



## Occurrence and removal of estrogens in Brazilian wastewater treatment plants



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### HIGHLIGHTS

- The occurrence of four endocrine disrupting chemicals was evaluated.
- The removal efficiency of four hormones in low-cost plants was examined.
- Estrogen occurrence showed a wide variation in influent and effluent samples.
- Estrone showed the highest occurrence in the influent and the effluent samples.
- WSP treatment was observed to be less effective for removing estrogens.

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### ABSTRACT

This paper evaluated the occurrence and removal efficiency of four estrogenic hormones in five biological wastewater treatment plants (WWTPs), located in the State of Ceará, Brazil. The five WWTPs comprised: two systems consisted of one facultative pond followed by two maturation ponds, one facultative pond, one activated sludge (AS) system followed by a chlorination step, and one upflow anaerobic sludge blanket (UASB) reactor followed by a chlorination step. Estrogen occurrence showed a wide variation among the analyzed influent and effluent samples. Estrone (E1) showed the highest occurrence in the influent (76%), whereas both 17 $\beta$ -estradiol (E2) and 17 $\alpha$ -ethynylestradiol (EE2) presented a 52% occurrence, and the compound 17 $\beta$ -estradiol 17-acetate (E2-17A), a 32% one. The occurrence in the effluent samples was 48% for E1, 28% for E2, 12% for E2-17A, and 40% for EE2. The highest concentrations of E1 and EE2 hormones in the influent were 3050 and 3180 ng L<sup>-1</sup>, respectively, whereas E2 and E2-17A had maximum concentrations of 776 and 2300 ng L<sup>-1</sup>, respectively. The lowest efficiencies for the removal of estrogenic hormones were found in WWTP consisted of waste stabilization ponds, ranging from 54 to 79.9%. The high-rate systems (AS and UASB), which have chlorination as post-treatment, presented removal efficiencies of approximately 95%.

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### 1. Introduction

Endocrine disrupting chemicals (EDCs) are a heterogeneous group of substances characterized by their potential to interfere with endocrine system functions in wildlife and humans (Sanfilippo et al., 2010). The presence of these contaminants in aquatic environment and their potential effects on living organisms have become a growing concern in recent years, which requires identification, analysis and characterization of risks in different environmental matrices (Dolar et al., 2012).

Estrogenic hormones excreted by humans are emerging contaminants which can reach the aquatic environment via wastewaters

release. The presence of such micropollutants in surface waters poses various questions concerning their degradation and their potential adverse effects on the sexual and reproductive systems in wildlife, fish and humans (Chang et al., 2011; Gabet-Giraud et al., 2010; Jobling et al., 1998; Purdom et al., 1994).

The occurrence of estrogens in wastewaters and surface waters has been investigated in numerous studies (Chang et al., 2011; Coleman et al., 2010; Gabet-Giraud et al., 2010; Lundstrom et al., 2010; Radjenovic et al., 2009), in which both natural (estrone and 17 $\beta$ -estradiol) and synthetic (17 $\alpha$ -ethynylestradiol) varieties were identified as the main compounds responsible for estrogenic activities in wastewater treatment plants (WWTPs). Many studies have also reported that the elimination of some EDCs in WWTP can be ineffective (Carballa et al., 2004; Dolar et al., 2012; Moon et al., 2008), as a result,

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they are found in surface water, groundwater, and even drinking water (Al-Odaini et al., 2010; Rahman et al., 2009). Therefore, it is important to develop reliable wastewater treatment technologies which can efficiently remove these emerging contaminants at trace level concentrations.

Estrogen removal in WWTPs is a very complex procedure since it depends on numerous design aspects (sludge age, hydraulic retention time (HRT), organic loading rate (OLR), etc.), environmental conditions (sunlight, temperature, pH, toxic compounds, etc.), type of sludge and operational conditions (Clara et al., 2005). There are several studies worldwide which have assessed the behavior of micropollutants in WWTPs. However, most of them refer to activated sludge systems, which are most commonly used in development countries (Andersen et al., 2003; Baronti et al., 2000; Carballa et al., 2004; Fernandez-Fontaina et al., 2012; Joss et al., 2006; Kanda and Churchley, 2008). In contrast, there are only a few published reports on the concentrations or removal of EDCs in Brazilian WWTPs (Brandt et al., 2013; Froehner et al., 2010; Queiroz et al., 2012; Ternes et al., 1999b), especially those which uses waste stabilization ponds (WSP) technology.

In the state of Ceará, biological processes are widely used, being 62% of these wastewater treatment systems consisted of stabilization ponds (Brandão, 2000). Because of the great applicability, low capital and operational costs of WSP in hot-climate developing countries, such as Brazil, it is important to evaluate the micropollutants removal efficiency of these systems (Coleman et al., 2010) and compare them with the systems commonly used in other countries, such as activated sludge systems and anaerobic reactors (Carballa et al., 2007; Czajka and Londry, 2006; Paterakis et al., 2012).

