



Assessment of the efficiency of photocatalysis on tetracycline biodegradation

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ABSTRACT

The use of photocatalysis to improve the biodegradability of an antibiotic compound, tetracycline (TC) was investigated. The toxicity of TC and its degradation products were also examined. The Sturm test was conducted to assess the biodegradability of by-products formed in the photocatalytic process. The toxicity of tetracycline and its by-products was evaluated using a dehydrogenase inhibition test, which showed a decrease in toxicity during photocatalysis. However, the Sturm test results indicated that, like tetracycline, the by-products are not biodegradable. Possible structures of these by-products were determined using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS). It was found that, during the photocatalytic process, the TC aromatic ring is not opened and the structure of the identified by-products is quite similar to that of tetracycline. A reaction pathway is proposed.

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1. Introduction

The extensive use of pharmaceuticals has led to the pollution of numerous environmental matrixes worldwide. Several classes of pharmaceuticals such as lipid regulators, antibiotics and hormones are detected in surface water, groundwater, sewage water, and sometimes in drinking water [1–6].

Tetracycline (TC) represents a major proportion of the antibiotics currently in use; Sarmah et al. reported that, in 2000, tetracyclines were the most widely used antibacterial compounds in the United Kingdom [7]. A report of the French Agency for Food Safety revealed that tetracycline represented more than half of the 1348.87 tons of antibiotics sold in 2007 in France [8]. Tetracycline is prescribed not only for humans but also in aquaculture and for livestock to treat and prevent bacterial infections. After administration, a significant part of the antibiotic is not completely metabolized, so it is excreted by humans into wastewater or by animals through excrement, which is then spread onto agricultural soil fertilized with animal slurry. Antibiotics can have adverse effects on living organisms. They can increase the resistance of bacteria to drugs, spread antibiotic resistance genes in the environment, have genotoxic effects on microorganisms and thus threaten human health [9–11].

Conventional water and wastewater treatment plants are unable to remove antibiotics and other pharmaceutical compounds

completely. Consequently, antibiotics are discharged in the effluent into the environment [12–14] so that people, aquatic organisms and flora become exposed to them. For these reasons, it is essential that future research focuses on the investigation of appropriate treatment methods that can be integrated into water and wastewater facilities [15].

During the last decade, advanced oxidation processes (AOPs) have been shown to be an alternative for the removal of recalcitrant and non-biodegradable compounds and the most popular AOPs studied are heterogeneous photocatalysis with semiconductors, ozonation and the Fenton process [16]. Among the catalysts used in heterogeneous photocatalysis, TiO₂ has been gaining attention for its strong photoinduced oxidation power [17]. However, although heterogeneous photocatalysis has been used successfully to eliminate bio-recalcitrant organic compounds, among the drawbacks of AOPs, one is the formation of intermediates and final products. It appears that, in some cases, the by-products are more toxic than the parent compounds [18–20]. Thus, it is clearly important to identify the structure of by-products and assess their toxicity during AOPs.

On the other hand, the complete mineralization of contaminants within the AOPs has been observed by several authors [15]. However, this is costly due to the quantity of energy and chemical reagents consumed during the oxidation process [21]. One solution to minimize the cost is to use AOPs to convert the initially persistent organic compounds into more easily biodegradable ones and then apply a biological treatment. This approach has been studied by several authors [22–25]. However, few articles have been devoted to assessing the ability of heterogeneous photocatalysis to improve tetracycline biodegradability [26,27] and none of them has focused

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Table 1
Reactor compositions in the Sturm test.

Reactor	Process evaluated	Composition
1	CO ₂ production when there is no inoculum	Sturm media, pollutants, NaClO, TOC = 30 mg/L
2	Inoculum respiration	Sturm media, inoculum
3	Biodegradability of sodium benzoate	Sturm media, inoculum, sodium benzoate (TOC = 45 mg/L)
4	Biodegradability of tetracycline	Sturm media, inoculum, tetracycline (TOC = 41 mg/L)
5, 6	Biodegradability of tetracycline by-products	Sturm media, inoculum, tetracycline by-products (TOC = 32 mg/L)

on the identification of tetracycline by-products formed during the photocatalytic treatment.

