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**Two-phase ozonation for the removal of estrone, 17 β -estradiol and 17 α -ethinylestradiol
in water using ozone-loaded Decamethylcyclopentasiloxane**

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Shortened title: Removal of EDCs in wastewater with ozone-loaded D5.

Abstract

Ozonation has been proven effective for the removal of endocrine disrupting chemicals (EDCs) in water. However, conventional ozonation processes are still limited by ozone low solubility and stability in water. These limitations may be overcome by mixing a prior ozone-loaded non-polar solvent with the aqueous solution. This two-phase ozonation process combines Liquid-liquid extraction and Ozonation (LLO). Decamethylcyclopentasiloxane (D5) was chosen as the solvent to be charged with ozone for its non-toxicity, reusability and high ozone solubility. A concurrent LLO column reactor was designed for the simultaneous degradation of three endocrine disrupting chemicals (EDCs) in water: Estrone (E1), 17 β -estradiol (E2) and 17 α -estradiol (EE2). Results showed that 98% of EDCs were removed effectively from a solution initially concentrated at 1 mg L⁻¹. The efficacy of the degradation depends essentially of the column feeding flow rate, the ozone dose and the operating pH. The

generation of byproducts during EDCs degradation by LLO was also investigated in this study. For a complete removal of both EDCs and byproducts, the dose of ozone had to be doubled.

Keywords: Ozone, Liquid/Liquid extraction- Ozone (LLO), Endocrine disrupting chemicals (EDCs), Decamethylcyclopentasiloxane (D5).

1. Introduction

Recently, the effects of Endocrine Disrupting Chemicals (EDCs) on human health and the aquatic life have received considerable attention (Reis, Tabei et al. 2014). In both cases, exposure to EDCs has been associated with several diseases involving the reproductive (Xin, Susiarjo et al. ; Topper, Walker et al. 2015; Uzumcu and Zama 2016), immune (Bado-Nilles, Techer et al. 2014; Dietert 2015; Teitelbaum, Belpoggi et al. 2015; Heindel and Zoeller 2016) and neurological (Rebuli and Patisaul 2016; Rosenfeld 2016) systems. Furthermore, EDCs are ubiquitous since they have been detected in almost all water matrices including treated and untreated wastewaters (Hamid and Eskicioglu 2012; Nie, Qiang et al. 2012; Manickum and John 2014; Belhaj, Baccar et al. 2015; Komesli, Muz et al. 2015), surface waters (de Vlaming, Biales et al. 2007; Jiang, Yan et al. 2012; Esteban, Gorga et al. 2014), groundwaters (Campbell, Borglin et al. 2006; Shi, Hu et al. 2013), and even drinking waters (Li, Ying et al. 2010; Bach, Dauchy et al. 2012). Nevertheless, wastewater treatment plants (WWTPs) have been identified as the major source of EDCs (Ternes, Stumpf et al. 1999; Jackson and Sutton 2008; Adams, Quraishi et al. 2014).

In the field of water policy, EDCs are considered as emergent environmental contaminants of concern. The European Union Priority Substances Directive 2013/39/EU has introduced a “*watch list*” as a new mechanism for identifying priority substances in the future. One pharmaceutical (diclofenac) and two EDCs (E2 and EE2) were highlighted in the first *watch*

list. Although these three substances were not designated as priority substances so far, their regulation is not ruled out in the future (Committee 2013). E2 and EE2, in addition to E1, are very potent estrogenic compounds as shown by *in vitro* (Jarošová, Bláha et al. 2014) and *in vivo* (Folmar, Hemmer et al. 2000; Alvarez, Shappell et al. 2013) studies. Therefore, effective methods for their removal from contaminated aquatic environments are required. Key physicochemical properties of E1, E2 and EE2 are shown in **Table I**.

Ozone-based technologies have proved being very effective in treating wastewaters containing EDCs (Esplugas, Bila et al. 2007; Pereira, Postigo et al. 2011; Umar, Roddick et al. 2013). However, this approach shows two limitations that lead to an over-consumption of ozone (Ward, Tizaoui et al. 2005; Tizaoui, Bickley et al. 2008). Firstly, the solubility of ozone in water is relatively low (0.2 mg/L in water per mg/L in gas). Secondly, the presence of free radical scavengers in water, such as carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-), leads to a wasteful consumption of ozone.

Using prior ozone-loaded solvents was proved to be suitable to go beyond these limitations (Ward, Tizaoui et al. 2003). The general process involves contacting contaminated water with an immiscible ozone-enriched solvent. Contaminants undergo more rapid degradation and even complete removal (Ward, Tizaoui et al. 2005). Hence, after reaction, the solvent can be recovered and reused. Thereby, replacement costs may be minimized by careful handling of the material.

Previous works in this field have involved the fluorocarbon solvents FC40 and FC77 (Bhattacharyya, Van Dierdonck et al. 1995; Ward, Tizaoui et al. 2003; Gromadzka and Świetlik 2007). However, in more recent researches, Decamethylcyclopentasiloxane (D5) has been identified as a more efficient solvent for the LLO process comparing to fluorocarbons (Ward, Tizaoui et al. 2003). The solubility of ozone was demonstrated to be 10 times greater

in D5 than in water. Additionally, it was demonstrated that D5 is resistant to ozone attack. Any detectable detriment was identified after exposing the solvent to ozone for 100 hours (Ward, Tizaoui et al. 2003; Ward, Tizaoui et al. 2005). Hence, D5 is considered suitable for LLO due to its limited water solubility ($17 \mu\text{g L}^{-1}$) and its low toxicity (LD_{50} oral rats = 2 g kg^{-1}) (Brooke, Crookes et al. 2009), as shown in **Table II**. Moreover, it was proven that D5 showed essentially no acute long-term toxicity to aquatic organisms when tested at concentrations up to its water solubility limit. In addition, D5 is not classified as a carcinogenic, mutagenic, or reprotoxic compound (Brooke, Crookes et al. 2009).

The aim of this study is to evaluate the efficacy of the removal of E1, E2 and EE2 from aqueous solutions by two-phase ozonation, using D5 as the ozone-loaded solvent. The effects of different operating parameters (ozone dosage, D5/water volume ratio, flow rate and pH), on the efficacy of the process, were studied. However, the transformation of EDCs into hazardous byproducts can be a side concern for the LLO process. Therefore, the removal of EDCs byproducts during the degradation was also investigated.

2. Materials and methods

2.1. Chemicals

E1, E2 and EE2 with purity higher than 99% were purchased in powder form from Sigma–Aldrich (Dorset, UK). Stock solutions of 1 g L^{-1} in methanol were prepared for each EDC. They were stored in a freezer at $-21 \text{ }^{\circ}\text{C}$. Working solutions were prepared daily by an appropriate dilution of the stock solutions. D5 was purchased from Dow Corning, UK. Potassium indigo trisulfonate (Indigo) was purchased from Acrós Organics. HPLC grade acetonitrile and methanol were purchased from Fisher Scientific (Loughborough, UK). Ultra pure water was obtained from a Milli-Q water purification system (Millipore Q system, 18 MX cm, Bedford, MA, USA) for solution preparation and LC/MS grade water was obtained from Fisher Scientific (Loughborough, UK).

2.2. Experimental set-up for LLO

In this study, the applied LLO process is composed of three sections as shown in **Figure 1**:

i. Dissolution of ozone in solvent:

Ozone was generated using a BMT 803 Ozone Generator (BMT-Messtechnik, Germany) from compressed oxygen cylinder (BOC, UK). The concentration of ozone in gas, displayed in g m^{-3} NTP, was recorded by a BMT 963 Ozone Analyser (BMT-Messtechnik, Germany). The gas stream was then totally diverted through a diffuser into a Dreschel bottle (max capacity of 250 mL) containing 40 mL of D5, under 300 rpm magnetic stirring. During ozone dissolution process, the concentration of ozone in D5 was followed until it reached equilibrium. The same measurements were realized for different initial concentrations of ozone in gas (4 - 12 g m^{-3} NTP). The samples of ozone-loaded D5 were drawn into a graduated gastight syringe (Samco Interchangeable, 28.21 mm Di and max capacity of 60mL) from the bottom of the Dreschel bottle. The concentration of the dissolved ozone in D5 was determined by colorimetric indigo method (Nobbs and Tizaoui 2014).

ii. Liquid-liquid extraction column (reactor):

The syringe containing the O_3 -loaded D5 and another similar syringe supplied with EDCs solution were horizontally mounted on their respective syringe pumps (KDS-100-CE) and connected to the bottom of the contact column. The flow rate of each pump was accurately adjusted. The contact glass column (L 30 cm x Di 1cm) was vertically mounted and fed with the two syringes in a concurrent flow. The first section of the column (2 cm) was packed with fixed glass beads of 6 mm diameter in order to promote the mixing of the two phases.

iii. Analysis techniques:

The degradation rate of EDCs was determined along the column at different sampling points fitted with drain valves and situated around 2 cm, 9 cm, 13 cm and 30 cm from the bottom. Aliquots of 4 mL were collected in each point. Immediately after sampling, the reaction was stopped by flushing out the excess of ozone by air bubbling. Then, the aqueous phase was recovered by a simple gravity separation between D5 and water. The supernatant, was selectively extracted with a micropipette and analysed.

