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Short Communication

Degradation of Enrofloxacin Antibiotic under Combined Ionizing Radiation and Biological Removal Technologies

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Enrofloxacin degradation by ionizing radiation and *cupriavidus metallidurans* resistance were investigated. Analysis by nuclear magnetic resonance spectroscopy (NMR) and fourier transform infrared spectroscopy (FTIR) were performed to monitor changes in metabolic profiles of enrofloxacin after irradiation at 50 KGy. The obtained result confirms that ionizing radiation process leads to the cleavage of three aromatic cycle, the cyclopropyl and the disparition of carboxyl acid function flowed by the formation of NH functions and aliphatic hydrocarbons chain. In addition, irradiation process is able to reduce the biological inhibition activity of enrofloxacin and leads to the growth of *C.metallidurans* at high concentration of enrofloxacin (1mg/ml). Consequently, the combined processes of ionizing radation followed by microbial removal of enrofloxacin seem to be the best solution for the treatment of effluents containing enrofloxacin antibiotics.

Keywords: Enrofloxacin, Ionizing radiation, *Cupriavidus metallidurans*, NMR, FTIR

1. INTRODUCTION

The occurrence of antibiotics in the aquatic environment has raised concern regarding their potential impact on drinking water quality. Antibiotics are widely used in human and veterinary medicines for disease treatment. They are also largely used in animal operations for growth promotion and for disease prophylaxis. These antibiotics can be introduced to different environments as parent compounds or by-products mainly by an excretion, disposal of unused or expired drugs and accidental spills [1 , 2, 3]. Consequently, A variety of antibiotics have been detected in wastewater effluents and natural waters at ng/L to low μg/L levels [4,5].

Antibiotics were recently classified as a priority risk group due to their high toxicity to algae and bacteria at low concentrations and their potential to cause multi-resistance amongst natural bacterial populations [6]. One of the most frequently detected pharmaceuticals in water and in urban wastes is ennrofloxacin known as a commercial name of Baytril. Enrofloxacin is a fluoroquinolone, synthetic antibiotics and like other FQs, its exhibits a broad spectrum of antibacterial activity, against both Gram-positive and Gram-negative bacteria, in diseased animals. Enrofloxacin is used to prevent and treat *pneumonia* and *E.coli* bacterial diarrhea syndrome in cows and pigs. Due to these many problems, a lot of researchers have been carried out to remove antibiotics.

A variety of recent investigations have been performed on the treatment of wastewater containing antibiotics, generally on biological processes for the degradation of antibiotics. A combined anaerobic– aerobic treatment system was recently used for the treatment of a high-strength pharmaceutical wastewater. However, this biological combination system showed no significant antibiotic removal (less than 10% percent removal) (Zhou et al., 2006). Electrochemical treatment is one of promising technologies for the organic toxic polluant degradation. This method is environmentally friendly and does not form new toxic wastes. In anodic oxidation, organic pollutants are directly destroyed by reaction with hydroxyl radical (HO•) formed at the anode surface from water oxidation [7,8].

Recent advances shown that radiolytic degradation using gamma radiation is used for better removal efficiency of water pollutants [9]. Gamma irradiation using ^{60}Co source can produce radiolysis of water, resulting in the production of radicals such as the hydroxyl radical ($^{\cdot}OH$), hydrogen radical ($^{\cdot}H$), and solvated electron (e^{-}). These radicals play a role in degrading antibiotics. The use of ionizing radiation is an especially effective and fast method, wich does not requires the use of additional reagent, and can be based both on oxidative and reductive radical processes.

The aim of this work is to investigate the efficiency ionizing radiation treatment of enrofloxacin which is widely detected in the environment. This work will aim to determine the best combination of irradiation and biodegradation for the treatment of enrofloxacin.

2. EXPERIMENTAL METHODS

2.1. Chemicals

Enrofloxacin is a fluoroquinolone antibiotic sold by the Bayer Corporation under the trade name Baytril (Fig1.).

