

1 **Improving biodegradability of soil washing effluents using**
2 **anodic oxidation**

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18 **Abstract**

19 In this work, a combination of electrochemical and biological technologies is proposed to
20 remove clopyralid from Soil Washing Effluents (SWE). Firstly, soil washing was carried
21 out to extract clopyralid from soil. After that, four different anodes —Ir-MMO, Ru-MMO,
22 pSi-BDD and Carbon Felt (CF) — were evaluated in order to increase the biodegradability
23 of the SWE. CF was selected because was the only one able to transform the pesticide to a
24 more biodegradable compounds without completely mineralizing it. Finally, biological
25 oxidation tests were performed to determine the aerobic biodegradability of the SWE
26 generated. From the obtained results, it was observed that at the beginning of the
27 electrolysis the toxicity slightly increased and the biodegradability decreases. However, for
28 electric current charges over $2.5\text{A}\cdot\text{h dm}^{-3}$ the toxicity drastically decreased, showing an
29 EC_{50} of 143 mg L^{-1} , and the BOD_5/COD ratio increased from 0.02 to 0.23.

30

31 **Keywords**

32 Clopyralid; electrochemical oxidation; biological treatment; integrated processes

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35 **1. Introduction**

36 In the 40's of the 20th century, the use of pesticides and fertilizers started to increase
37 exponentially. Consequently, the crops productivity drastically increased and in turn, the
38 World population underwent a very large increase. Unfortunately, agrochemicals have also
39 a dark side and nowadays they are known to be related to severe health and environmental
40 problems (Rodrigo et al., 2014). Thus, an important fraction of the herbicides and
41 pesticides applied to crops may be transported to many other places, causing many
42 environmental and health problems or limiting the use of water reservoirs for human or
43 animal supply (Geed et al., 2017; Nawab et al., 2003; Verma et al., 2014).

44 Clopyralid (3,6-dichloro-2-pyridine-carboxylic acid) is a widely used herbicide that
45 belongs to the pyridine chemical group. This herbicide persists in soil under anaerobic
46 conditions and with low microorganisms content (Corredor et al., 2006). Clopyralid
47 presents high solubility in water and because of that, it has been detected very frequently in
48 the environment and even in drinking water (Berberidou et al., 2016).

49 The high water solubility of clopyralid makes very easy its extraction from polluted soils
50 through a conventional Soil Washing (SW), without being necessary the addition of
51 surfactant to the washing fluid. This is very interesting since the use of surfactants
52 generates highly-polluted effluents containing complex mixtures of dissolved organics,
53 surfactants and inorganic salts (Chair et al., 2017b). For these reasons, the application of
54 SW to soils polluted with hydrosoluble pollutants becomes a simple and low cost option
55 for their remediation (Chair et al., 2017a). Once the pesticide is extracted from the soil, the
56 soil pollution problem becomes a wastewater problem, because the pollution is then
57 associated to the washing fluid and not to the soil.

58 Nowadays, the biological treatment is the most widespread method to treat almost any type
59 of wastewater. It possesses various important advantages, because they are cost effective,
60 well studied and can be easily modified according to wastewater characteristics (Ganzenko
61 et al., 2014; Liu et al., 2010). Unfortunately, they are not always effective for the treatment
62 of wastewater polluted with complex organic molecules (such as the SW effluents
63 containing anthropogenic herbicides). In fact, it is observed very frequently the presence of
64 slowly biodegradable and even bio-refractory pollutants in the soil. When working with
65 slowly biodegradable or complex chemicals such as herbicides, the acclimatization stages
66 and the application of long solids retention times seem to be the keys to enhance the
67 biological removal (Carboneras et al., 2017). Anyway, sometimes the results obtained are
68 not as good as desired, being necessary to combine or substitute the biological treatment by
69 other type of treatments.

70 In the literature, Advanced Oxidation Processes (AOPs) are frequently proposed for this
71 purpose (Barrera-Díaz et al., 2014). The oxidation conditions obtained in those processes
72 are harsh, because of the formation of hydroxyl radicals (Oller et al., 2011), one of the
73 most powerful oxidants known nowadays (Ganzenko et al., 2014). A variety among the
74 AOPs are the electrochemical AOPs, which entail the processes in which hydroxyl radicals
75 are produced from anodic oxidation (AO) of water or cathodic reduction of oxygen (Sires
76 et al., 2014). In the literature, the electrochemical oxidation (EO) processes using a wide
77 variety of electrodes has become a reference in recent years for the depletion of persistent
78 organic pollutants, such as the herbicides, contained in wastewater. Different electrodes
79 have been used for AO processes. Among others, the Boron doped diamonds (BDD),
80 Mixed Metal Oxides (MMO) and carbon felt (CF) stand up. Although EO processes has
81 been proved to be a very efficient way to remove persistent pollutants, they present a
82 considerable drawback, the intensive energy consumption required to complete the total

83 mineralization. Therefore, the cost associated to this process is very high (Oller et al.,
84 2011).

85 Taking into account the advantages and downsides of each technology, a good option
86 seems to be the combination of both processes. In this way, electrochemical process could
87 be applied as a pre-treatment or post-treatment depending on the initial toxicity and
88 biodegradability of the effluent. When the effluent is not toxic, the electrochemical process
89 can be implemented as pre and post-treatment, whereas when dealing with toxic effluents
90 the electrochemical technology must be always carried out as pre-treatment, in order to
91 remove the toxic pollutants before the biological treatment. Thus, when the
92 electrochemical process is applied as a pre-treatment, the main objective would be the
93 rupture of the organic molecule into more biodegradable ones and/or the removal of toxic
94 compounds. On the contrary, when the electrolysis is applied as a post-treatment, the aim
95 would be the total elimination of the refractory molecules, which were not removed by the
96 biological treatment.

97 In this context, the aim of this study was to improve the biodegradability and to reduce the
98 toxicity of the effluents generated by a SW process. Firstly, the soil polluted with a
99 commercial herbicide based on clopyralid underwent SW producing a Soil Washing
100 Effluent (SWE). After that, anode material and operating conditions of AO were optimized
101 in order to get a more biodegradable SWE and this was verified by using some biological
102 tests carried out to evaluate the toxicity and biodegradability of the effluent generated.

103

104 **2. Materials and Methods**

105 *2.1. Soil and Soil-Washing Fluid*

106 The soil used in this work was a clay soil. Main characteristics can be found elsewhere
107 (Risco et al., 2015). It was polluted with Lontrel[®] 72, a commercial herbicide based on
108 clopyralid. (72% w/w) supplied by Dow AgroSciences. To prepare the polluted soil, 1.0 g
109 of clopyralid was dissolved in 0.5 dm⁻³ of water and this solution was spiked over 1.0 kg of
110 clay soil.

111 *2.2 Experimental procedure*

112 As Soil-Washing Fluid (SWF) a synthetic groundwater was used. It was made by
113 dissolving 3.3355 g of MgSO₄·7H₂O, 0.6590 g of NaCl, 0.6510 g of NaNO₃, 0.1277 g KI
114 and 1.2485 g CaCO₃ in 5,0 dm³ of deionized water (Chair et al., 2017a). The Soil-Washing
115 consists of mixing the polluted soil and 5.0 L of synthetic groundwater for 6 hours and
116 then, letting it settle for 12 additional hours in order to separate the soil from the aqueous
117 phase. The SWE generated was filtered through a 0.45 µm glass fibre filter (Whatmann
118 GF/C) then used in the electrochemical pre-treatment. The soil washing effluent presented
119 a clopyralid concentration of 180 mg dm⁻³, which means a recovery of about 90% of the
120 pesticide spiked in the soil.