2.3. *Analytical methods*

2.3.1. Spectrophotometry: Quantification of dissolved O₃ in D5

The indigo method was used for the analysis of dissolved ozone in D5. Thereby, the concentration of dissolved ozone in D5 was determined indirectly. When contacted with the ozonated D5, indigo aqueous solution is rapidly discolored. Being nonmiscible phases, the difference in absorbance between an unreacted sample of indigo solution and that of an ozone-reacted sample of indigo solution is the basis for determining the concentration of dissolved ozone in D5. A volume of a solution of indigo at a known concentration was drawn into the gastight syringe containing a volume of ozone-loaded D5. The valve of the syringe was closed. The syringe was then shaken for 1 min to ensure good mixing of the two liquid phases. After reaction, the aqueous phase was collected and its absorbance was measured at 600 nm. All measurements of absorbance were made relative to a blank of deionized water. The concentration of indigo in water was determined based on a calibration curve priorly determined.

2.3.2. HPLC: Quantification of EDCs in water

The HPLC analysis was performed using an Agilent 1200 Series HPLC system, equipped with an on-line-degasser, a quaternary pump, an autosampler and a thermostated column compartment. Reverse phase chromatographic separation of the EDCs was achieved by a

Hypersil GOLD C18 column (150 x 4.6 mm x 5 μ m) (Thermo Scientific, Hertfordshire, UK) that was thermostatically held at 30 °C. Agilent ChemStation software was used for the control of the HPLC system and data acquisition.

Both Fluorescence detector (model G1321A, Agilent, USA) and Diode Array detector (model G2180BA, Agilent, USA) were used for the detection of EDCs. Fluorescence excitation and emission wavelengths were set at λ_{ex} 200 nm and λ_{em} 315 nm for the detection of both E2 and EE2 while the diode array detector was fixed at λ_{DAD} 200 nm for E1 detection.

The total run time was 6 min and the injection volume was 20 μ L using a mobile phase flow rate of 1 mL min^{-1} . EDCs in the aqueous phase were quantified using external calibration methods and their identification was based on the EDCs' respective reference standard retention time ($\text{RT}_{\text{E2}} = 4.05$ min, $\text{RT}_{\text{EE2}} = 4.82$ min and $\text{RT}_{\text{E1}} = 5.05$) min when eluted with 50:50 (v/v) acetonitrile: Milli-Q water. Calibration curves of E1, E2 and EE2 were generated by serial dilutions of the three EDC stock solutions using Milli-Q water and secondary treated wastewater to cover a concentration range of 1 – 10,000 $\mu\text{g L}^{-1}$. The standard curves were calculated by linear regression of the plots concentration versus peak area. The resulting calibration curves were linear with r^2 values > 0.998 . Checks of the calibration curves have also been carried out routinely.

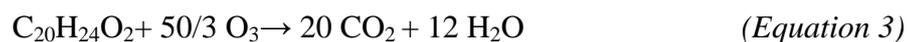
2.3.3. LC-MS/MS: Detection of byproducts in water

Tandem mass spectrometer (Agilent, Triple Quad LC/MS/MS, 6410) was used to detect the LLO process byproducts. The compounds were identified by their (m/z) ratios. Detection was performed with an electrospray (ESI) interface in the negative ionization (NI) mode. Source parameters were set at a gas temperature of 300°C, a gas flow of 10.0 L/min and a nebulizer pressure of 50.0 psi. Scan mode was used to identify the byproducts and Single ion monitoring (SIM) mode was used to quantify them in samples. Instrument control, data

acquisition and evaluation were realized by means of Agilent MassHunter software. Optimum mass spectrometric parameters were firstly set for the detection of EDCs. The results are shown in **Table III**.

2.4. LLO method

Equations 1, 2 and 3 give the overall ozonolysis reaction of E1, E2 and EE2. At the stoichiometric O₃/EDCs molar ratio, 47 moles of ozone are suggested for the complete mineralization of 3 moles of EDCs (1 mol E1 + 1 mol E2 + 1 mol EE2).



In a typical ozonation process, the required dose of ozone is directly bubbled through the solution of EDCs in water. In LLO, ozone is priorly dissolved in a solvent that is then mixed with EDCs aqueous solution. For the aim of this study, a working solution was prepared with Milli-Q water spiked with EDCs at a concentration of 1 mg L⁻¹ of E1, E2 and EE2. During the treatment of this solution by LLO, the effect of EDCs/ozone molar ratio, total flow rate, D5/water volume ratio and pH were studied.

The effect of residence time of ozone and EDCs in the contact column on the process efficacy was studied by varying the total flow rate between 0.5 mL min⁻¹ and 4 mL min⁻¹. D5/water volume ratio (1:1) and stoichiometric O₃/EDCs molar ratio were both kept constant for a solution of EDCs of 1 mg L⁻¹.

The effect of ozone dose on the removal of EDCs in water was investigated for a solution of EDCs of 1 mg L⁻¹ at pH 6 and at ambient temperature (~ 25 °C). Solvent/water volume ratio was kept constant (1:1) by feeding up the column under the same flow rates (1 mL min⁻¹,

each). At zero dose of ozone, the distribution of EDCs in D5 could be determined in the D5/water system. The distribution coefficient (K_D) of an EDC between the organic phase (D5) and the aqueous phase at equilibrium could be determined by liquid-liquid extraction (LLO with zero dose of Ozone), according to **Equation 4**.

$$K_D = \frac{C_{org_eq}}{C_{aq_eq}} = \frac{1}{Rv} \left(\frac{C_{aq0}}{C_{aq_eq}} - 1 \right) \quad (\text{Equation 4})$$

Where: K_D is the distribution coefficient of a contaminant;

R_v is the volume ratio between D5 and water;

C_{aq0} is the initial concentrations of a contaminant in water;

C_{aq_eq} is the final concentrations of a contaminant in water at equilibrium;

C_{org_eq} is the final concentration of a contaminant in D5 at equilibrium.

Then, ozone concentration in the solvent was changed to provide the following O_3 /EDCs stoichiometric molar ratios: 0.25, 0.5, 1, 2, 4 and 8.

In a continuous process as ours, setting a D5/ water volume ratio could be carried out by setting this ratio between the flow rate of D5 and the flow rate of water. A special attention was accorded to keep the stoichiometric O_3 /EDCs molar ratio constant for all applied D5/water volume ratios. For this aim, the concentration of ozone in D5 was varied anti-proportionally to the variation of the flow rate of ozone-loaded D5. The concentration of EDCs solution was kept constant.

The effect of pH on the degradation of EDCs was investigated for pH 2, 6 and 12 by adjusting the initial pH of the EDCs solution (1 mg L^{-1}). For this aim, solutions of HCl (1 M) and NaOH (1 M) were used.

3. Results and discussion

3.1. *Ozone solubility in D5*

The solubility of ozone in water and in non-polar solvents has been widely investigated in the literature (Ward, Tizaoui et al. 2003; Biń 2004; Biń 2006). In the present study, ozone solubility (S_{O_3}) was calculated in terms of relative concentration between ozone concentration in gas (C_g) and ozone concentration in solvent (C_{D5}) according to **Equation 5**.

$$S_{O_3} = C_{O_3}(D5) / C_{O_3}(g) \quad (\text{Equation 5})$$

Where: S_{O_3} is the solubility of ozone in D5 (mg L^{-1} D5 per mg L^{-1} gas).

During bubbling ozone in D5, the concentration of dissolved ozone in D5 was determined as a function of time by indigo method as described in **Section (2.3.1)**. The same experiment was conducted for different concentrations of ozone in gas in the range of 4 - 12 g Nm^{-3} . The results are presented in **Figure 2**.

Figure 2.a shows that the dissolution of ozone in D5 was very rapid with equilibrium being reached within 5 min. **Figure 2.b** shows that the concentration of ozone in D5 at equilibrium increases linearly with increasing the concentration of ozone in the gas phase. Henry's law predicts that ozone solubility in D5 should increase linearly with ozone concentration in the gas phase (Ward, Tizaoui et al. 2003). The linearity of the data indicates that ozone solubility in D5 is 1.769 mg L^{-1} in D5 per mg L^{-1} in gas at $25 \text{ }^\circ\text{C}$. A similar value was found in the literature ($1.71 \pm 0.09 \text{ mg L}^{-1}$ in D5 per mg L^{-1} in gas) (Nobbs and Tizaoui 2014).

