

Full Paper

Optimization of the Electro-Fenton Process for the Elimination of Oxytetracycline Antibiotic from Water: Degradation/Mineralization Kinetics

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Received: 30 December 2022 / Received in revised form: 24 April 2023 /

Accepted: 25 April 2023 / Published online: 30 April 2023

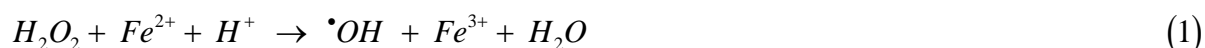
Abstract- The Electro-Fenton process is an electrochemical advanced oxidation method based on electrocatalytic in situ production of hydroxyl radicals ($\bullet\text{OH}$). The decontamination of water polluted by Oxytetracycline (OTC) as being a toxic and persistent organic contaminant was carried out by the Electro-Fenton process (EF). Using a platinum anode and a carbon felt, many experiments have been carried out while studying the effect of several parameters on the efficiency of EF such as the applied current, the concentration of the catalyst, and the initial concentration of OTC. The degradation kinetics were studied and monitored by HPLC during electrolysis using an OTC initial concentration of 0.03 mM, 500 mA as applied current, and 0.1 mM of Fe^{2+} as optimal parameters. As for the mineralization, the chemical oxygen demand COD gave a reduction rate greater than 97% for 500 mA and 0.2 mM of Fe^{2+} . The identification of some intermediate compounds was carried out by HPLC and LC-MS/MS.

Keywords- Oxytetracycline antibiotic; Electro-Fenton process; Oxidative degradation; Mineralization; Intermediates

1. INTRODUCTION

Several emerging refractory pollutants of chemical origin appeared in the environment in the last century following intensive industrialization. These so-called biorecalcitrant substances accumulate over time, persist, and therefore cause enormous ecological danger and contribute to adverse health effects [1,2]. They include pesticides, dyes, and pharmaceutical products such as drugs, including antibiotics, which take a large place at the level of consumption or at the level of detection in the aquatic environment [3,4]. Tetracyclines are the second most widely produced and used group of antibiotics worldwide [5–7]. In this family, we find Oxytetracycline (OTC), the most widely used prophylactic antibiotic in human and veterinary medicine due to its low cost, its broad-spectrum activity, and its high efficiency in the culture of larvae and shrimp [8–10]. Its polyaromatic and cyclic structure promotes its chemical stability and its half-life in the ecosystem is long enough to classify it as a persistent pollutant Table 1 [11]. Its high consumption has contributed to the appearance of its residues in the aquatic environment at concentrations of low doses. They were 0.11 µg/L in surface waters, 0.52 µg/L in tributaries, and 0.17 µg/L in effluents [12]. OTC has been widely detected in water intended for drinking water stations with a concentration of 0.38 to 2 mg/L [13]. It has also been shown that the toxicity of this antibiotic is moderate for the activated sludge organisms [14], and that due to the complete absence of regulations setting the minimum threshold for this drug, it is able to affect the growth of aquatic plants and make bacteria more resistant [15].

Unfortunately, conventional treatments applied in sewage treatment plants do not eliminate these residues [16], hence the need to apply other more efficient technologies to degrade these refractory compounds such as advanced POA oxidation processes. Several studies report the use of these processes for the degradation of OTC whether in a homogeneous or heterogeneous system such as photo-Fenton [17,18], anodic oxidation [19], pulsed radiolysis [20], UV/H₂O₂ [21,22], (UV/TiO₂/H₂O₂) [23], heterogeneous photocatalytic with different metals as catalyst [24–27]. Indeed, no study has been carried out for the degradation of OTC by Electro-Fenton, especially since this process has confirmed its effectiveness in the almost total degradation of several drugs into organic compounds CO₂, H₂O and inorganic ions in according to the evolution of the chemical oxygen demand COD as a function of the electrolysis time [28–32]. EF is the Fenton reaction assisted by electrochemistry. Its principle is based on the *in-situ* production of Fe²⁺ and / or H₂O₂, the whole of which constitutes the Fenton reagent and which ensures the formation of hydroxyl radicals •OH in an acidic medium as being powerful oxidants, extremely reactive and non-selective according to the reaction 1 [33].

