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Efficacy and mechanistic insigh Endocrine Disruptor degradation using atmospheric air plasma

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1 **Efficacy and mechanistic insights into Endocrine Disruptor degradation using atmospheric**

2 **air plasma**

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Abstract

Endocrine disruptors are a class of contaminants found in water and process effluents at low concentrations. They are of concern due to their high estrogenic potency. Their presence in the environment has led to the search for effective techniques for their removal in wastewater. For this purpose, an atmospheric air plasma reactor was employed for the study of the degradation of three endocrine disruptor chemicals (EDC) namely; bisphenol A (BPA), estrone (E1) and 17β-estradiol (E2) within a model dairy effluent. Identification of the plasma induced active species both in the gas and liquid phases were performed. Also studied was the influence of an inhibitor, namely tertiary butanol, on the degradation of the EDCs. The results demonstrate that air plasma could successfully degrade the tested EDCs, achieving efficacies of 93% (k=0.189 min^{-1}) for BPA, 83% (k=0.132 min^{-1}) for E1 and 86% (k=0.149 min^{-1}) for E2, with the process following first order kinetics. The removal efficacy was reduced in the presence of a radical scavenger confirming the key role of oxygen radicals such as ·OH in the degradation process. The intermediate and final products generated in the degradation process were identified using UHPLC-MS and LC-MS. Based on the intermediates identified a proposed degradation pathway is presented.

Keywords: Atmospheric air plasma, endocrine disruptors, degradation.

1. Introduction

Endocrine disrupting compounds (EDCs) are organic chemicals which are either excreted endogenously from humans and animals or are derived from uses in clinical practices [1]. An EDC is defined as an exogenous agent that alters the function(s) of the endocrine system, leading to adverse health effects in organisms [2]. Endocrine disruptors vary widely in their structure and have numerous uses in everyday items including; electrical appliances, clothing, furniture and cosmetics. They can also include pesticides (e.g., DDE, dicofol), plastics (bisphenol A, phthalates), food preservatives (UV-filters), hormonal agents and phytoestrogens. The presence of EDCs has been widely detected in surface waters, process effluents and sewage sludge treatment plants (STP). Hartmann et al.[3] has reported that the main source of animal-derived estrogens (60–70%) in the human diet is milk and dairy products. Estrogen content is mostly distributed in the fat phase because of its solubility. Food processing operations do not typically affect the hormone patterns. Animal fecal and urinary excretions may also result in the occurrence of estrogens in dairy effluents [4]. Recently, free and conjugated forms of estrogens including 17β-estradiol and estrone have been detected in milk and milk products [3]. Inappropriate disposal, leaching and poor removal by conventional wastewater treatment processes (WWTP) are identified areas of concern. Various studies have reported on the occurrence of these emerging contaminants, including those from dairy and meat process effluents [5, 6].

When effluents containing chemicals are used for agricultural crops they can be transported to surface or ground waters. Colbron *et al.*[7] reported that EDCs are associated with reproductive and sexual abnormalities in animals and wildlife. The effects of EDCs include; fish feminization, changes in reproduction and behavior, decrease in number of spermatozoids,

64   increase in breast cancer rates and an increase of certain anomalies in the human reproductive

65   system [8-10]. With particular concern to human health, it is paramount that these chemicals are

66   effectively treated before entering drinking water supplies, however for such hydrophobic

67   contaminants, conventional treatment may be ineffective. Biological processes have been used

68   for their treatment however, such chemicals have been found to be resistant and/or toxic to

69   microbes and consequently they cannot be readily treated by biological processes [11][10].

70   Consequently, such treatment processes may not reach the required regulatory limits for these

71   contaminants. It is reported that the regulatory limits for all EDCs is <0.0001 mg/L [12].

72   Chlorination of EDCs and its chlorinated products have been reported to elicit estrogenic activity

73   [13]. Moreover, chlorination leads to the production of disinfection by-products (DBPs) that may

74   be residually present in the treated effluent and pose a potential risk to consumers. Several

75   authors had reported on the removal of these chemicals by advanced oxidation process (AOPs)

76   such as ozonation [14], UV/H2O2 [15] and TiO2 photocatalysis [16]. However, in the case of the

77   later, an additional step for the removal of the reagents used and incomplete mineralization might

78   increase the operational costs. As a result, there is a need to find a sustainable, effective and

79   economical process for degrading and removing EDCs from effluents.

80   Recently, dielectric barrier discharge non-thermal plasma (DBD-NTP) oxidation has

81   emerged as a promising technology for eliminating organic micropollutants with high removal

82   rates and environmental compatibility [17] . Plasma is a partially or wholly ionized state which

83   consists of positively and negatively charged ions, free electrons, free radicals and intermediate

84   highly reactive species, atoms, molecules and UV photons [18, 19]. Plasma can be generated

85   either in the gas or liquid phase simultaneously [20]. The electron impact dissociation of oxygen

86   and water molecules leads to generation of numerous active species such as $O^{\bullet}$, $^{\bullet}OH$, $N^{\bullet}$, $HO_2^{\bullet}$,

87   N_2^*, N^*, OH^-, O_2^-, O^-, O_2^+, N_2^+, N^+, O^+, and O_3, H_2O_2 following subsequent chemical reactions

88   [21, 22]. These species react with chemicals causing oxidation. Moreover, pollutant degradation

89   could be initiated by UV light or shock waves. Recently, several authors have reported on the

90   potential of DBD plasma technology for the removal of toxic chemicals from wastewaters such

91   as pollutant dyes [23], pesticides [24, 25], mycotoxins [26], volatile organic compounds [27, 28]

92   and antibiotics [29]. Recently Gao *et al.,*[30] reported on the degradation of estrogenic endocrine

93   disruptors by DBD plasma technology. A DBD plasma source combined with a Pt–TiO_2

94   photocatalyst was developed by Chen et al.[31] which demonstrated effective degradation of

95   17β-estradiol. The application of DBD plasma to the treatment of bisphenol A and tributylin was

96   studied by Hijosa-Valsero et al. [32], demonstrating that plasma can be an alternative AOP for

97   the removal of persistent and toxic pollutants from water and wastewater. Abdelmalek and co-

98   authors [33] used a gliding arc discharge plasma reactor to study the degradation of the

99   endocrine disruptor bisphenol A with ferrous ions. They found that both hydroxyl radicals and

100 hydrogen peroxide are responsible for the degradation effect and the addition of ferrous ions (II)

101 allowed for greater mineralization via the production of additional hydroxyl radicals, according

102 to the Fenton reaction.

103   The selection of an oxidation process may depend upon many factors such as the degree

104 of degradation, the by-products formed in the process, the total cost, safety and reliability of the

105 process [34]. Most studies to date on pollutant degradation using plasma have been carried out in

106 the absence of competing organic matter. In this study, a novel high voltage and low-frequency

107 DBD prototype reactor was employed which utilized atmospheric air as the inducer gas for the

108 degradation of EDCs in a model dairy effluent. Furthermore, identification of degraded products

109 and possible mechanisms of EDC degradation in the presence of dairy effluent is also studied.

2. Materials and Methods

2.1. Materials

Analytical grade standards of Bisphenol A (BPA), Estrone (E1) and 17β-Estradiol (E2) of purity (>96 %), HPLC grade methanol, acetonitrile, ethyl acetate, ammonium hydroxide solution (32%) puriss p.a. (NH4OH), acetic acid (AcOH), formic acid (HCOOH), sodium acetate (NaOAc), ammonium formate, tert-butyl alcohol(TBA) and LC-MS grade water were obtained from Sigma-Aldrich (Ireland).

2.2. Sample Preparation

A model dairy effluent was prepared by dissolving 4 g of skim milk powder, 0.4 g of milk fat and 0.01% of NaOH per liter of distilled water [35]. The model effluent was used to overcome inherent variability in commercial effluent composition. Large particulate matter was removed by filtering the model effluent through a Whatman (UK) filter paper and a 0.45 μm membrane (Millipore). Each endocrine disruptor (BPA, E1 and E2) was dissolved in acetonitrile to obtain a standard stock solution with the concentration 100mg/L. The prepared stock solution was diluted and spiked with the filtered model effluent to obtain a concentration of 2 mg/L.

2.3. Experimental procedure

2.3.1 Atmospheric air plasma treatment

The experimental set-up employed for this work is shown in **Fig.1**. The experimental apparatus consisted of two aluminium plate electrodes of circular geometry (outer diameter = 158 mm) which were covered with dielectric materials of 2 mm thickness for ground electrode and 10mm thick acrylic sheet for the high voltage electrode. For each experiment, 20 mL of dairy effluent spiked with EDC's (at an initial concentration of 2 mg/L) was added to petri dish and placed

132   within a polypropylene (PP) container of dimensions 310 mm × 230 mm × 22 mm which acts as

133   a closed reactor and as an additional dielectric barrier. This container was further sealed inside a

134   high barrier Cryovac BB3050 film in order to prevent loss of reactive species generated during

135   plasma treatment. The voltage was delivered through a step-up transformer (Phenix

136   Technologies, Inc., USA) whose primary winding received an input at 230 V, 50 Hz and

137   delivered a high voltage output in the range 0-120 kV_{RMS}. Plasma treatment was performed at

138   varying voltage (60-80 kV) and treatment durations (0-15 min). Treatment of samples was

139   carried out in duplicate at ambient temperature (16-18 ℃). After processing, containers were

140   stored at room temperature of 16-18 ℃ for 24 h to ensure that the generated and contained

141   reactive species reacted with the samples. Ozone concentrations were measured using short-term

142   ozone detection tubes obtained from Gastec (Product No. 18M, Gastec, Japan). These tubes

143   contain a reagent which changes color after coming into contact with the specified gas and are

144   calibrated for specific sampling volumes. Ozone concentrations were measured immediately

145   following treatment. The emission spectra of the discharge were acquired with a computer

146   controlled Ocean Optics spectrometer (HR2000+), to which the light from the plasma is coupled

147   via an optical fibre [36]. Further details regarding gas measurement methodology for ozone and

148   the optical emission spectroscopy experiments can be found elsewhere [37]. The conductivity of

149   the effluent was measured using a conductivity meter (model CON-BTA, Vernier Software &

150   Technology Inc.) at ambient temperature (16℃) and turbidity was measured by using a Hach

151   2100 P, ISO USA turbidity meter. The pH of all the samples were measured after 24h storage

152   using a calibrated glass electrode ORION pH meter (model 420A, Thermo Fisher Scientific Inc.)

