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Research Article

Endocrine Disruptor Degradation by UV/Chlorine and the Impact of Their Removal on Estrogenic Activity and Toxicity

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Recently, chlorination disinfection technology applying ultraviolet radiation (Cl/UV) has received attention as an advanced oxidative process (AOP) for the generation of highly oxidant species. Many studies have evaluated its effects on pathogen inactivation, contaminant removal, and formation of disinfection by-products (DBPs). However, the degradation of three endocrine disruptor chemicals (EDCs), 17β-estradiol (E2), 17α-ethinylestradiol (EE2), and bisphenol-A (BPA), associated with simultaneous disinfection and estrogenic activity and ecotoxicity assessments has not yet been reported. Compound degradation increased with increasing chlorine concentrations (2 mg·L^{-1} chlorine), with pseudo-first-order kinetics 1.86×10^{-2} s^{-1}, 3.06×10^{-2} s^{-1}, and 3.09×10^{-2} s^{-1} for BPA, E2, and EE2, respectively. The degradation kinetics in a WWTP effluent significantly decreased to 4.94×10^{-2} min^{-1}, 4.75×10^{-2} min^{-1}, and 4.84×10^{-2} min^{-1}, for BPA, E2, and EE2, respectively. However, 45% TOC removal and disinfection of *E. coli* and total coliform bacteria (TCB) were observed in 10 min of treatment. The yeast estrogen screen (YES) revealed that the treatment did not form by-products with estrogenic activity, demonstrating cleavage or mineralization in the phenolic group, common to all assessed compounds. High cell growth inhibition and mortality for *Raphidocelis subcapitata* and *Ceriodaphnia dubia*, respectively, were observed during the photodegradation process. Thus, the formed DBPs may be responsible for the observed toxicity and should be taken into account in WWTP treatments in order to monitor the formation of chlorinated by-products.

1. Introduction

Wastewater treatment plants (WWTPs) are not designed to remove contaminants of emerging concern (CECs), leading to environmental detection at low concentrations (ng·L^{-1}-μg·L^{-1}) in aquatic systems [1]. Recently, several chemical compounds displaying estrogenic activity have received attention as environmental contaminants, due to their possible effects on aquatic organisms and human health [2]. Particularly, certain endocrine disruptors (EDCs), such as the estrogens 17α-ethinylestradiol (EE2) and 17β-estradiol (E2), and the plasticizer bisphenol-A (BPA) have been detected in wastewater treatment plant (WWTP) effluents, responsible for endocrine disruption in fish (feminization) inhabiting postwastewater release areas [3, 4].

Advanced oxidative processes (AOPs) have been applied in an attempt to remove these contaminants from the environment, with the advantage of using highly reactive radicals, based on the formation of the hydroxyl radical ($^{\cdot}$OH) for contaminant degradation [5, 6]. The generation of hydroxyl radicals by AOPs for wastewater treatment has been reported using conventional oxidants, such as

H_2O_2/UV [7], ozone/UV [8], and TiO_2/H_2O_2/UV [9]. Only recently has the photolysis of chlorine species been proposed as an AOP for the generation of the $^{\cdot}OH$ radical in water [10, 11]. The UV/Cl combination takes advantage of both processes and accelerates CEC degradation rates [12]. In addition to the $^{\cdot}OH$ radical, the UV/Cl process can also form reactive chlorine species (RCS), such as the chlorine atoms $Cl^{\cdot}$ and $Cl_2^{\cdot-}$ [13]. These species display oxidation potentials of 2.47 V and 2.0 V, respectively, lower than the $^{\cdot}OH$ radical (2.8 V) [14]. They are, however, more selective and quicker to react with electron-rich portions [15]. The UV/chlorine process has been efficiently applied in the elimination of pharmaceutical compounds and personal care products (PPCPs), such as carbamazepine [16], ibuprofen [14], ronidazole [12], and 17β-estradiol [17]. However, the use of UV/Cl as an AOP for the simultaneous removal of three EDCs (E2, EE2, and BPA), associated with kinetic, estrogenic activity removal and ecotoxicity assessments, has not yet been reported. In addition, many studies involving AOPs for wastewater treatment are carried out with only one compound at concentrations much higher than those detected in the environment and often use very simple matrices, such as ultrapure water without any background contamination, making it difficult to extrapolate the obtained data to real situations.

Complete compound mineralization may not occur during AOPs applied to water treatment, and EDC oxidation may form products with estrogenic activity [17]. In addition, another concern regarding the use of chlorine is the formation of disinfection by-products (DBPs), with recognized cytotoxic and genotoxic action [12]. Thus, it is important not only to identify the main transformation products but also to assess whether these intermediates display estrogenic activity and toxicity towards aquatic organisms.