121 The electrolyses tests were performed in an in-house electrochemical flow cell described
122 elsewhere (Canizares et al., 2005). The effluent was stored in a thermo-regulated glass tank
123 of 5.0 L. The effluent circulation through the flow-cell was obtained using a peristaltic pump
124 working at a flow rate of 15 mL s⁻¹. The electrical current was applied using a DC Power
125 Supply (FA-376 PROMAX). All experiments were performed in galvanostatic mode. Four
126 types of anodes were tested: p-Si Boron Doped Diamond (BDD) manufactured by Adamant
127 (Switzerland), Ruthenium based Mixed Metal Oxides (Ru-MMO) and Iridium based Mixed

128 Metal Oxides (Ir-MMO) manufactured by Tiaano (India) and Carbon Felt (CF) Sigracell®
129 GFA6EA manufactured by ElectroChem Inc. (USA) and presented an specific surface area
130 of 35.3 m² g⁻¹ and 17700 m² m⁻², areal weight 500 g m⁻², porosity 95% and roughness 30
131 μm. An AISI 316 steel cathode was used in all the experiments performed.

132 *2.3. Analytical techniques*

133 Clopyralid concentration was measured by HPLC, using an Agilent 1260 Infinity
134 equipment. The column was an Eclipse Plus C-18 (4.6mm x 100mm; 3.5μm). The mobile
135 phase was a mixture of 30% methanol and 70% formic acid (0.1%). The detection of the
136 herbicide was achieved at 280 nm and the flow rate was set at 1 mL min⁻¹. The injection
137 volume was 20.0 μL and the retention time was around 2.7 minutes. The reactions
138 intermediates of clopyralid were identified by gas chromatography – mass spectrometry
139 (GC-MS) and HPLC. Before using GC-MS, a freeze dryer was used in order to remove the
140 water contained in the samples. The equipment was operated at -50°C and 0.02 bar. Once
141 removed the water, ethyl acetate was added to the freeze-dried samples. The organic phase
142 was analysed by GC-MS using a Thermo Scientific DSQ II Series Single Quadrupole GC-
143 MS (Coolvauum Technologies) with a NIST05-MS library. The column was a polar TR-
144 WAXMS (30 m x 0.25 mm x 0.25 mm). The temperature ramp was 40°C for 1 min, 5°C
145 for min 2 up to 250°C. Acids were identified by a HPLC equipped with a Zorbax SB-Aq,
146 column (4.6mm x 150mm), the mobile phase consist of 4 mM H₂SO₄ at 210 nm. The
147 identifications of the intermediates was conducted by the retention time comparison and
148 internal standard addition methods using standard solutions (Özcan et al., 2010).

149 The inorganic ions contained in soil washing effluent were determined by ionic
150 chromatography in a Metrohm 930 Compact IC Flex device. TOC and nitrogen
151 concentrations were monitored using a MultiN/C 3100 analytic Jena analyser. Organic

152 carbon compounds were converted to carbon dioxide and then detected by a non-dispersive
153 infrared detector. COD was measured using a Spectroquant[®] test CSB from Merck. All the
154 samples were measured in duplicate.

155 BOD₅ test were performed in Oxitop IS 6-Var. This measurement detects the oxygen
156 consumed by the microorganisms when oxidizing the organic matter contained in the
157 sample. According to the literature a trace minerals solution was added (Eaton et al., 2005).
158 The samples were inoculated with 1 mL of activated sludge taken from a conventional
159 WWTP described elsewhere (Rodríguez Mayor et al., 2004) to provide an adequate
160 inoculum to degrade the organic matter. A Strathtox respirometer by Strathkelvin
161 instruments was employed to measure the oxygen consumption rate by bacteria over a
162 period of 30 minutes. The tests were carried out using the same mineral solution and
163 conventional activated sludge as in the BOD₅ tests. Before the respirometric tests, the
164 activated sludge was aerated overnight in order to ensure the full consumption of the
165 substrate contained in the sludge sample and therefore the endogenous conditions.
166 Additionally a blank reference test was performed to isolate the oxygen consumption rate
167 caused by the inoculum added.

168 Water samples toxicity were determined along the different stages of the electrochemical
169 pre-treatment by means of a BioTox[™] Kit supplied by Aboatox. The inhibitory effect of
170 the sample on the light emission of luminescent bacteria, *Aliibivrio fischeri*, formerly
171 *Vibrio fischeri* (Urbanczyk et al., 2007), is measured with a luminometer (Junior LB 9509
172 of Berthold Technologies). This test provides a rapid, easy to use method for measuring
173 toxicity of aqueous samples (Rodriguez et al., 2013). The sample toxicity was evaluated as
174 EC₅₀ by measuring the effective concentration at which 50% of the light is lost due to the
175 toxicity (Salles et al., 2010; Sarria et al., 2002).

176 *2.4. Electrode characterization*

177 In order to characterize the electrode before and after the AO process scanning electron
178 microscopy (SEM), Brunauer, Emmett and Teller (BET) and Raman spectroscopy
179 techniques were used. SEM images were obtained by using a FEI QUANTA 250. With
180 regard to the BET technique, porosity measurements were carried out using a Quadrasorb
181 Model 2SI sorptometer apparatus with N₂ at the sorbate at -196 °C . The samples were
182 outgassed at 180 °C under vacuum (5×10^{-3} Torr) for 12 h prior analysis. Raman spectrums
183 were obtained by means of a SENTERRA spectrometer with a grating of 600 lines per mm
184 and a laser wavelength of 532 nm at a very low laser power level (< 1 mW), to avoid any
185 heating effect. Raman spectroscopy is considered a very interesting tool in the study of
186 carbon materials due to its quickness and trustworthiness (Lavin-Lopez et al., 2017).

187

188 3. Results and Discussion

189 3.1 Soil washing and Anodic oxidation of the Soil Washing Effluent

190 SW is an efficient technology for extracting pollutants from soil and transferring them into
191 a SWE, which should be subsequently treated as a wastewater. As clopyralid is highly
192 soluble in water, no addition of surfactant (or any other chemical) was required in the
193 formulation of the Soil Washing Fluid (SWF). In previous tests (Chair et al., 2017b)
194 different ratios SWF/soil (w/w) were evaluated and it was found that using a ratio of 0.50 g
195 SWF g⁻¹ soil allows us to extract up to 90% of the clopyralid contained in the soil,
196 producing in this case a SWE with a concentration of 180 mg L⁻¹ of herbicide. The
197 remaining 10% seems to be irreversible adsorbed onto the soil, because the extraction
198 percentage does not improved with larger ratios. The SWE does not only extract the
199 pesticide but also drags anions and cations contained in the clay soil and Table 1 shows the
200 ion concentrations of the soil washing effluent generated.

201 [TABLE 1 NEAR HERE]

202 The most important information attained is that because of these salts, the conductivity of
203 this SWE is high enough (1200 mS cm⁻¹) to apply an AO process without adding any other
204 salts.

205 The efficiency of AO processes is related to the anode material used and the current
206 density applied. It is well-known that various materials lead to soft oxidation conditions
207 promoting the formation of many reaction products, while other materials generate a harsh
208 reactive media for which carbon dioxide is the main or even the only final product. In this
209 work, it is aimed to select an anode capable of breaking the bio-refractory herbicide into
210 more simple and biodegradable molecules. For this reason, four anodes were evaluated.
211 Three of them are typically identified in the first group (Ru-MMO, Ir-MMO and CF) while

212 the other (p-Si BDD) was selected in this study for comparison purposes, because it is
213 known to be the best anode to attain mineralization of wastewater. Results of the AO
214 depends on the applied current density, as well. Low values are known to lead to soft
215 oxidation while high values lead to harsh conditions. Hence, the simultaneous selection of
216 the anode material and the operating current density can be very important to get a suitable
217 AO-biological oxidation combined technology.