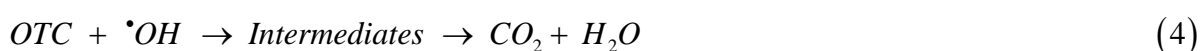


The Fe²⁺ reagent is produced in the solution according to reaction 2, while H₂O₂ is generated by bi-electronic reduction of molecular oxygen from a saturated solution of oxygen at the beginning of the electrolysis (reaction 3).



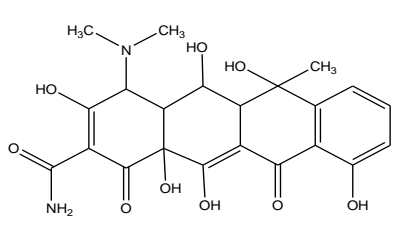
Electro-Fenton is known for its simple principle based on the intervention of certain experimental parameters necessary to achieve good efficiency in the degradation of organic pollutants.

The OTC degradation in the Electro-Fenton process follows a mechanism based on the hydroxyl radicals ($\cdot OH$) generation during the decomposition of hydrogen peroxide H_2O_2 in the presence of iron. Once electrolysis begins, OTC reacts instantly with hydroxyl radicals and produces intermediates which undergo a series of oxidation reactions until the OTC is completely mineralized into H_2O and CO_2 (Eq. 4).



In the present work, the degradation of OTC solution was investigated by applying the Electro-Fenton process. The main aim of this study is to analyze how the degradation process of OTC can be affected by initial concentrations of OTC, applied current and the iron II catalyst concentration. The second objective focuses on the identification of a certain number of OTC intermediate products using HPLC and liquid chromatography-mass spectrometry analyzes.

Table 1. Proprieties of Oxytetracycline molecule

Compound	Structure	Molecular weight g/mol	Solubility g/L
$C_{22}H_{24}N_2O_9 \cdot HCl$		496.89	0.313 at 25 °C

2.2. MATERIALS AND METHODS

2.1. Chemicals

OTC ($C_{22}H_{24}N_2O_9 \cdot HCl$ > 98% purity) was obtained from Sigma Aldrich, sulfate pentahydrate (purity 99%) and Na_2SO_4 (purity 99%), used as a catalyst source and inert supporting electrolyte respectively were provided from Acros Organics. The initial pH of the solutions was adjusted using analytical grade sulfuric acid and sodium hydroxyd from Sigma Aldrich and Fluka, respectively. Mercuric sulfate $HgSO_4$, silver sulfate $AgSO_4$ and potassium

dichromate K_2CrO_7 were purchased from Hach Lange Europe, Belgium. All solutions were prepared in ultra-pure water.

2.2. Electro-Fenton process

The degradation of the antibiotic studied by the Electro-Fenton process was carried out in a cylindrical electrochemical cell with a capacity of 250 ml and in the presence of three electrodes; i) Working electrode (Cathode) made of carbon felt of dimensions (10 cm×8 cm×0.5 cm) supplied by Carbone-Lorraine and characterized by its flexibility, resistance and ease of handling. It is placed on the wall of the cell. ii) Platinum electrode (anode) with a surface area of 5 cm² placed in the center of the cell and surrounded by the cathode and iii) Reference electrode which is a saturated calomel electrode KCl, placed in a compartment separated from the other two electrodes by a fritted glass. The current applied between the anode and the cathode is imposed by a Potentiostat / Galvanostat instrument PGZ301 Voltalab. All experiments were conducted at room temperature.

2.3. Analytical determinations

The measurement of the chemical oxygen demand (COD) was carried out by the Lovibond® Vario-MD200 photometer. After oxidation with $K_2Cr_2O_7$ in an acid medium, the quantity of oxygen necessary for the oxidation of organic and mineral matter at 148°C for 2 h was obtained [34]. The COD values were measured by colorimetry using a DR/125 spectrophotometer (Hach, USA).