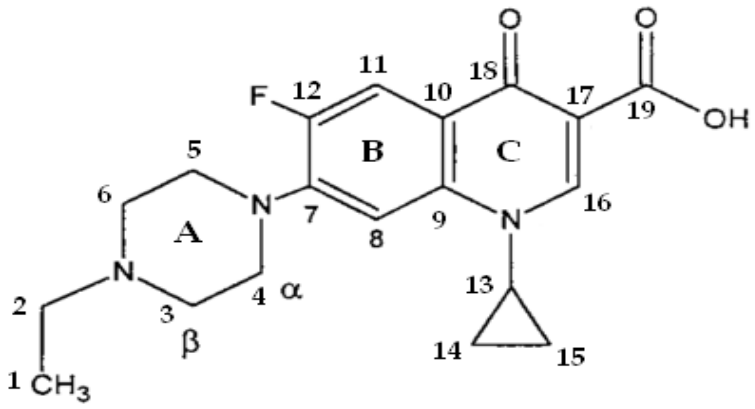

Figure 1. Chemical structure of Enrofloxacin (1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid)

2.2. ɣ-Radiation treatment

The Tunisian gamma irradiation facility (at Sidi Thabet) is designed for the preservation of foodstuff and sterilisation of medical devices. The source consists of eight encapsulated ^{60}Co pencils with a diameter of 9.7 mm and an overall length of 452 mm. The starting activity of the source was 99.162 kCi. The installation is equipped with a stainless steel telescopic source rack that allows obtaining a linear source of approximately 900 mm height. The source pencils are distributed circularly on a diameter of 140 mm for the upper source rack and of 80 mm for a lower one. The source rack comprises 20 housings allowing sources loading for several years. These sources are stored in dry condition in a cylindrical shield container in which they were transported. Three independent solution of Enrofloxacin (0.02 mg/mL) were exposed to gamma radiation dose of 50 kGy at a dose rate of 22.21 Gy/min and at room temperature (27±2 °C).

2.3. Antimicrobial effect of Enrofloxacin

Whatman filter paper is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish LB solidified with agar and sterilized in a hot air oven. The loop used for delivering the antibiotics is made of 20 gauge wire and has a diameter of 2 mm. This delivers 15 µL of treated and untreated enrofloxacin antibiotic (1 mg/ml) to each disc.

2.4. Nuclear Magnetic Resonance analysis (NMR)

Nuclear Magnetic Resonance (NMR) spectra were recorded using $CDCl_3$ as a solvent on Bruker DPX-300 spectrometer capable of producing 300 MHz Radio Frequency (RF). The chemical shifts in δ (ppm) were recorded from Tetramethyl silane (TMS) for ^{13}CNMR. FTIR spectrum of the compound was recorded using Perkin Elmer Spectrum 100 instrument.

2.5. Electrolytic system

Electrochemical measurements were performed using a computer controlled by Potentiostat/Galvanostat model PGZ 100 associated to "Volta-Master 4" software. A conventional three electrodes cell (100 cm3) thermoregulated glass cell was used (Tacussel Standard CEC/TH). The anode was a square plate of BDD electrode with effective surface area of 1 cm^2, whereas the cathode was a platinum electrode, and the gap between electrodes was 1cm. A saturated calomel electrode (SCE) was used as a reference.

Galvanostatic electrolysis was carried out with a volume of 100 ml aqueous solution of initial COD_0 (2742 mg/L). The range of applied current density was 20 to 70 mA/cm^2 and samples were taken, at predetermined intervals during the experiment, and submitted for analysis. All tests have been performed at different current density in magnetically stirred and aerated solutions. In all cases sodium chloride was added to the electrolytic cell, at 1g/L.

Chemical Oxygen Demand determinations were made following the titrimetric method, according to standard methods [15]. The Chemical Oxygen Demand (COD) values were determined by open reflux, a dichromate titration method.

The maximum absorbance of Enrofloxacin samples were determined by a UV Vis spectrophotomètre (UV-1700 Pharmas pec, Shimizu) witz a spectrometric quartz cell (1 cm pat lent).