153   at ambient temperature (16 ℃). Total organic carbon (TOC) of the dairy effluent was measured

154 using a digestion unit DRB 200, Hach, USA and double beam UV-visible spectrophotometer

155 Hach, DR 2400, USA.

156 **2.4. Sample Extraction**

157 The plasma treated model effluents were firstly extracted by solid-phase extraction (SPE). The

158 extraction was carried out using a solid phase cartridge (Supelclean™-ENVI™-18), previously

159 preconditioned by flushing with acetonitrile followed by methanol (3×3 mL). In succession, the

160 cartridges were washed with water ($3 \times$ mL) and then the sample was passed through the

161 cartridges at a flow rate of 10 mL/min with the aid of a vacuum. These cartridges were dried and

162 elution was performed with methanol (3×3 mL). The methanol extracts were evaporated to

163 dryness under nitrogen stream and re-suspended in 1.5 mL of acetonitrile.

164 **2.5. Analytical Methods**

165 Standard curves for the EDC's were established using standard solutions ranging between 0.05

166 mg/L and 5 mg/L using acetonitrile as solvent. The linear correlation coefficients (r^2) were

167 0.997, 0.998 and 0.997 for BPA, E1 and E2 respectively. The quantification of EDCs was

168 determined using a HPLC system which consisted of a Waters 600 Satellite connected to a

169 Waters 996 PDA detector and Waters auto-sampler (Waters, Ireland). Separation was carried out

170 on a Phenomenex Gemini-Nx C18 column (Phenomenex, U.K.), 5 μm particle size (250 mm $\times$

171 4.6 mm). The mobile phase consisted of 70% acetonitrile and 30% water, and the flow rate was

172 set at 0.6 mL/min. The detector wavelength was set at 210-400 nm. Chromatographic data was

173 collected and processed using Empower2 software (Waters, Ireland). The SPE recoveries

174 obtained in the present study were found to be 97% for BPA, 92% for E1 and 94% E2

175 respectively.

176 The degradants were analyzed using an Acquity UHPLC coupled to a Quattro Premier XE triple

177 quadrupole instrument operating in electrospray ionization (ESI) mode (all from Waters,

178 Milford, MA, USA). Separation was carried out in an Acquity BEH C_8 analytical column (2.1 x

179 50 mm, particle size 1.7 µm) maintained at 30ºC. A binary gradient system was used to separate

180 analytes comprising of mobile phase A, 5mM 0.24 mM ammonium formate in MeOH /H_2O

181 (80:20, v/v) and mobile phase B, 5 mM of ammonium formate in MeOH. The gradient profile

182 was linear from 80% A to 30% A over 5 min and 0% A at 6.5 min then 2 min at 0% A followed

183 by 2 min for re-equilibration at 80% A. The UHPLC-MS system was controlled by MassLynx™

184 software and data was processed using TargetLynx™ software (all from Waters). A Full scan

185 mode was used to acquire MS spectra of the intermediates with a scan range of *m/z* 80–500.

186 Transformation products were also identified using LC–MS. The organic acids in the dairy

187 effluent were quantified by an ion chromatograph (ICS 3000, Dionex, USA) at 30 °C.

188 **2.6. Determination of nitrite, nitrate and hydrogen peroxide**

189 Nitrite concentrations in the effluent were quantified by employing the Griess reagent (N-(1-

190 naphthyl) ethylenediamine dihydrochloride) spectrophotometric method [38]. This was

191 accomplished by the addition of 100 μl sample, trichloroacetic acid and Griess reagent. The

192 reaction mixture was incubated at 37 °C for 30 min, after which the absorbance was determined

193 at 548 nm using a UV–visible spectrophotometer (Shimadzu UV-1800, Shimadzu Scientific

194 Instruments Kyoto, Japan). A calibration curve was prepared using a standard solution of sodium

195 nitrite. Nitrate concentrations were determined according to the procedure of Lu *et al*.[39].

196 Hydrogen peroxide concentrations were determined according to the procedure of Bohem *et*

197 *al.,*[40].

198 **2.7. Degradation kinetic modelling study and data analysis**

199 The removal efficiencies (η) of EDC's were calculated according to the following equation:

$$\eta = \frac{C_0 - C}{C_0} \times 100 \qquad (1)$$

200 Where η is removal efficiency of each EDC, C is the concentration of EDCs at time 't' and C_0

201 the initial concentration of the EDC. Plasma degradation of EDCs in aqueous media followed

202 first order kinetics [25, 41] as described in the following equation

$$C = C_0 \exp(-kt) \qquad (2)$$

203 Where k is the degradation rate constant (min^{-1}) of the reaction and t is the treatment time (min)

204 and energy yield was calculated according to the procedure of Jiang *et al*.[42].

205 **3. Results and discussion**

206 **3.1. Current-Voltage characteristic of plasma source**

207 **Fig.2.** shows the typical current-voltage waveform for the discharges within the reactor at the

208 applied voltages. It can be observed that the applied voltage was sinusoidal and total current is

209 the combination of displacement current and several current pulses associated with filamentary

210 micro-discharges. A stable discharge was found at the high voltages employed even at the large

211 discharge gap of 2.2 cm. The dielectric used acts a charge trapping agent and also prevents

212 arcing [43]. Further details regarding the electrical characteristics of the discharge using

213 capacitance-voltage Lissajous patterns can be found in a previous publication [44].

214 **3.2. Evaluation of removal efficiency and degradation kinetics**

215 The endocrine disruptors, namely estrone (E1), 17β-estradiol (E2) and bisphenol A (BPA) were

216 identified using chromatographic retention time data. The EDCs were shown to degrade

10

217 significantly by >25% and >42% following plasma treatment times of 5 and 15 min respectively,

218 for all applied voltages (**Fig.3.**). A significant increase in removal efficiencies was also achieved

219 with increases in applied voltage from 60 kV to 80 kV. The maximum removal efficiencies

220 achieved were 83.57±0.45%, 86.52±0.75 % and 93.90±0.92% for E1, E2 and BPA respectively,

221 after 80 kV for 15 min of plasma treatment. Similar results were observed for the degradation of

222 methylene blue and pesticides by non-thermal plasma [23, 25]. In order to confirm the effect of

223 the plasma process parameters and systematic analysis for removal of contaminants, the

224 degradation was modelled using a first order kinetic model **Fig.4**. It is evident from **Table 1** that

225 the first order kinetic model is in reasonable agreement with the experimental data, with

226 correlation coefficients >0.90. Increases in applied voltage from 60 kV to 80 kV, resulted in an

227 increase in the rate constant values (k) from 0.045 to 0.189 min^{-1} for all three EDCs.

228 Atmospheric air plasma are known to produce a variety of excited and active species, such as

229 $O^{\bullet}$, $^{\bullet}OH$, $N^{\bullet}$, $HO_2^{\bullet}$, N_2^*, N^*, OH^-, O_2^-, O^-, O_2^+, N_2^+, N^+, and O^+ [43] in the gas phase or at the

230 liquid interface, which can dissolve into solutions leading to contaminant oxidation. The active

231 species generated not only react with the target contaminant but also with the degraded products.

232 During plasma treatment EDCs are oxidized through an attack by the active species such as the

233 ozone molecule, the hydroxyl radical and hydrogen peroxide. Ozone reacts selectively with

234 certain functional groups, whereas an indiscriminate reaction occurs with the hydroxyl radical

235 [45]. The degradation of a contaminant by plasma depends on many factors such as sample

236 concentration, sample volume, electrode gap, sample chemistry, reactor type, discharge gas and

237 sample viscosity. The electrode gap is an important plasma process parameter for DBDs. In the

238 present study, a stable discharge was achieved at the very high voltages employed facilitating a

239 large discharge gap of 2.2 cm. The physical processes occurring during air breakdown and the

240 micro-discharges formed in the gap govern the resultant chemistry [46]. This novel plasma

241 design of high voltage, low frequency and large gap atmospheric air DBD has demonstrated

242 rapid treatment efficacies for a range of processes [12,14,15,16].

243 The chemical structure of the contaminant is the dominant factor for the target's persistence as it

244 influences the chemical stability during the degradation reaction [47]. The degradation behavior

245 of E1, E2 are found to be similar due to their comparable chemical structure, whereas a slight

246 difference was observed for BPA. The high degradation rates obtained are due to the effective

247 generation of large quantities of active species during the discharge coupled with the retention of

248 the species within the confines of the reactor post discharge. Similar reports were observed for

249 ozonation of phenolic pharmaceutical compounds by Boyd *et al* [48]. The plasma active species

250 specifically attack the high electron density carbon (C=C) double bond, activated aromatic acids

251 and non-protonated amines. This is due to the donation of an electron by the hydroxyl group to

252 the benzene ring which activates the aromatic system and facilitates oxidative attack by ozone

253 [48]. In our study, a closed reactor setup was employed by placing the samples in the

254 polypropylene (PP) container. These reactors were stored for 24 h to ensure that the generated

255 and contained reactive species reacted with the samples, post plasma treatment. The plasma

256 species also diffuse to regions outside of the discharge zone within the reactor. The degradation

257 of EDCs was found to be greater inside the discharge zone, which is due to the presence of all

258 the reactive species (electrons, ions, free radicals, etc.) when compared to the remote regions

259 where meta-stables are only found.   Nevertheless, EDC degradation was found to occur with

260 exposure to either a direct discharge or the retained afterglow [36].

261   A brief comparison of the different methods of EDC removal is summarized in **Table 2**.

262 It is observed that 70% of E1 can be removed by chlorination for a contact time of 15 min.

263   However, the chlorination process introduces the risk of the generation of disinfection by-

264   products [49]. More than 80% of E2 could be removed via the photo-Fenton process, however

265   the additional step required for the removal of the reagents employed would increase the

266   operational costs [50]. Zhang *et al*. [51] and Ohko *et al*.[16] demonstrated that photo-catalysis

267   with TiO_2 to be efficient in the elimination of E1 and E2. However, these approaches require

268   significant contact times to achieve satisfactory removals. Ozonation has been reported as an

269   efficient oxidation process for the removal of EDCs (>90%), however the process can also

270   generate toxic products [52, 53]. Plasma can be an alternative process which can be used for

271   removal of pollutants. The advantage of plasma over ozone is that, plasma contains over 100

272   reactive species in humid air (including ozone), many of which are more active. Gao *et al*. [30]

273   achieved 100 % removal of E2 using a DBD plasma reactor, however an increase in

274   concentration to 900 µg/L reduced the removal to 64% after 30 min of plasma treatment. In

275   another study, a synergetic system which combined a DBD plasma discharge and Pt-TiO2

276   achieved 99.8% efficiency with 72% achieved with the DBD reactor alone for a treatment time

277   of 30 min and an initial concentration of 400 µg/L [31]. Furthermore, a 96% degradation of

278   BPA, at an initial concentration of 1 mg/L, was found for a DBD reactor using helium as the

279   carrier gas [32].