218 AO test were performed at 5.0, 15.0 and 60.0 mA cm⁻² using the Ru-MMO and the Ir-
219 MMO electrodes (Figure 1). As observed, there are almost no transformation of clopyralid,
220 indicating both MMO electrodes were unable to transform the clopyralid at the current
221 densities studied. Initially, this was an expected result for the Ir-MMO but not for the Ru-
222 MMO, for which mediated oxidation by chlorinated species was expected. This behaviour
223 could be explained because of the low concentration of oxidant precursors contained in the
224 electrolyte as compared to most works found in the literature (in this work, no additional
225 supporting electrolyte was used but only the salts drawn during the SW process).

226

227 [FIGURE 1 NEAR HERE]

228

229 Regarding to the pSi- BDD electrode, the results obtained in these test carried out at the
230 three same current densities are presented in Figure 2.

231

232 [FIGURE 2 NEAR HERE]

233

234 A very rapid removal of clopyralid is observed at the three current densities applied and
235 the herbicide was fully mineralized (total depletion of TOC) in all cases. These trends
236 agrees with the expected oxidative capacity of the AO with pSi-BDD, owing to the larger
237 generation hydroxyl radicals. They also indicate that the most efficient conditions are
238 attained by working at the lowest current density, because the electric charges required for
239 this depletion are the lowest.

240 In order to get deep inside the transformation taking place when operating with pSi-BDD
241 anodes, the samples taken over the electrolyses were analysed by HPLC. The obtained
242 results (not shown) revealed that BDD anode did not generate any appreciable concentration
243 of reaction intermediates (only 2 peaks were observed, all of them close to the detection limit
244 of the analytical technique), which indicates that the pesticide was completely mineralized,
245 almost directly. This was ratified by the TOC measurements (see Figure 2b). As can be seen,
246 the TOC decreases at the same rate as the clopyralid, indicating the complete oxidation to
247 carbon dioxide. Similar results were previously reported in the literature in many works
248 focused on the oxidation with diamond anodes of low concentrated wastewater. This can be
249 explained because DDB anodes present high O₂ evolution overpotencial, which favors the
250 complete oxidation of the clopyralid to carbon dioxide (Gheraout et al., 2011; Moreira et
251 al., 2017).

252 However, taking into account the goal of this work, this result was adverse, because here
253 the interest is to combine electro and bio technologies in order to minimize the high
254 electrical consumption of AO. This fact motivated the trial with carbon felt (CF) anodes.

255 However, before this study, because of the porosity of the CF electrode, the potential
256 adsorption of the clopyralid over the electrode surface was determined. To do that, pieces of
257 CF electrodes were submerged in soil washing effluents containing different clopyralid

258 concentrations ranging from 25 to 200 ppm during three days. From the obtained results, it
259 can be concluded that the clopyralid was not absorbed on the CF electrode. Once ensured
260 the absence of clopyralid adsorption on the CF electrode, the AO tests using CF were
261 performed. CF presents a low O₂ evolution overpotencial, and theoretically only permit the
262 partial oxidation of organics (Ghernaout et al., 2011; Moreira et al., 2017). The clopyralid
263 and TOC removal obtained during the AO with CF are presented in Figure 3. These tests
264 were carried out at 5.0 and 10.0 mA cm⁻² because at larger current densities the CF electrode
265 undergoes severe degradation (it is known to be electrochemically burned at the cell
266 potentials originated at larger current densities). As can be seen, a significant transformation
267 of the clopyralid was obtained. However, the TOC presented a slower removal rate and it
268 was not completely removed, indicating a transformation, but not a complete mineralization
269 of the clopyralid. The transformation of the pesticide into other more oxidized organic
270 compounds made the CF the most interesting electrode to perform the pre-treatment. In
271 comparing the effect of the current density, it should be pointed out that similar trends were
272 observed when working at 5 and 10 mA cm⁻². However, at 5mA cm⁻², the larger
273 chromatographic areas for the intermediates were obtained. For this reason, the test carried
274 out at the current density of 5 mA cm⁻² was selected for the subsequent studies related to the
275 biological characterization.

276

277 [FIGURE 3 NEAR HERE]

278

279 In order to verify the transformation taking place when using CF as anode, GC-MS and
280 HPLC analyses were carried out identifying the main 3 compounds. Two of them, 2,5 –
281 dichloropyridine and 2-picolinic acid, showed the typical behaviour of intermediates, while

282 the third one, acetic acid, behaves as final product. Figure 4 presents the additive area of all
283 the reaction intermediates detected versus the electric charge applied, as well as the
284 corresponding area of the final product, acetic acid. As can be seen, the total concentration
285 of intermediates increase during electrochemical pre-treatment reaching a maximum value
286 for electric charges passed of about $2.5 \text{ A}\cdot\text{h dm}^{-3}$.

287

288 [FIGURE 4 NEAR HERE]

289

290 The pH and conductivity were also monitored during the AO pre-treatment. Figure 5
291 shows the changes in the pH and conductivity when treating the SWE using a CF anode.
292 pH value evolved from a neutral value to 3.44. This can be explained in terms of the AO
293 process taking place on the electrode surface, but also on the acidification due to the acetic
294 acid dissociation in the liquid bulk (Fernández et al., 2011). Because of the subsequent
295 biological treatment, it was necessary to adjust the pH to 7.0 after the electrolysis in order
296 to perform the biological tests and toxicity analyses. Regarding to the conductivity, its
297 value increased at the beginning of the treatment and then it was kept almost constant
298 along the electrolysis tests. As seem, values are high enough to perform the electrolysis at
299 not low but still suitable cell voltages (within the range of 13-18 V).

300

301 [FIGURE 5 NEAR HERE]

302

303 *3.2. Electrode characterization*

304 SEM pictures showed that CF electrodes had not changed significantly during the AO
305 process when working at 5 mA cm^{-2} of current density. The surface of the carbon felt
306 electrodes only suffer low corrosion effects, even after working for several hours and
307 deposits of minerals salts on the surface of the electrodes. Similar results were obtained by
308 (Panizza & Cerisola, 2009).

309 BET analysis were used to determine the CF pores sizes. From these tests, the average pore
310 radio values of the electrodes before and after the treatment were 20.94 \AA and 16.34 \AA , very
311 similar values that indicates a small clogging effect of the electrode pores during the
312 electrolysis. As indicated previously in the SEM photos, mineral salts deposits could have
313 partially blocked the pores of the electrode surface.

314 Raman spectrum and the most characteristics Raman parameters corresponding to the carbon
315 felt electrodes before and after the electrochemical treatment were identified. In the CF
316 electrodes used in this work two different peaks, G and D, appeared. G peak was located
317 around 1580 cm^{-1} and indicated the movement of carbon atoms linked by sp^2 bonds, whereas
318 D peak was located at 1350 cm^{-1} and it measures the presence of imperfections in the carbon
319 felt structure. The ratio between the intensities of two peaks (I_D/I_G) is commonly used to
320 determine how the structural disorder increase in the material (Lavin-Lopez et al., 2017).

321 From the results obtained in the Raman spectra corresponding to samples before and after
322 the AO process at $3.82 \text{ A}\cdot\text{h}\cdot\text{dm}^{-3}$ of electrical charge, the ratio I_D/I_G ratios were determined.
323 The I_D/I_G increased from 1.17 cm^{-1} to 1.20 cm^{-1} when the AO process took place indicating
324 that the CF electrode had suffered a slight corrosion. In this sense Raman spectrum
325 corroborated the results obtained with SEM pictures and BET analyses, indicating that after
326 the AO process hardly influenced the surface of the CF electrode.

327

328 *3.3. Toxicity and biodegradability evaluation*

329 Once the SWE underwent the AO with CF at 5 mA cm^{-2} , the changes in the toxicity and
330 biodegradability were evaluated. To do that, EC_{50} , BOD_5 , COD and OUR (oxygen uptake
331 rate) test were determined.