In this work, the effect of heterogeneous photocatalysis was evaluated in terms of the change in toxicity and the improvement in biodegradability of tetracycline. The biodegradability of tetracycline photoproducts was assessed using the Sturm test and some of them were identified using LC–ESI–MS/MS. The toxic effects of tetracycline and its by-products were evaluated on *Pseudomonas* and compared.

2. Experimental

2.1. Materials

Tetracycline hydrochloride with a purity of over 99% was purchased from Sigma–Aldrich. Titanium dioxide (AEROXIDE® TiO₂ P25, S_{BET} 50 m²/g) was purchased from Evonik Degussa GmbH (Frankfurt, Germany). Analytic reagents were obtained from Merck and ethylenediaminetetraacetic acid (EDTA) from Sigma–Aldrich. The bacteria *Pseudomonas aeruginosa* came from the Collection of the Pasteur Institute (CIP) A22.

2.2. Photochemical experiments

Tetracycline photocatalysis degradation was carried out in an annular reactor with an emission source, a medium mercury lamp (TQ 718 Z1 700 W) purchased from Heraeus, at its center. Irradiation below 290 nm was filtered by a Duran cooling tube surrounding the lamp. The volume of the reactor was 950 mL and the concentration of TiO₂ suspension was 30 mg/L. In order to detect and analyze intermediates, high initial tetracycline concentration (67 mg/L), higher than those detected in real effluent (ng/L) [3], was used.

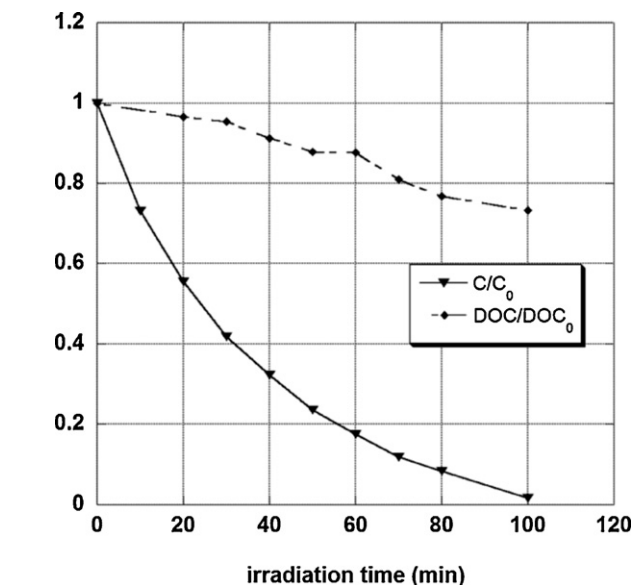


Fig. 1. TC and DOC concentration during the photocatalytic treatment ($C_0 = 67$ mg/L, $DOC_0 = 40$ mg/L, $[TiO_2] = 30$ mg/L).

During the irradiation, the solution was shaken and continuously bubbled with gaseous oxygen. Every 10 min, aliquots were taken to determine the tetracycline residual concentration, total organic carbon, and toxicity. The photocatalytic treatment was stopped when the tetracycline concentration reached 1 mg/L. The solution was filtered through glass micro filters (GF/B, $D = 1$ μ m, Whatman) and stored in glass bottles for the Sturm test.

2.3. Analytic methods

The residual concentration of tetracycline was measured by HPLC (Model 600E, Waters) using a Nova Pack C18 reverse phase column (150 mm \times 3.9 mm, I.D. 4 μ m, Waters). A mobile phase isocratic elution program was applied with two solvents; EDTA 10^{-3} M (in water) and methanol ($V_{EDTA}/V_{methanol} = 72/28$, pH of EDTA = 6.6) at a flow rate of 1 mL/min. The detection was performed with a UV detector (Model 486, Waters) at 357 nm. Dissolved organic carbon (DOC) was monitored with a Shimadzu 5000 TOC analyzer.