280   The removal efficiency may be characterised by the amount of EDC degraded per unit of

281   energy (yield). The energy yield depends on the type of discharge reactor, initial concentration

282   and nature of the compound [41]. The energy yield of EDC degradation was calculated and the

283   results were shown in **Fig.5**. With increasing applied voltage from 60 kV to 70 kV, the energy

284   yield decreased from 1295×10^{-6} to 1249×10^{-6} g/kW h for BPA, 777×10^{-6} to 737×10^{-6} g/kW h for

285   E1, 1006×10^{-6} to 810×10^{-6} g/kW h for E2 and 805×10^{-6} to 652×10^{-6} g/kW h for BPA/TBA,

286 respectively. Comparing with previous studies on EDC degradation using DBD reactors [30, 31]

287 our design was found to have slightly low energy yield values. This might be due to a high initial

288 concentration of the solution, the volume of samples treated and the presence of competing

289 organic matter as reactive sinks. The degradation of diuron in aqueous solution by DBD plasma,

290 by Feng et al.[54], reported high energy yield values of 0.16 g/kW h. Similarly, Reddy et al.[41]

291 also reported a high energy yield values of 0.298 g/kW h for the mineralization of endosulfan in

292 an aqueous medium by DBD plasma. AOP technologies which employ chemical addition are

293 typically high energy consumption and environmentally hazardous [42]. For example, Jantawasu

294 *et al.* [55] obtained a 78% decomposition of methyl orange by nanocrystalline mesoporous-

295 assembled TiO2 photocatalysis, after a 4 h treatment. The energy cost and treatment time were

296 rather high when compared to non-thermal plasma. As compared to other common AOPs, such

297 as UV/H_2O_2 and ozone/H_2O_2 the primary benefit of DBD plasma is the ability to generate UV

298 light and oxidizing species, ozone, hydroxyl radicals, etc. without chemical addition or the use of

299 UV lamp [42].

300 **3.3. The role of active species**

301 The plasma discharge was characterized using optical emission spectroscopy (OES). From **Fig.6,**

302 it can be seen that small peaks of $^{\bullet}OH$ were recorded at 295-300 nm. Emissions from N_2 and

303 excited species of N_2^+ exhibited distinct peaks in the UV region [56]. A high-intensity

304 metastable singlet oxygen (O) was recorded at around 750 and 780nm which is similar to

305 reported studies for DBDs operating at atmospheric pressures in air [57]. As expected the

306 atmospheric discharge is an effective source of reactive nitrogen and oxygen species (RNOS).

307 Plasma species such as superoxides, hydroxyl radicals, and peroxynitrites are short lived

308 whereas, hydrogen peroxide is relatively more stable even up to 24 hours. Such active species

14

309   play an important role in the degradation of EDC's. Ozone is a widely applied strong oxidizing

310   agent for the treatment of wastewater. Misra *et al.*[20] reported that ozone is one the most stable

311   active species generated in DBD with a high oxidation potential of 2.02V. The ozone reaction

312   with EDCs takes place through molecular and radical reactions. The ozone concentrations

313   measured after 10 min of plasma treatment were found to be 1600, 2400 and 3100 ppm (within $\pm$

314   10% errors) for applied voltages of 60, 70, and 80 kV respectively. The mechanism of ozone

315   degradation of contaminants can be either by direct oxidation or an indirect one, by converting

316   into hydroxyl radicals. The direct reaction is predominant in acidic environments while at high

317   pH indirect oxidation takes place. However, both reactions can occur simultaneously and ozone

318   may react with unsaturated functional groups present in organic molecules. Electron dissociation

319   of water molecules leads to the formation of $\cdot$OH which further forms H_2O_2 in both air and water.

320   In order to study the function of hydroxyl radicals ($^\cdot$OH) during DBD plasma treatment

321   tertiary butanol (4% v/v), a hydroxyl radical scavenger was added to the BPA solution with the

322   degradation efficiency reported as a function of treatment time and applied voltage (see **Fig.4**).

323   The data shows that the process efficiency was reduced by ~20% with the addition. Similar

324   observations were reported by Gao *et al.,*[30] and Mehrvar *et al.,* [58]. The addition of the

325   radical scavenger also resulted in a decrease in the rate constant from 0.189 min^{-1} to 0.098 min^{-1}

326   at 80 kV. The results infer that tertiary butanol competes with BPA to consume the generated

327   hydroxyl radicals and can restrain the interaction between $^\cdot$OH and the BPA molecule.

328   **Fig.7(a)** shows the TOC removal efficiencies of all three EDC's as a function of

329   treatment time. The initial concentration of EDC spiked dairy effluent corresponds to TOC

330   concentrations of 1125.44, 1245.9 and 1243.7 mg/L for BPA, E1 and E2 respectively. As shown,

331   the TOC removal efficiencies of all three EDC's, linearly increased with time, reaching a

332 maximum removal of 21.5%, 17.8% and 22.3% for BPA, E1 and E2 respectively, after 15 min of

333 plasma treated at 80 kV. Interestingly, all the tested EDCs were effectively degraded more than

334 80 % within 15 min however, the TOC removal efficiencies achieved were not more than 30%,

335 which can be attributed to the formation of low-molecular weight compounds such as oxalic acid

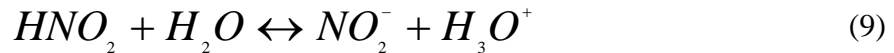336 and formic acid before being completely mineralized [59].

337   **Fig 7(b)** shows the evolution of pH as a function of treatment time and applied voltage

338 for the treated samples, with pH values changing from 6.95 to 5.41. The decrease in pH values is

339 due to the formation of nitric acid, nitrous acids and intermediate organic acids (oxalic and

340 formic acid) that originate from the nitrogen in the air and the degraded EDCs. It was known that

341 these organic acids have large acidic dissociation constants, which lead to significant reductions

342 in solution pH [29]. The conductivity of the model effluent with EDCs is shown in **Fig.7 (c)**. It

343 was observed that the conductivity increased with increases in treatment time and applied

344 voltage due to the generation of ionic species. The conductivity of the control sample was 750

345 µS/cm, which increased to 1660 µS/cm after plasma treatment for 15 min at 80 kV. An increase

346 in the conductivity of brewery effluent and dye solution with plasma treatment was demonstrated

347 by previous authors [60, 61]. The increased ionic strength will reduce ozone solubility in the

348 bulk solution because of the salting-out effect, which would also likely affect the degree of

349 oxidation. The dissolution of nitrite and nitrate in water which are inevitably produced by

350 electric discharges can also induce significant changes in the pH and electrical conductivity of

351 the effluent. The dissolution of nitrite to form nitric acid can be explained as follows.

$$N_2 + e^- \rightarrow 2N^\cdot + e^- \tag{3}$$

$$O_2 + e^- \rightarrow 2O^\cdot + e^- \tag{4}$$

$$N^{\cdot} + O^{\cdot} \rightarrow NO_2 \qquad (5)$$

$$NO + O \rightarrow NO_2 \qquad (6)$$

$$NO_2 + OH^{\cdot} \rightarrow HNO_3 \qquad (7)$$

352

353   Nitrate and Nitrite concentrations were measured in the model effluent sample, without

354 the presence of EDCs to ensure no interaction from their presence with the reagents used. Nitrite

355 can be formed not only by the degradation of the effluent but also by the electrical discharge in

356 air. **Fig. 8 (a) (b)** shows the temporal variations of nitrite and nitrate concentrations in the plasma

357 treated dairy effluent. The concentrations of nitrite and nitrate after 15 min treatment were found

358 to be 56.50, 57.62, 66.86 mg/L and 114.87, 146.30, 163.59 mg/L for applied voltages of 60, 70,

359 and 80 kV respectively. These values are substantially lower than the maximum permissible

360 concentration (MPC) for nitrates or nitrites in dairy effluents [62]. The formation of nitrite can be

361 explained from Eq (19-20), where formation of nitrate is attributed to the dissociation of nitric

362 acid formed by the sequence of reactions (21) and (22) [63].

$$NO + OH \rightarrow HNO_2 \qquad (8)$$

$$HNO_2 + H_2O \leftrightarrow NO_2^- + H_3O^+ \qquad (9)$$

$$NO_2^- + H_2O_2 + H_3O^+ \rightarrow ONOOH + 2H_2O \qquad (10)$$

$$ONOOH \rightarrow HNO_3 \qquad (11)$$

363   It was also known that nitrate is formed from nitrite oxidation and also an intermediate.

364   The transient character of nitrite ions was observed previously [64, 65]. About 30% of nitrites

365   were converted to nitrates after 24 h of storage in the tank contacting with air [66].

366   The concentration of hydrogen peroxide (H_2O_2) is presented in **Fig.8.(c)**, the

367   concentrations of H_2O_2 increased linearly with respect to time for all voltages with

368   concentrations in the range of 2074 to 16810 mg/L. The high amounts of H_2O_2 in the treated

369   effluent are due to the retention of reactive species in the gas phase in contact with the treated

370   liquid for extended periods of time. Earlier work by our group achieved stable concentrations of

371   H_2O_2 in PBS solutions for extended storage times of several weeks in a closed reactor [40].

372   The concentrations of formic and oxalic acid in the dairy effluent were determined by ion

373   chromatography and the results are presented in **Fig.9**. The concentrations of oxalic acid

374   measured after 15 min treatment were found to be 200.09, 231.31 and 242.42 mg/L for applied

375   voltages of 60, 70, and 80 kV respectively. As shown in **Fig.9**, the concentration of both organic

376   acids increased with treatment time and all applied voltages. Interestingly, for all the organic

377   acids measured oxalic acid was found to be the most abundant in the treated effluent. It was also

378   observed that the initial formation of formic acid was rapid, however after 10 min, the

379   concentration was found to decrease. This phenomenon is attributed to mineralization of organic

380   acids. The decrease in pH presented in **Fig.7. (b)** also, explains the formation of organic acids as

381   byproducts. Similar results in the formation of organic acid as by-products has been

382   demonstrated by Kim et al., [59] in plasma-treated aqueous antibiotic solutions.