3. RESULTS AND DISCUSSION

3.1 Effect of irradiation on enrofloxacin antibiotic degradation

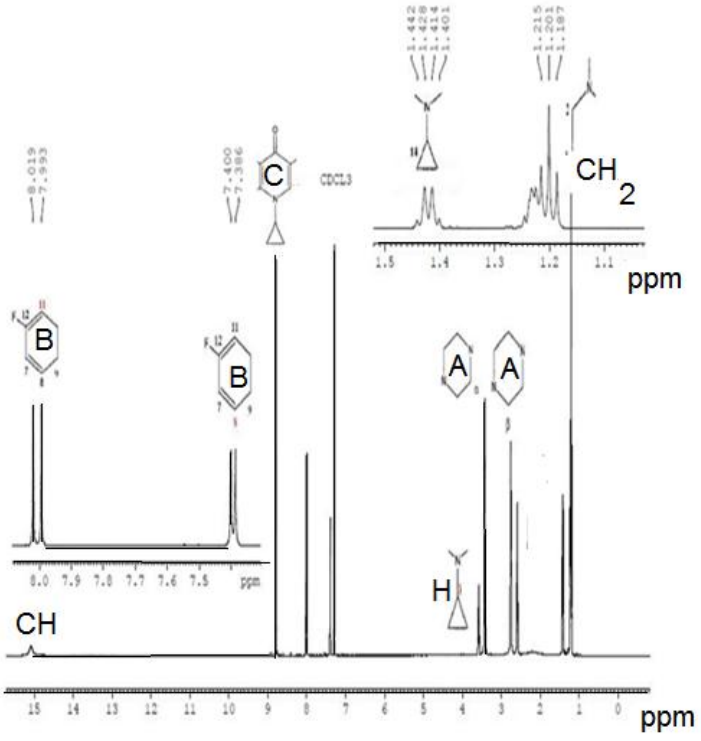

Figure 2. ^1H NMR spectra of enrofloxacin.

The ^{1}H NMR spectrum of irradiated enrofloxacin at 50 KGy comparing to the control showed the disparition of alcool function at 15 ppm. This result is confirmed by the FTIR analysis (figure 3).

Typically the alcool function present a vibration band at the region of 3000 cm-^{1} [10]. Observed only in control enrofloxacin (figure 4). In addition, peak observed on 1700 cm^{-1} corresponding to C=O function is absent on the irradiated enrofloxacin. Thus, the result obtained confirms the disparaition of carboxylic acid function (C_{19}) and probably the cleavage of the cycle C. The disparition of majority of peaks in the region of 600 to 900 cm^{-1} and the region around 1650 cm^{-}1 (figure 4) corresponding respectively to aromatic CH and C=C function [11], let supposed and confirms the cleavage of cycle C and B.