For the identification of tetracycline by-products, samples taken during the photocatalytic experiments were analyzed by LC–MS/MS on a Thermo Fischer system including an autosampler thermostated at 15 °C, a high performance liquid chromatograph equipped with a quaternary pump which can work at low speed, a mass spectrometer with an electrospray ionization (ESI) source, a triple quadrupole analyzer and a photomultiplier electron detector (TSQ Quantum Discovery). The column used for the LC separation was a Uptisphere ODB 120 Å (150 mm \times 2.1 mm I.D., 3 μ m, Interchim). The eluent was an 80/20 mix of water (0.1% formic acid) and acetonitrile (0.1% formic acid). Mass spectra were obtained as an average of 50 scans, each requiring 0.02 s. ESI source conditions were as follows: positive mode, heated capillary temperature 350 °C; sheath gas (N₂) 40 psi, auxiliary gas (N₂) 15 psi, spray

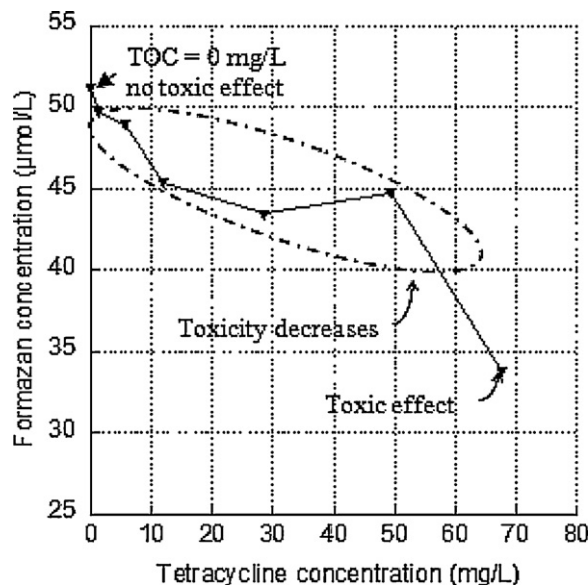


Fig. 2. Effect of tetracycline remaining in solution and by-products on formazan formation.

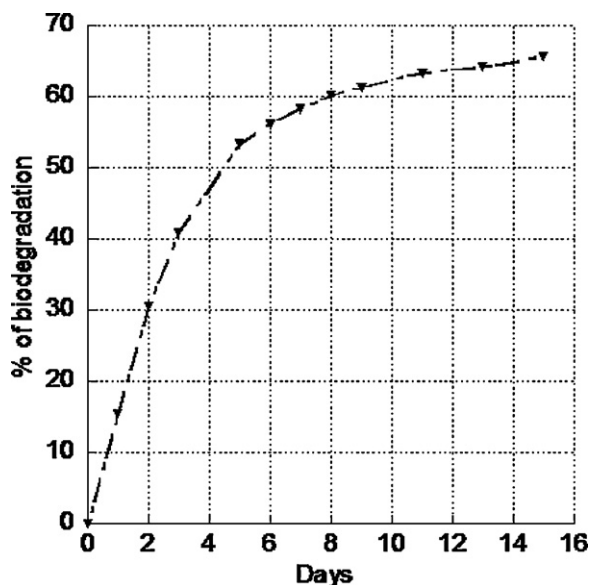


Fig. 3. Percentage of benzoate biodegradation in the Sturm test.

voltage 3500V, tube lens offset voltage 100V. The signal was obtained in full scan mode, full scan MS/MS mode and MRM mode. In order to optimize the sensitivity, collision energies were varied and the product ions with the highest sensitivity were selected for the detection and the confirmation.

2.4. Toxicity test

An acute toxicity test using *P. aeruginosa* was set up to assess the change in toxicity during the photocatalytic degradation of tetracycline. This test measures the dehydrogenase activity in the *P. aeruginosa* bacterial strain and uses an organic electron acceptor (2-p(iodophenyl)-3(p-nitrophenyl)5-phenyltetrazolium chloride (INT)) to measure the disruption in the respiratory chain [28]. In contact with electrons, INT is reduced to the formazan salt. The effect of tetracycline and its by-products was thus evaluated

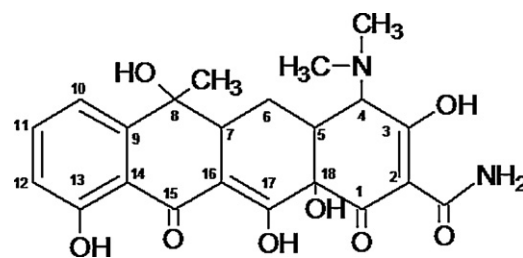


Fig. 4. Tetracycline structure.

by monitoring the formation of formazan, which depends on the flow of electrons in the bacterial respiratory chain.