Based on the obtained calibration curve, the concentration of dissolved ozone in D5 could be set by simply controlling the concentration of O_3 in the gas phase.

3.2. *Effect of the Total Flow Rate*

The effect of the total flow rate in LLO was studied to optimise both the D5/water emulsion and the contact time between O₃ and EDCs. The O₃/EDCs molar ratio was set at the stoichiometric value for an initial concentration of 1 mg L⁻¹ of EDCs in water at pH 6. The total flow rate (feeding flow rate of D5 + feeding flow rate of water) was varied between 1 mL min⁻¹ and 8 mL min⁻¹. For all experiments, D5/ water volume ratio was kept constant (1:1).

During their passage through the mixing glass beads, D5 and water break up into small droplets, greatly increasing the total surface area of each phase. Rapid flow rates increase the contact surface area between the two phases which would enhance EDCs mass transfer into O₃-enriched D5. Low flow rates increase the residence time of O₃ and EDCs in the contact column. In theory, long residence time increases the removal of the contaminants. Therefore, the optimal total flow rate should conciliate between a good mass transfer of EDCs into O₃-enriched D5 and a sufficient contact time between O₃ and EDCs.

Figure 3 shows that the removal of EDCs by LLO is favored by relatively low flow rates with a maximum efficacy at a total flow rate of 2 mL min⁻¹ (velocity based on empty column = 1.53 m/h). When the total flow rate increases from 2 to 8 mL min⁻¹, the average removal rates of EDCs decrease respectively from 97% to 73%. A total flow rate of 2 mL min⁻¹ (i.e. each phase has a flow rate of 1 mL min⁻¹) was considered suitable for the designed LLO process and was set for the rest of the experiments.

3.3. *Effect of ozone / EDCs molar ratio*

In order to determine the dose of ozone required for a complete degradation of EDCs, two series of ozonation were carried out for O₃/EDCs molar ratios between 0.25 and 8 times the stoichiometric value. The dissolution of ozone was performed in D5 (LLO) in the first series of experiments and in water (conventional ozonation) in the second. For LLO, a working

solution was prepared at a concentration of 1 mg L^{-1} . D5/ water volume ratio was kept constant (1:1) in all experiments. For the conventional ozonation, all the calculations were done on the basis of a concentration of 1 mg L^{-1} of EDCs in the total volume of ozone-enriched water mixed with EDCs aqueous solution. All operating conditions were kept constant through all the investigation (total flow rate 2 mL min^{-1} and pH 6 at 25°C).

Figure 4 compares the degradation rates of EDCs achieved by conventional ozonation and LLO, as a function of O_3/EDCs molar ratio. In the two series of experiments, the degradation of EDCs in water increases proportionally to O_3/EDCs molar ratio but with different rates. The quasi-total removal of EDCs ($98 \pm 5 \%$) is achieved at the stoichiometric O_3/EDCs molar ratio in LLO while it requires a double dose of ozone in the conventional ozonation process. Thus, for (O_3 / EDCs) molar ratios that are 4 and 2 times lower than the stoichiometric value, EDCs removal rates are higher by LLO than the theoretically predicted values (25% and 50%, respectively). This suggests that LLO treatment enhances the ozonation process by combining other mechanisms with oxidation. As evoked by Ward et al. (Ward, Tizaoui et al. 2004), there are two key factors that can enhance or hinder LLO process: the interfacial mass transfer and the effectiveness of promoted chemical reaction pathways. In a preliminary work, the mass transfer of the EDCs into D5 was investigated and their distribution coefficients were determined (Ben Fredj, Nobbs et al. 2015). The results showed EDCs to distribute into D5 with different distribution coefficients: E1 ($K_{E1}=2.66$) highly distributed in D5, followed by EE2 ($K_{EE2}=1.67$) and by E2 ($K_{E2}=0.61$) at pH 6 and at 20°C (Ben Fredj, Nobbs et al. 2015).

Accordingly, a correlation between the degradation percentage and the interfacial mass transfer of EDCs can be established basing on the results of LLO in **Figure 4**. In fact, it is noticed that more an EDC distributes in ozone-loaded D5, more effective its degradation by LLO is, comparing to the conventional ozonation. For O_3/EDCs molar ratio that is at 0.5 time the stoichiometric value, the degradation of EDCs increases in LLO comparing to the

conventional ozonation by 31.47% for E1, 26.10% for EE2 and 15.07% for E2. This trend could be correlated with the distribution of the EDCs into ozone-loaded D5, expressed by: ($K_{E1} = 2.66$) > ($K_{EE2} = 1.67$) > ($K_{E2} = 0.61$). Accordingly, it is expected that ozonation reactions would occur majorly in the organic phase constituted with D5. In this case, ozone would react directly under its molecular form (Bhattacharyya, Van Dierdonck et al. 1995). According to the literature, the oxidation of contaminants can occur either in organic phase (**Figure 5.a**) or in the interface between organic and aqueous phases (**Figure 5.b**) (Ward, Tizaoui et al. 2003). The reaction site has been correlated with the distribution of ozone and the contaminants between the two phases. Contaminants with low polarity and high K_D values (e.i. E1 and EE2) easily diffuse into the solvent where oxidation is expected to take place (Bhattacharyya, Van Dierdonck et al. 1995). For contaminants characterized by a low K_D value (e.i. E2), the oxidation reaction can take place in the interface solvent/ water. In this case, the ozone-enriched solvent constitutes only an ozone reservoir (Gromadzka and Świetlik 2007). During the process, ozone is gradually released from the solvent into the water.

3.4. *Effect of D5/ Water volume ratio*

In LLO, the required volume of solvent is a key parameter to define the cost effectiveness of the process. Ideally, a maximum removal of EDCs in water is obtained with the minimum volume of D5. The effect of D5/water volume ratio was investigated in the range of (1:5 - 2:1). The variation of D5/water volume ratio was monitored by varying simultaneously the flow rates of O_3 -loaded D5 and of EDCs-spiked water while keeping the total flow rate constant (2 mL min^{-1}). A special attention was given to set the concentration of dissolved O_3 in D5 in order to keep the O_3 / EDCs molar ratio always at the stoichiometric value.

Figure 6 shows that, for a stoichiometric O₃/EDCs molar ratio, the removal of EDCs varied slightly in function of applied D5/water volume ratios. The trend of the removal of EDCs by LLO seems not to be influenced by the employed volume of D5 ($\pm 8\%$). Based on the results of **Section (3.3)**, O₃/ EDCs molar ratio is proven to be more influential than D5/water volume ratio in LLO. Therefore, the required dose of ozone for the total removal of EDCs can be dissolved in a very small volume of D5 (e.g. D/water volume ratio of (1:5) without noticeably altering the effectiveness of the EDCs' removal. Economically, this can lead to a higher efficiency of the process.

3.5. *Effect of pH*

Two main oxidants (molecular O₃ and ·OH radical) may act in ozonation reactions, depending on the solution pH (Bila, Montalvão et al. 2007). The formation of ·OH radicals, a less selective oxidant, is favored by increasing the pH. At low pH, oxidation is expected to occur largely through molecular O₃, which reacts with specific bonds of high electronic density (i.e. double bonds) and specific functional groups (Hoigné and Bader 1976). O₃ reacts selectively with molecules containing functional groups such as amino groups, activated aromatic systems (i.e., phenolics) and double bonds (Harrison Joseph F. and Peggy 2000; von Gunten 2003). Phenolic selectivity of O₃ is specifically interesting in the case of E1, E2 and EE2, where phenolic rings are responsible of their estrogenicity (Bennett 2003). Ozone reacts highly with phenolic moieties of EDCs with second order rate constants of $1.5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for E1, $2.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for E2 and $1.8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for EE2 and with their corresponding phenolates ($4.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, $3.7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ and $3.7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) (Huber, Ternes et al. 2004; Deborde, Rabouan et al. 2005). Hence, in the two-phase ozonation system, EDCs with high K_D would react with molecular ozone directly in the solvent which is mostly expected for the case of E1, E2 and EE2. This means that in LLO, the oxidation of EDCs is principally

governed by molecular ozone (O_3). If $\cdot OH$ contributed in EDCs oxidation, the removal rates would be highest at pH12 where hydroxyl radicals are predominant.