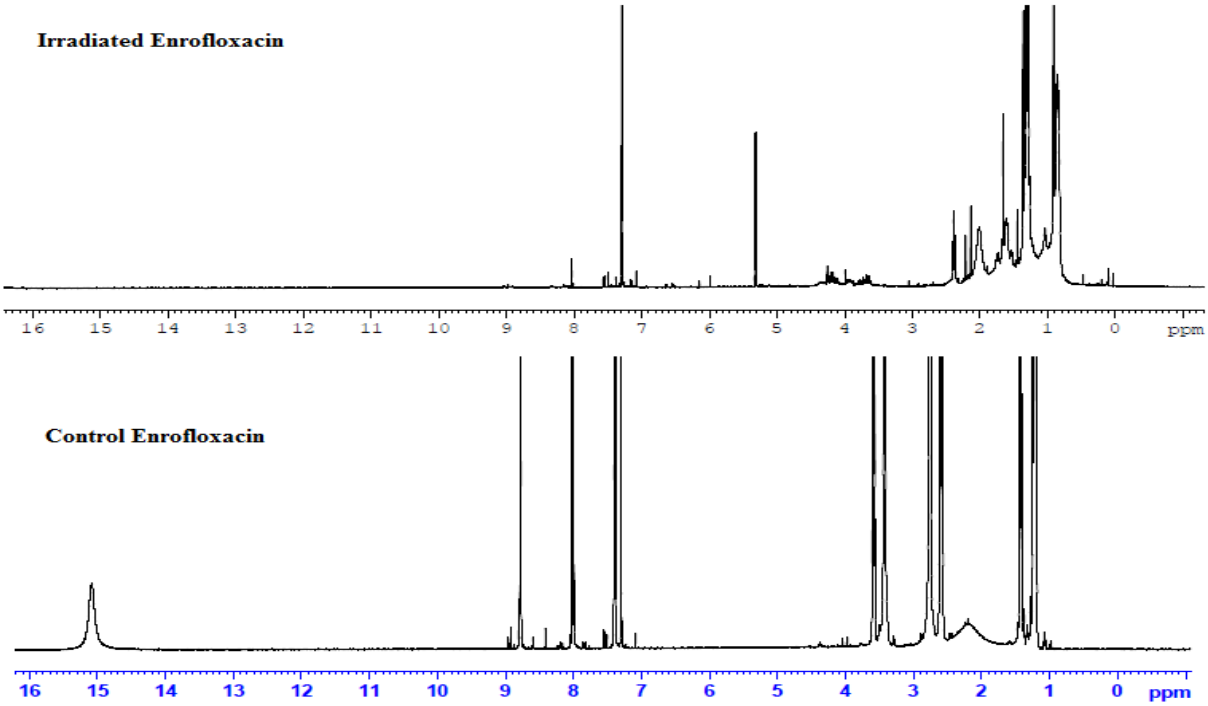

Figure 3. ^{1}H NMR superposition spectra of enrofloxacin: Control and irradiated enrofloxacin at 50kGy

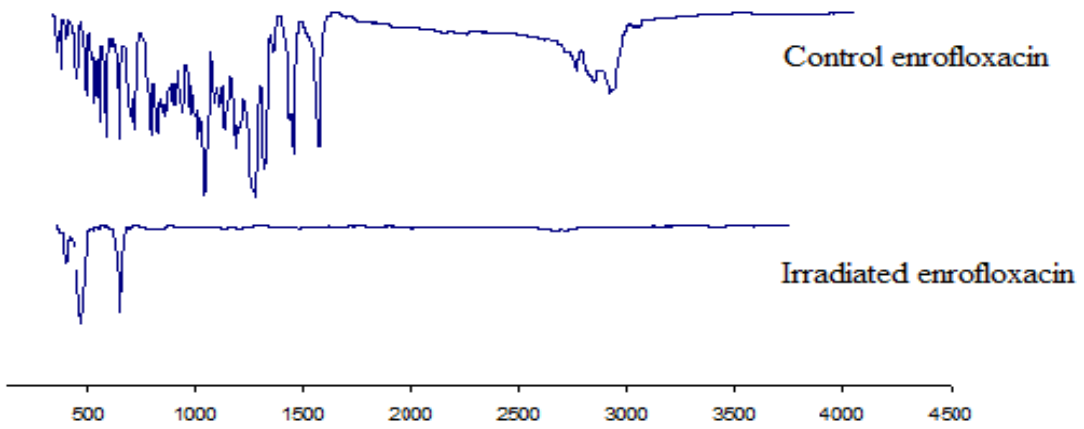

Figure 4. FTIR spectra of control and irradiated enrofloxacin at 50 kGy

Further investigation using ^{13}C NMR (figure 5) showed the disparition of the peaks under 110 ppm corresponding to the carbons hybridation sp^2 (C_{19}, C_{18}, C_{12}, C_9, C_{10}, C_{11}, C_7 and C_{16}). This result confirm the cleavage of the cycle C and B, and the disparition of the carboxylic acid function