0.5 mL of each sample taken during photocatalysis treatment and 0.5 mL of pure tetracycline solution (67 mg/L) were added to 3 mL of a *P. aeruginosa* cell suspension in an assay tube. After 19 h, the volume in each tube was adjusted to 6 mL with TRIS (tris(hydroxymethyl)aminoethane) buffer solution and 1 mL of INT (2 g/L) was added. The mixture was maintained at 30 °C for 30 min and then 8 mL of methanol was added. A control without pollutant was also carried out. Finally, solutions were centrifuged at 1000 rpm, and then the absorbance of the supernatant at 480 nm was determined in comparison with the control absorbance.

2.5. Sturm test

The Sturm test (ISO 9439) was used to evaluate the biodegradability of tetracycline and its photoproducts. It was prepared according to the method described by Stavrakakis et al. [29]. The Sturm test system is composed of six reactors ($V = 1$ L) aerated with CO_2 -free air for 28 days and of $\text{Ba}(\text{OH})_2$ traps after each reactor. The composition of the reactors is given in Table 1. The CO_2 released from the biooxidation of the tested compounds is trapped by $\text{Ba}(\text{OH})_2$ and then precipitated as BaCO_3 . The amount of CO_2 produced was determined by titrating the remaining $\text{Ba}(\text{OH})_2$ with HCl 0.05 N in presence of phenolphthalein solution. Active sludge used as the inoculum (30 mg/L) came from a municipal sewage treatment plant located in Nantes, France (population equivalent 600,000).

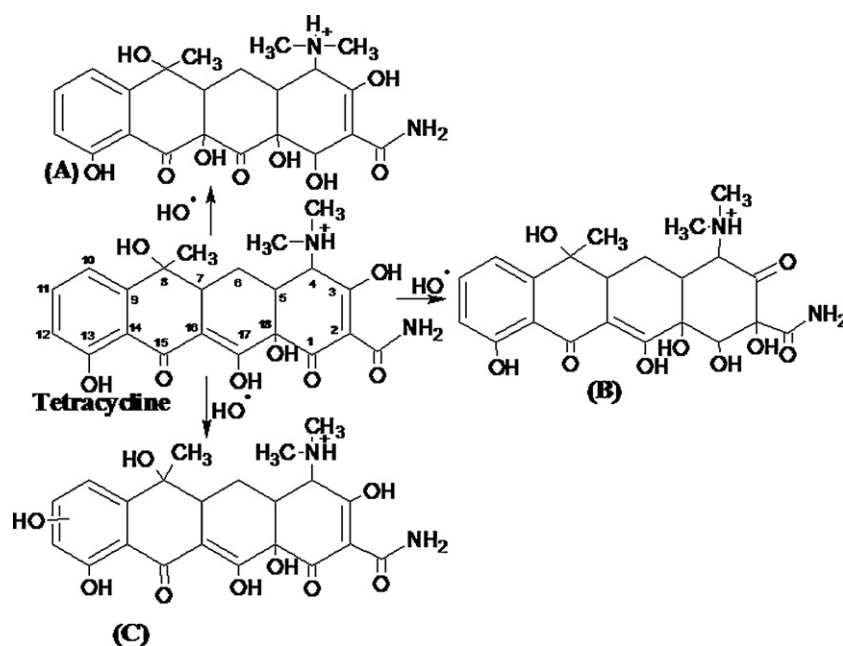


Fig. 5. Formation pathway of the parent ions with an m/z of 461.1.

Table 2
Transition and collision energies of compounds formed during photocatalytic degradation.

Compound	Molar weight (g/mol)	Parent ion (m/z) $[M+H]^+$	Product ion (m/z)	Collision energy (V)
Tetracycline	444.1	445.1	410.1 427.2	20 13
By-product 1	430.1	431.1	242	41
By-products 2	460.1	461.1	396.1 426.1	18 20
By-products 3	476.1	477.1	443.1 442	18 24
By-products 4	458.1	459.1	459.9 442.1	17 18
			-	-

3. Results and discussion

3.1. Photocatalytic degradation of tetracycline

Increasing irradiation time led to a decrease in tetracycline concentration in solution, however, at the same time, a smaller fall in the DOC was observed (Fig. 1). At the beginning of the photocatalytic treatment, the DOC of the solution was about 40 mg/L but, after 80 min of treatment, just 24% of the initial DOC had been removed.