The dissociation of the phenolic moieties into phenolate moieties is pH-dependant. Hence, the degree of molecular dissociation of the EDCs defines their distribution into ozone-loaded D5. Based on the principle “like dissolves like”, the non-dissociated EDCs ($pH < 9.5$) have higher affinities to non-polar organic phase, so they are more distributed to the organic solvent (ozone-loaded D5). On the opposite, when EDCs become ionised ($pH > pK_a$), they preferentially distribute to the aqueous phase (Ben Fredj, Nobbs et al. 2015). Therefore, increasing the pH enhances the reactivity of ozone towards phenolate moieties of EDCs in one hand and hinders their distribution into ozone-loaded D5 in the other hand. Studying the effect of pH on the removal of EDCs can lead to a better understanding of the pH-driven effects on LLO. Experiments were conducted on a solution of EDCs concentrated at 1 mg L^{-1} . The ozone dose was set at the stoichiometric O_3/EDCs molar ratio. The D5/ water volume ratio was set at 1:5. The aqueous solution of EDCs was set at different initial pH values (2, 6 and 12) and the results are shown in **Figure 7**.

Figure 7 shows similar trends of the removal rates of the three EDCs by LLO as function of pH. As pH increased from 2 to 6, the removal rates of all EDCs remained in the range between 65% and 95%. A further increase of pH to 12 resulted in a significant drop of the removal of EDCs to a range between 15% and 55%. At pH 12, EDCs are mostly found in the dissociated molecular form. Eventhough ozone is supposed to react more with phenolate moieties, the removal of EDCs decreases at pH12. This could be explained by a low mass transfer of EDCs into ozone-loaded D5. In our previous work (Ben Fredj, Nobbs et al. 2015), we found that the distribution coefficients of the three EDCs remain constant at pH values between pH2 and pH9 but drops drastically for higher pHs. At pH12, the distribution coefficients of E1, E2 and EE2 were found to approach zero. It was noticed that the trend of

the removal of EDCs by LLO is similar to that of their distribution coefficients into D5 as function of pH. Based on this, the LLO process seems to be mainly driven by the transfer of EDCs into the ozone-loaded D5. More the EDCs distribute into the organic solvent, better their removal from the aqueous phase is. Acid to neutral pHs are shown to be more suitable for the removal of EDCs by LLO.

3.6. EDCs byproducts

The degradation of EDCs in water (1 mg L⁻¹ at pH6) was performed at the optimal conditions defined previously: a total flow rate of 2 mL min⁻¹, a stoichiometric O₃/EDCs molar ratio and a D5/water volume ratio of (1:5) at 25°C. **Figure 8** shows the removal of EDCs along the contact column during LLO. All EDCs were removed very rapidly and an overall removal of the EDCs of 86±2% was obtained at the first sampling point of the column (2.16 cm), which corresponded to 51 s of reaction time. At the top of the column, the final removal of the three EDCs was around 93±3%. This suggests that the removal of EDCs take place mainly in the inlet section of the column.

E1, E2 and EE2 have shown to be efficiently removed from water by LLO (Stoichiometric O₃/EDCs molar ratio, D5/water volume ratio (1:5), total flowrate (2 mL min⁻¹) and pH 6. However, byproducts have been detected by means of HPLC - MS / MS by ESI (-). The (m/z) ratios of the major peaks identified before the degradation of E1 (m/z 269), E2 (m/z 271) and EE2 (m/z 295) by LLO are depicted in **Figure 9.a**. The main byproducts of the degraded EDCs are: BPE1 (m/z 285), BPE2 (m/z 287) and BPEE2 (m/z 311). The simultaneous screening of EDCs and their byproducts in water after LLO is presented in **Figure 10.b**. Based on their nominal (m/z) values, the three EDCs are believed to follow the same degradation pathway. In fact, all the generated byproducts differ in one oxygen mass unit comparing to their respective parent molecules. A second phenolic group is added to the molecule of each EDC. This new (—OH) group can be fixed on the molecular structure of

each EDC at different sites. The obtained (m/z) values may be related to four molecular structures for each byproduct (Pereira, Postigo et al. 2011). The four possibilities are presented in **Figure 10**.

The obtained byproducts may confer a certain estrogenic activity to the final effluent. In the literature, it was reported that the estrogenic activity of E1, E2 and EE2 during ozonation process was completely removed by adding over stoichiometric doses of ozone (Hashimoto, Takahashi et al. 2006). Other authors suggested that a small modification of the phenolic ring in the molecular structure of EDCs could be effective in reducing their estrogenicity (Huber, Ternes et al. 2004). However, investigations about the estrogenicity of disinfected waters containing E2 showed that a certain estrogenic activity remained after water ozonation due to the newly formed oxidation byproducts (Alum, Yoon et al. 2004; Bila, Montalvão et al. 2007; Guedes Maniero, Maia Bila et al. 2008) even after the application of relatively high doses of ozone (Bila, Montalvão et al. 2007).

In the present study, the dose of ozone that guarantees the complete removal of both EDCs and their byproducts throughout the LLO process was investigated. For a solution of EDCs initially concentrated at 1 mg L^{-1} , different O_3/EDCs molar ratios were employed for the degradation experiments by LLO. For each dose, the amount of the generated byproducts was determined by LC-MS/MS by ESI (-) under SIM mode. The peak area relative to each EDC and to each byproduct was measured. **Figure 11** shows that all the byproducts of the three EDCs present a maximum concentration in the final effluent when an O_3/EDCs molar ratio of 0.06 time the stoichiometric value was used. An overall removal of 90% of this amount was realized with twice the stoichiometric dose of O_3 . It was noticed that an O_3/EDCs molar ratio twice the stoichiometric value enabled the removal of both EDCs (98 %) and their byproducts (90%) in the final effluent.

4. Conclusion

The aim of this study was to assess the efficiency of the removal of selected potent EDCs (E1, E2 and EE2) from water, using ozone as a powerful oxidant. A two-phase ozonation system was investigated using D5 as ozone-loaded solvent. The effect of different operating parameters on the process efficacy was studied.

For a solution of EDCs initially concentrated at 1 mg L^{-1} , working under optimal operating conditions defined by a total flow rate of 2 mL min^{-1} , a D5/water volume ratio of (1:5) and an ozone/EDCs molar ratio of twice the stoichiometric value leads to the removal of more than 98% of all EDCs. Experiments also showed that under the same conditions, more than 90% of generated oxidation byproducts were also removed in water. However, further investigation is needed to evaluate the efficacy of the process in terms of estrogenicity removal. For this aim, the estrogenic activity of the final effluent could be evaluated by YES (Yeast Estrogen Screen) tests.

5. Acknowledgment

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Table I. Physic-chemical properties of E1, E2 and EE2 (Lai 2000; Schäfer 2003; Kimura, Toshima et al. 2004; Kwon, Liljestrand et al. 2006; You, Zhao et al. 2009).

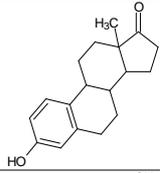
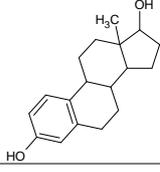
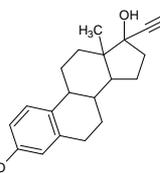
| Compound name | Molecular formula | CAS no. | Structure | MW (g mol ⁻¹) | Solubility in water (mg L ⁻¹) | pKa | Log K _{ow} |
|----------------------------|------------------------------------------------|---------|------------------------------------------------------------------------------------|---------------------------|-------------------------------------------|-------|---------------------|
| Estrone (E1) | C ₁₈ H ₂₂ O ₂ | 53-16-7 |  | 270 | 13 | 10.34 | 3.13 |
| 17β-estradiol (E2) | C ₁₈ H ₂₄ O ₂ | 50-28-2 |  | 272 | 3.6 | 10.23 | 4.01 |
| 17α-ethynylestradiol (EE2) | C ₂₀ H ₂₄ O ₂ | 57-63-6 |  | 296 | 4.8 | 10.25 | 3.67 |

Table II. General physico-chemical properties of decamethylcyclopentasiloxane

(D5) (Brooke, Crookes et al. 2009).