It is also important to determine the concentrations and fate of estrogenic compounds in Brazilian WWTPs since there are limited studies on these compounds in wastewater systems, receiving bodies (water and sediments) and drinking water (Jardim (Jardim et al., 2012; Montagner and Jardim, 2011; Moreira et al., 2009, 2011; Sodré et al., 2010a, 2010b)). Despite the significant increase in research on the subject, the occurrence of several micropollutants in environmental matrices has not been established yet for many countries, mainly due to the difficulties and costs associated with the chemical analyses (Virkutyte et al., 2010). In fact, in Brazil, there are only a small number of studies reporting the occurrence of a few compounds.

Therefore, the main objective of this paper was to determine the occurrence and removal of four EDCs in five Brazilian real scale WWTPs (specially low costs treatment technologies), i.e. three waste stabilization ponds systems, an activated sludge system followed by a chlorination step and a UASB reactor also followed by a chlorination step. This would provide important insights into the technology which can most effectively remove these compounds and, therefore, help to guide current environmental legislations. The paper does not focus on either removal mechanisms, which are well reported in literature and depend on the treatment technology, or estrogenic activity, which was out of the scope of the present investigation.

## 2. Materials and methods

### 2.1. Reagents and material

Solid-phase extraction (SPE) was carried out by using 200 mg Oasis® HLB cartridges (Waters, Milford, MA, USA) and HPLC-grade methanol purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade water was produced using a Milli-Q purification system (Millipore, Bedford, MA, USA). Standards for estrone (E1, 99% purity), 17 $\beta$ -estradiol (E2, 98% purity), 17 $\alpha$ -ethynylestradiol (EE2, 99.4% purity) and 17 $\beta$ -estradiol 17-acetate (E2-17A, 98.8% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock EDC standard solutions, at a concentration of 1000 ng mL<sup>-1</sup>, and the appropriate working standard solutions were prepared in methanol and stored in amber glass bottles at 4 °C.

### 2.2. Sample preparation

The first step of sample preparation involved performing wastewater filtrations under vacuum through 0.45  $\mu$ m glass-fiber filters to remove suspended particulate matter and avoid SPE cartridge clogging. The pH of each sample was then adjusted to 3.0 by addition of 50% (v/v) HCl, after which the analytes were extracted with a Speed Mate 12-port SPE vacuum manifold (Applied Separations, Allentown, PA, USA).

The SPE cartridges were initially preconditioned with 10 mL of methanol, and subsequently with 10 mL of Milli-Q water. The samples, typically 500 mL, were then loaded onto the cartridges at a flow rate lower than 2 mL min<sup>-1</sup>. The cartridges were then dried for 30 min under vacuum and eluted with 4 mL of methanol. Extracts collected in amber glass flasks were dried in an oven at 45 °C. The dry residues were derivatized by the addition of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) for 30 min at 60 °C. The derivatives were cooled to room temperature and resuspended in 500  $\mu$ L of methanol. The solutions were then transferred to sealed cap vials and analyzed by gas chromatography–mass spectrometry (GC/MS).

### 2.3. GC/MS analysis

EDC determinations were performed on a Focus GC instrument interfaced to a DSQ II mass spectrometer and controlled by the software XCalibur (Thermo Electron SA, Madrid, Spain). The GC instrument was equipped with a Supelco SLB™-5MS capillary column (silphenylene polymer, which is virtually equivalent in polarity to 5% diphenyl/95% methyl siloxane; 30 mm  $\times$  0.25 mm I.D.  $\times$  0.25  $\mu$ m film thickness), and helium (>99.999% purity) was used as the carrier gas at a flow rate of 1.2 mL min<sup>-1</sup>.

The injection port temperature was maintained at 250 °C, and the oven temperature program varied as follows: ramp up to 150 °C from 50 °C (at a rate of 40 °C min<sup>-1</sup>), ramp up to 270 °C at a rate of 40 °C min<sup>-1</sup> (held for 1 min), ramp up to 280 °C at a rate of 10 °C min<sup>-1</sup> (held for 5 min), and ramp up to 290 °C at a rate of 10 °C min<sup>-1</sup> (held for 1 min). Sample injection was performed in splitless mode using an injection volume of 1  $\mu$ L. The mass spectrometer was operated in electron positive impact ionization mode at 70 eV, with a full scanning range of 50–650 m/z, and ion source and transfer line temperatures of 290 °C. This method was developed from previous studies (Liu et al., 2004; Mol et al., 2000; Shimada et al., 2001; Tan et al., 2007; Ternes et al., 1999b).