In this context, the aim of the present study was to (1) assess the effectiveness of the degradation of three endocrine disruptor chemicals (E2, EE2, and BPA) and simultaneous disinfection by UV and UV/Cl radiation, (2) evaluate the effects of different operational parameters (chlorine concentrations, UV radiation, and WWTP effluent matrix), (3) determine estrogenic activity and toxicity during the process, and (4) determine reaction kinetics.

2. Material and Methods

2.1. Reagents. BPA, E2, and EE2 (98% purity) were purchased from Sigma-Aldrich (São Paulo, Brazil). A stock solution was prepared containing a mixture of the three compounds at 1 g·L^{-1} each in acetonitrile (Tedia, São Paulo, Brazil). After mixing, the solution was maintained at 4°C and diluted in the assessed aqueous matrices (ultrapure or wastewater) before each experiment, to a final concentration of 100 μg·L^{-1} of each compound. Purified water was obtained from a Milli-Q system (Millipore Corporation). Sodium hypochlorite (NaCl 5% *v*/*v*) was provided by Sigma-Aldrich. Chlorophenol red-β-D-galactopyranoside (CPRG) was supplied by Merck. KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4$, $Fe_2(SO_4)_3$, L-leucine, L-histidine, adenine, L-arginine–HCl, L-methionine, L-tyrosine, L-isoleucine, L-lysine–HCl, L-phenylalanine, L-glutamic acid, L-valine, L-serine, thiamine, pyridoxine, calcium pantothenate, inositol, D-glucose, aspartic acid, L-threonine, copper sulfate (II), and KOH pellets were supplied by Sigma-Aldrich. Biotin and absolute ethanol were supplied by Merck.

2.2. Photodegradation Set-Up. The photolysis and Cl/UV process experiments were carried out in a cylindrical glass reactor comprising a total volume of 500 mL, with a lamp placed on the center line, magnetic stirrers positioned at the bottom of the reactor, and a water recirculation system to maintain a constant temperature of 25°C, which did not influence the degradation process. Lamps at 6 W, emitting radiation in the UVA (λmax = 356 nm and 6.80 mW cm^{-2}) and UVC (λmax = 254 nm and 14.79 mW cm^{-2}) spectra, were used. The radiant fluxes at 254 and 356 nm were measured with a radiometer (Cole-Parmer Instrument Co.; model 9811-50). The UV lamps were heated for at least 30 minutes before the beginning of each experiment.

EDCs (100 μg·L^{-1}) were spiked in ultrapure water and a WWTP effluent. The UV/Cl process was performed at different initial chlorine concentrations, ranging from 0.2 to 2 mg·L^{-1} at pH 7. Samples (1 mL) were collected every 30 seconds in the ultrapure water experiment, while wastewater aliquots were removed every 5 minutes. At the end of the process, sodium thiosulfate was added to all the samples to stop the reactions, followed by filtering through a 13 mm diameter and 0.22 μ pore precleaned nylon syringe filter and storage at 4°C in the dark. Control EDC degradation tests by UV direct photolysis and dark chlorination were also conducted in a similar manner. Disinfection evaluations were carried out by determinations of *Escherichia coli* and total coliform bacteria (TCB) before, during, and after WWTP phototreatments.

The WWTP effluent was collected after secondary biological treatment from the Fiocruz WWTP, located in Rio de Janeiro, Brazil. The WWTP treatment was based on an activated sludge system with a flow rate of 512 m^3·day^{-1}, sludge retention time between 18-30 days, and hydraulic holding time of 16-24 h. Samplings were carried out at the end of the treatment, followed by effluent characterization (pH = 7.39; turbidity = 27.0 NTU; TSS = 22.0 mg·L^{-1}; TOC = 20.31 mg·L^{-1}; $[Cl^-]$ = 53.0 mg·L^{-1}; $[PO_4^{3-}]$ = 1.2 mg·L^{-1}; $[SO_4^{2-}]$ = 21.1 mg·L^{-1}; total nitrogen = 12.46 mg·L^{-1}; $[NO_2^-]$ = 0.4 mg·L^{-1}; and $[NO_3^-]$ = 1.2 mg·L^{-1}). Effluent physical-chemical characterization was performed following *Standard Methods for the Examination of Water and Wastewater* [18]. All measurements were conducted in triplicate using analytical-grade chemicals and ultrapure water.