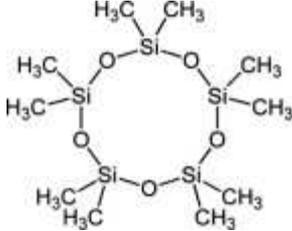
| Molecular Structure | Property | Value |
|-----------------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------------|
|  | Molecular formula | C ₁₀ H ₃₀ O ₅ Si ₅ |
| | CAS No. | 541-02-6 |
| | Density (kg m ⁻³) | 0.955 at 20 °C |
| | Viscosity (cp) | 3.9 at 25 °C |
| | Molar mass (g mol ⁻¹) | 370.77 |
| | Flash point (°C) | 70 |
| | Water solubility (µg L ⁻¹) | 17 at 25 °C |
| | Vapour pressure (Pa) | 11 at 20 °C |
| | Interfacial tension with water (mN m ⁻¹) | 18.9 at 20 °C |

Table III. Chromatographic parameters for E1, E2 and EE2 analysis by LC-MS/MS.

| EDC | Product ions | Fragmentor voltage | Quantitative (m/z) transitions | Collision energy | Confirmative (m/z) transitions | Collision energy |
|-----|--------------|--------------------|--------------------------------|------------------|--------------------------------|------------------|
| E1 | m/z 169 | 160 V | 269 → 145 | 40 V | 269 → 143 | 60 V |
| E2 | m/z 271 | 160 V | 271 → 145 | 40 V | 271 → 183 | 40 V |
| EE2 | m/z 295 | 160 V | 295 → 145 | 40 V | 295 → 159 | 35 V |

Figures

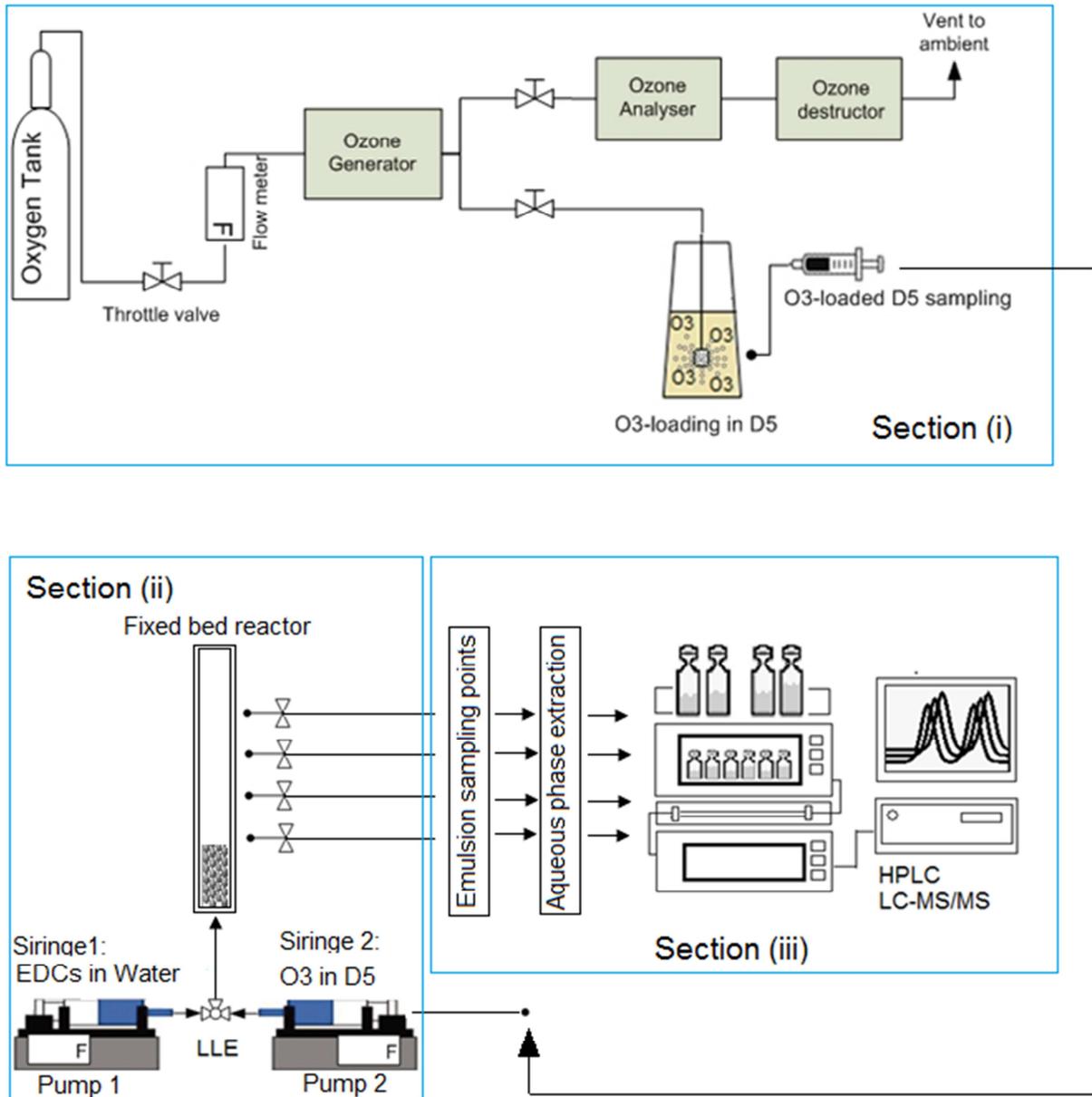


Figure 1. Diagram of two-phase ozonation process for the removal of E1, E2 and EE2 in water, using Decamethylcyclopentasiloxane as solvent.

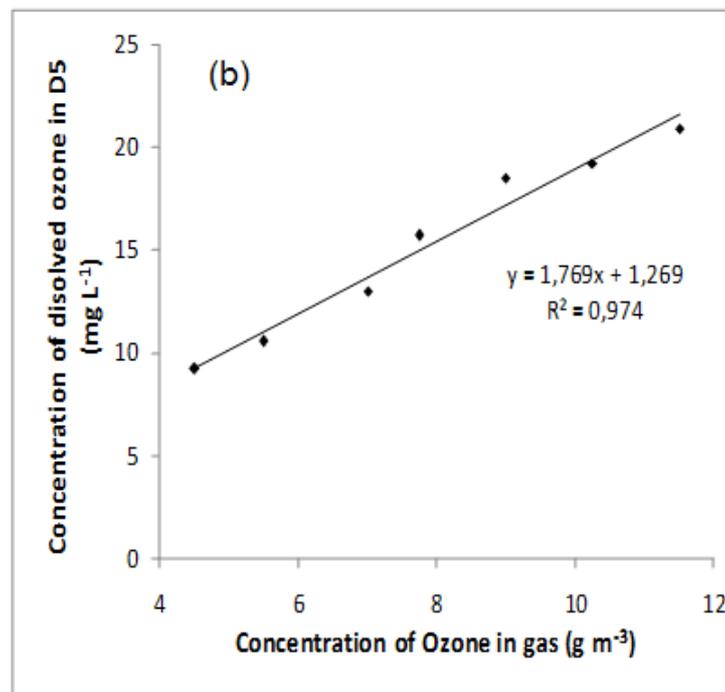
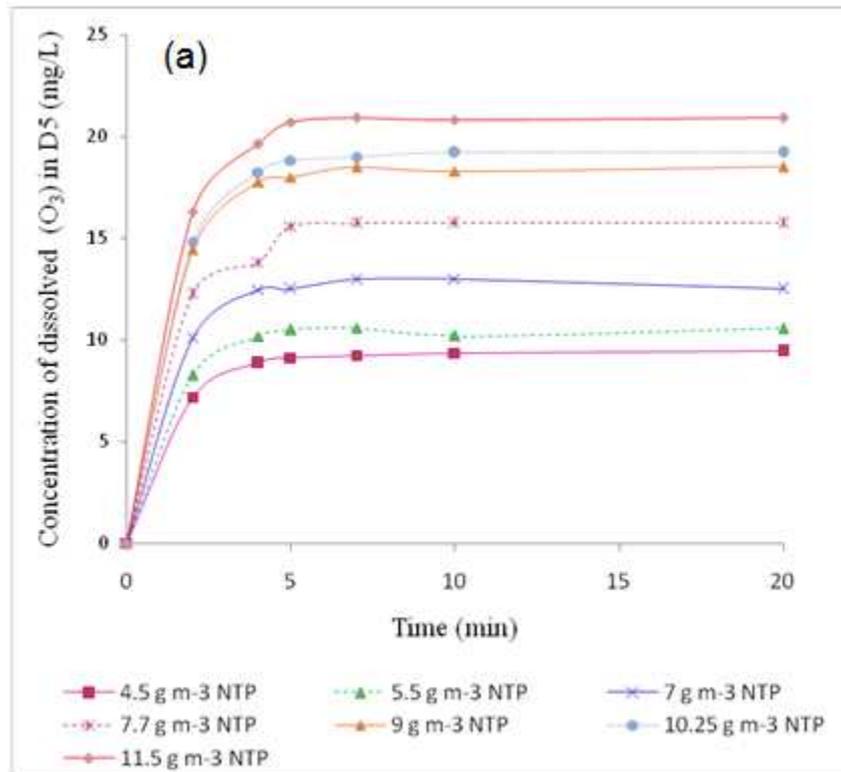


Figure 2. Concentration of dissolved ozone in D5 as a function of the concentration of ozone in gas (4 - 12 g m⁻³ NTP) at 25°C (a) as a function of dissolution time and (b) at equilibrium.

