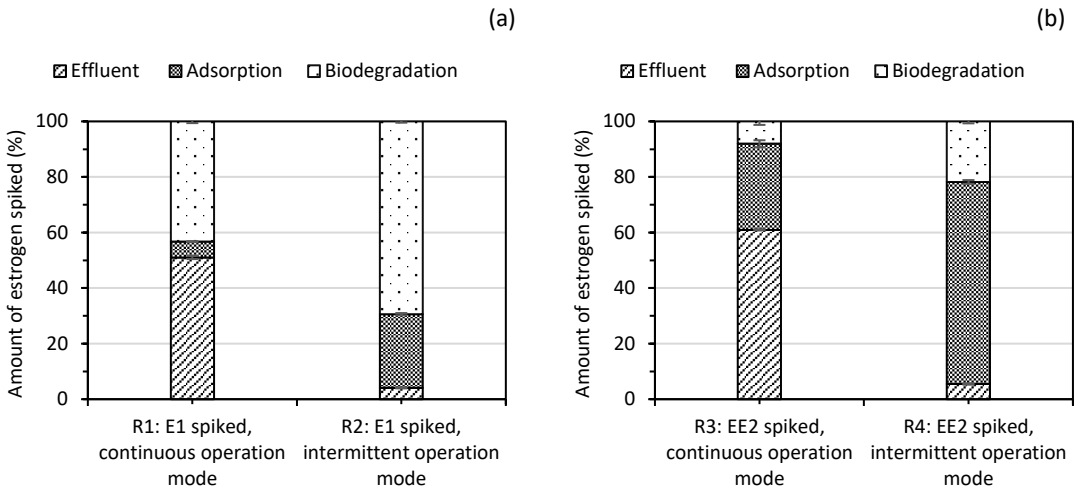

Figure 4 Mass balance for the CO and IO of UASB reactors for the treatment of synthetic wastewater contaminated with (a) E1 and (b) EE2. (Note that, for some of the experimental points, error bars are too small to be visible in the figure)

In the presented study, higher values of k_{biol} coefficients determined at the end of the assay (after 21 days) were achieved using the IO (0.80 ± 0.02 L g_{VSS}^{-1} d^{-1} for E1 and 0.10 ± 0.01 L g_{VSS}^{-1} d^{-1} for EE2) compared to the CO (0.31 ± 0.03 L g_{VSS}^{-1} d^{-1} for E1 and 0.015 ± 0.004 L g_{VSS}^{-1} d^{-1} for EE2). These findings indicate that the IO favours the E1 and EE2 biodegradation in wastewater using UASB reactors. The beneficial effect of IO can be justified by the feedless periods, during which the microbial population present in the sludge is deprived of easily degradable substrates present in the feed and are forced to degrade complex substrates, such as estrogens (Couras et al., 2014). The comparison of the results of k_{biol} coefficients obtained in the present with previous results reported by Alvarino et al. (2016, 2014) (0.01-0.04 L g_{VSS}^{-1} d^{-1} for E1 and 0.02-0.04 L g_{VSS}^{-1} d^{-1} for EE2) evidence that higher values for E1 and similar values for EE2 were achieved in the present study using the CO of UASB reactor. It is important to highlight that in the present study the estrogens concentration entering the UASB reactors were higher than the concentrations reported in the literature (1-10 μg L^{-1}). This may influence the biodegradation kinetics since the

pseudo-first order kinetics are driven by the concentration of estrogens in the reactor (Alvarino et al., 2016, 2014; Arias et al., 2018; Moya-Llamas et al., 2018). Moreover, the high concentration also influences the behaviour of the estrogens removal by microorganisms, which might be due to primary metabolism instead of cometabolism. Thus, in order to assess the removal of estrogens by the cometabolism of microorganisms, studies using initial concentrations similar to those found in WWTP samples should be taken into account.

Moreover, to gain a deeper understanding on the performance for a longer period of IO of UASB reactors on the estrogens´ removal (by adsorption and biodegradation), further research should be undertaken. In this context, different operating cycles of IO of UASB reactors and different feeding and feedless periods should be studied. In addition, the adaptation of biomass to estrogens should be assessed, for instance through the characterization of the microbial community composition by 16S amplicon sequencing.