2.3. Estrogenic Activity by the Yeast Estrogen Screen (YES) Assay. The *in vitro* YES assay was performed according to Routledge and Sumpter [19] with modifications. Samples treated with 2 mg·L^{-1} of free chlorine for 0-, 2-, and 5-minute photodegradation times were analyzed. The YES assay was performed in a sterile 96-well flat-bottom microplate under a laminar flow. Samples and E2 standard solutions were serially diluted in ultrapure water at a 1 : 2 ratio. After dilution, a 10 μL sample volume was immediately transferred to the microplate, and 190 μL of the assay

medium (fresh growth medium, recombinant yeast, and CPRG) was added to each well. Ultrapure water was used as a negative control. The microplates were then sealed, homogenized using a shaker (IKA, model MS-3), and incubated for 72 hours at 30°C. Absorbances were determined at 575 nm (for colour) and 620 nm (for turbidity) using a SpectraMax M3 plate reader (Molecular Devices). The dose-response curves of the positive control (E2) were fitted to a symmetric logistic function using the Origin 6.0 software package (Microsoft, USA). The EC_{50} mean value from E2 was determined from the dose-response curve. For the samples, estradiol equivalents (EQ-E2) were calculated by interpolating the curve data from the E2 standard curve at a range varying from 2724 to 1.33 ng·L^{-1}. The limit of detection (LOD) and limit of quantification (LOQ) were 8.8 ± 4.0 ng·L^{-1} and 26.0 ± 12.0 ng·L^{-1}, respectively. The EC_{50} mean value from 17β-estradiol was of 41.0 ± 2.9 ng·L^{-1}. During the YES assay, absorbance control at 620 nm was used to evaluate whether inhibition of yeast growth occurred due to toxicity of the samples according to Equation (1) as described by Frisch et al. [20].

$$\text{Toxicity} = 1 - \left(\frac{\text{ABS}_{620\,\text{sample}}}{\text{ABS}_{620\,\text{control negative}}}\right). \quad (1)$$

2.4. Chronic Toxicity Assay. Chronic toxicity tests were performed with two organisms, the algae *Raphidocelis subcapitata* [21] and the microcrustacean *Ceriodaphnia dubia* [22]. The Cl/UVC experiments were performed at a 2 mg·L^{-1} chlorine concentration and pH 7.0, and the ecotoxicological tests were performed at 0, 2, and 5 minutes.

2.5. Analytical Determinations. The BPA, E2, and EE2 concentrations were quantified by HPLC/FLU (Agilent Technologies 1200 series) equipped with a C18 column (ZORBAX Eclipse Plus 5 μm, 4.6 × 250 mm) at an emission wavelength of 310 nm and excitation wavelength of 230 nm. The isocratic mobile phase consisted of ultrapure water (pH 3, adjusted using hydrochloric acid) and acetonitrile (50 : 50, *v*/*v*%), at a flow rate of 1.2 mL·min^{-1} and injection volume of 100 μL. The limits of quantification (LOQ) were 2.8, 0.72, and 0.88 μg·L^{-1} for BPA, E2, and EE2, respectively, while the limits of detection (LOD) were 0.84, 0.22, and 0.26 μg·L^{-1}.

Total coliform bacteria (TCB) and *E. coli* were quantified by the Colilert method [23]. TOC determinations were carried out in filtered samples (nylon 0.2 μm filters) using a Shimadzu TOC-V SCN analyzer. Total suspended solid (TSS) was determined by the gravimetric method. Turbidity was measured by the nephelometric method using a portable Hach turbidimeter (2100P/1991–1998). pH was measured with a digital pH meter (Marte MB-10). Anions were analyzed by ion chromatography (Metrohm on a Personal IC Model 2.790.010) using a Metrosep A Supp 4/5 Guard 4 precolumn and Metrosep A Supp 5 150/4.0 column. Free chlorine concentrations were determined using a Pocket Colorimeter II (Hach) kit.