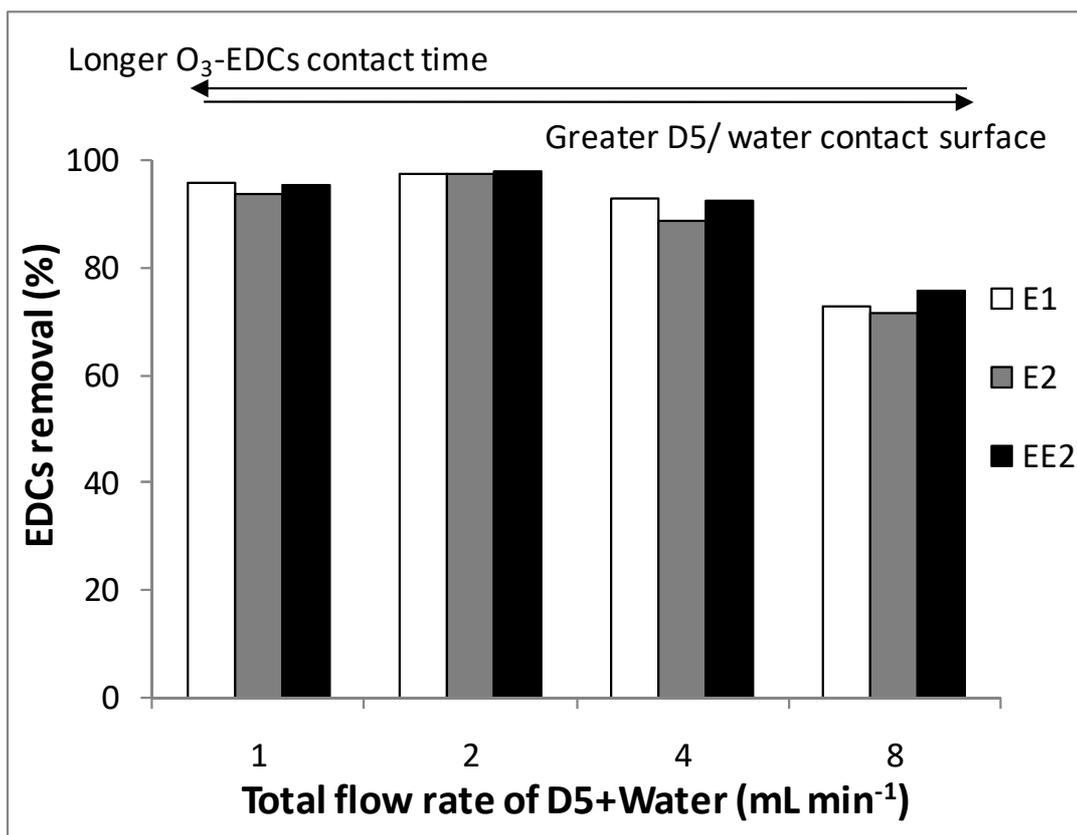


Figure 3. Effect of the total flow rate along the LLO contact column on EDCs removal. Initial concentration of EDCs (1 mg L^{-1}), stoichiometric O_3/EDCs molar ratio, D5/water volume ratio (1:1) and pH 6 at 25°C .

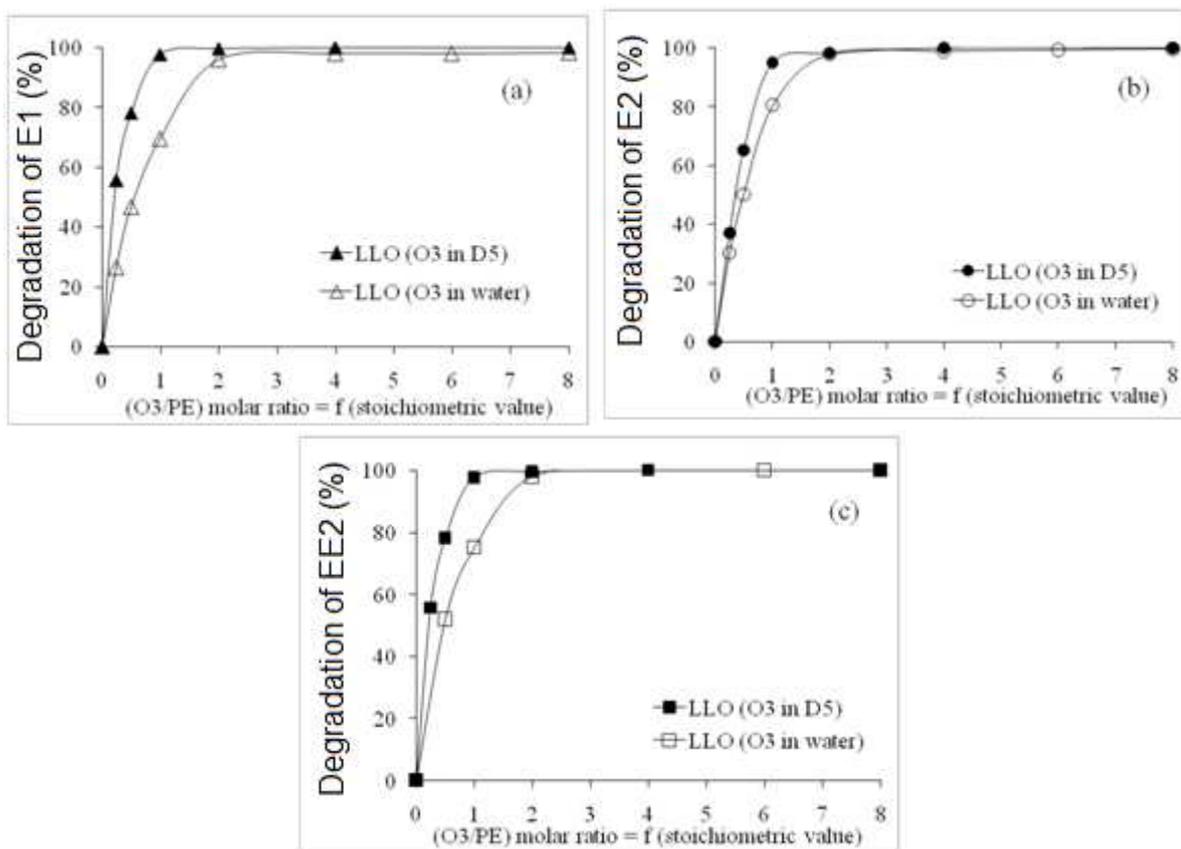


Figure 4. Effect of O₃/EDCs molar ratio on the degradation rates of (a) E1, (b) E2 and (c) EE2 by LLO (O₃ in D5) and (O₃ in water). Initial concentration of EDCs (1 mg L⁻¹), D5/water volume ratio (1:1), total flow rate 2 mL min⁻¹, pH6 at 25°C.

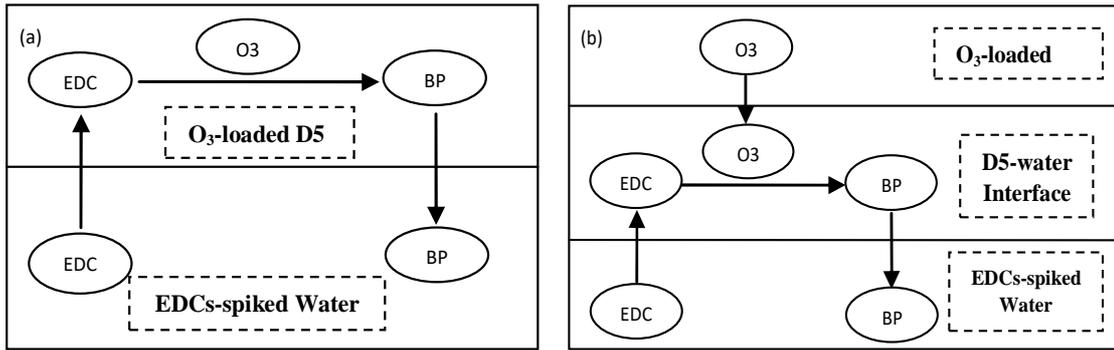


Figure 5. Oxidation sites of EDCs in LLO (Bhattacharyya, Van Dierdonck et al. 1995).