332 The parameter EC_{50} gives information about the toxicity of electrolysed effluents. This
333 parameter refers to the concentration causing and inhibition over the 50% of the
334 individuals. Figure 6 presents the evolution of the EC_{50} along the AO treatment. As can be
335 seen, initially the toxicity slightly increased, as indicate the EC_{50} trend. However, when the
336 applied charge was higher than $2.5 \text{ A}\cdot\text{h}\cdot\text{dm}^{-3}$ the toxicity decreased, showing an increasing
337 EC_{50} value. At the maximum applied charge ($3.83 \text{ A}\cdot\text{h}\cdot\text{dm}^{-3}$), the EC_{50} achieved its
338 maximum value, 143 mg L^{-1} , a value that triplicate the initial one. This means that the first
339 stages of the AO do not reduce the toxicity. However, after the application of a minimum
340 current charge, the toxicity decreased.

341

342 [FIGURE 6 NEAR HERE]

343

344 The EC_{50} is an important parameter because it informs about toxic and inhibitory effects in
345 the microbial population. However, toxicity/inhibitory effects and biodegradability are
346 completely different. Because of that, the biodegradability of the samples was also
347 measured as oxygen uptake rate (OUR) according to the literature (De Lucas et al., 2005;
348 De Lucas et al., 2007) and the obtained results are also presented in Figure 6. The OUR of
349 each sample was measurement using a respirometer. Each sample was analysed twice, in
350 order to ensure the reproducibility. When low electrical charges were applied, the

351 biodegradability decreased. This could be explained because of the oxidation of the
352 biodegradable substrates initially contained in the sample and because of the slight increase
353 in the toxicity at the beginning of the electrolysis previously described due to the presence
354 of two chemicals presenting acute toxicity: 2,5 – dichloropyridine and 2-picolinic acid,.
355 Then, when the applied electric charge increased, the substrates were transformed into
356 more biodegradable ones, such as acetic acid, at the same time that the toxicity decreased,
357 leading to a sample presenting higher biodegradability. Additionally, a partial
358 dehalogenation of the organic molecules was ratified by IC, accounting to about 20% of
359 the initial chlorine contained in the organic molecule of the clopyralid. With regard to
360 Figure 6, it must be highlighted that grey area corresponds to negative respiration rates.
361 These negative respiration rates, which mean respiration rates lower than those obtained in
362 the reference test, can only be explained because toxic/inhibitory effect of the reaction
363 intermediates generated during the electrolysis. These results indicates that, in order to
364 improve the biodegradability of the soil washing effluents via electrolysis with CF anodes,
365 the required electric charged should be higher than $2.5 \text{ A}\cdot\text{h dm}^{-3}$. As can be seen both, the
366 biodegradability and the toxicity, trends drastically changes when the applied charge
367 exceeds $2.5 \text{ A}\cdot\text{h dm}^{-3}$.

368 In the literature the biodegradability of the effluents are usually measured as the
369 BOD_5/COD ratio, being considered the effluents as easily biodegradable when its
370 BOD_5/COD ratio exceeds 0.4 (Zaghdoudi et al., 2017) (Pulgarin et al., 1999). In this work,
371 the biodegradability after the AO process, measured as BOD_5/COD ratio, increased from
372 0.02 to 0.23 as can be seen in Figure 6. The minimum value assigned to biodegradable
373 samples was not reached, but the biodegradability was significantly improved.

374

375 **4. Conclusions**

376 In conclusion, MMO electrodes are not be able to transform clopyralid, whereas BDD
377 anodes completely oxidize the clopyralid to carbon dioxide, without the generation of any
378 reaction intermediates. Conversely, CF anodes are able to degrade clopyralid generating
379 reaction intermediates such as 2,5 – dichloropyridine and 2-picolinic acid and acetic acid
380 as final product. When using CF anodes, and applying low electric charges, the
381 toxicity/biodegradability of effluents were hardly affected. However, when applying
382 electric charges over 2.5 Ah dm^{-3} , the biodegradability of the pre-treated effluents
383 increases at the same time that toxicity decreases.

384

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Table 1. Anions and cations concentrations in the soil-washing effluent.

Cation	Concentration (mg dm⁻³)	Anion	Concentration (mg dm⁻³)
Na ⁺	106.17	SO ₄ ²⁻	234.52
Ca ²⁺	64.35	NO ₃ ⁻	134.71
Mg ²⁺	32.69	Cl ⁻	83.98
K ⁺	3.41		

Figure 1. Clopyralid and TOC transformations during the AO process using Ru-MMO and Ir-MMO electrodes at different current densities, open symbols correspond to clopyralid and closed symbols to TOC. (Ir-MMO electrode: Clopyralid: \circ 5 mA cm⁻² Δ 15 mA cm⁻² \square 60 mA cm⁻². TOC: \bullet 5 mA cm⁻², \blacktriangle 15 mA cm⁻², \blacksquare 60 mA cm⁻²). (Ru-MMO electrode: Clopyralid: \diamond 15 mA cm⁻², \star 60 mA cm⁻². TOC: \blacklozenge 15 mA cm⁻², \star 60 mA cm⁻²).

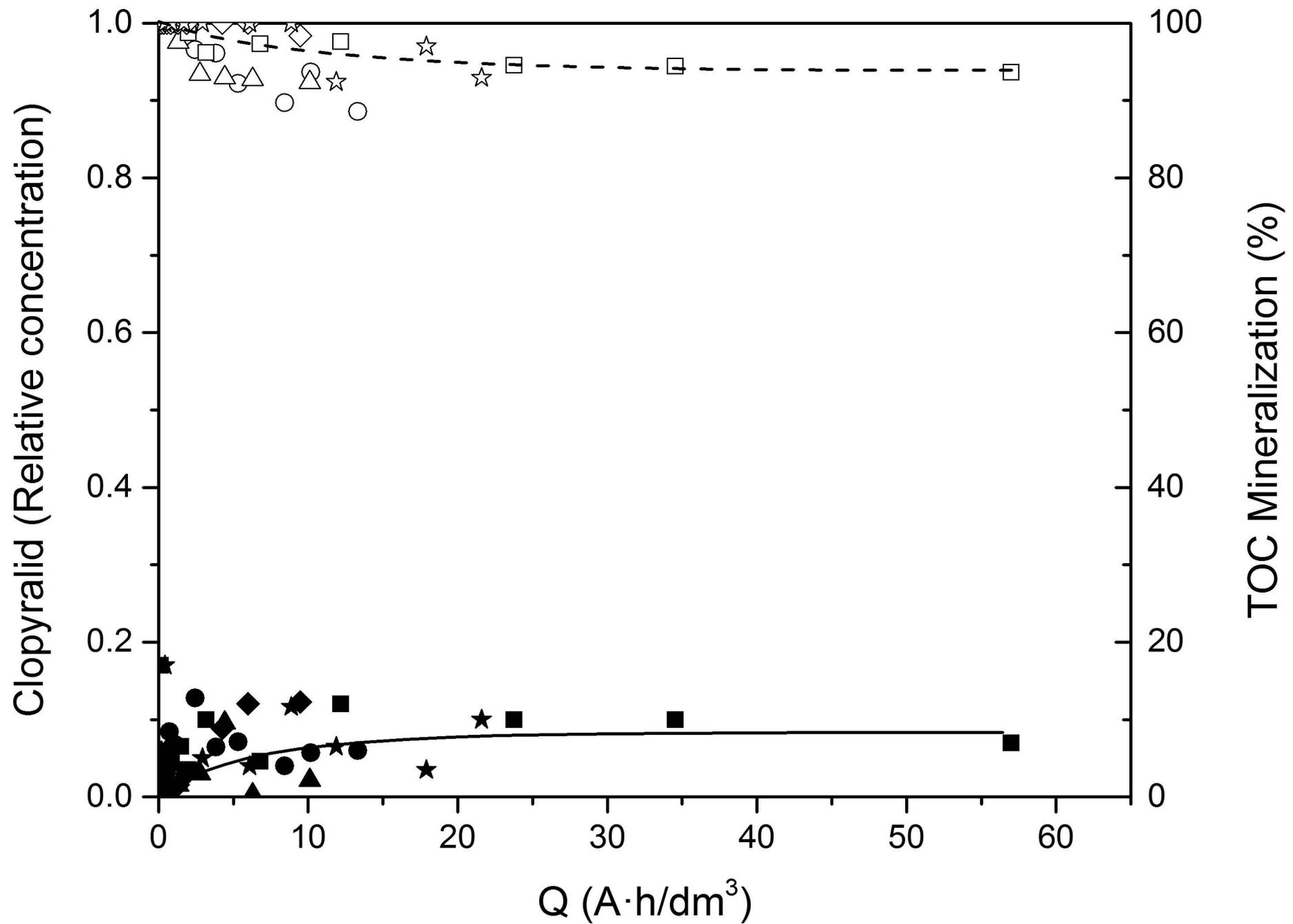
Figure 2. Clopyralid and TOC transformations during the AO process using pSi-BDD electrode at different current densities. (Clopyralid: \circ 5 mA cm⁻² Δ 10 mA cm⁻² \square 15 mA cm⁻². TOC: \bullet 5 mA cm⁻², \blacktriangle 10 mA cm⁻², \blacksquare 15 mA cm⁻²).