383   **3.4. Degradation mechanism of Endocrine disruptors**

384   The intermediate products were identified using UHPLC-MS in both the positive and negative

385   modes. It has been reported that the negative ion mode is preferred for the identification of target

18

386 estrogens [67]. In this study, the maximum concentrations of EDCs were spiked to identify the

387 intermediate products (see supplementary material Table S1 and S2). The MS spectrum (see

388 supplementary Fig.S4) of Bisphenol A showed a deprotonated major molecular ion at m/z 227

389 and a minor ion at m/z 134 and the fragmentation spectra showed peaks at m/z 213 and m/z 93

390 which were similar to the published reports of Debrode et al.[52]. The intermediate product ions

391 are identified at m/z 243, m/z 260, m/z 275, m/z 221. It was observed that the peak area of the

392 product ion m/z 227 decreased with plasma exposure time. Conversely, an increase in the peak

393 area of other products was seen in the 60 kV sample. Major peaks at m/z 275 and m/z 319 were

394 also observed. These might be 3-nitro-bisphenol A and 3,3'-dinitrobisphenol A, the nitrite

395 compounds formed by reactive nitrogen species [33, 68]. The fragments ions at m/z 243, m/z 149

396 and m/z 134 could result in ring opening. These might be due to hydroxylation of BPA via direct

397 reaction between plasma active species such as ozone and hydroxyl radicals. The formation of

398 monohydroxy BPA was further confirmed by fragmented peak ions at m/z 228 and m/z 93. The

399 reaction further proceeded to yield dihydroxy BPA m/z 261. The peak identified in the MS

400 spectra at m/z of 241 is an intermediate product, likely to be diketone, formed by the selective

401 reaction of ozone with an electron rich site. The loss in a carbonyl group would lead to another

402 peak at m/z 213[M-H]$^-$. However, the peak at m/z 276.10 would correspond to dicarboxylic acid

403 formed by initial attack of .OH via hydrogen abstraction. Other peaks identified in the MS

404 spectrum were observed at m/z 219 and m/z 235. Similar degradation products were detected in

405 ozonation of bisphenol A by Debrode et al.[52] and Tay et al.[69]. Additional peaks at m/z 232

406 [M-H]$^-$ and m/z 189 [M-H]$^-$ with a loss of CO_2 were also evident. The fragments at m/z 149,

407 136,134,110 could result from a chain fragmentation of BPA. Based on previous reports [70, 71]

408 the fragments at m/z 110 are intermediates of catechol, orthoquinone and hydroquinone. The

409   reaction of ozone and hydroxyl radicals with the phenol ring of BPA results in similar reaction

410   by-products of phenol. These products were identified with samples treated for 70 kV for 5 min.

411   A large number of peaks observed in the chromatograms are polar compounds such as acid and

412   aldehydes. In the present study, oxalic and formic acid were identified which, is similar to the

413   findings by Garoma *et al.*,[72].

414   The degraded products and intermediates of E1 and E2 are identified from the MS

415   spectrum. It is known that E1 and E2 differ by two mass units, with molecular weights of

416   270.166 g/mol for E1 and 272.18 g/mol for E2. The structural difference is due to the presence of

417   a ketone group in position 17 of E1 and a hydroxyl group in the same position of the E2

418   molecule [8]. As mentioned plasma species target high electron rich cites such as the ortho and

419   para position of EDCs to form hydroxylated derivatives. The deprotonated ion at *m/z* 285 and

420   *m/z* 287 were identified as 2-hydroxy estrone and 2-hydroxyestradiol (2OH-E2) or 6-

421   hydroxyestradiol (6OH-E2). These products were also identified for ozonation [14, 73], photo-

422   Fenton reaction [74] and photo degradation [75] of E2. In addition to the major fragments at *m/z*

423   287 other peaks at *m/z* 259,183,158,134 were also identified in the MS spectrum of E2. The

424   oxidation of E2 by reactive species will yield diketone E2 and the initial attack of •OH via

425   hydrogen abstraction leads to opening of the aromatic ring which is converted to dicarboxylic

426   acids. This was confirmed by the major peak at *m/z* 319 and other ions at *m/z* 262, 162,135. The

427   MS spectra shows a peaks at *m/z* 303,287,259,175 and *m/z* 287, 259, 162, 134 these compounds

428   represent the 2-hydroxyestradiol or its resonance structure 10e-17b-dihydroxy-1,4-estradien-3-

429   one (DEO)[16]. A fragment ion peak *m/z* 259 [M-H]$^-$ was observed in the MS spectra formed by

430   the elimination of the carbonyl group from compound *m/z* 288 and consecutive elimination of

431   carbonyl and oxygen at *m/z* 303. As previously reported by Gao *et al.,*[30] a number of other

432   intermediates at *m/z* 336, *m/z* 352, *m/z* 368 with molecular weights greater than E2 are also

433   observed in the spectrum. In addition to the peak at *m/z* 368, a few other fragments at *m/z*, 336,

434   292 and 274 were also observed. In addition to the fragment ion peak *m/z* 368, the product ion at

435   *m/z* 352 is identified as the deprotonated molecule ([M-H]$^-$ with other fragment ions 336,319,292

436   and 274. Similar fragments as *m/z* 319,292,275 were also seen for the deprotonated molecule at

437   *m/z* 336. The fragments ion at peak *m/z* 319 is formed by the loss of oxygen, where *m/z* 291 [M-

438   H]$^-$ might be formed by the loss of the carboxyl group and *m/z* 275 [M-H]$^-$ could be attributed to

439   the consecutive loss of CO_2 and H_2O. The loss of H_2O from the ion at *m/z* 352 [M-H]$^-$ and loss of

440   O_2 from ion *m/z* 368[M-H]$^-$ would yield the ion *m/z* 336. These results are similar to previous

441   studies [8, 30, 76, 77]. Another peak was identified at *m/z* 317(2-nitro 17β-estradiol), this

442   compound might be an intermediate formed by the reaction of nitrogen species. Besides, these a

443   few other peak ions at *m/z* 208, 131, and 90 were also identified. These peaks would be chain

444   fragmented products of E2 which result in further oxidation to oxalic acid and formic acid.

445   Based on the intermediates formed possible degradation pathways for the tested EDC's

446   are proposed. The pathway for BPA (I) to (II) can occur through hydroxylation or reaction

447   between BPA and ozone and hydroxyl radicals as shown in **Fig.10** pathway 1. A similar reaction

448   for E1(I) to (II)) and E2(P) to (P1) is also proposed for the degradation pathway of E1 and E2 see

449   **Fig.11(a)** and **Fig.11(b)**. The most probable attack of plasma active species is against one of the

450   ortho positions (with respect to phenolic hydroxyl group) of the aromatic ring of E1 and E2. The

451   most probable site for the initial addition of the ·OH radical is at the C2 atom leading to the

452   formation of 2-hydroxy estrone (II) and 2-hydroxyestradiol (2OH-E2)(P1) or 6-hydroxyestradiol

453   (6OH-E2) [30, 76]. It is reported that the estrogenic activity of hydroxylated EDCs is much

454   lower than that of the parent compound [73, 78]. The degraded products such as hydroxylated

455 EDCs are further attacked by ·OH radicals on the aromatic ring. The increase in the addition of

456 hydroxylated structures involves ring opening, leading to oxidized products to form diketone

457 intermediates (II) to (III) and (P1) to (P2) which convert to dicarboxylic acids (III) to (IV) and

458 (P2) to (P3) to (P4). These dicarboxylic acids further oxidize to smaller products. The reaction

459 (IV) to (V) and (P4) to (P5) to (P6) is formed either by direct reaction of ozone or indirect

460 oxidation by hydroxyl radicals. Degradation of these EDCs by plasma is initiated with the

461 phenol-moiety oxidation [16]. Thus, this involves oxidation reactions to form a resonance

462 structure P7 (DEO) with a low estrogenic activity and which is favored by an attack by ·OH and

463 ·OOH radicals [16, 79]. The deprotonation of the BPA molecule or reaction with plasma reactive

464 species at electron rich sites by ·OH abstraction at the side chains leads to the formation of S1

465 compound (**Fig.10** pathway 2). The reaction is proceeded by cleavage of the C-C bond resulting

466 in S2 and phenol radicals (S3) [69]. Moreover the phenol radical reacts with ·OH to form

467 hydroquione (S4) or catechol (S5) and further orthoquinone (S6) which has relatively weak

468 estrogenic activity compared with BPA [80, 81]. The intermediate product (S2) undergoes both

469 direct and indirect reaction with ozone and hydroxyl radicals to form 4-isopropenylphenol (S7)

470 and 4-hydroxyacetophenone (S8). However, these compounds are similar to products identified

471 by Ike *et al*. [82] which showed a lower estrogenic activity compared to BPA. The results

472 obtained in this study suggest that atmospheric air plasma technology could be applied for the

473 removal of hazardous chemicals from processing effluents, with efficient degradation pathways

474 that mitigate the formation of biologically active intermediates.

475 **4. Conclusion**

476 This study demonstrates that atmospheric air plasma can effectively degrade endocrine

477 disruptors in a model dairy effluent. The removal efficiency achieved at 80 kV for 15 min was

22

478 found to be more than 80% for all EDCs tested. The degradation kinetics followed a first-order

479 kinetic model. The rate constants were found to increase with voltage and treatment time. The

480 plasma discharge under atmospheric air conditions was monitored using optical emission

481 spectroscopy and metastables measured using H_2O_2, nitrates or nitrite assays. The addition of

482 tertiary butanol reduced the removal efficiency by 20% which reveals the major role of $\cdot OH$ in

483 the degradation process. The oxidative decomposition of EDCs by plasma species leads to

484 intermediate products which were identified by UHPLC-MS and LC-MS. Based on the

485 intermediates formed a degradation mechanism was proposed for all three EDCs. The use of eco-

486 friendly gasses and fast removal rates make this technology a potential approach for industrial

487 application. This work provides an efficient method for degradation of contaminants in water and

488 has the potential to lead to novel applications for environmental protection.