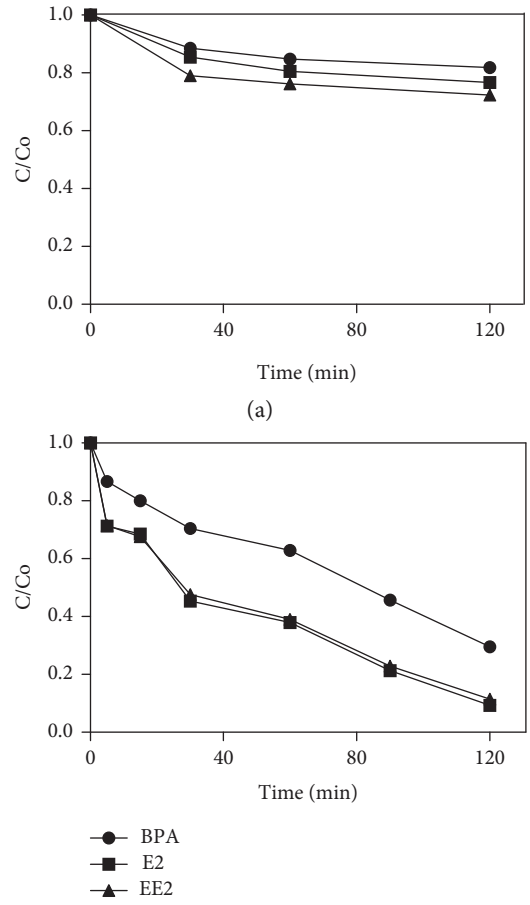

Figure 1: EDC degradation by photolysis in ultrapure water: (a) UVA; (b) UVC; initial EDC concentrations: 100 μg·L^{-1}, UVC = 14.79 mW cm^{-2}, UVA = 6.80 mW cm^{-2}, T = 25°C, pH = 7, reaction time = 120 min.

3. Results and Discussion

3.1. EDC Degradation by Cl/UV. Figure 1 displays the photolysis results for BPA, E2, and EE2 under UVA and UVC radiation. The results demonstrate different removal efficiencies for each compound. BPA presented lower degradation values than the assessed estrogens for both irradiation sources, with degradations between 18 and 70% for the UVA and UVC lamps after 120 min, respectively. This can be explained by the BPA's structure, since the more complex the molecule and the higher number of phenols, the more difficult it is to degrade [24]. All compounds displayed higher degradation rates in the UVC radiation treatment compared to UVA radiation. The maximum photolysis removal was of 89% for E2 and EE2 under UVC radiation in 120 min, while removal in the same conditions for UVA was of approximately 28%. Carvalho et al. [25] observed E2 removals (5 mg·L^{-1}) of 88% and 75%, respectively, by UVC and UVA photolysis. Similar results were obtained by Liu et al. [26], when evaluating E2 degradation, where 60% degradation using UVC radiation was obtained, while degradation under UVA radiation was negligible. Li Puma et al. [27]