4. Conclusions

In the present research, an innovative process for wastewater treatment contaminated with estrogens was proposed based on the IO of UASB reactors. In this context, the performance of the CO and IO of UASB reactors was investigated regarding the removal of E1 and EE2 from synthetic wastewater. Higher E1 and EE2 removal efficiencies by biodegradation and adsorption were achieved (above 95%) under the IO compared to the CO (ranging from 39% to 49%). Additionally, the IO of the UASB reactor enhanced 2.6 and 5.0 times the values of biodegradation kinetic coefficient, k_{biol}, for E1 and EE2, respectively. The improvements of the IO compared to the CO are attributed to the adaptation of the microbial population to complex substances that are retained in the sludge, as estrogens.

Results attained in this investigation indicated that the IO of UASB reactors can be a promising, sustainable, and robust alternative to aerobic processes coupled to UASB reactors for the removal of estrogens from wastewaters. It is expected that the increase of the feedless period for the IO mode allows the development of a microbial population involved in the biodegradation of E1 and EE2. Thus, the studies presented in this research represent a step forward in the knowledge about the performance of UASB reactors on the removal of two estrogens, E1 and EE2.

Abbreviations

CO	Continuous operation
COD	Chemical oxygen demand
E1	Estrone
E2	17β-estradiol
EDC	Endocrine-disrupting chemical
EE2	17α-ethinylestradiol
FLD	Fluorescence detection
HPLC	High-performance liquid chromatography
HRT	Hydraulic retention time
IC	Inorganic carbon
IO	Intermittent operation
K_d	Specific sorption coefficient
K_{ow}	Octanol/water partition coefficient
LOD	Limit of detection
LP	Liquid phase
MBR	Hybrid aerobic membrane bioreactor
OC	Organic carbon
OLR	Organic loading rate
SP	Solid phase
SRT	Solids retention time
TSS	Total suspended solids
UASB	Upflow anaerobic sludge blanket
ULE	Ultrasonic liquid extraction
VSS	Volatile suspended solids
v_{up}	Upflow velocity
WWTP	Wastewater treatment plant

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Supplementary material

Impact of UASB reactors operation mode on the removal of estrone and 17α-ethinylestradiol from wastewaters

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Materials and Methods

Table S1 Feeding schedule for each UASB reactor.

Reactor	Cycle																				
	I				II			III				IV			V				VI		
	Day																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
R1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
R2	■	□	□	□	■	□	□	■	□	□	□	■	□	□	■	□	□	□	■	□	□
R3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
R4	■	□	□	□	■	□	□	■	□	□	□	■	□	□	■	□	□	□	■	□	□

Legend:

■ = 12 hours feed with HRT of 30 h.

□ = 72 hours feedless with HRT of 30 h.

Results

Table S2 Main characteristics of sludge from UASB reactors.

Parameter [a]	Reactor							
	R1: E1 continuous operation		R2: E1 intermittent operation		R3: EE2 continuous operation		R4: EE2 intermittent operation	
	Initial [b]	Final [c]	Initial [b]	Final [c]	Initial [b]	Final [c]	Initial [b]	Final [c]
TSS (g L^{-1})	20.4 ± 0.7	3.82 ± 0.07	23 ± 1	6 ± 0.1	29 ± 2	5 ± 1	25 ± 2	6.4 ± 0.2
VSS (g L^{-1})	16.1 ± 0.5	3.1 ± 0.2	17.5 ± 0.7	4.6 ± 0.1	21 ± 2	4.1 ± 0.7	19 ± 1	5.1 ± 0.2
pH	5.62	6.10	5.35	7.49	5.53	6.90	5.03	7.00
OC (%)	41.9 ± 0.8	42 ± 1	42.3 ± 0.8	40.9 ± 0.1	42 ± 1	41 ± 0.6	44.3 ± 0.4	44.8 ± 0.2
IC (%)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

[a] IC, inorganic carbon; LOD, limit of detection; OC, organic carbon; TSS, total suspended solids; VSS, volatile suspended solids.

[b] Real sludge before inoculation.

[c] Mixture of sludge and effluent collected inside of the UASB reactor.