In the chromatograms of the samples taken at 30 min and at 80 min, new peaks with different retention times were observed (data not shown) i.e. new compounds were formed. At the end of the photocatalytic treatment (80 min), these intermediate products were still in the solution. This is in accordance with the level of DOC found in solution at the same time.

The AOPs produce intermediates or by-products of degradation [30,31] and, in some cases, their total mineralization is too expensive to achieve in terms of time and materials [32]. Zhao et al. [33] studied the photocatalysis of oxytetracycline (OTC) with 5A and 13X zeolite loaded with TiO_2 . They have assessed the mineralization rate by measuring the dissolved organic carbon (using a TOC analyzer), and with 15 wt% $TiO_2/5A$, about 31% of the initial DOC ($TOC_0 = 22.5$ mg/L) was removed in 10 h whereas OTC was removed in 200 min. For 10 wt% $TiO_2/13X$, about 15% of DOC was removed in 120 min and then the DOC removal rate slowly reached 16.00% after 8 h.

As the DOC concentrations remaining in solution are high compared to the TC degradation, it is clear that the photocatalytic degradations tested in our study do not lead to the mineralization of the initial compound. This is not a drawback since the goal of the present research was to study the potential of photocatalysis to transform TC into other compounds which could be less toxic and more biodegradable. When total mineralization is not obtained, the key point is to be able to evaluate the potential adverse effects of by-products. In our case, the biodegradability of the TC by-products and their toxicity toward *P. aeruginosa* dehydrogenase activity were examined, with a view to optimizing parameters or recommending additional treatments if required.

3.2. Toxicity test

The formation of formazan was monitored to assess the effect of tetracycline and its by-products. Fig. 2 presents the changes in formazan concentration in assays containing *Pseudomonas*, INT and samples taken during irradiation as a function of the remaining tetracycline concentration. In the control experiment, corresponding to *P. aeruginosa* cells and INT, the maximum concentration of formazan formed was 51 $\mu\text{mol/L}$. In the presence of pure tetracycline solution, the INT reduction led to a formazan concentration of about 32 $\mu\text{mol/L}$; this corresponds to a decrease of 40% of the control assay due to the disruption of the bacterial metabolism. Tetracycline disrupted the dehydrogenase activity of *P. aeruginosa*, thus there were fewer electrons in the respiratory chain and

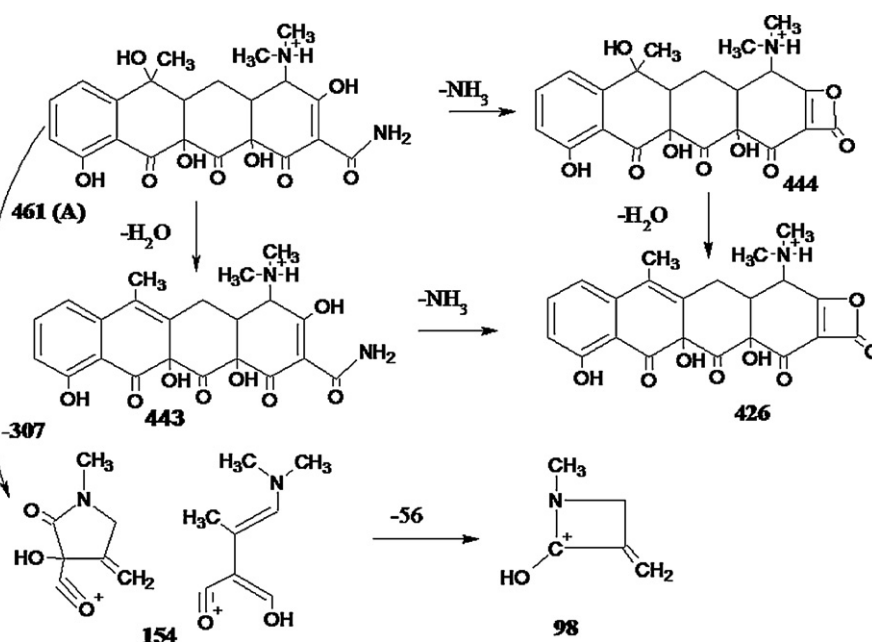


Fig. 6. Fragmentation pattern of compounds with m/z values of 461.1.