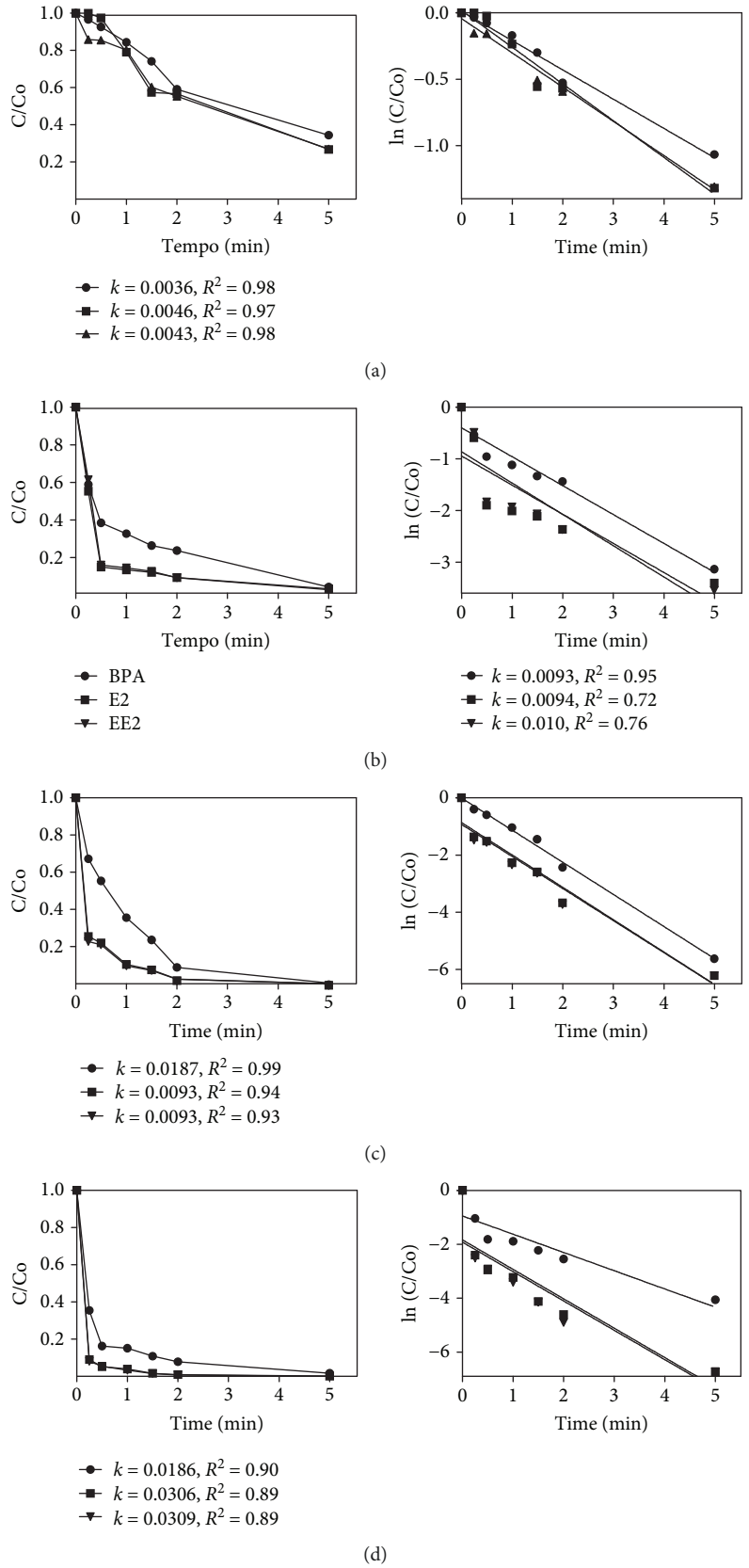

Figure 2: EDC photodegradation and respective pseudo-first-order rate constants (seconds) by Cl/UVC in ultrapure water under different chlorine concentrations: (a) 0.2 mg·L^{-1}; (b) 1 mg·L^{-1}; (c) 1.5 mg·L^{-1}; (d) 2.0 mg·L^{-1}. Initial EDC concentrations: 100 μg·L^{-1}, UVC irradiance = 14.79 mW cm^{-2}, T = 25°C, pH = 7, reaction time: 5 minutes.

demonstrated that 20 and 25% E2 and E22 were removed using UVA radiation after 180 minutes of treatment, increasing to 60% when using UVC radiation, for both compounds. Kondrakov et al. [28] observed 100% photolysis (UVC) of BPA (50 mg·L^{-1}) in 130 minutes. A study carried out by Frontistis et al. [29] reported that EE2 degradation follows the order UVC>UVA, with compound removal of 47 and 17% under UVC and UVA radiation, respectively, after 60 minutes. The direct photolysis process occurs based on photon absorption by compound molecules, where the maximum absorption wavelength of each compound and the wavelength of each lamp show a direct influence on compound degradation rates [30]. BPA displays a more intense UV absorption spectrum at around 230 nm [31], while E2 and EE2 show poor absorption from 290 to 350 nm [32]. The higher UV absorption of E2 and EE2 is observed in the 200-300 nm range, with absorption peaks at 230 and 280 nm, which explains the higher degradation rates for UVC radiation (200-280 nm) compared to UVA radiation (315-400 nm) [33].

EDC photodegradation under different chlorine (0.2 to 2 mg·L^{-1}) concentrations and their respective degradation kinetics are displayed in Figure 2. While UVC treatment showed pseudo-first-order rate in minutes, UVC/Cl was in seconds. The combination UVC with chlorine degrades EDCs 24 times faster than UVC alone. Increasing chlorine concentrations increased compound degradation, with approximately 99% EDC removal in 3 minutes at 2 mg·L^{-1} of chlorine. The only chlorine concentration where EDCs were not totally removed was 0.2 mg·L^{-1}, leading to 66, 74, and 75% removals for BPA, E2, and EE2, respectively. The compounds followed pseudo-first-order rate constants, with BPA, E2, and EE2 degradation rates of 1.86×10^{-2} s^{-1}, 3.06×10^{-2} s^{-1}, and $3.09 \times 1\,0^{-2}$ s^{-1}, respectively, at 2 mg·L^{-1} of chlorine (Figure 2(d)). The lowest degradation kinetics were 3.6×10^{-3} s^{-1}, 4.6×10^{-3} s^{-1}, and 4.3×10^{-3} s^{-1} for BPA, E2, and EE2, respectively, at 0.2 mg·L^{-1} of chlorine. The degradation kinetics for all tested chlorine concentrations followed the increasing order of EDCs: E2 ≅ EE2 > BPA. Similar results were observed for ibuprofen degradation with increased degradation, from 6.1×10^{-4} s^{-1} to 3.1×10^{-3} s^{-1}, with increasing chlorine concentrations (10 mM to 100 mM). However, the increase in kinetic constants is more gradual with increasing chlorine dosages, due to the scavenger effect of reactive species caused by excess free chlorine (Equation (2)) [14].