The Waters 2695 coupling photodiode detector was used to follow the evolution of the OTC concentration by inverse HPLC. The optimum wavelength of 270 nm was selected with a C18, 25 cm, 5 μm, 4.6 mm (id) thermosupersin at 40 °C. A sample volume of 100 μl was specified to carry out its analysis isocratically using a mixture of pH=2.2 and a mobile phase composed of acetonitrile 46:54 (v/v) with a flow rate of 1.5 ml/min.

The identification of the intermediates was performed by LC-MS/MS (AB SciexAPI 3200 QTRAP®, quadripolar triplex instrument) in negative ion mode and by the analysis software ®Version 1.5. A micro-syringe (Hamilton corporation, Reno, USA) was used to perform direct infusions into the ion source with a flow rate of 20 μl/min. the ESI model is characterized by - 4.5 kV voltage spray, sheath gas (N₂) average flow 20 ml/min, heated capillary temperature 300°C, device nebulizer gas curtain and gas average adjusted to 10 and 30 ml/min, respectively.

3. RESULTS AND DISCUSSION

3.1. Effect of experimental parameters on the degradation of Oxytetracycline (OTC) in aqueous solution

3.1.1. Effect of applied current

The importance of the applied current on the oxidative OTC degradation was studied in the presence of 0.15 mM drug concentration and 0.1 mM Fe^{2+} . The pH of the solution is maintained at 3. From the results of Figure 1, the degradation kinetics of OTC is accelerated by increasing the applied current from 100 to 500 mA. This can be explained by the acceleration of reactions 1 and 3 to produce a sufficient quantity of hydroxyl radicals [35,36] to achieve total disappearance of the product, moreover the OTC was eliminated after 10min for the currents 100, 300 and 400 mA and after 5min for the current 500 mA. For this purpose, the current 500 mA seems the optimal current for a better degradation of the OTC.

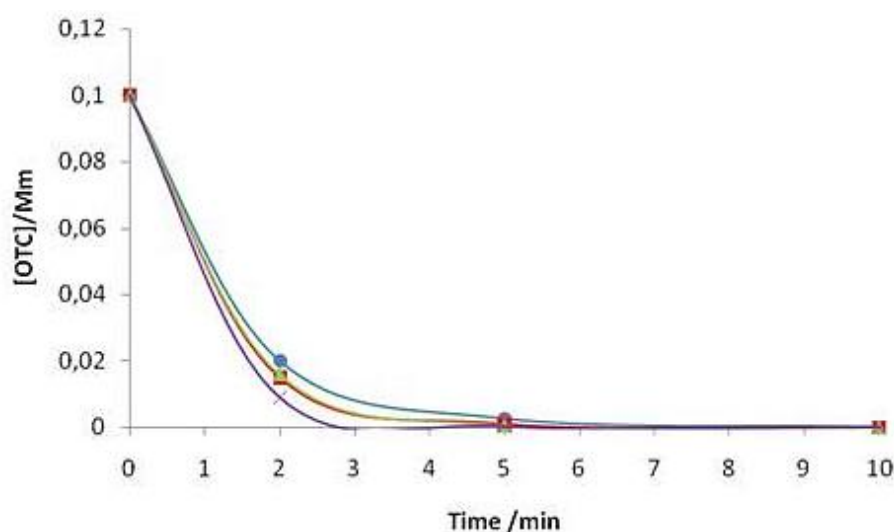


Figure 1. Influence of applied current on the concentration of Oxytetracycline by Electro-Fenton. Applied current I (mA): 100 (-●-), 300 (-■-), 400 (-▲-), 500 (-×-)

3.1.2. Effect of Initial catalyst concentration

In order to note the importance of the concentration of iron as a catalyst in the oxidation of Oxytetracycline, experiments were carried out by choosing different concentrations varying from 0.1 to 0.5 mM. Recall that the electrolysis was performed at an optimum current of 500 mA.