### 2.4. Quality assurance and quality control

The validation of the optimized method, as well as the determination of recoveries and occurrences in the wastewater samples, was conducted according to Brazilian standard DOQ-CGRE-008 (INMETRO, 2006). Linearity (*L*), limits of detection (LOD) and quantification (LOQ), recovery, and repeatability (*R*) were investigated. LOQ and LOD were calculated from the calibration curve and the spiked recoveries, whereas repeatability was determined from the relative standard deviation (RSD) of 10 replicated samples. Analysis of each wastewater sample was performed in triplicate and was accompanied by the analysis of one blank laboratory reagent. Recoveries were determined by the extraction, derivatization and analysis of two replicated spikes at an estimated detection limit (500 ng L<sup>-1</sup>) for each target analyte.

### 2.5. Sample collection

Duplicate grab wastewater samples were collected between April 2010 and April 2012 from five full-scale WWTPs located in the State of Ceará, a semi-arid zone in Brazil. Additional details regarding these WWTPs are given in Table 1. For each WWTP, five influent and effluent samples were analyzed in order to determine the estrogens removal.

**Table 1**  
Details of the wastewater treatment plants studied.

WWTP	Treatment technology	Resident inhabitants	Additional information <sup>a</sup>
A	Waste stabilization pond (WSP) (1 facultative + 2 maturation)	23,870	Working depth (facultative) = 1.8 m; HRT (facultative) = 11 days; working depth (maturation) = 1.5 m; HRT (maturation) = 5 days (each pond); total HRT = 21 days; mean flow rate = 59.4 L s <sup>-1</sup>
B	Waste stabilization pond (WSP) (1 facultative + 2 maturation)	3,000	Working depth (facultative) = 1.5 m; HRT (facultative) = 10 days; working depth (maturation) = 1.5 m; HRT (maturation) = 4.2 days (each pond); total HRT = 18.4 days; mean flow rate = 7.6 L s <sup>-1</sup>
C	Waste stabilization pond (WSP) (1 facultative)	12,705	Working depth = 2 m; HRT = 26.4 days; mean flow rate = 49.6 L s <sup>-1</sup>
D	Activated sludge (AS) with post-chlorination disinfection	2880	Mean flow rate = 2.7 L s <sup>-1</sup> ; solid retention time = 30 days
E	Upflow anaerobic sludge blanket (UASB) with post-chlorination disinfection	1100	Working height = 4.5 m; HRT = 7 h; mean flow rate = 91.7 L s <sup>-1</sup>

<sup>a</sup> Design values.

However, to calculate the occurrence of estrogens in influent and effluent wastewater, all the 25 samples were considered. The design value of the hydraulic retention time (HRT) of each plant was not considered because most of the systems are old and the real HRT is unknown but shorter due to sludge accumulation. Especially in ponds, the accurate determination of real HRT demands tracer studies, which was outside of the scope of this study. Data details of temperature and total precipitation are given in Table 2.

The samples were collected in glass flasks, transferred to 1 L amber glass bottles and preserved by the addition of 10 mL L<sup>-1</sup> formaldehyde (Cavalcante et al., 2010). Then, the samples were transported to the laboratory in cooling boxes and were prepared for analysis within 48 h. Formaldehyde was also used in the method validation, and no interference was observed.

### 3. Results and discussion

#### 3.1. Validation of proposed method

Table 3 presents validation data and determined recovery rates for the four target analytes, with LOQ and LOD in the ranges of 37–121 and 11–60 ng L<sup>-1</sup>, respectively. Recoveries were calculated for

**Table 2**  
Mean temperature and total precipitation for the 5 days prior to the sampling dates at the Fortaleza sewage treatment plants.

WWTP	Date	Mean temperature (°C)	Total precipitation (mm)
A	May 05/10	27	5.4
	Dec 06/10	29.7	0
	Oct 10/11	27.92	0.04
	Dec 13/11	28.1	0
	Dec 20/11	28.14	1
B	Apr 28/10	28.4	2.3
	Jul 29/11	26.83	0
	Mar 07/12	27.86	0.16
	Mar 28/12	26.6	23.3
	Apr 19/12	25	6
C	May 19/10	28.6	0
	Jul 14/11	26.2	8.1
	Dec 13/11	28.1	0
	Dec 20/11	28.14	1
	Jan 24/12	27.64	4.38
D	Jun 10/10	27.7	1.3
	Jul 17/11	25.6	11.2
	Nov 22/11	28	0
	Mar 07/12	27.8	0.16
	Mar 28/12	23.3	26.64
E	Nov 22/11	28	0
	Mar 07/12	27.8	0.16
	Mar 28/12	26.6	23.3
	Apr 19/12	25	6
	May 19/12	27	4.6

wastewater influent and effluent samples after correcting for background concentrations of target analytes, as determined from analyses of unspiked samples. Recoveries were above 60% for three of the four analytes under optimized SPE conditions, and standard deviations were generally within 0.04%–0.58% of the measured recoveries on these analytes, even in wastewater influent. The small variation in recovery among analytes demonstrates the reliability of the method used, and the range of repeatability was below 0.30% (RSD).