$$\begin{aligned}
&HO^{\cdot} + HOCl \longrightarrow ClO^{\cdot} + H_2O: \quad K_1 = 2.0 \times 10^9 M^{-1} s^{-1},\\
&HO^{\cdot} + OCl^{-} \longrightarrow ClO^{\cdot} + OH^{-}: \quad K_2 = 8.8 \times 10^9 M^{-1} s^{-1},\\
&Cl^{\cdot} + HOCl \longrightarrow H^{+} + Cl^{-} + ClO^{\cdot}: \quad K_3 = 3.0 \times 10^9 M^{-1} s^{-1},\\
&Cl^{\cdot} + OCl^{-} \longrightarrow Cl^{-} + ClO^{\cdot}: \quad K_4 = 8.2 \times 10^9 M^{-1} s^{-1}.
\end{aligned} \tag{2}$$

When the Cl/UVC process was tested in the WWTP matrix (Figure 3), degradation rates decreased significantly

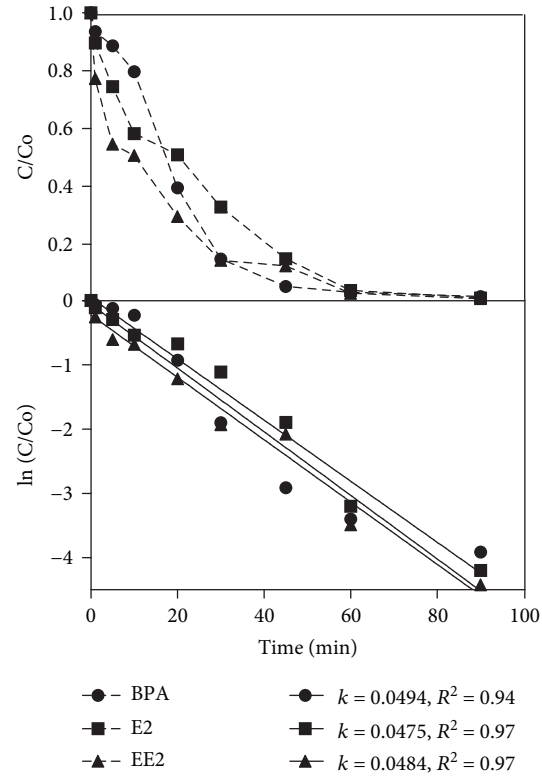

Figure 3: EDC photodegradation and respective pseudo-first-order rate constants (minutes) in WWTP by Cl/UVC. Initial EDC concentrations: 100 μg·L^{-1}, initial chlorine concentration = 2 mg·L^{-1}, UVC irradiance = 14.79 mW cm^{-2}, T = 25°C, pH = 7, reaction time: 90 minutes.

to 4.94×10^{-2} min^{-1}, 4.75×10^{-2} min^{-1}, and 4.84×10^{-2} min^{-1} for BPA, E2, and EE2, respectively. On the other hand, 45% TOC removal and disinfection of *E. coli* and total coliform bacteria (TCB) were observed at the end of the treatment (Table 1). Rott et al. [34] studied BPA (0.77 μg·L^{-1}) degradation in WWTP by the Cl/UV process, reaching 90% removal at 3 mg·L^{-1} chlorine. At the same initial chlorine concentrations, using residual water instead of ultrapure water, Wang et al. [35] observed a 30% decrease in the Cl/UV process reaction kinetics concerning carbamazepine degradation. This can be explained by the presence of different substances in the matrix (organic matter, carbonates, sulfates, chlorides, and nitrogen compounds; see Table 1), mainly carbonates, which act as scavenger agents for the radicals ($^{\cdot}$OH and Cl$^{\cdot}$) formed during the process [14, 36]. The reaction between carbonates (HCO_3^-) and the $^{\cdot}$OH/Cl$^{\cdot}$ radicals generates $CO_3^{\cdot-}$, which has a lower oxidant potential [14]. On the other hand, chloride ions (Cl$^-$) quickly react with HO$^{\cdot}$ and generate HOCl$^{\cdot-}$, which then dissociates into $^{\cdot}$OH and Cl$^{\cdot}$, thus neglecting the influence of Cl$^-$ in the Cl/UV process [37, 38]. Ammoniacal nitrogen can significantly reduce the efficiency of the Cl/UV process, since nitrogen can rapidly convert chlorine to chloramine, which displays a lower oxidation capacity than chlorine and decreases the formation of $^{\cdot}$OH radicals, since the free chlorine concentration is reduced [17]. In

Table 1: Physical-chemical and microbiological evaluation during EDC photodegradation in WWTP by Cl/UVC. Initial EDC concentrations: 100 $\mu g \cdot L^{-1}$, initial free chlorine concentration = 2 $mg \cdot L^{-1}$, UVC irradiance = 14.79 mW cm^{-2}, $T = 25^{\circ}C$, pH = 7, and reaction time: 90 minutes.