493

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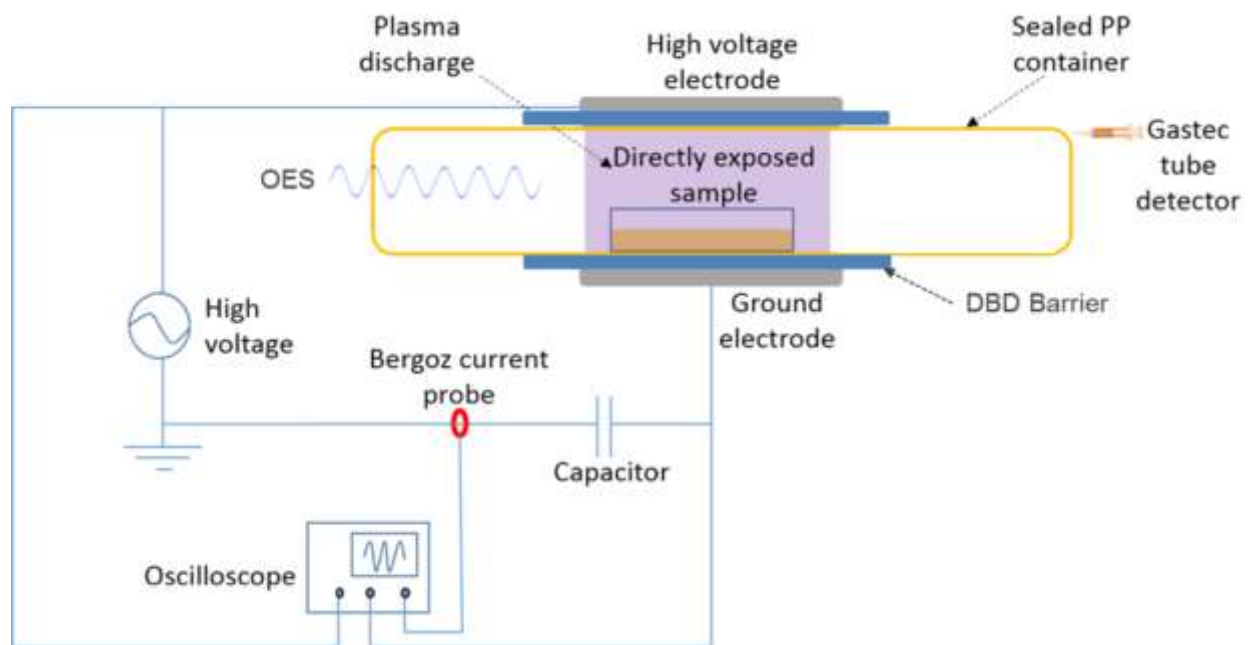699 biodegradation products of bisphenol-A, Environmental toxicology 17 (2002) 457-461.
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707 **Fig. 1**. Schematic of the experimental setup with electrical and optical diagnostics.

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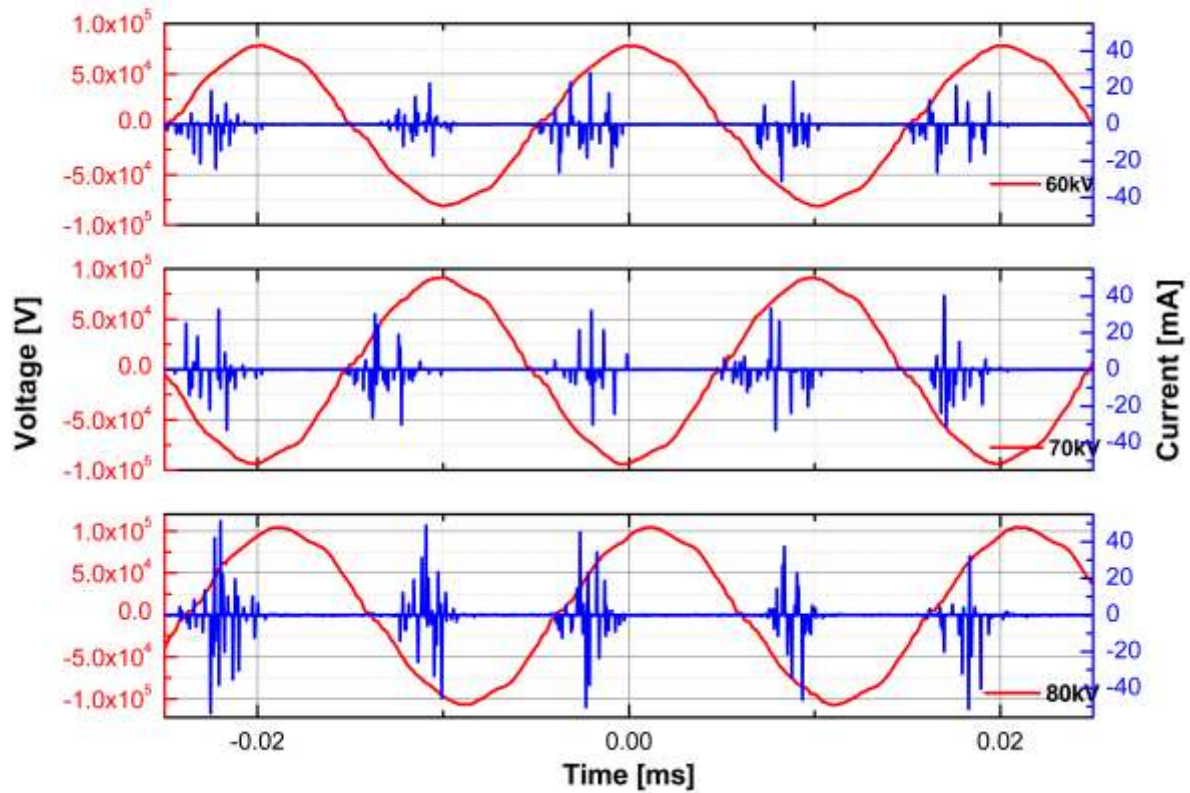
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716 **Fig.2.** Representative current –voltage charecteristics of the discharge.

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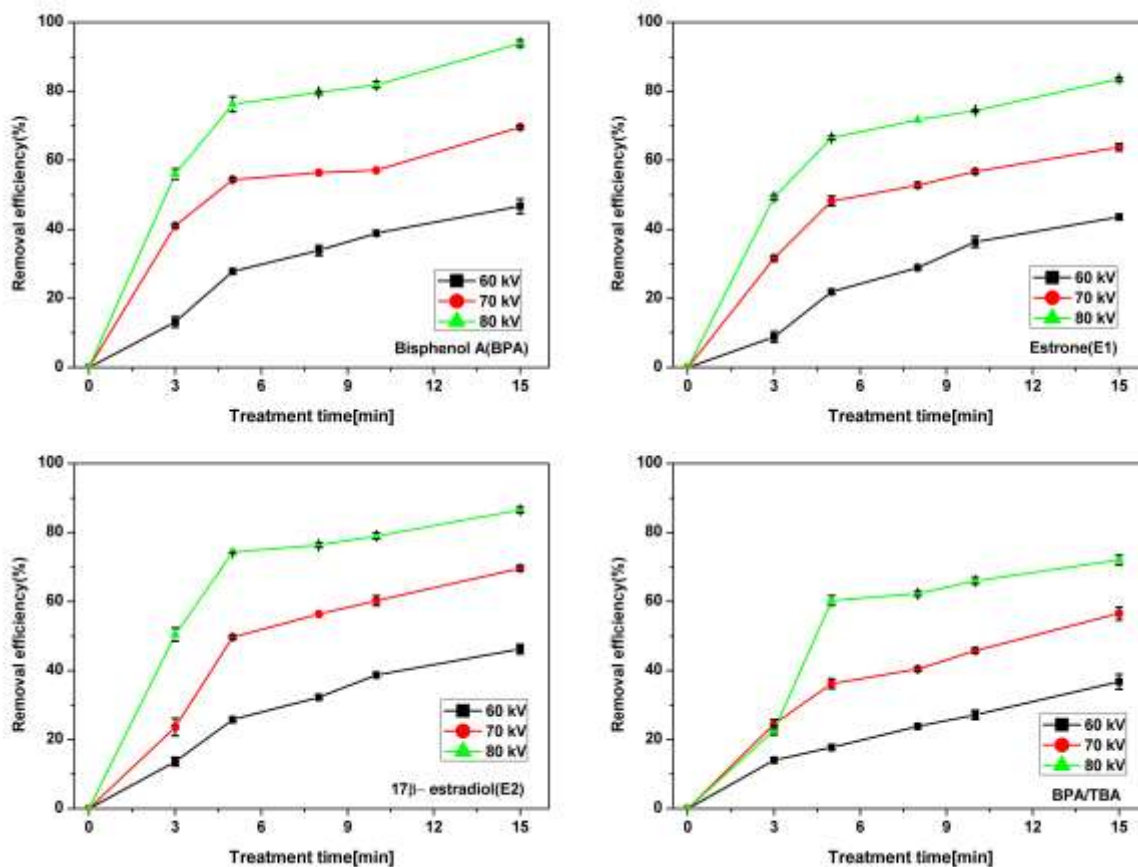
Fig.3. Percentage degradation of endocrine disruptors in model effluent. BPA/TBA refers to