Andrási et al. (2011) found LOQ values similar to those obtained in the present work, ranging from 1.88 to 37.5 ng L<sup>-1</sup>. Additionally, the LOQ obtained for E1, E2 and EE2 determined by this method were lower than those found by Quintana et al. (2004) (10–17 mg L<sup>-1</sup>), who also used GC/MS for identification. On the other hand, the recoveries of the analytes with the Oasis® HLB cartridge were smaller than those presented by other authors who used the same cartridge in their recovery assays, in which sanitary effluent added with low estrogens concentrations was used as matrix (Benijts et al., 2003; Laganà et al., 2004; Salvador et al., 2007; Trenholm et al., 2006). Nevertheless, despite the low recoveries achieved in the current work, the values were acceptable since they showed a good repeatability in all samples, indicating the consistency of the method.

Vega-Morales et al. (2012) reported a recovery ranging from 88 to 98% for analytes at 500 ng L<sup>-1</sup>. However, Abegglen et al. (2009) reported a recovery of 77% for EE2, a value close to the obtained in this study (69%) with the polymeric cartridge. Pedrouzo et al. (2009) also found recoveries similar to those of the present study. These authors used sewage as matrix, Oasis® HLB cartridge and estrogens at a concentration of 300 ng L<sup>-1</sup>, and obtained recovery efficiencies of 51% (E1), 61% (E2), 26% (E2-17A) and 52% (EE2).

#### 3.2. Occurrence of estrogens in influent and effluent samples

The distribution of estrogen concentrations which were measured in raw and treated wastewater from all five WWTP ( $n = 25$ ) is shown in Fig. 1. In addition, Table 4 shows the occurrence and concentration of estrogens in influent and effluent samples, with these data having an elevated variation. The highest estrogen occurrence in the influent was recorded by E1 (at 76%). Johnson and Sumpter (2001) reported that

**Table 3**  
Validation of the proposed method.

Compound	L	R (RSD%)	LOD (ng L <sup>-1</sup> )	LOQ (ng L <sup>-1</sup> )	Recovery <sup>a</sup> (%)
E1	0.9937	0.10	35.38	117.92	76 (±0.04)
E2	0.9928	0.13	11.28	37.58	66 (±0.11)
E2-17A	0.9930	0.10	18.90	62.99	42 (±0.50)
EE2	0.9970	0.28	60.43	121.44	69 (±0.58)

<sup>a</sup> Calculated from samples spiked at 500 ng L<sup>-1</sup> (wastewater influent and effluent), numbers in parentheses represent the standard deviation for the analysis of two replicate samples.

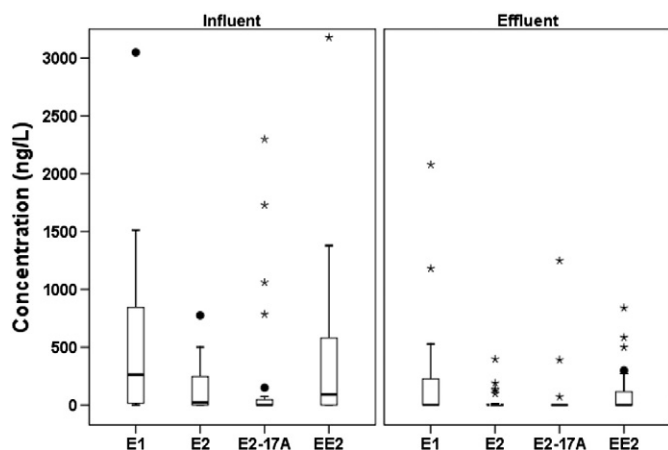


Fig. 1. Influent and effluent estrogens concentrations.

the origin of E1 in influent sewage is unclear; it may be a byproduct of E2 biodegradation or, alternatively, largely due to E1 sulfonide or glucuronide deconjugation in the sewer system, especially in treatment plants which are located a large distance away from households. These authors suggest that E1 is the most environmentally important estrogen when compared to E2 since it is more frequently detected in higher concentrations than E2.

E2 and EE2 both recorded a 52% occurrence, and E2-17A presented a 32% occurrence. The relatively low occurrence of estradiol-based compounds may be the result of their fast degradation to estrone (Servos et al., 2005). Miège et al. (2009) reported EE2 to be present in 91% of raw wastewater at  $\text{ng L}^{-1}$  concentration level. Thus, although EE2 was expected to be found in more influent samples, it was only present in half of the samples in the current work.