According to the results of Figure 2, the best degradation was obtained for the 0.1 mM concentration since the total elimination of the product is reached after 10 min of the electrolysis, unlike the other concentrations 0.2 and 0.5 mM whose yield was less effective since the antibiotic was eliminated after 30 and 40 min, respectively. In other words, using a high concentration of the iron catalyst can negatively affect the efficiency of the process due to the presence of the parasitic reaction that can take place between the iron ions and hydroxyl radicals (reaction 5) [34,37,38].



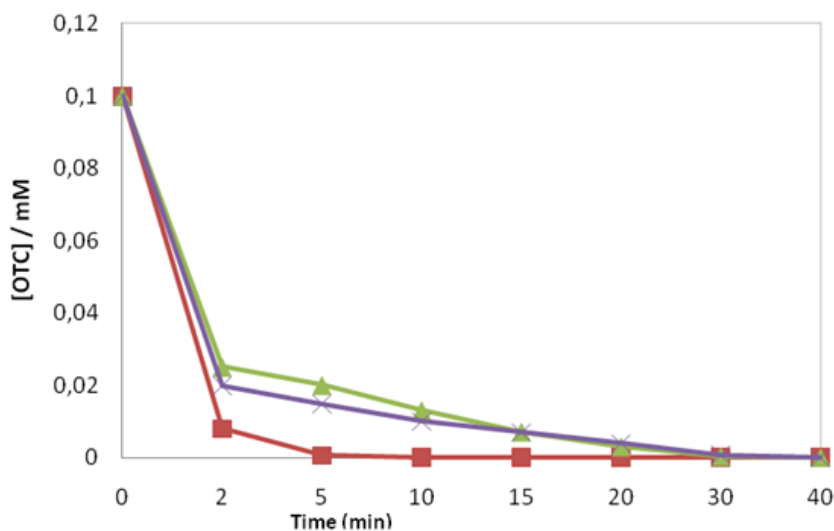


Figure 2. Influence of catalyst concentration Fe^{2+} on the concentration of Oxytetracycline by Electro-Fenton. $[\text{Fe}^{2+}] = 0.1 \text{ mM}$ (-■-), 0.2 mM (-▲), 0.5 mM (-×-)

3.1.3. Effect of Oxytetracycline concentration

It is quite logical that the drugs concentration in industrial effluents and hospital discharges changes over time, which means that the study of the Electro-Fenton process efficiency is important when the concentration of the antibiotic concerned varies. The degradation kinetics with different concentrations of Oxytetracycline 0.03, 0.1 and 0.2 mM were studied at ambient temperature, pH 3 under the optimum conditions previously found 500 mA and an iron concentration equal to 0.1 mM. Figure 3 shows the results of this study.

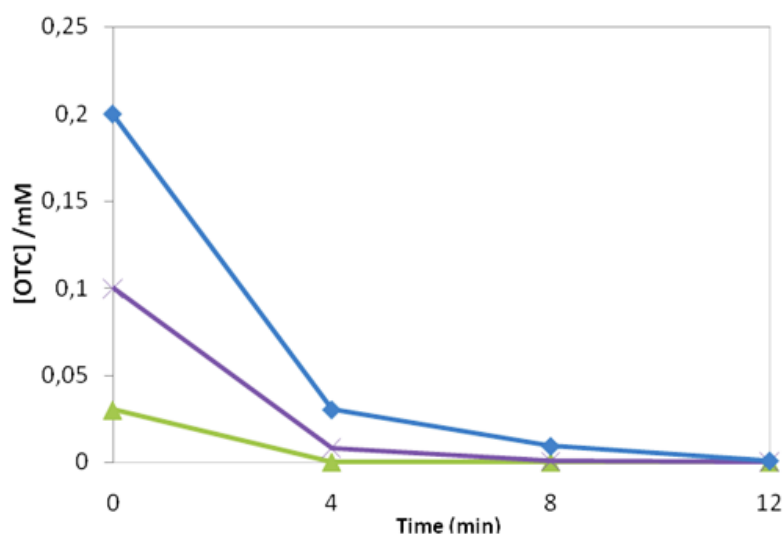


Figure 3. Influence of Oxytetracycline concentration during Electro-Fenton $[\text{OTC}] = 0.03 \text{ mM}$ (▲), 0.1 mM (×), 0.2 mM (■)

According to the monitoring of concentrations during electrolysis, it is now clear that the EF process remains applicable in a wide range of concentrations. The OTC degradation is fully completed after 4, 8 and 12 min of electrolysis for 0.03, 0.1 and 0.2 mM concentrations, respectively. So, the electrolysis time for a complete oxidation is shorter for low concentrations, it can be explained by the weak competition between the OTC molecules and the intermediate by-products formed during the treatment with hydroxyl radicals in situ [39].