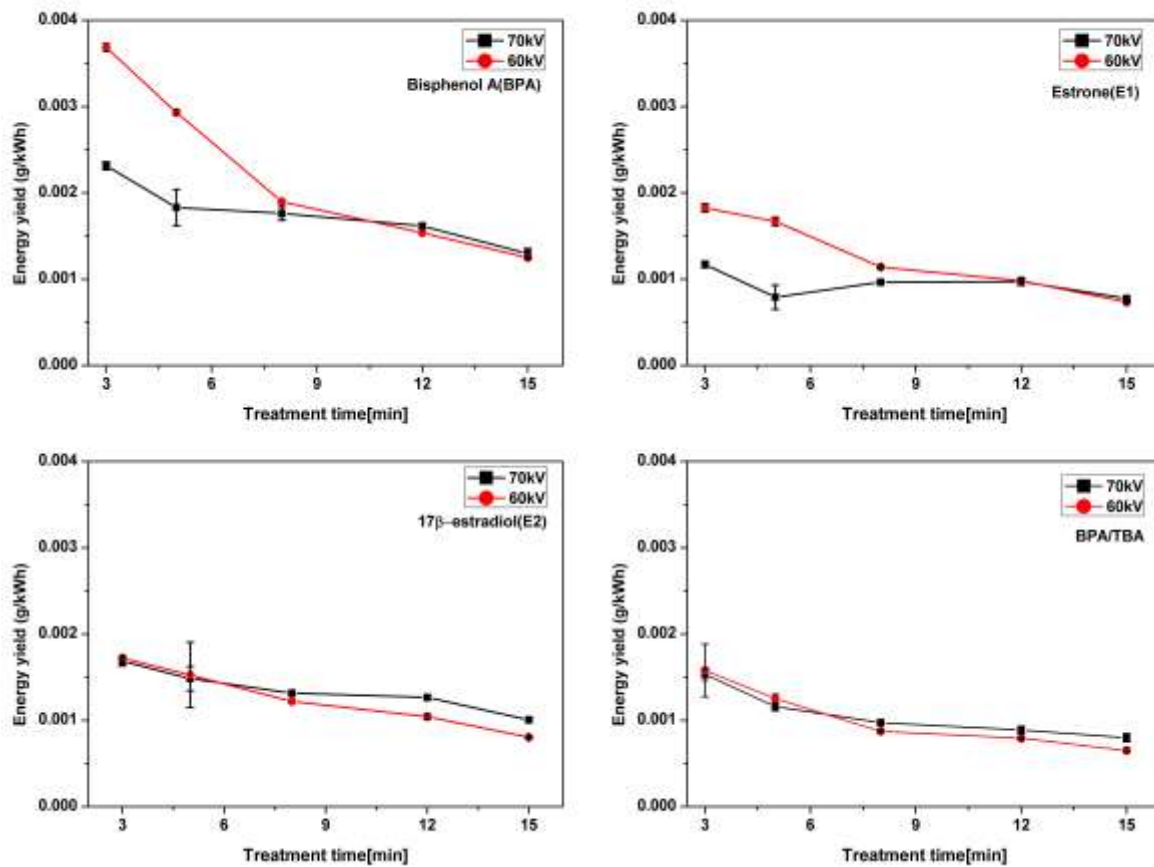degradation in presence of radical scavenger tertiary butanol alcohol.

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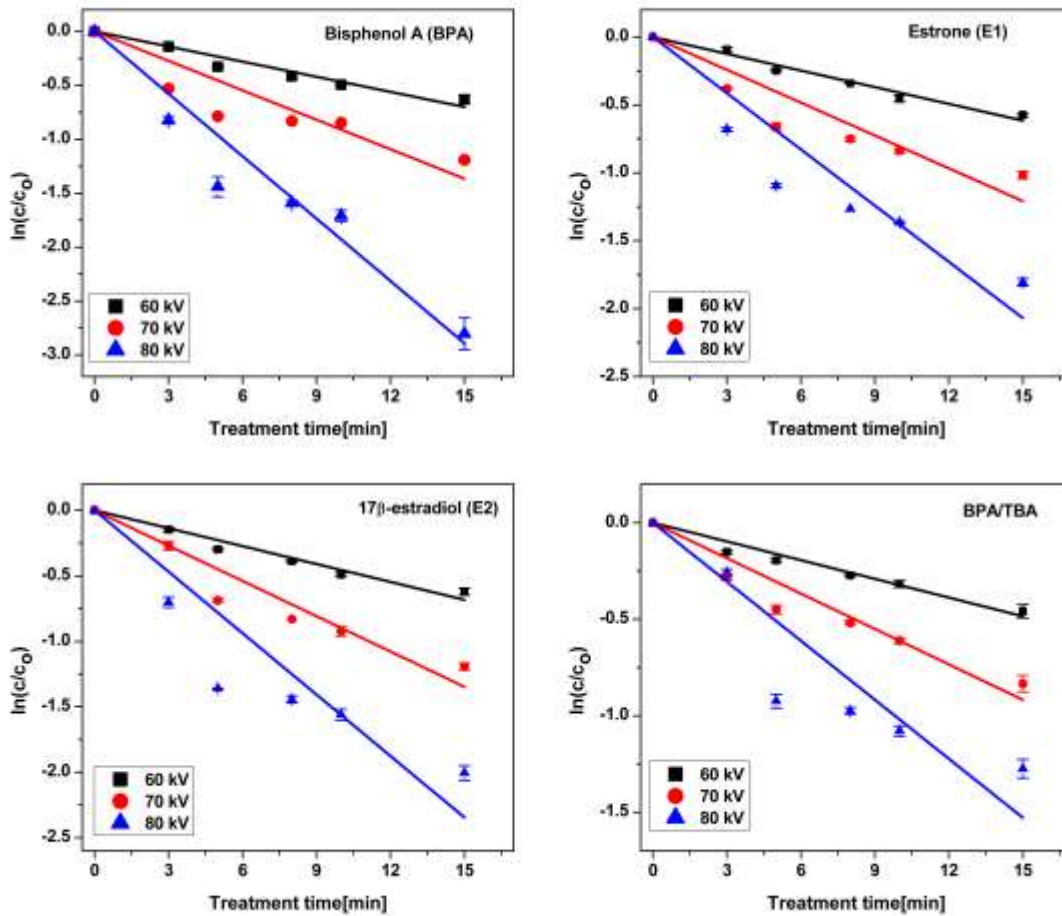
Fig.4. First order kinetics plots of endocrine disruptor degradation in model effluent. BPA/TBA

refers to degradation in presence of radical scavenger tertiary butanol alcohol.

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Fig.5. Evolution of energy yield with treatment time under different applied voltages. BPA/TBA

refers to degradation in presence of radical scavenger tertiary butanol alcohol.

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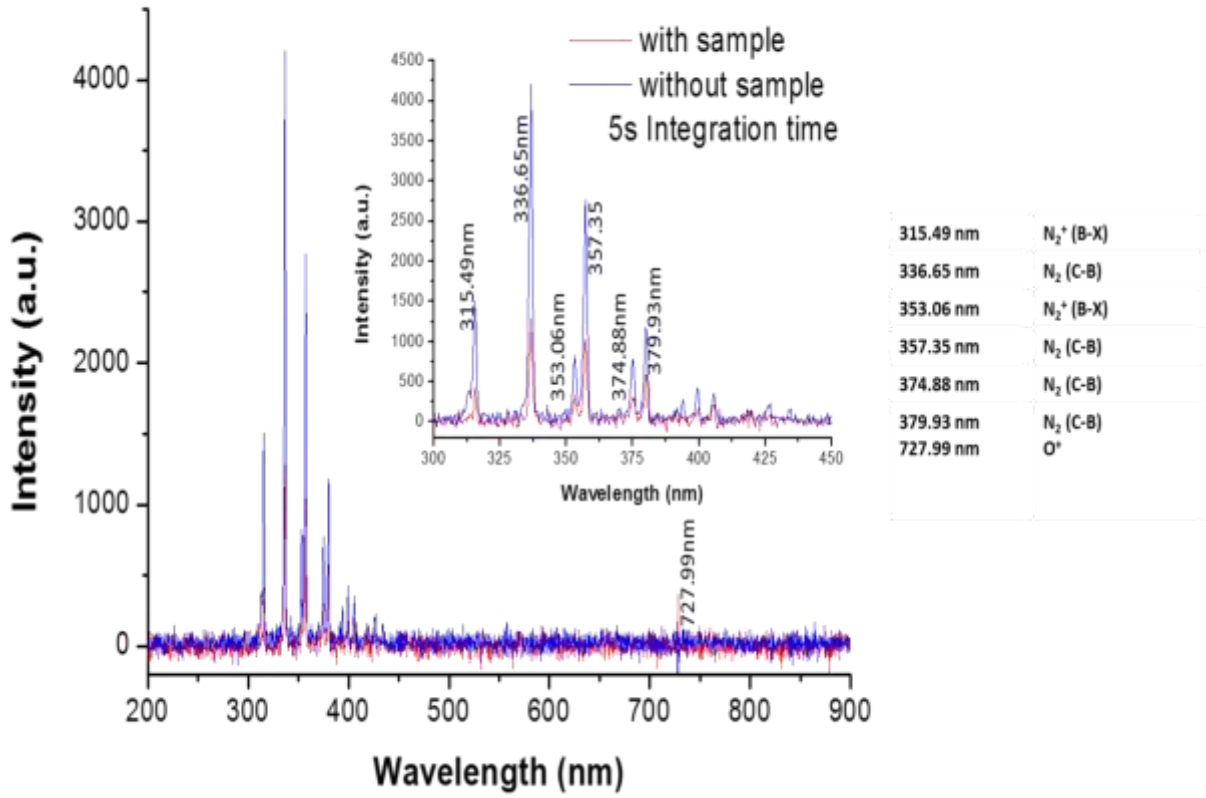
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744 **Fig.6.** Typical Optical Emission Spectrum (OES) of the dielectric barrier discharge in air.