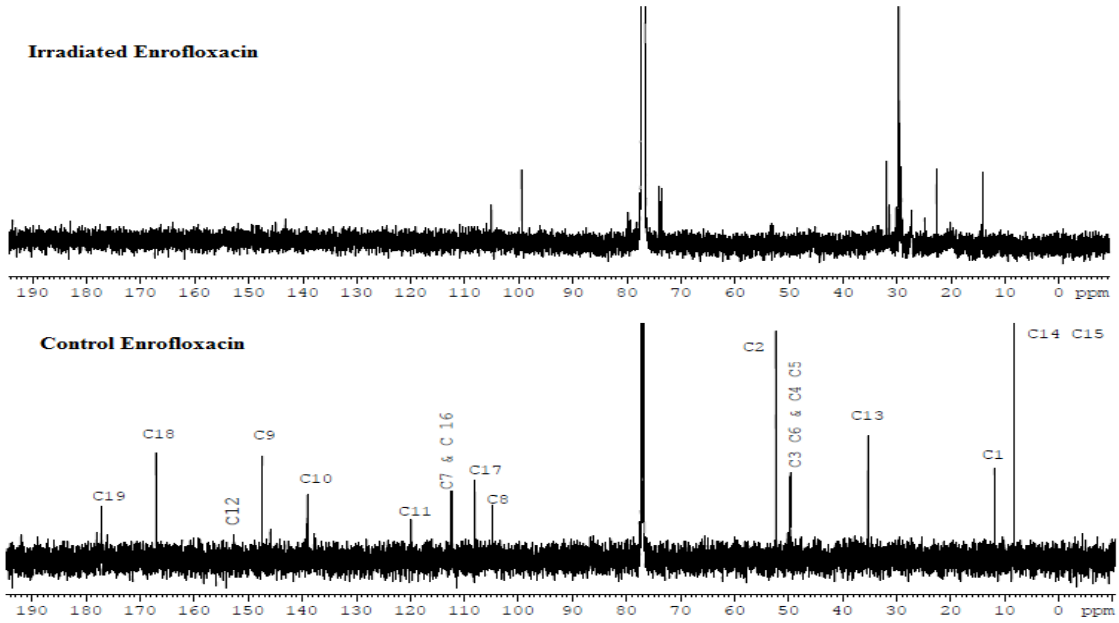

Figure 5. ^{13}C NMR superposition spectra of enrofloxacin: Control and irradiated enrofloxacin at 50 kGy

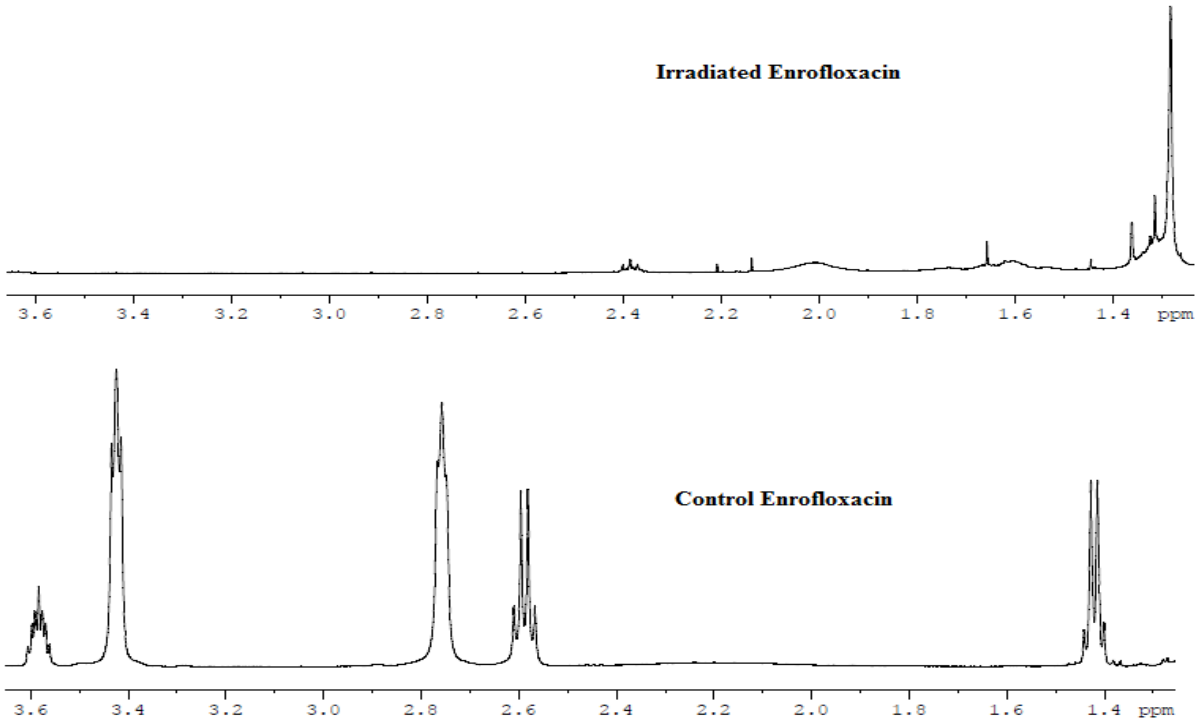

Figure 6. Superposition ^{1}H NMR region 1.4 - 3.6 ppm

For the piperazine (cycle A), the examination of ^{1}H NMR spectrum in specific region around of 1.4-3.6 ppm (figure 6), confirm the disparition of these peaks (2.7 and 3.42 ppm) and probably the cleavage of the piperazine cycle flowed by formation of NH functions indicated by the apparition of peaks in the region around 100 ppm in ^{13}C NMR spectrum (figure 5), and the region around 700-900 cm^{-1} observed in the FTIR analysis (figure 4). In addition, the figure 7 showed the disparition of the multiplet and the quadruplet respectively in the 3.6 ppm and 1.4 ppm shifts related to the cyclopropyl. This result confirms likewise the cleavage of the cyclopropyl.