		$t = 0$	$t = 10$ min	$t = 45$ min	$t = 90$ min
Physical-chemical parameters ($mg \cdot L^{-1}$)	Total organic carbon (TOC)	21.31	17.37	13.92	11.83
	Chloride	53	54	55	57
	Nitrite	0.4	0.3	0.4	0.4
	Nitrate	1.2	1.3	1.2	1.2
	Phosphate	1.2	1.2	1.3	1.3
	Sulfate	21	25.8	32.4	31.1
Microbiological parameters (MPN/100 mL)	Total coliform bacteria	50.5×10^4	0	0	0
	Escherichia coli	17.05×10^4	0	0	0

addition, ammoniacal nitrogen can be oxidized into nitrite and nitrate by $^{\cdot}OH$, leading to higher consumption rates of the main oxidizing agent responsible for organic pollutant degradation [39].

3.2. Estrogenic Activity Reduction. Although target compound degradation is the specific AOP goal, it is also necessary to examine the estrogenic potency of the treated solutions, which may contain by-products with estrogenic activity. Thus, the *in vitro* YES assay was performed with the sample obtained in the best treatment condition determined herein, according to Figure 2 (2 $mg \cdot L^{-1}$ of chlorine and UVC irradiation). None of the samples were toxic to *Saccharomyces cerevisiae* cells under assay conditions. Estrogenic activity removal is shown in Figure 4. The YES assay determined an estrogenic activity of 265.0 ± 6.0 $\mu g \cdot L^{-1}$ of estradiol equivalents (EQ-E2) for the sample obtained at the initial treatment time ($t = 0$ min). All compounds were removed after 2 and 5 min of treatment (Figure 2(b)) and estrogenic activity was reduced to values below the method limit of quantification ($<26.0 \pm 12.0$ $ng \cdot L^{-1}$), demonstrating treatment efficiency concerning compound removal, with no formation of estrogenic by-products.

Wu et al. [40] have suggested that EDC estrogenic activity is reduced when these compounds undergo the chlorination process, since the phenolic ring is preferably oxidized by chlorine, consequently reducing estrogenic activity [41]. Rott et al. [34] observed an 80% decrease in total estrogenic activity for BPA and nonylphenols after treatment with Cl/UVC (3 $mg \cdot L^{-1}$ of chlorine) in residual water. Li et al. [17] observed the reduction of E2 estrogenic activity (500 $\mu g \cdot L^{-1}$) in ultrapure water and residual water by the Cl/UVC process and reported 97.2 and 78.3% reduction, respectively, after 5 minutes of treatment with 10 $mg \cdot L^{-1}$ of chlorine. In the same study, Δ9(11)-dehydro-estradiol, a DBP with greater affinity for the estrogen receptor than estrone (E1), was detected, which may act as an endocrine disruptor in the environment [17]. Thus, the decreased estrogenic activity observed herein demonstrates that cleavage or mineralization of the phenolic group, common to all three assessed compounds (BPA, E2, and EE2), is justified due to the facility of the aromatic ring to undergo electrophilic substitutions, making phenol highly susceptible to hydroxyl radical oxidation [2, 17, 42].

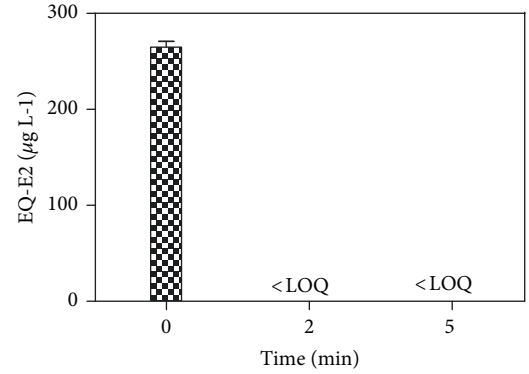

Figure 4: Estrogenic activity reduction using a combined Cl/UVC treatment containing BPA, E2, and EE2 in solution. Initial EDC concentrations: 100 $\mu g \cdot L^{-1}$, initial chlorine concentration = 2 $mg \cdot L^{-1}$, UVC irradiance = 14.79 mW cm^{-2}, reaction time = 5 min. <LOQ = below the limit of quantification.