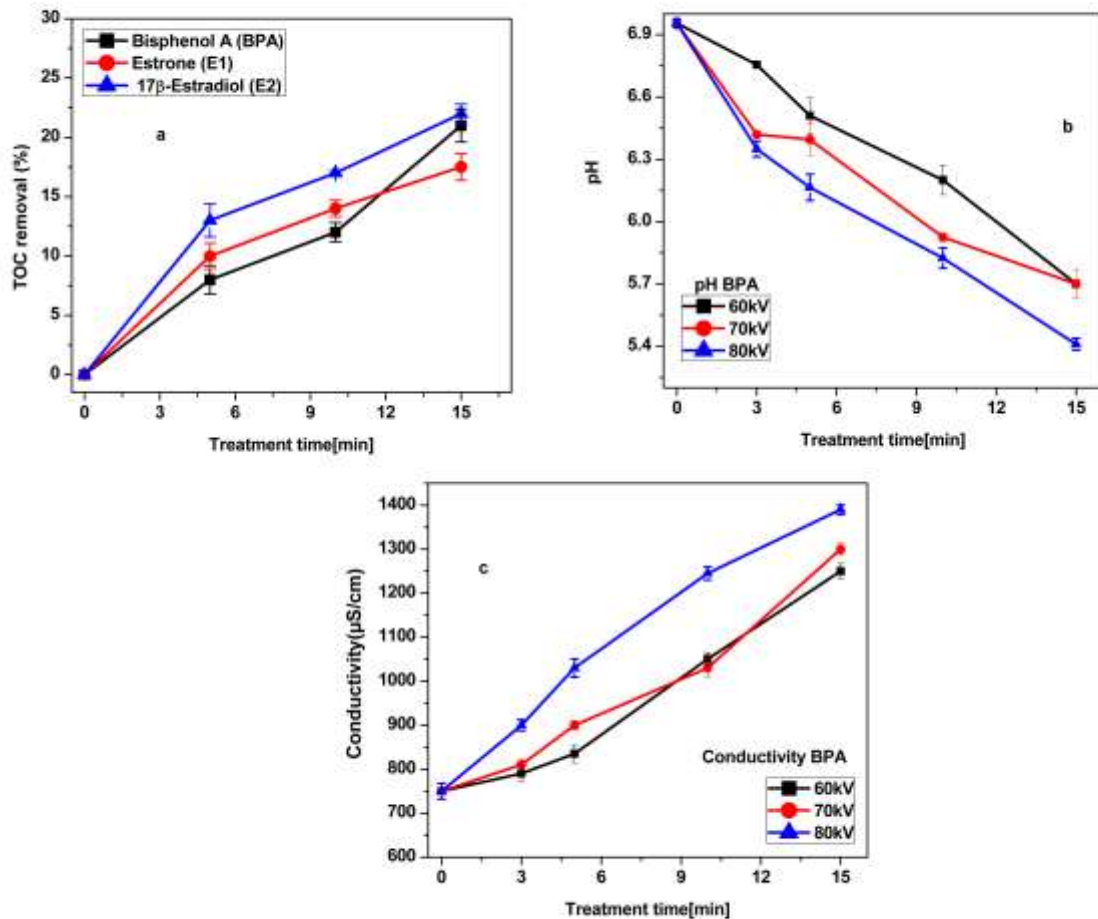745 Operating voltage 80 kV.

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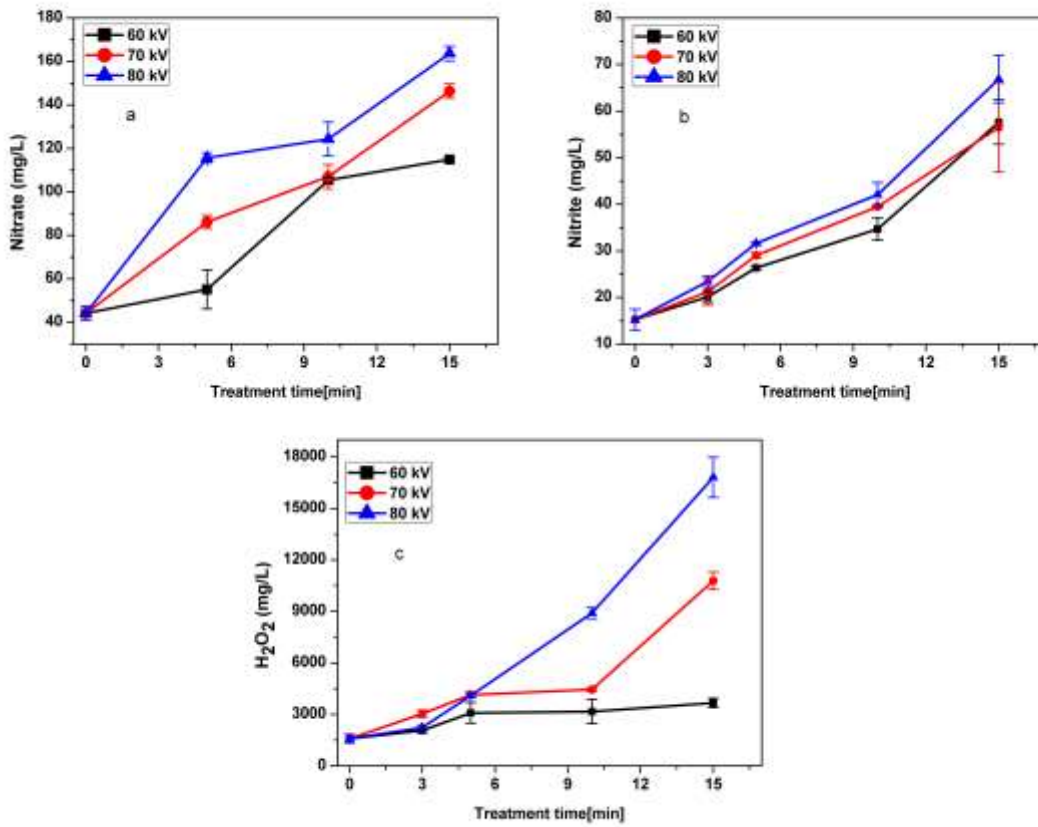**Fig.7.** (a) TOC removal efficiencies of EDC's after plasma treatment, (b) and (c) shows

evolution of pH and conductivity after plasma treatment of dairy effluent with Bisphenol A

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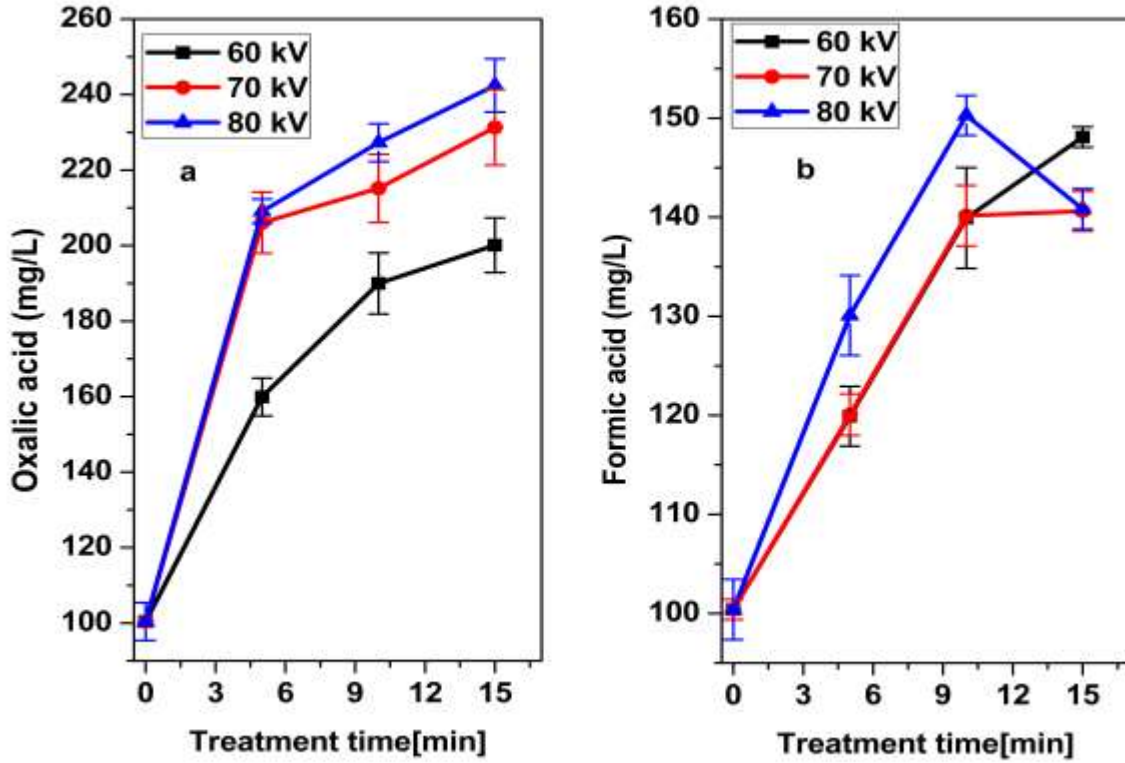761   **Fig. 8.** Variation of nitrite, nitrate and H_2O_2 concentration in plasma treated dairy effluent.