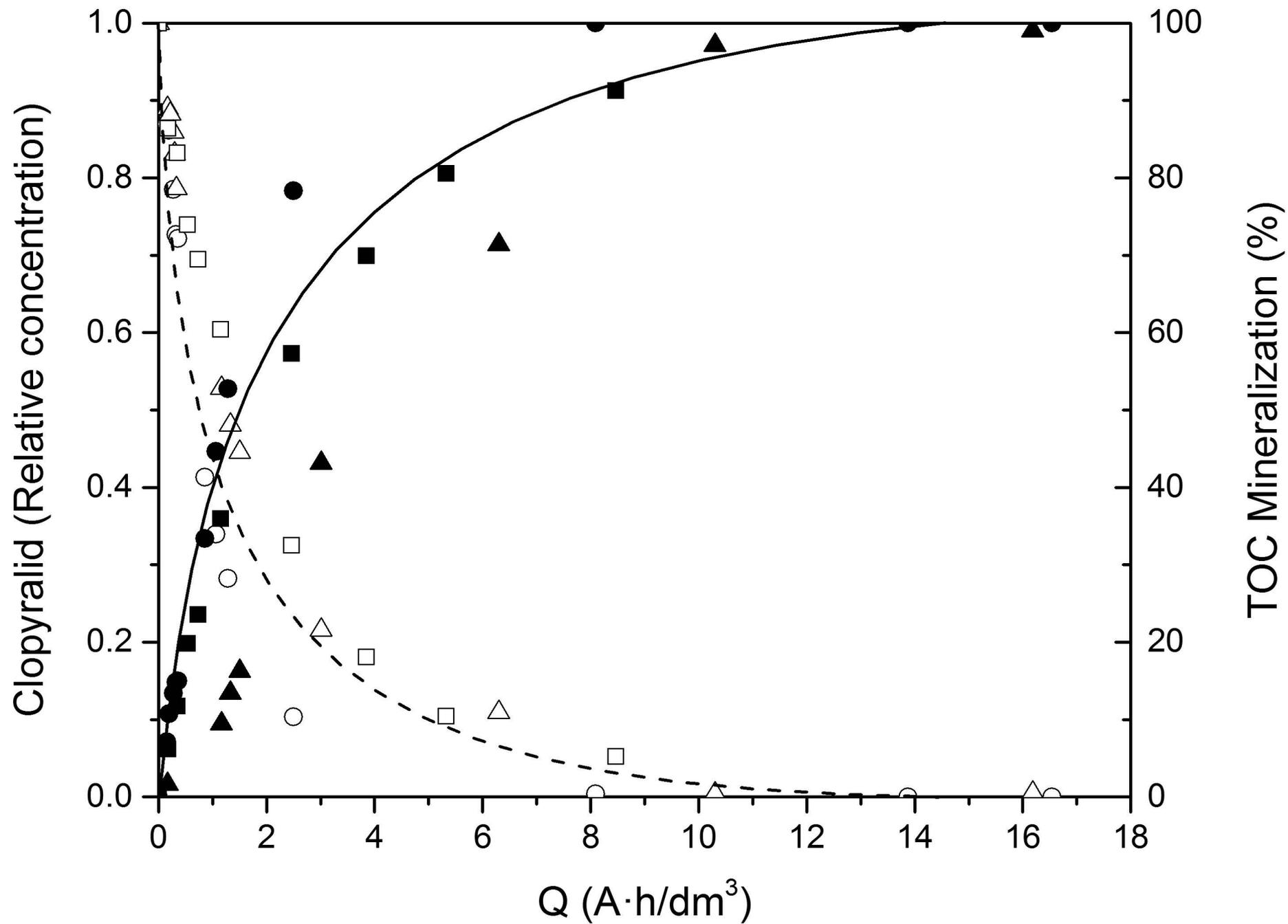
Figure 3. Clopyralid and TOC transformations during the AO process using CF electrode at different current densities. (Clopyralid: \circ 5 mA cm⁻² Δ 10 mA cm⁻² \square 15 mA cm⁻². TOC: \bullet 5 mA cm⁻², \blacktriangle 10 mA cm⁻², \blacksquare 15 mA cm⁻²).

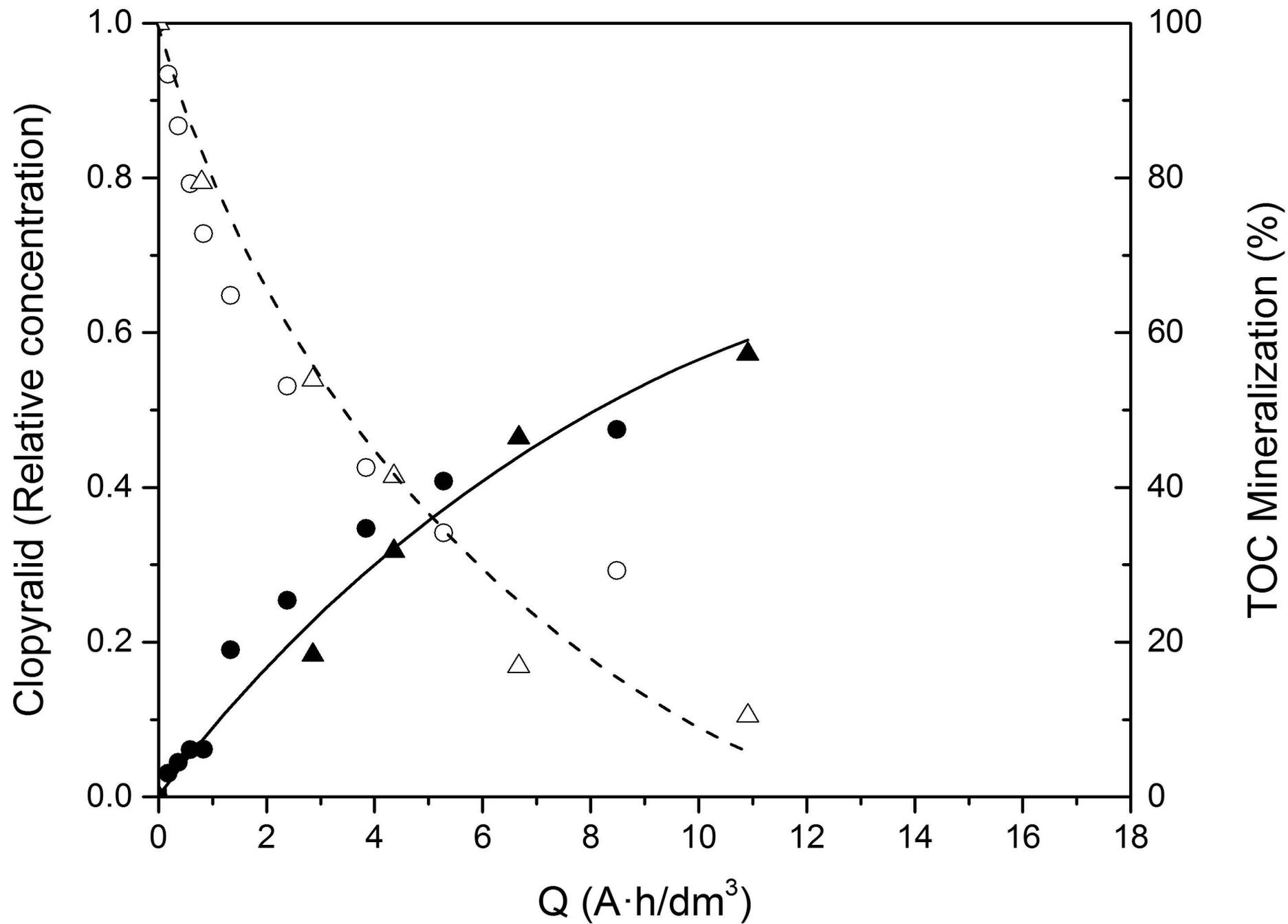
Figure 4. AO products peaks evolution along the AO process with CF anode at 5 mA cm⁻².