3.2. Influence of operating parameters on the mineralization of Oxytetracycline in an aqueous medium

3.2.1. Influence of current

To study the Oxytetracycline mineralization in water by the Electro-Fenton process, it is necessary to carry out numerous experiments with different current values in order to define the optimal current to achieve total mineralization of the antibiotic. This wide current range varies from 60 mA to 600 mA. It should be remembered that the iron concentration is equal to 0.1 mM, pH 3 and 0.05 mM for Na_2SO_4 .

According to Figure 4, during electrolysis, the COD values decrease, explaining the degradation of Oxytetracycline and its derivatives generated during the treatment. In fact, the COD reduction rate increases as the electrolysis continues, it reached after 6 hours 62%, 87%, 94%, 98% and 90% for currents 60, 100, 300, 400 and 600 mA, respectively. On the other hand, for the 500 mA current, almost total mineralization (> 97%) was obtained after only 4 hours of treatment. Therefore, this current made it possible to save time and avoid unnecessary overconsumption with a good mineralization yield.

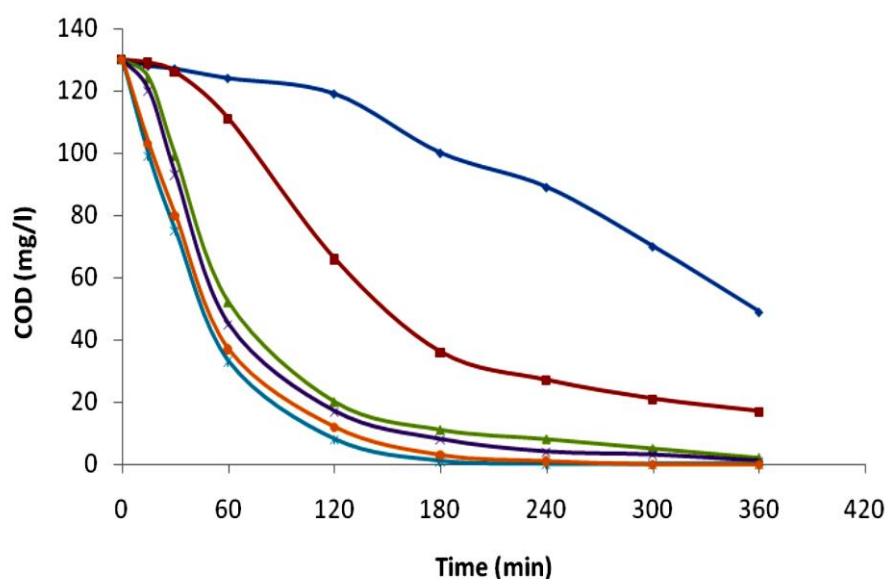


Figure 4. Influence of applied current on Oxytetracycline mineralization. I (mA) = 60 (- •-), 100 (- - -), 300 (- ▲), 400 (- x -), 500 (- x -), 600 (- ▪)

3.2.2. Influence of catalyst concentration

To assess the influence of iron concentration on the efficiency of Oxytetracycline mineralization in water by Electro-Fenton, varying concentrations of 0.1 mM to 0.5 mM were used. The results obtained are in Figure 5. This figure illustrates well the effect of catalyst concentration on the efficiency of drug mineralization by Electro-Fenton. In fact, the use of different concentrations gave a common reduction rate greater than 97% after 3 hours of electrolysis. The difference is at the level of the first hour, the mineralization rate has already reached 91% for 0.2 mM while for 0.1 and 0.5 mM it is 85%.