In this work, the influent samples showed higher estrogens concentrations when compared to other works (Gabet-Giraud et al., 2010; Lishman et al., 2006), possibly caused by the low precipitation (<27 mm) and high temperatures (25–29 °C), resulting in low dilutions of estrogens. The highest concentrations of E1 and EE2 in the influent were 3050 and 3180  $\text{ng L}^{-1}$ , respectively, whereas the concentrations of E2 and E2-17A were 776 and 2300  $\text{ng L}^{-1}$ , respectively (Table 4). In all cases, the lowest concentrations were below the LOD.

The occurrences of each hormone in the effluent samples were 48% for E1, 28% for E2, 12% for E2-17A, and 40% for EE2 (Table 4). In terms of effluent concentration, Ternes et al. (1999b) found estrone (E1) to be the highest of all measured estrogens, whereas E2 was near the

detection limit of  $1 \text{ ng L}^{-1}$ . The relatively high concentration of E1 in the present investigation (a mean value of  $242 \text{ ng L}^{-1}$ ) is interpreted to have been caused by the conversion of E2 and EE2 into E1 before it could be transformed further (Carballa et al., 2004; Czajka and Londry, 2006; Shi et al., 2004; Ternes et al., 1999a).

### 3.3. Removal of estrogens from the aqueous phase

The main goal of this work was to provide important insight into the technology that can most effectively remove EDCs. The paper does not deal with mechanisms, which are very difficult to establish in real scale systems and depend on the technology applied. However, some discussion about the known published mechanisms was made in order to explain the occurrence and efficiency of WWTPs. Estrogen removal efficiencies in each of the five WWTPs were individually analyzed and calculated in this study (Table 5), as described below.

#### 3.3.1. Removal of E1

The mean E1 removal in WWTP A, which utilized one facultative and two maturation ponds, was approximately 65% (Fig. 2). On the other hand, WWTP B (which operates with the same technology) had an average removal efficiency of only 41.4%, suggesting that the operational and design conditions might have directly affected the treatment performance (Table 1).

WWTP C operated only one facultative pond and was less efficient when compared to systems A and B, with a mean removal efficiency of only 31%. This result suggests that maturation ponds might be important for estrogen removal.

WSPs are well known for their UV-disinfection capacity and have recently been reported to be effective photo-oxidizers of EDC. In fact, Coleman et al. (2010) found that E1 was completely degraded by UV light. The photo-oxidative potential in ponds can be increased by both the presence of humic substances and high oxygen concentrations, with the latter contributing to the formation of reactive oxygen species (Davies-Colley et al., 1999). Thus, as the oxygen concentration in maturation ponds is higher than in facultative ponds, it is expected that the photo-oxidative potential will also be higher. WWTPs with a polishing step may also theoretically improve their performance by estrogen volatilization. However, compared to volatile organic pollutants (e.g. chlorinated hydrocarbons, aromatics) with a Henry's law constant (H) of approximately  $10^3$ , estrogenic hormones have a small H (approximately  $10^{-7}$ – $10^{-11}$ ), which makes these compounds less susceptible to volatilization under normal pressure and temperature conditions (Hamid and Eskicioglu, 2012). Coleman et al. (2010) found E1 removal efficiencies which ranged from 100 to 82% after secondary treatment in two WSPs located in Australia, which each system with two ponds.

WWTP D employed an AS treatment and a chlorination step, and was effective in removing E1, with a mean efficiency of ~84%. The chlorine dose added to disinfect the effluent was  $2.5 \text{ mg L}^{-1}$ . The AS treatment process is known to be effective in removing estrogenic hormones and other lipophilic contaminants from the wastewater aqueous phase (Baronti et al., 2000; Leusch et al., 2006). Consequently, E1 was only found in one effluent sample at a concentration of  $261 \text{ ng L}^{-1}$ . Elsewhere, E1 has been detected at a maximum concentration of  $350 \text{ ng L}^{-1}$  after AS treatment and seasonal chlorine disinfection (Atkinson et al., 2012).

WWTP E, which uses a UASB followed by chlorination post-treatment, showed a 100% removal of E1 from wastewater. Similar results were also found by Salgado et al. (2010), who investigated the removal of E1 in UASB reactors. de Mes et al. (2008) assigned adsorption as the responsible for a 32–35% loss of E1 and E2 from the liquid phase. In both WWTPs D and E, the complementary abiotic removal of E1 by chlorine must also be considered (Deborde et al., 2004; Hu et al., 2003; Lee et al., 2004).