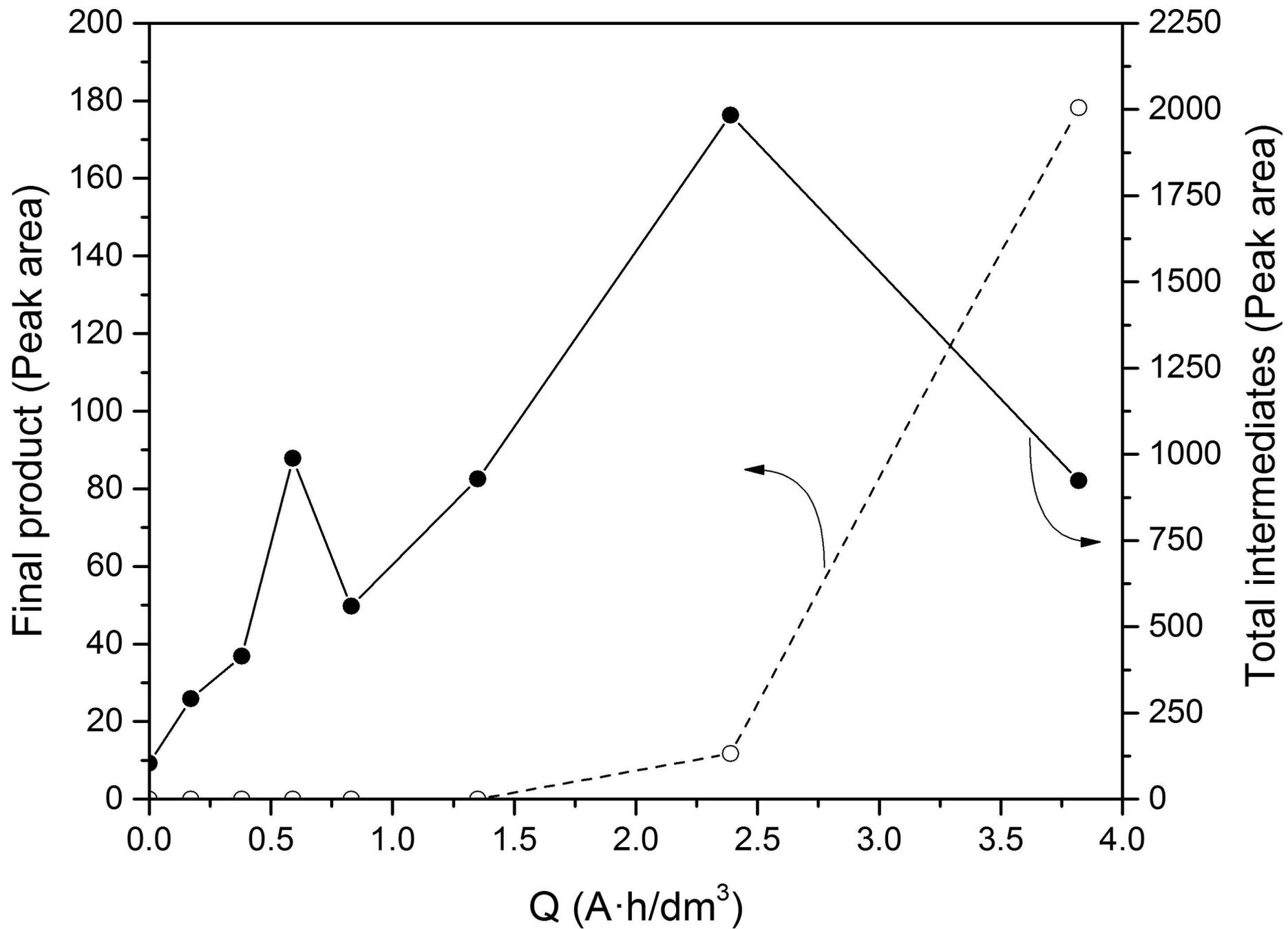
Figure 5. Conductivity and pH evolution during AO process with CF.

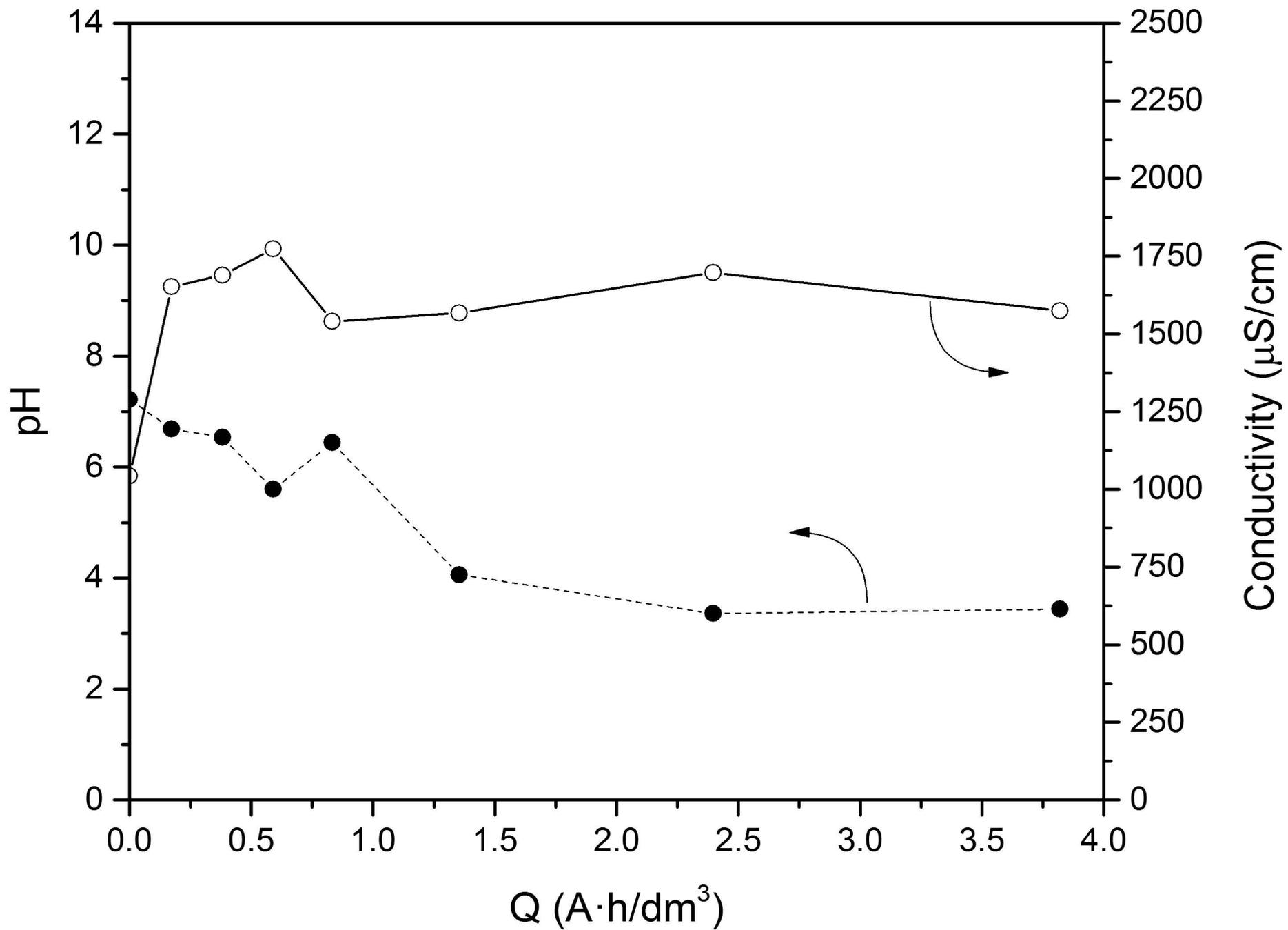
Figure 6. Changes in the EC₅₀ \circ , oxygen uptake rate \bullet and BOD%/COD ratio (bar diagram) during AO process.