3.2. Resistance of C.metallidurans to Enrofloxacin anttibiotic

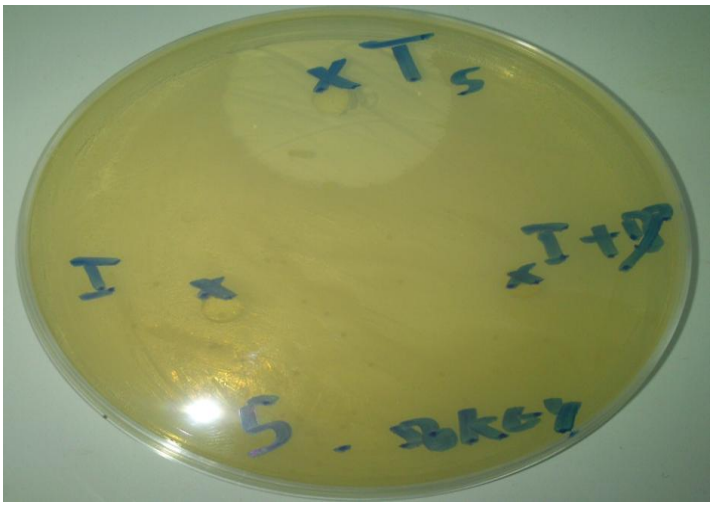

Figure 7. Zone inhibition observed in presence of enrofloxacin control (Ts) and irradiated enrofloxacin (S-50 kGy) with *C.metallidurans*.

The suceptiblity of *C.metallidurans* to the enrofloxacin is tested by disc method. The Figure 7 showed a zone of inhibition around the disc impregnated with untreated enrofloxacin antibiotics; beyond this zone, an unaffected area of normal growth of *C.metallidurans* is observed in contact of irradiated enrofloxacin at 50 kGy. Thus, we can conclude that the irradiation process is able to reduce the biological inhibition activity of enrofloxacin and led to the growth of *C.metallidurans* at high concentration of enrofloxacin (1mg/ml).

3.3. Effect of current density

The influence of the current density on the COD removal during the electrochemical oxidation of enrofloxacin 8 ppm at the electrode BDD. Degradation assays were performed using different current densities: 20, 40, 60 and 70 $mAcm^{-2}$. The results presented in Fig. 8 show that COD removal rates slightly increase with current density. Increasing the current density until 70 $mA.cm^{-2}$ resulted in an enhancement of the oxidation rate [9]. After 5h time of electrolysis, the COD percent removal

increased from (68.2 ± 5.4) % to (98 ± 5) % when the current density increased from 20 to 70 $mA.cm^{-2}$.

Figure. 9 represents the Effect of current density on the decay of absorbance at 280 nm for enrofloxacin antibiotic. The absorbance at 280 nm of antibiotic was increasing with the current density value. At 70 $mA.cm^{-2}$ was the maximum absorption value. With the increase of degradation time, the maximum absorption peak at 70 $mA.cm^{-2}$ rapidly decreased and finally disappeared after 130 min.

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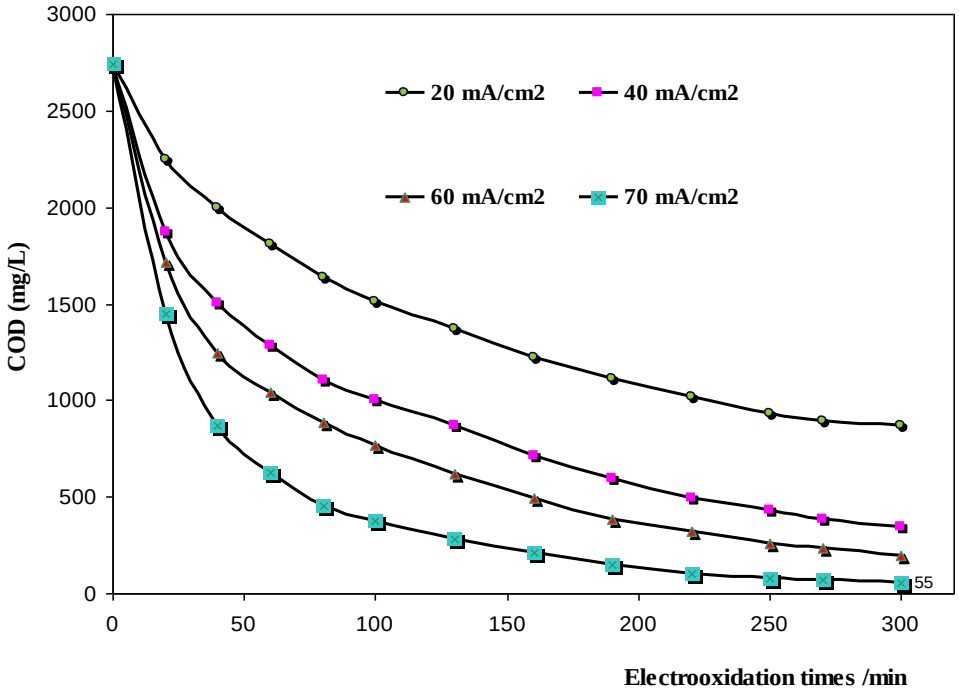