**Table 4**  
Occurrence of estrogens in influent and effluent samples.

Estrogen	Influent concentration					Occurrence (%)
	Mean ( $\text{ng L}^{-1}$ )	RSD (%)	Min ( $\text{ng L}^{-1}$ )	Max ( $\text{ng L}^{-1}$ )	n	
E1	566	125	<LOD	3050	25	76
E2	143	139	<LOD	776	25	52
E2-17A	268	2	<LOD	2300	25	32
EE2	421	166	<LOD	3180	25	52
Estrogen	Effluent concentration					Occurrence (%)
	Mean ( $\text{ng L}^{-1}$ )	RSD (%)	Min ( $\text{ng L}^{-1}$ )	Max ( $\text{ng L}^{-1}$ )	n	
E1	242	193	<LOD	2080	25	48
E2	48	177	<LOD	397	25	28
E2-17A	87	291	<LOD	1250	25	12
EE2	124	176	<LOD	176	25	40

LOD = limit of detection and RSD is the relative standard deviations.



**Table 5**  
Average removal of estrogens in WWTPs.

Compound	Average removal (%) <sup>a</sup>					Removal for each compound <sup>b</sup>
	A	B	C	D	E	
E1	62	41	31	84	100	63.6
E2	81	81	62	96	87	81.4
E2-17A	100	100	26	100	100	85.2
EE2	30	99	58	100	94	75.8
Global Removal <sup>c</sup>	68.25	79.75	44.25	95	95.25	–

<sup>a</sup> Removal was considered 100% when the concentration was <LOD or <LOQ.

<sup>b</sup> Removal considered for each compound in all treatments.

<sup>c</sup> Average for all estrogens.

### 3.3.2. Removal of E2

The mean E2 removal efficiencies in WWTPs A, B and C were 95%, 100% and 81%, respectively (Fig. 3). This behavior was expected, given that ponds are generally very effective at removing E2, with apparent removals ranging from 80% to 98% (Lishman et al., 2006; Servos et al., 2005). However, as observed previously, systems B and C only removed low percentages of E1. One possible explanation for this would be the conversion of E2 into E1, with systems B and C being unable to degrade E1 efficiently.

WWTP D was 100% effective in degrading estrogen E2. The most likely hormone removal pathways for this technique might include biological degradation, abiotic removal (chlorination) and adsorption onto solids. Sorption and biodegradation are the two major removal mechanisms for 17 $\beta$ -estradiol in AS systems (Estrada-Arriaga and Mijaylova, 2010; Fang et al., 2003; Ren et al., 2007; Shi et al., 2004; Stevens-Garmon et al., 2011; Vader et al., 2000).

Several studies have pointed out the ability of some bacteria isolated from AS systems to convert these molecules (Ren et al., 2007; Shi et al., 2004; Vader et al., 2000; Yoshimoto et al., 2004). Joss et al. (2004) and Lee and Liu (2002) proposed a mechanism for aerobic or anaerobic estrogen degradation in WWTPs. According to them, 17 $\beta$ -estradiol was initially oxidized to estrone, which was then further oxidized to unknown metabolites, and finally converted (mineralized) to CO<sub>2</sub> and water.

WWTP E, which uses a UASB followed by chlorination post-treatment, showed an 87% removal of E2. Paterakis et al. (2012) reported E2 biodegradation under anaerobic conditions, although the complementary abiotic E2 removal by chlorine must also be considered as a viable mechanism (Hu et al., 2003). In the latter study, it was reported that E2 rapidly reacted with HOCl, with almost 100% of E2 removed after a 10 min reaction.

### 3.3.3. Removal of E2-17A

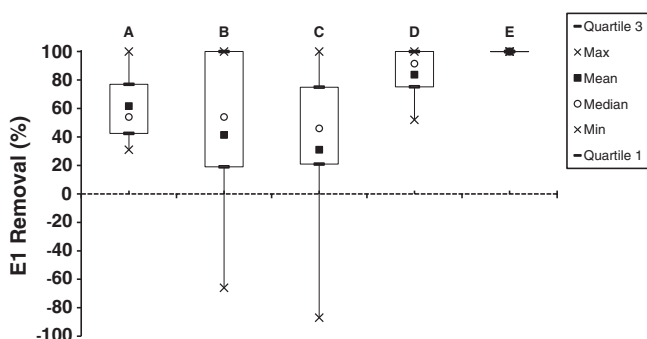
Regardless of the wastewater treatment method evaluated in this investigation, the compound E2-17A was removed with 100% efficiency (Fig. 4). Probably, the adsorption of the E2-17A molecule onto the sludge might

have played a key role, since its log *K*<sub>ow</sub> is very high (4.95) when compared to those for the E1 (3.13), E2 (4.01) and EE2 (3.67) molecules. However, Ren et al. (2007) described the sorption of E1, E2, estriol (E3) and EE2 onto sewage sludge as being deactivated by heat treatment. Although batch experiment data fitted well to a Freundlich isotherm, in contrast to previous studies, they observed sorption behavior to be independent of the *K*<sub>ow</sub> values of each compound. Nieto et al. (2008) studied the presence of E2-17A in sludge from sewage treatment plants and found values of 175–375  $\mu\text{g kg}^{-1}$ . The only system that removed a low proportion of E2-17A (26%, Fig. 4) was WWTP C. The reason for this system behavior still remains unclear.