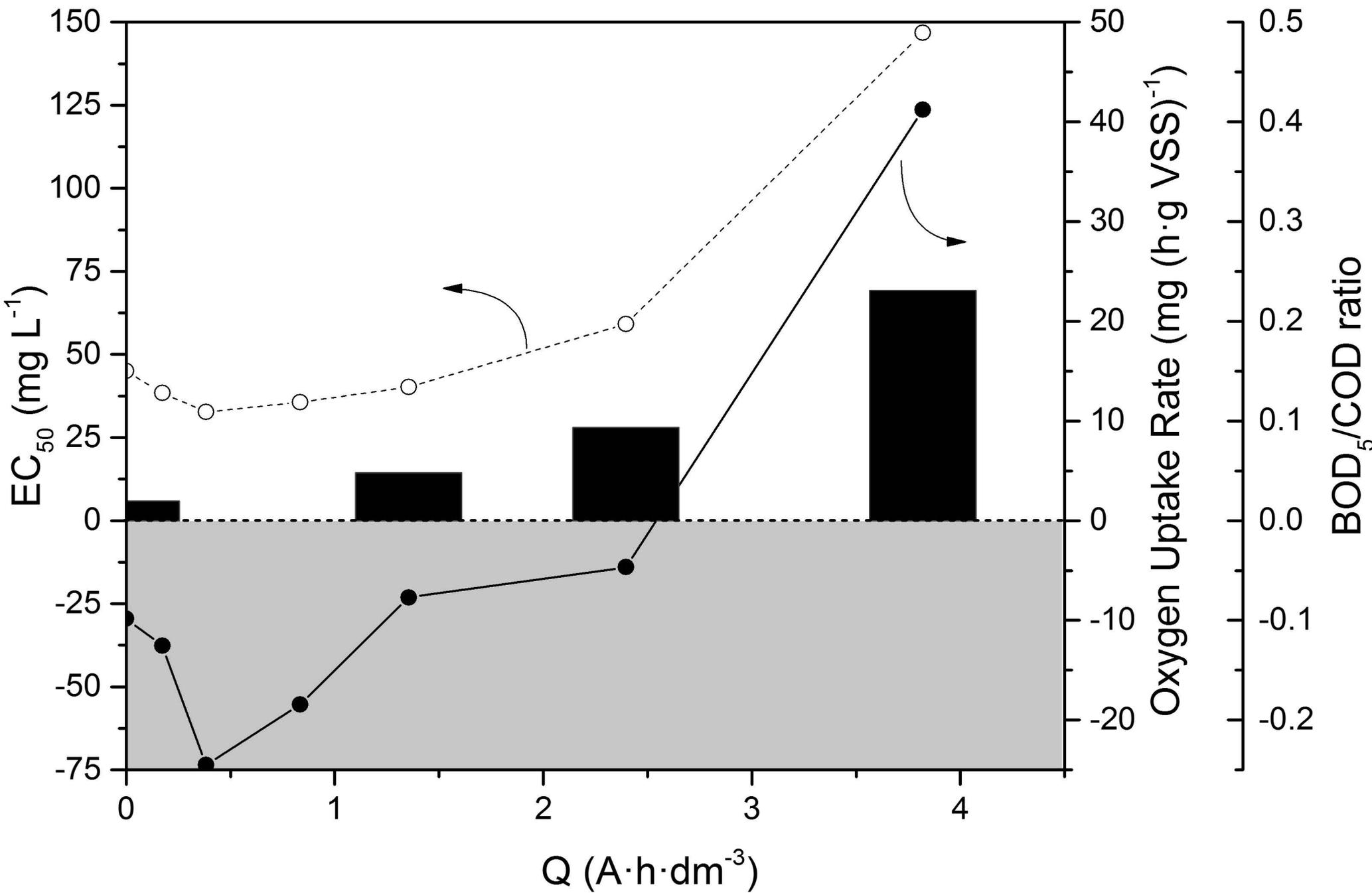












Supplementary Materials

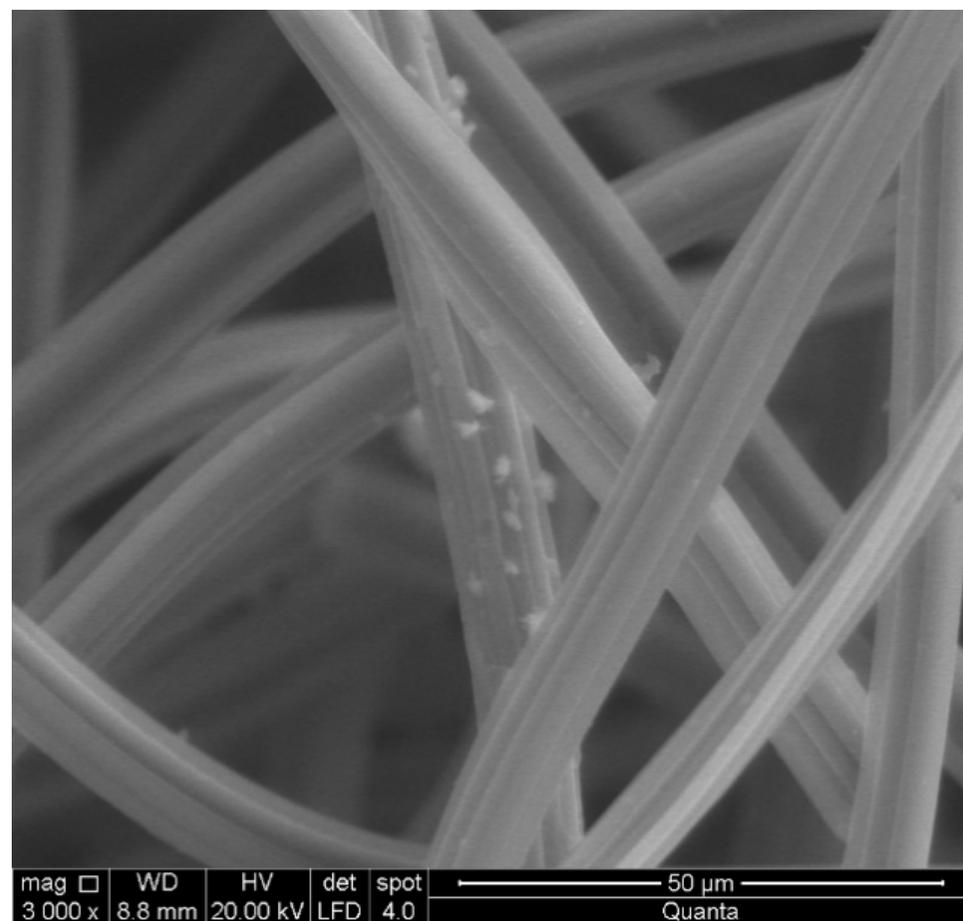
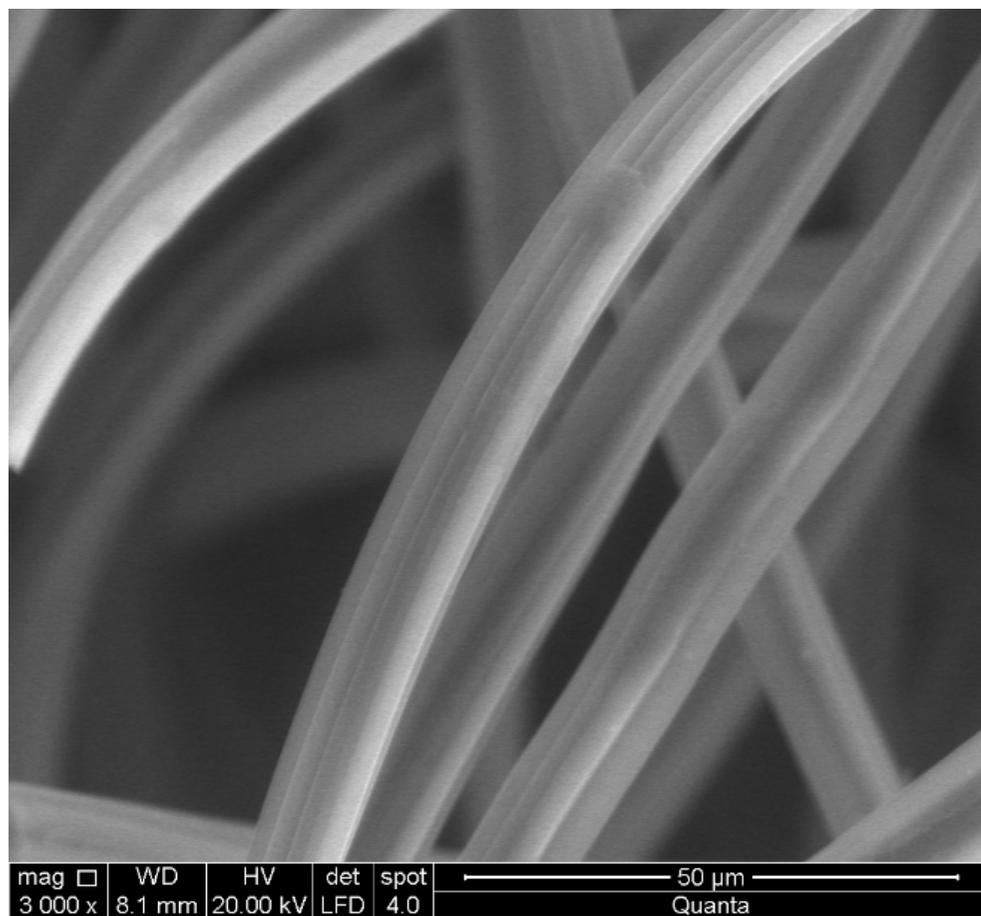


Figure SM1. SEM pictures of the