Figure 8. Influence of current density on the decay of COD during electro-oxidation of 2742 mg/L enrofloxacin antibiotic on BDD anode.

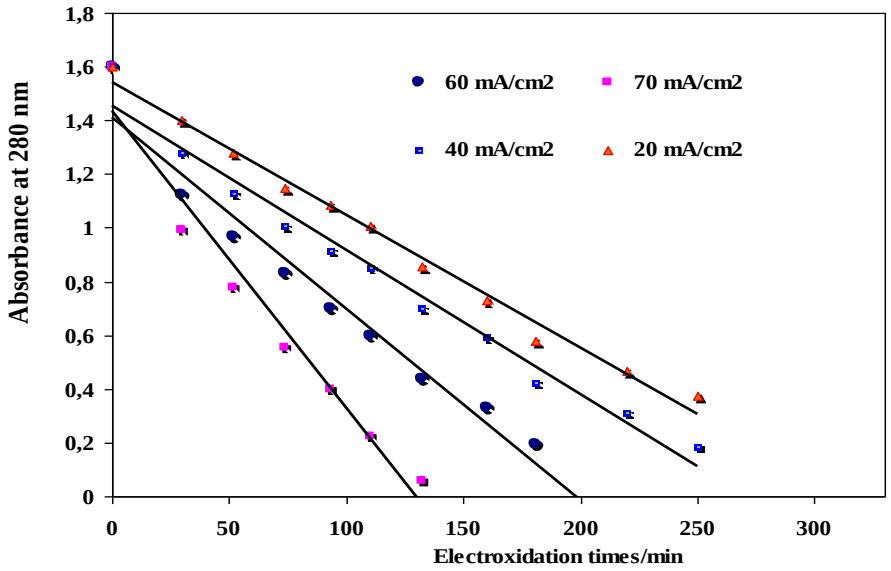

Figure 9. Effect of current density on the decay of absorbance at 280 nm (on the variations of UV spectra) during electro-oxidation of 2742 mg/L enrofloxacin antibiotic on BDD anode.

4. CONCLUSION

Conventionally, Conductive-diamond electrochemical oxidation, Ozonation and Fenton oxidation are commonly used for the degradation of enrofloxacin in waste water. Howerver, these techniques leads to the formation of refractory compounds [12, 13]. In addition, microbiological transformation of enrofloxacin by the fungus *Mucor ramannianus* led to disparaition of 78 % of this antibiotics with apparaition of three metabolites enrofloxacin *N*-oxide , *N*-acetylciprofloxacin and desethylene-enrofloxacin [14] .

In this work, we demonstrate that irradiation process of enrofloxacin at high dose of 50 kGy leads to the cleavage of three cycle: A, B and C, the cleavage of cyclopropyl and the disparition of carboxyl acid function flowed by the formation of NH functions and aliphatic hydrocarbons chain. Microbiological analysis showed that the antibacterial activity of enrofloxacin was completely removed. These results are helpful for antibiotics removal in the environment, and for exploring new technology for wastewater treatment.

Electrochemical oxidation using a BDD anode has been successfully applied to treat aqueous solutions containing enrofloxacin. The experimental results showed that: The removal rate of COD increases with applied current density due to the increase of the mass transport caused by oxygen evolution reaction, but decreases for higher values due to the improvement of this reaction.

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