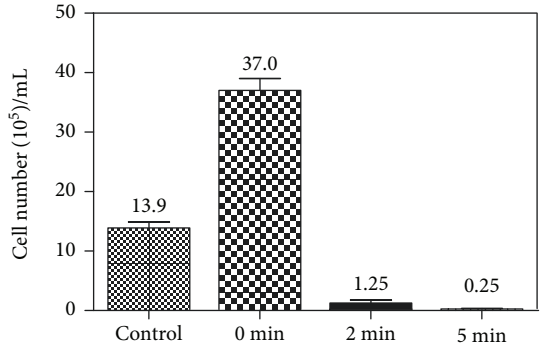

Figure 5: Chronic toxicity evaluation with algae Raphidocelis subcapitata using a combined Cl/UVC treatment containing different BPA, E2, and EE2. Initial EDC concentrations: 100 $g \cdot L^{-1}$, initial chlorine concentration = 2 $mg \cdot L^{-1}$, UVC irradiance = 14.79 mW cm^{-2}, reaction time = 5 min.

3.3. Toxicity Assessment. The results of the chronic toxicity test with the test organism *Raphidocelis subcapitata* in Cl/UV

photodegradation are displayed in Figure 5. Samples obtained during the photodegradation process at 2 and 5 min presented strong cell growth inhibition when compared to the control. The formation of intermediate compounds may have caused this toxicity [43], reaching 91% and 98% inhibition of exposed cell growth at the end of treatment. The initial time sample ($t = 0$ min) led to cell growth stimulus, explained by the presence of BPA, which acts as a stimulating agent concerning algae growth at the tested concentration [44]. However, this growth stimulus cannot be considered positive, since excess algae in natural environments can lead to eutrophication in aquatic environments. In addition, the chronic tests performed with *Ceriodaphnia dubia* demonstrated high organism sensitivity to the analyzed compounds, since EDC samples before the Cl/UVC treatment led to 60% mortality on the third test day and 100% on the fourth day (data not shown). This is explained by E2, EE2, and BPA synergistic effects, since these compounds when analyzed separately in other studies display EC_{50} values close to the EC_{50} value applied in the present study [45–47]. For posttreatment samples (2 and 5 min), organism mortality reached 100% on the third test day, which may indicate the formation of toxic chlorine compounds during UV treatment [48, 49]. Thus, a chronic reproduction analysis of this species could not be carried out, due to an acute effect in the initial trial.

Comparatively, under the same experimental Cl/UVC process conditions, according to Figures 2(d) and 4, the ?photocatalytic process completely removed all EDCs from the sample, as well as estrogenic activity, demonstrating that the formed DBP is responsible for the observed toxicity, mainly due to the formation of trihalomethanes (THMs) and halogenated acetic acids (HAAs), such as trichloromethane (TCM); 1,1,1-trichloropropanone (1,1,1-TCP); chloral hydrate (CH); 1,1-dichloro-2-propanone (1,1-DCP); dichloroacetic acid (DCAA); and trichloroacetic acid (TCAA) [14]. The combination of chlorine and UV promotes the formation of highly oxidizing species, which favors the opening of heterocyclic, aromatic, and phenolic rings, leading to higher DBP formation, unlike what occurs when the processes are carried out separately (photolysis and chlorination), due to nonopening of the rings [12]. Thus, the ?formation of the DBP occurs by halogenation of the aromatic ring with electrophilic substitution by chlorine in the *-ortho* and *-para* portions and eventual cleavage of the aromatic structure, in addition to radical $^{\cdot}OH/Cl^{\cdot}$ reactions in the phenolic groups [17, 50]. Chlorate formation may be a limiting factor for BPD formation, controlled by careful chlorine doses. In addition, BPD formation will depend on the competitive reaction between $Cl^{\cdot}$ and chlorides with dissolved organic matter (DOM) [48].

4. Conclusions

The Cl/UV process was efficient in removing endocrine disruptors BPA, E2, and EE2 and followed a pseudo-first-order kinetics. A chlorine concentration of 2 mg·L^{-1} under UVC radiation was the best condition to remove all EDCs in 180 seconds, at 1.86×10^{-2} s^{-1}, 3.06×10^{-2} s^{-1}, and 3.09×10^{-2} s^{-1} for BPA, E2, and EE2, respectively. The WWTP matrix adversely affected EDC removal, requiring 90 minutes for complete removal. However, the process was able to simultaneously promote the disinfection of *Escherichia coli* and total coliforms within 10 minutes of treatment. The *in vitro* YES assay demonstrated that by-products formed during the Cl/UVC process did not display an estrogenic activity. The results of the ecotoxicological tests indicate the importance of performing this assay in association with estrogenic activity analyses, since the Cl/UVC process was efficient in estrogenic activity removal, disinfection, and elimination but generated toxic DBP. Thus, WWTP treatments should be carefully assessed in order to monitor the formation of chlorinated by-products.

Data Availability

The YES estrogenic activity, toxicity, and photodegradation data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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