CF electrodes before and after the AO Process.

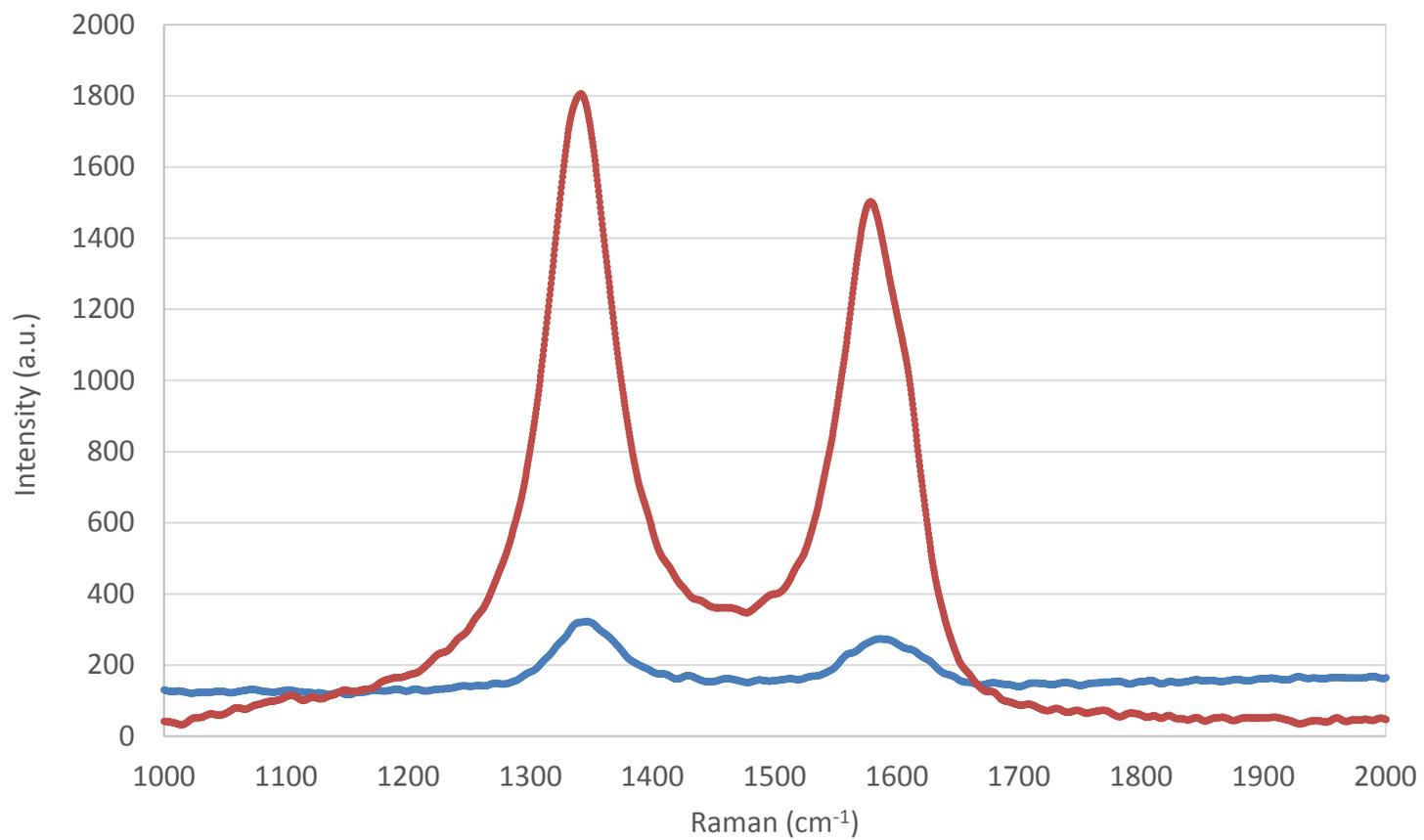


Figure SM2. Raman Spectra of the CF electrode before (blue line) and after (red line) the AO process.