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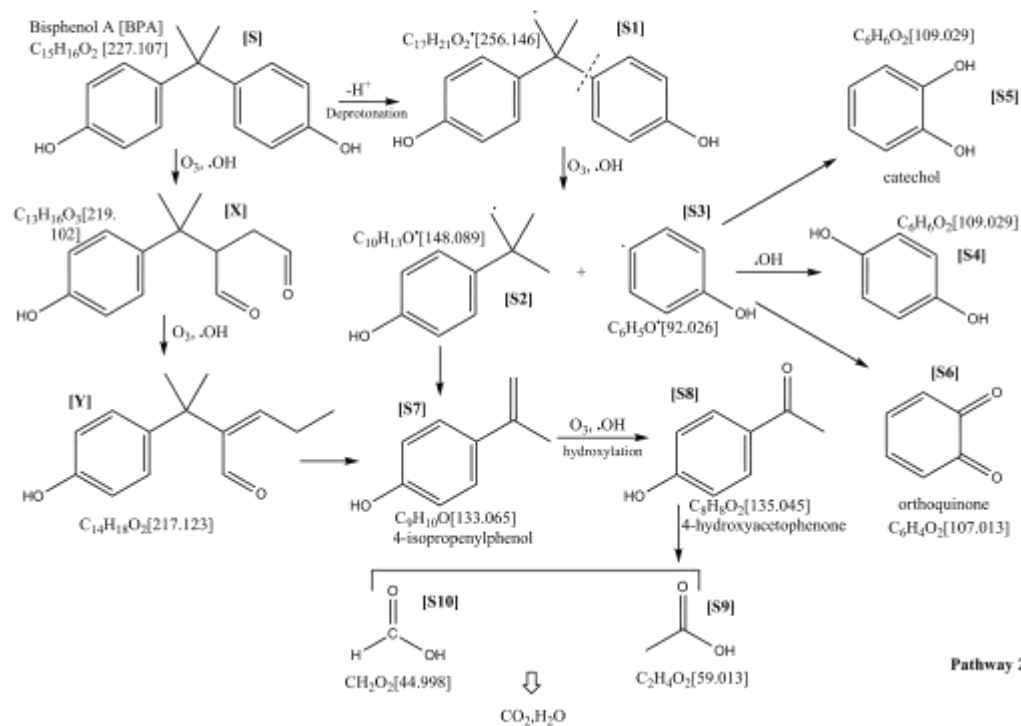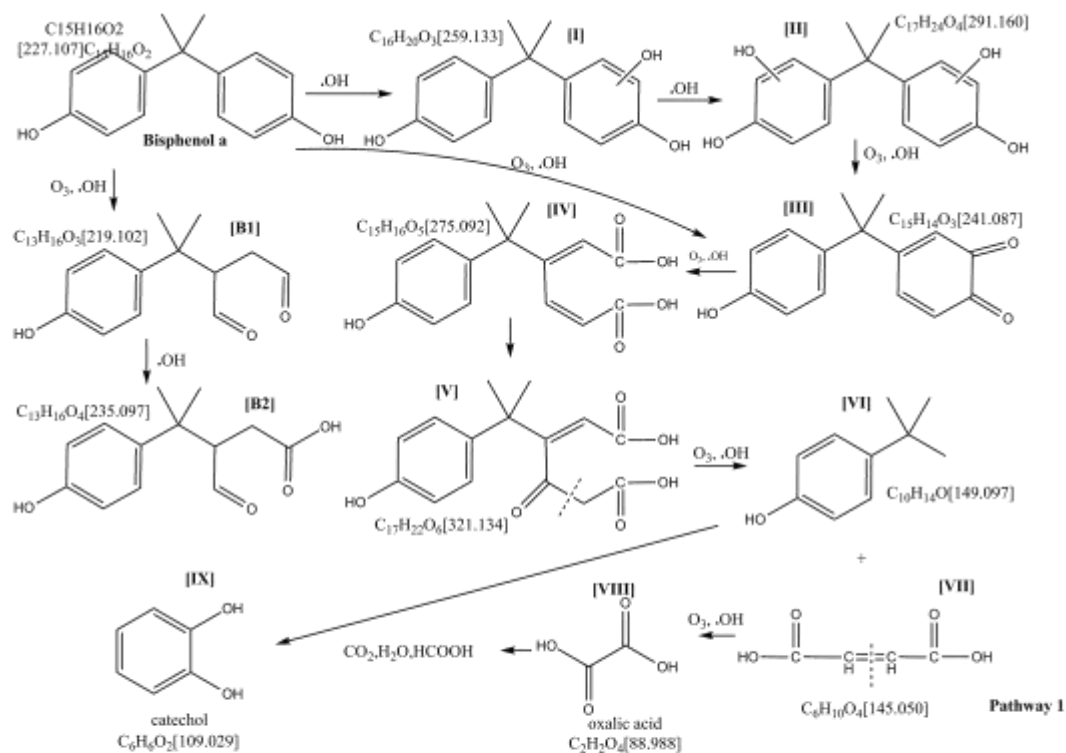
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Fig. 9. Variation of organic acid concentrations produced during degradation of plasma treated
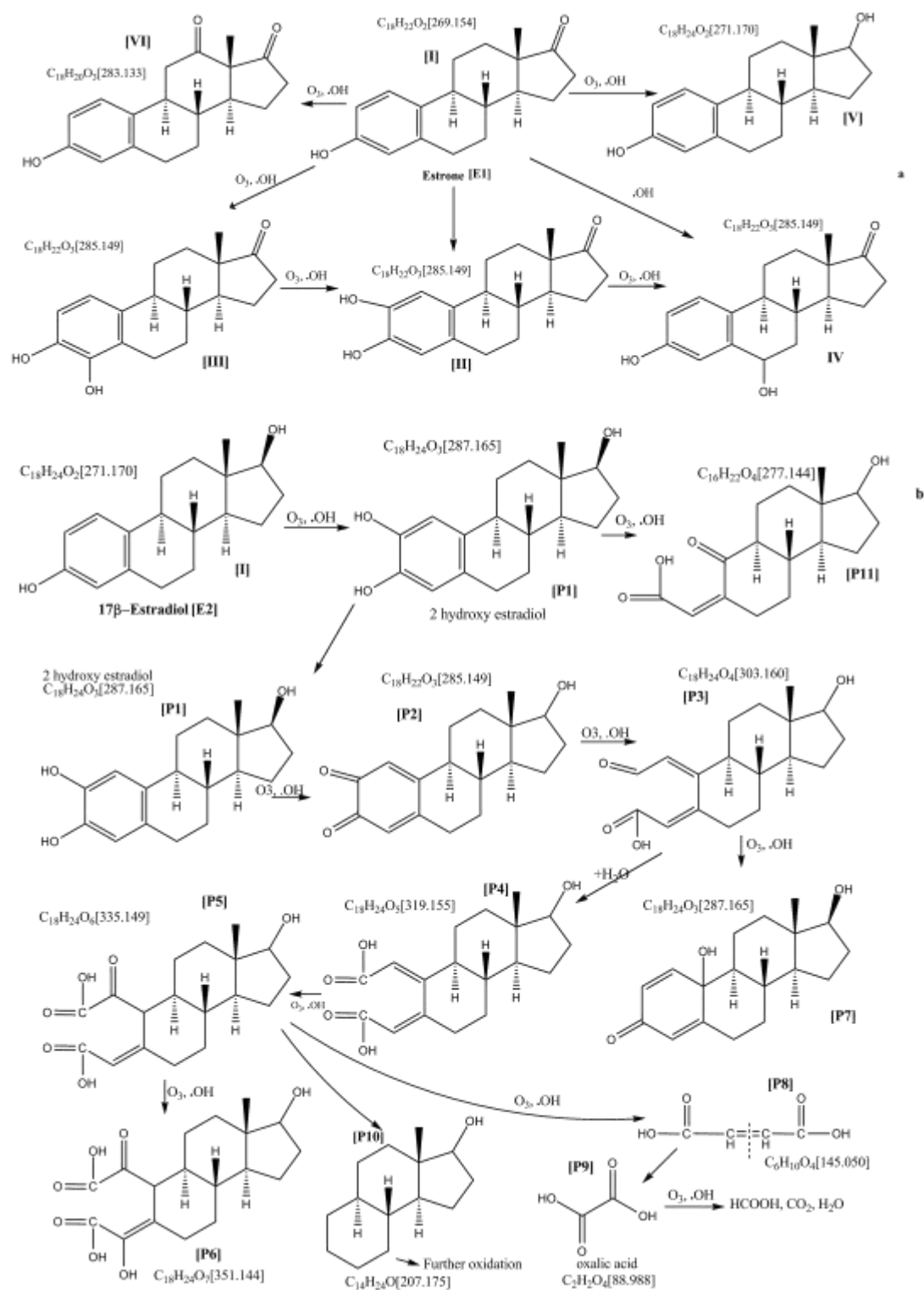
dairy effluent.

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Fig. 10. The proposed degradation pathways of Bisphenol A (BPA) with chemical formula and

monoisotopic mass of identified products

Fig. 11. The proposed degradation pathways of estrone (a) and 17β-estradiol (b) with chemical formula and monoisotopic mass of identified products

778 **Table 1**

779 **Model parameters for endocrine degradation based on first-order kinetics**

Endocrine disruptor	Voltage(kV)	Rate constant k (min^{-1})	R^2(Adj)
Bisphenol A (BPA)	60	0.045±0.003	0.97
	70	0.086±0.011	0.93
	80	0.189±0.016	0.97
Estrone (E1)	60	0.041±0.002	0.98
	70	0.076±0.009	0.93
	80	0.132±0.014	0.95
17β-estradiol (E2)	60	0.044±0.003	0.98
	70	0.087±0.086	0.96
	80	0.149±0.02	0.93
Bisphenol with TBA	60	0.031±0.012	0.99
	70	0.059±0.005	0.97
	80	0.098±0.015	0.91

780 Note: BPA/TBA refers to degradation in presence of radical scavenger tertiary butanol alcohol.

781   **Table 2**

782   **Removal efficiencies of estrogens using different oxidative technologies**

Process	Estrogens studied	Removal (%)	Treatment conditions	Other observations	reference
Chlorination	E1	70	1.0 mg/L contact time of 15 min	Matrix used was water with buffer solution.	[49]
Photo-Fenton	E2	86.4 98	pH-7.47 α-FeOOH-(5g/L)- 8 h. pH-3.07 α-FeOOH-(5g/L)- 8 h.	Matrix used was Milli-Q water at initital concentration of 272μg/L.	[50]
TiO$_2$/UV	E1, E2 E2	94 99	1g/L TiO2 and 1 h. 1g/L TiO2 and 30 min.	Matrix used was deionized water.	[51] [16]
Ozonation	E2, E1, EE2	100 94	0.38 mg/min 4 min (1.52 mg/L). 0.38 mg/min – 8 min(3.04	Matrix used was water at initial concentration of 10 mg/L, pH-9.	[53] [52]

			mg/L).	Matrix used was Milli-Q water with initial concentration of 100 µM and pH-6.5	
	BPA	100	Aqueous O$_3$(15-400 µM) treatment for 24 h		
Combination of AOP (US/O$_3$)	BPA	34.6	US intensity of 60W/cm^2.	Matrix used was deionized water at initial concetration of 100 ug/L	[83]
		63	O$_3$ flow rate of 10 mL/min		
		100	US/O$_3$ at 60W/cm^2 followed by O$_3$ treatment at flow rate of 10 mL/min		
DBD plasma	E2	100	Peak voltage of 12 kV at pH- 5.6 -30 min of treatment with ambient air as discharge gas	100 ug/L	[30]
		75		300 ug/L	
		64		600 ug/L	

		64		900 ug/L Matrix used was ultrapure water.	
DBD plasma combined with Pt– TiO$_2$ photocatalyst	E2	75 90 99.8	DBD plasma alone DBD+ TiO$_2$ DBD+Pt-TiO$_2$ Peak voltage of 12 kV-30 min of treatment with ambient air as discharge gas	Matrix used was ultrapure water at pH-5.6	[31]
Gliding arc plasma with ferrous ions	BPA	100 70	COD TOC At voltage of 10 kV-30 min of treatment. Under different working gases such as air, argon, oxygen/argon mixture	Matrix used was acidified osmosis water at pH-3 with initial concentration of 120 µM (28 mg/L)	[33]

			(20/80, v/v),		
DBD plasma	BPA	96	Voltage of 20 kV treatment time of 5 min with helium as discharge gas	Initial concetration of 1 mg/L	[32]

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