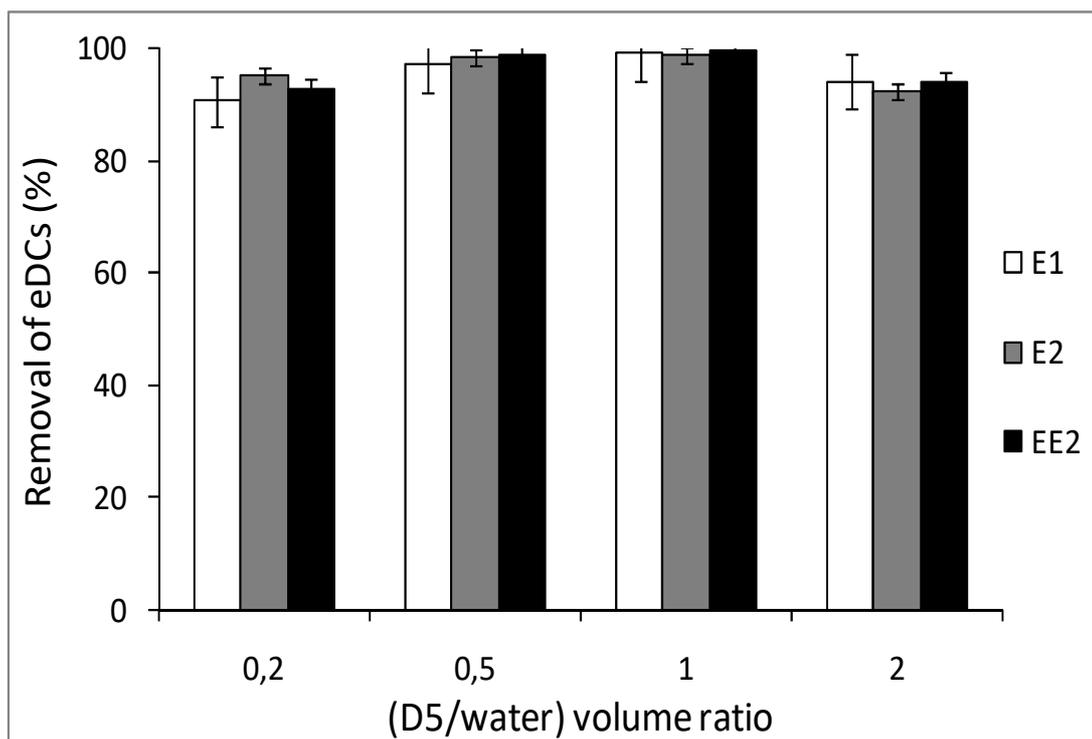


Figure 6. Effect of D5/water volume ratio (1:5 – 2:1) on the removal of EDCs by LLO. Initial concentration of EDCs (1 mg L^{-1}), stoichiometric O_3/EDCs molar ratio, total flow rate (2 mL min^{-1}) and pH 6 at $25 \text{ }^\circ\text{C}$.

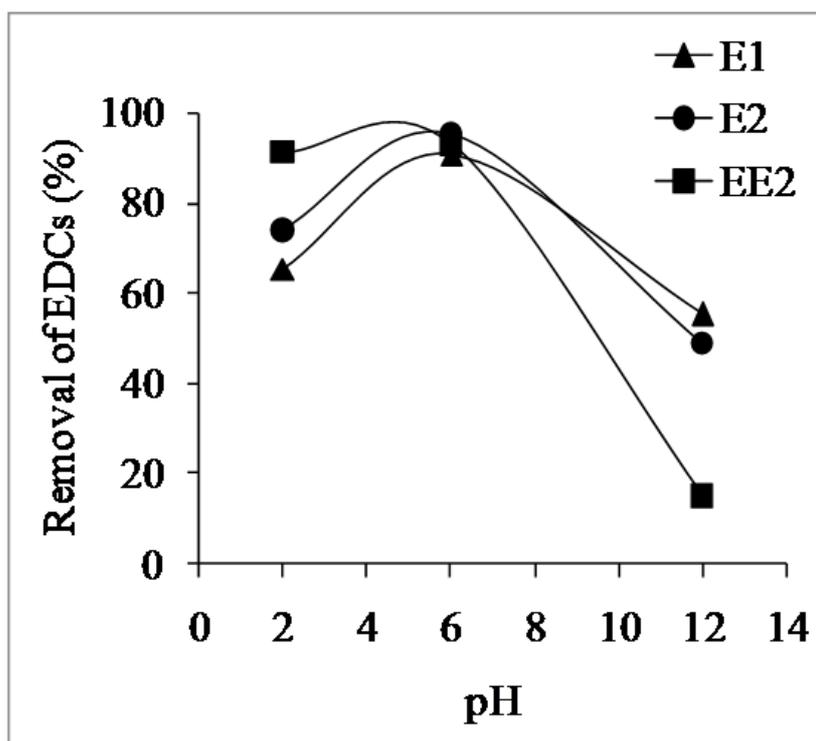


Figure 7. Effect of aqueous pH on the removal of EDCs by LLO at stoichiometric O_3 /EDCs molar ratio. Initial concentration of EDCs (1 mg L^{-1}), D5/water volume ratio (1:5) and total flow rate (2 mL min^{-1}).

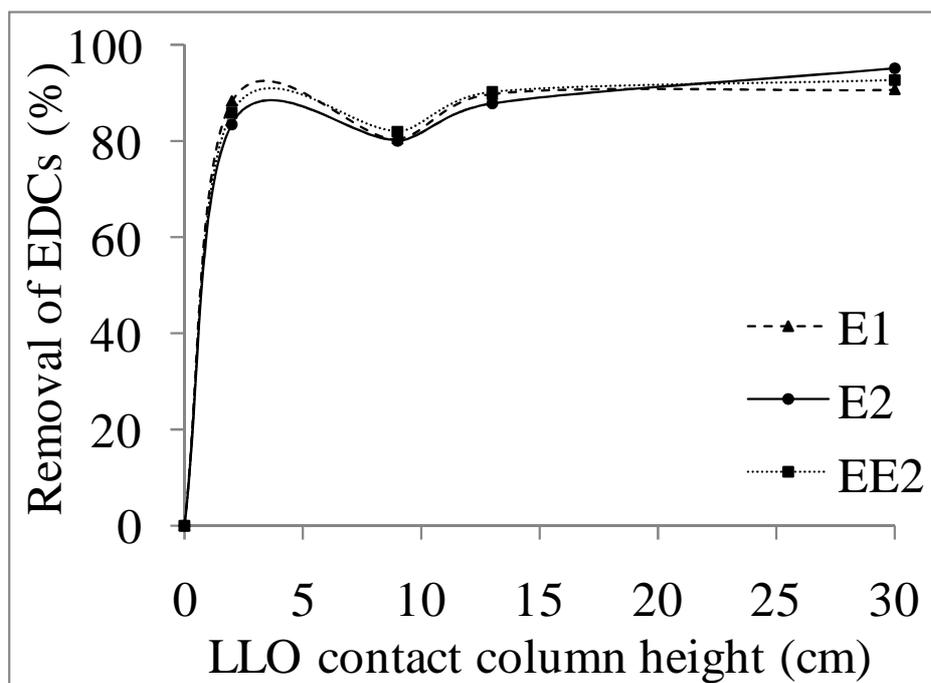


Figure 8. Removal of EDCs (1 mg L^{-1}) along the contact column during LLO process, under optimized operating conditions: Stoichiometric O_3/EDCs molar ratio, D5/water volume ratio of (1:5), total flow rate of 2 mL min^{-1} and pH6 at $25 \text{ }^\circ\text{C}$.