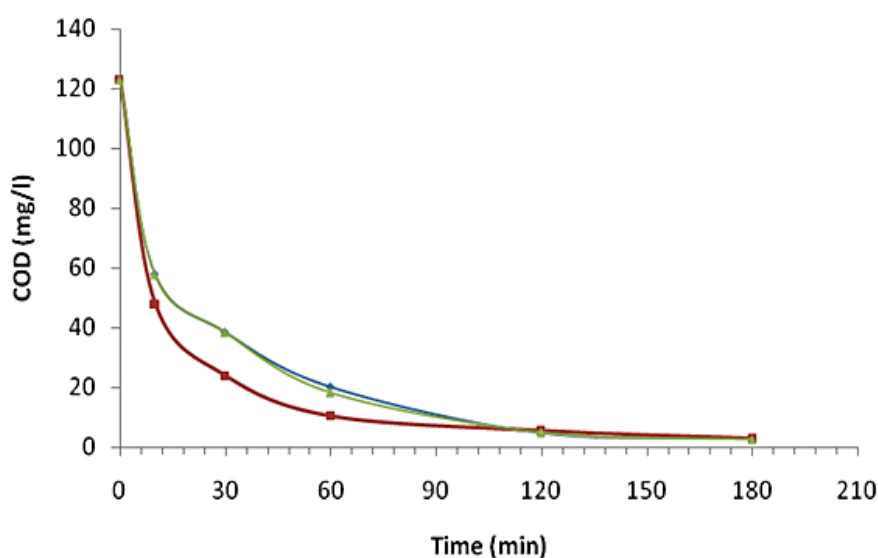


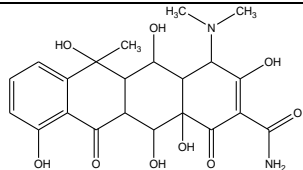
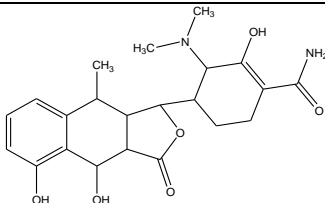
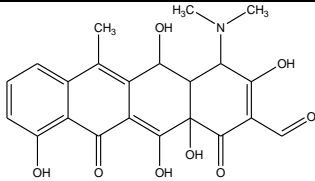
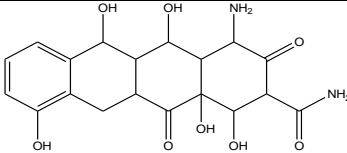
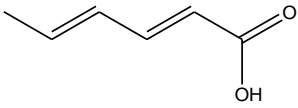

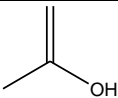
Figure 5. Influence of catalyst concentration on Oxytetracycline mineralization, $[\text{Fe}^{2+}] = 0.2$ mM (---), 0.1 mM (---), 0.5 mM (-▲)