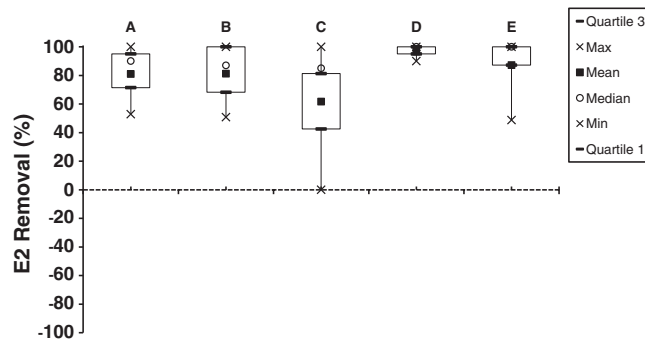
### 3.3.4. Removal of EE2

The removal of EE2 in WWTP A, which utilized one facultative and two maturation ponds, was only 29.7% (Fig. 5). This result is in agreement with Ying et al. (2008), who noted a similar EE2 removal of 25% and concluded that WSP do not effectively remove particular endocrine disruptors. Contrary to the above-mentioned results for WWTP A, the system B (involving same configuration – one facultative and two maturation ponds) removed 100% of EE2, which is close to the 90–95% range reported by Gomez et al. (2007). The difference in terms of EE2 removal between A and B might be attributed to the EDCs load. The influence of influent concentration on removal of EDCs for each type of process was also tested (removal vs. influent concentration). Thus, a tendency of higher influent concentrations values with lower removal was observed although the *p*-value was greater than 0.05 (Table 6). In this specific case, the average EE2 influent concentration was 358.8 and <121.44 ng L<sup>-1</sup>, for A and B, respectively. In addition, some design and operational parameters might have also played a role.

WWTP C removed 58.4% of EE2 using only one facultative pond. The absence of a maturation pond in this case suggests that it is an important factor. Williams et al. (2007) reported a 25% EE2 removal efficiency in WSP containing only a primary facultative pond, although they do not provide flow or population information.



**Fig. 2.** Estrone (E1) removal in 5 WWTPs.



**Fig. 3.** 17 $\beta$ -Estradiol (E2) removal in 5 WWTPs.

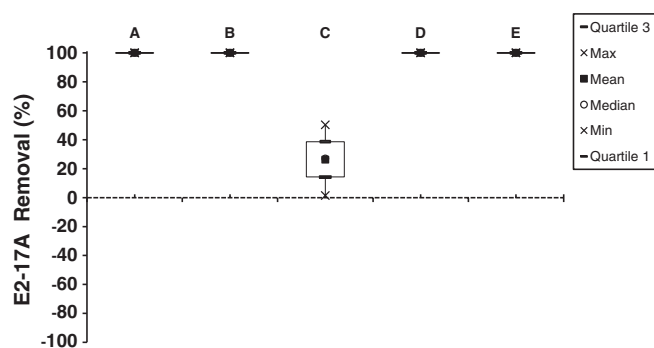


Fig. 4. 17β-Estradiol 17-acetate (E2-17A) removal in 5 WWTPs.

AS treatment in WWTP D exhibited a 100% efficiency in removing EE2 (Fig. 5), which is consistent with the high removal rates (>90%) in AS plants reported by Muller et al. (2008) and Joss et al. (2004). Yi et al. (2006) suggested that EE2 degradation may involve ammonium monooxygenase, the key enzyme that catalyzes nitrification. They showed that EE2 underwent mineralization in conventional bioreactors and produced unidentified metabolites. In contrast, Kanda and Churchley (2008) only measured a 3.2% removal efficiency of EE2 in a modern nitrifying AS, with this persistence to biodegradation also observed by Weber et al. (2005).

The removal of EE2 in WWTP E, which employed a UASB reactor followed by chlorination post-treatment, measured a mean efficiency of 94% (Fig. 5). Although EDCs can persist through the anaerobic sludge digestion process, with removals ranging from 10% to 48% (Ifelebuogu, 2011), Carballa et al. (2007) reported a 90% EE2 removal efficiency during the anaerobic digestion of sewage sludge. No EE2 was detected in the UASB effluent by de Mes et al. (2008).

### 3.3.5. Overall removal capacity of the WWTPs

Three different sewage treatment techniques (stabilization ponds, UASB, and AS followed by chlorination post-treatment) employed in five WWTPs in Brazil have been analyzed for their capacity to remove hormones from wastewater. Overall removal efficiencies (Fig. 6 and Table 5) showed that AS followed by chlorination post-treatment had an average estrogen removed of 95%, and UASB followed by chlorination post-treatment averaged a 95.25% removal. In contrast, the removal efficiency observed in stabilization ponds was approximately 64%. These data clearly indicate that AS and UASB followed by chlorination post-treatment are more effective to remove estrogens, with a low variability as denoted by a ~7% inter-quartile. Conversely, a larger difference of 29–31% was found between the first and third quartiles for WSP techniques.