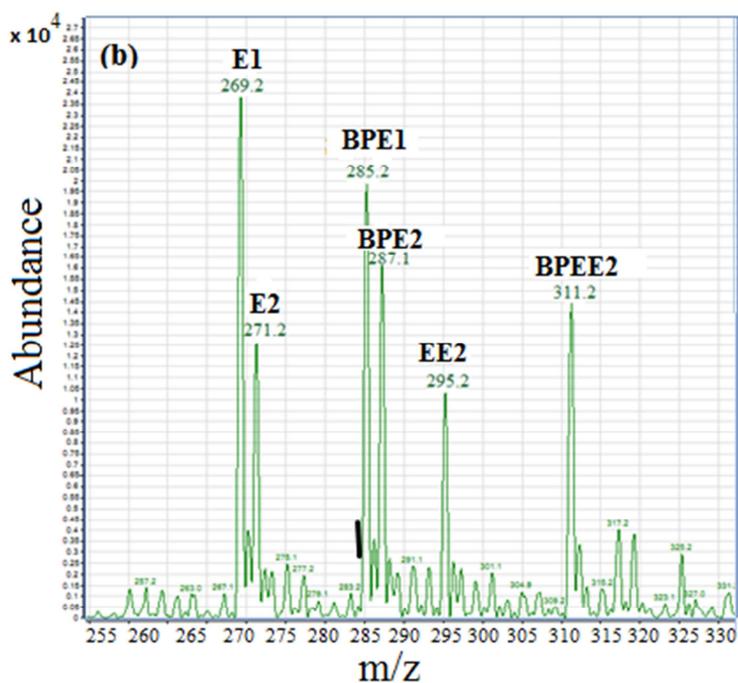
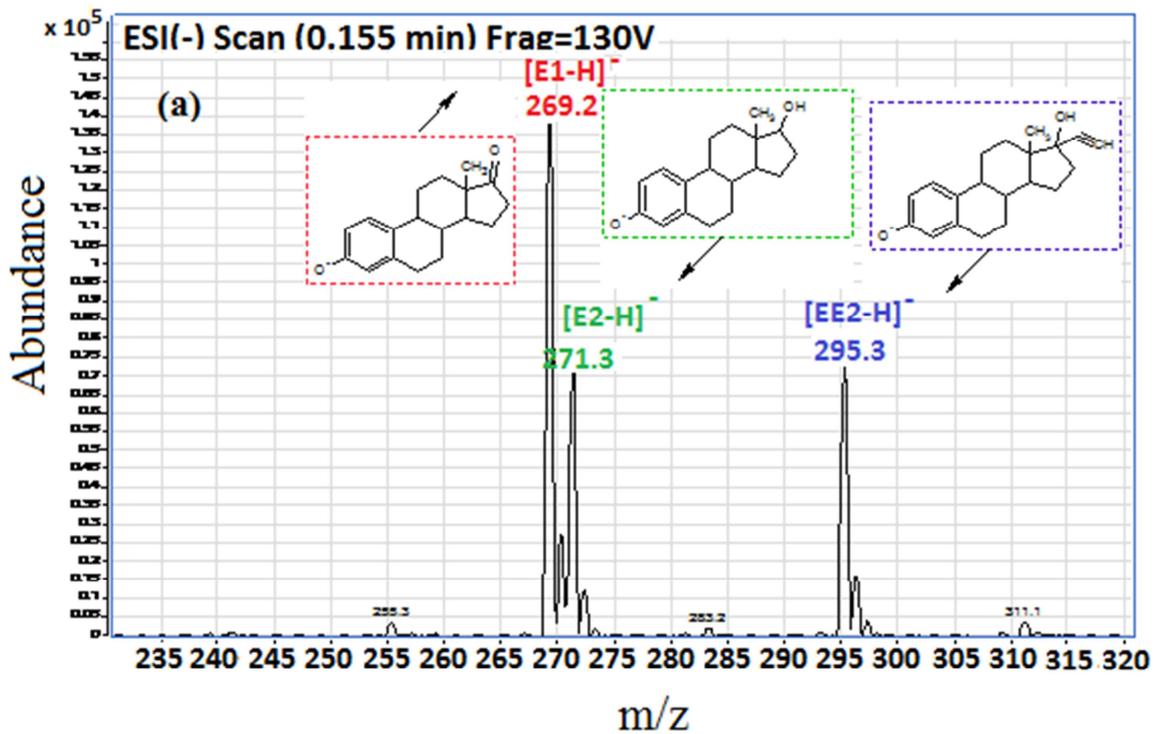
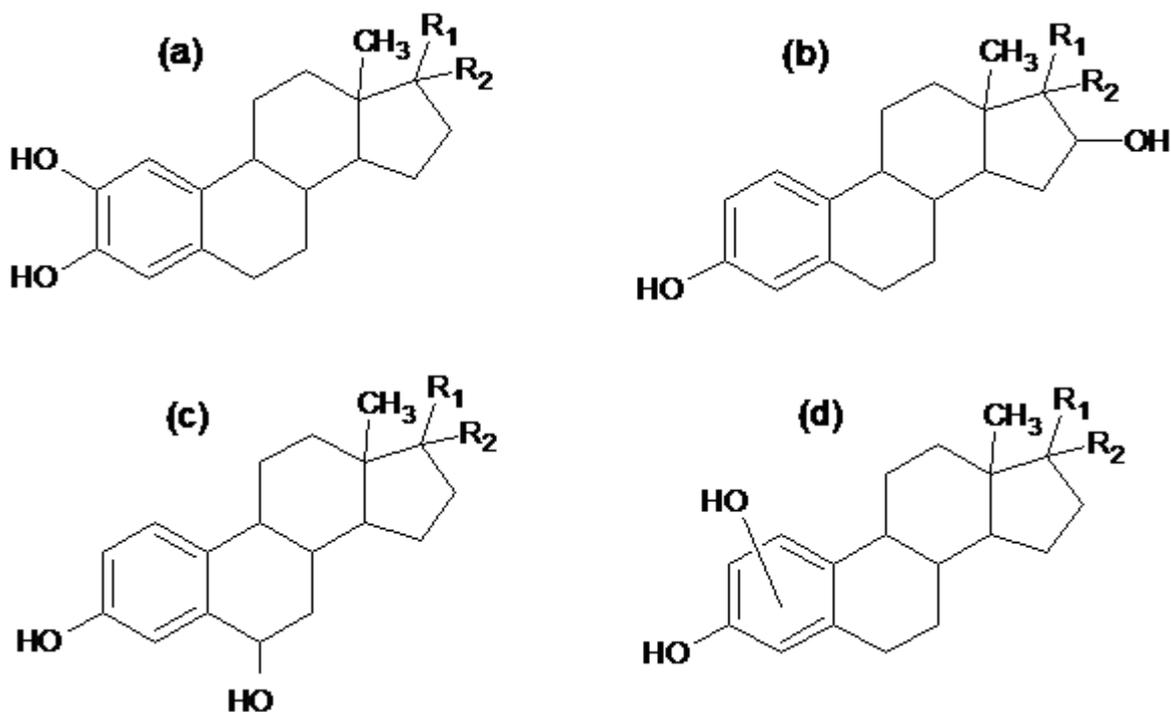


Figure 9. Qualitative mass spectra of (a) E1, E2 and EE2 before LLO and (b) their respective byproducts (BPE1, BPE2 and BPEE2) generated after LLO treatment using D5 as an ozone-loaded solvent. LC-MS/MS analysis was performed in Scan mode (ESI (-) and at a fragmentor voltage of 130V).



1

2 *Figure 10. Possible molecular structures of obtained oxidation byproducts of EDCs during*

3 *LLO process (m/z 285 for BPE1, m/z 287 for BPE2 and m/z 311 for EE2). R_1 is (=O) for E1,*

4 *(—OH) for E2 and for EE2. R_2 is (—H) for E1 and for E2 and (—C≡CH) for EE2.*

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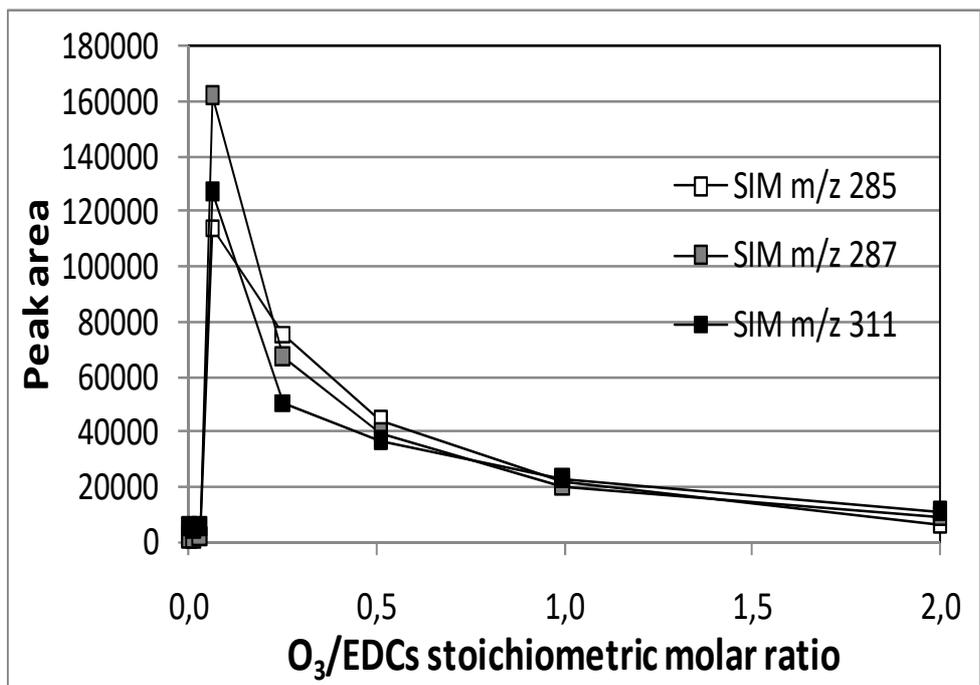
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13 *Figure 11. The removal of EDCs' byproducts generated during LLO process at different*

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$O_3/EDCs$ molar ratios.

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