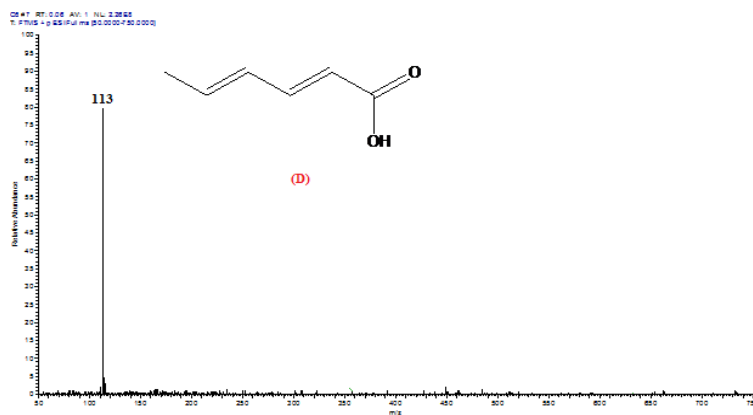
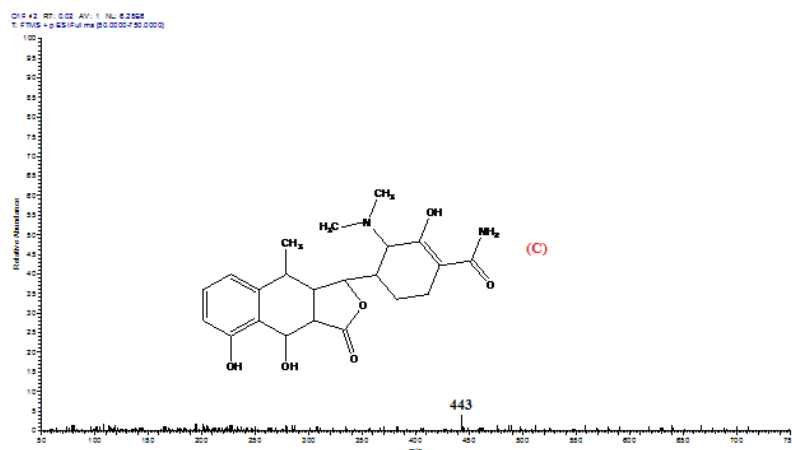
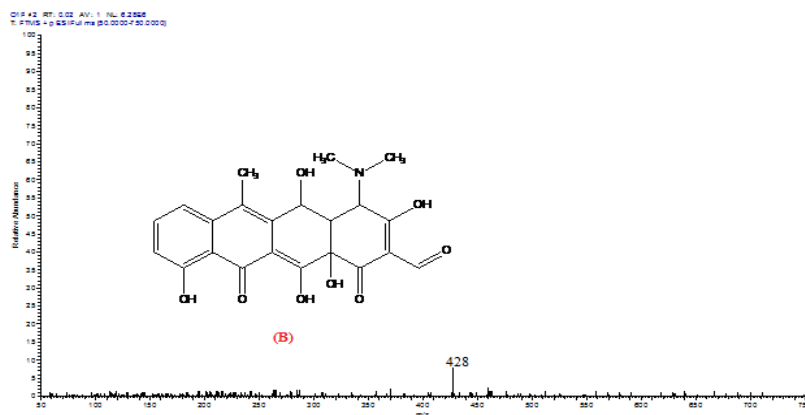
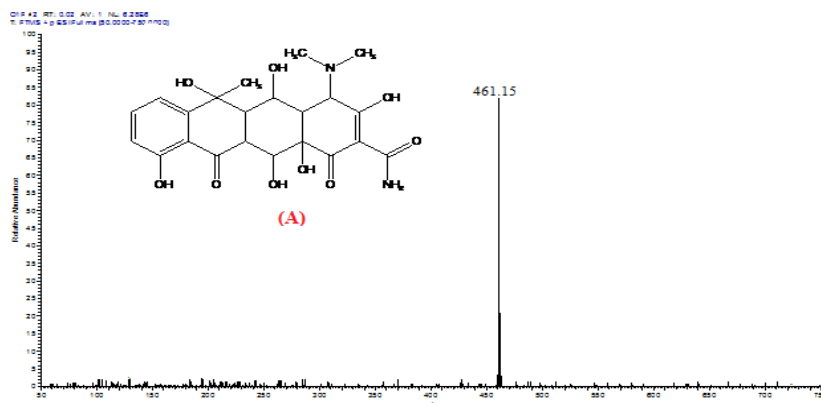
3.3. Identification of the reaction intermediates

The analysis by LC-MS/MS and HPLC was carried out in order to determine the intermediates formed during the first hour of electrolysis. The results obtained showed an increasing disappearance of the antibiotic accompanied by the formation of certain aromatic and aliphatic intermediates. Seven by-products of degradation, their ratio (m/z) and their chemical structures have been shown in Table 2 [40–43]. The mass spectra of the identified products are presented in Figure 6. Indeed, these results can be explained by the fact that the degradation of Oxytetracycline by the EF treatment generates at the start of the electrolysis of the aromatic products (compounds A, B, C, D, and F). The latter are attacked by hydroxyl radicals thus leading to the opening of aromatic rings and therefore the formation of aliphatic intermediates which are short carbon chains such as carboxylic acids such as sorbic acid (compound E) and acetic acid (compound G) [44]. It should also be noted that the action of hydroxyl radicals on the aromatic ring is easier than an aliphatic compound which is very resistant to oxidation, which explains the significant decrease in the efficiency of

mineralization at the end by compared to the rate observed at the start of treatment, this is due to their stability and their low reactivity with respect to hydroxyl radicals [44].