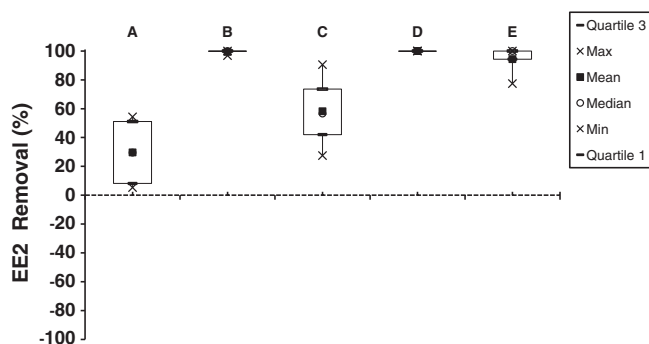


Fig. 5. 17α-Ethynylestradiol (EE2) removal in 5 WWTPs.

Table 6  
Correlation analysis of influent concentration on EDCs removal.

Estrogen		E1	E2	E2-17A	EE2
Removal (%)	Pearson correlation	0.231	−0.365	−0.320	−0.225
	Sig. (2-tailed)	0.278	0.079	0.127	0.291
	N	25	25	25	25

These results for AS systems are in agreement with Miège et al. (2009), who reported mean removal rates from the dissolved aqueous phase of around 80% for E1, αE2, and βE2. These authors created a database from scientific publications, in order to quantitatively assess the removal efficiency of EDCs in WWTPs. In their database, two types of ASP were considered: those which perform carbon removal (sludge age <10 days), and others that perform nitrogen removal (sludge age >10 days). In the current study, the system has a sludge age >10 days (Table 1).

Verlicchi et al. (2012) reported global removal efficiencies of micropollutants in 244 conventional AS systems (242 full-scale plants and 2 pilot ones) in the world (located in European countries, the Americas including Brazil, Asia and Australia). Conventional AS usually operates at an HRT ranging from 2 to 24 h and at an SRT generally of 2–20 days. The estrogen removals were between 67% and 80%. Froehner et al. (2011) reported hormone removal efficiencies of 73.1% in AS systems, 66.5% in UASB systems, and 56.5% for stabilization ponds. These researches presented the same pattern of removal efficiency (%) obtained for wastewater treatment plants in the present investigation.

In Table 6, it is possible to observe the removal for each compound in all treatments. E1 presented a 63.6% removal, whereas the other estrogens showed a better removal, ranging from 75 to 85%.

## 4. Conclusions

Some patterns of occurrence and removal of estrogens in 5 wastewater treatment systems in the State of Ceará, Brazil, were found. E1 showed the highest occurrence in the influent and the effluent. The sampling results suggest that this estrogen is excreted in major concentration by humans or others sources, and the compound was more persistent than the others since its global removal considering all treatments were smaller.

Systems using chlorination as post-treatment presented a better removal efficiency of compounds when compared to waste stabilization pond treatment. WSP treatment was observed to be less effective for removing steroid estrogens, particularly estrone. In some cases, it led to an increased concentration of estrone in the effluent.

Continued monitoring of these estrogens is necessary in order to improve wastewater treatment processes and to ensure the safety of drinking water. Monitoring should also be expanded to include conjugates

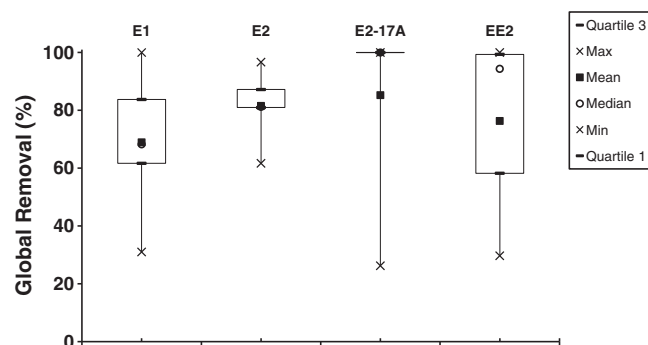


Fig. 6. Global removal of estrogens in 5 WWTPs.

and other matrices, such as sludge present in the systems analyzed. Besides, the effect of the air temperature and precipitation should also be assessed in WSP systems, although the operational conditions such as flow, sludge accumulation etc. cannot be fully controlled.

Finally, more specific study seems to be necessary to understand more about the mechanisms (biotic and abiotic) and microorganisms involved in the micropollutants removal, especially in low-cost wastewater technologies, in which experiments under controlled conditions are necessary.

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