Table 2. By-products identified using HPLC and LC-MS/MS during the degradation of OTC by EF

Intermediates			
compounds	m/z	Chemical formula	Reference
A (OTC) (C ₂₂ H ₂₄ N ₂ O ₉)	461.15		[41–43]
B (C ₂₂ H ₂₂ N ₂ O ₈)	443		[41]
C (C ₂₂ H ₂₁ NO ₈)	428		[43]
D (C ₂₂ H ₁₈ N ₂ O ₈)	404		[40]
E (C ₆ H ₈ O ₂) (Sorbic Acid)	113		[41]
F (BENZENE)	78		[40]
G (Acetic acid)	60		[40]



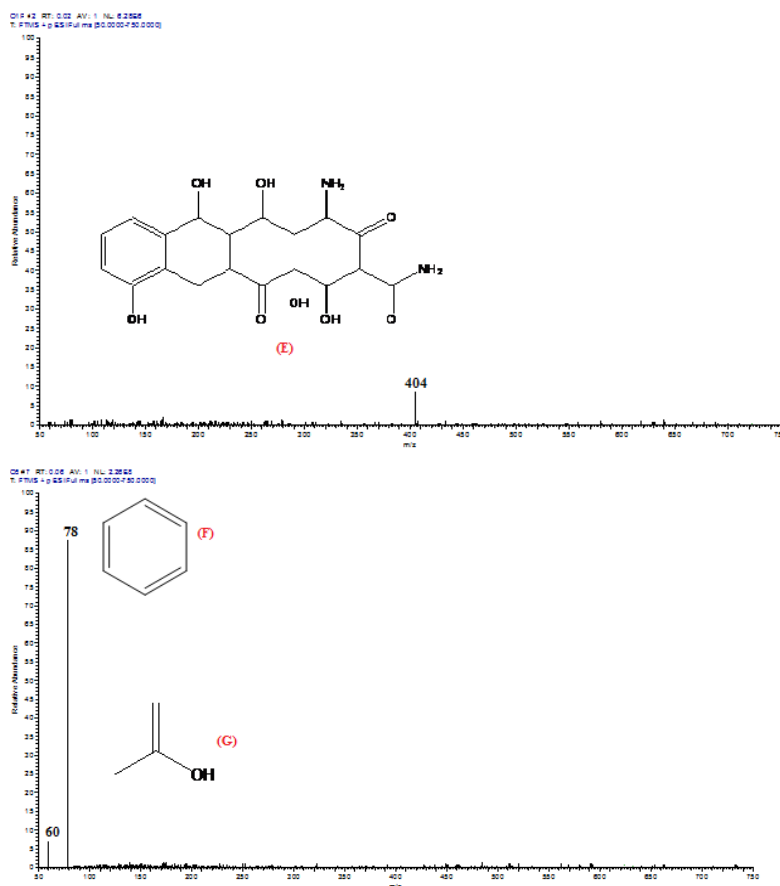


Figure 6. Mass spectra of the main degradation intermediates A-G

4. CONCLUSION

In our study, the EF process was used for two major objectives: the first is to degrade the drug Oxytetracycline as an organic matter polluting water and the second aims to determine the optimal conditions to achieve a mineralization rate greater than 95%. According to the results obtained, a current of 500 mA and an iron concentration of 0.2 mM seem to be the best conditions for effective reduction. The degradation kinetics were studied and monitored by HPLC during electrolysis using a 0.03 mM initial concentration of OTC, 500 mA as applied current and 0.1 mM of Fe^{2+} as optimal parameters. The identification of certain aromatic and aliphatic intermediates was carried out by HPLC and LC-MS/MS.

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