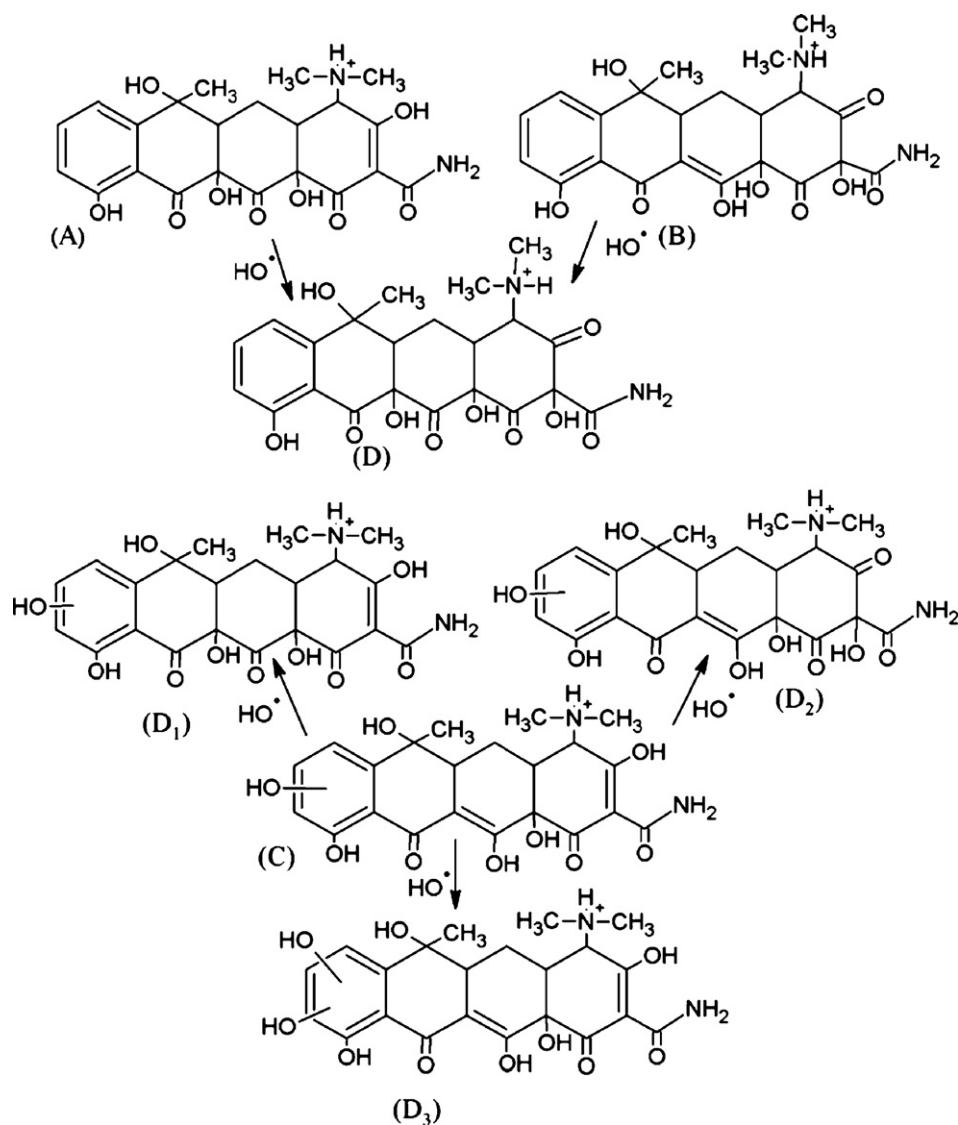


Fig. 7. Formation pathway of the parent ions with an m/z of 477.1.

less formazan was formed. During the photocatalytic process, the remaining concentration of tetracycline decreased and more formazan could be formed.

After the photocatalytic treatment, only 24% of the initial DOC was mineralized, so there were still several organic compounds in solution. However, in comparison with the beginning of the photocatalytic treatment, the formazan concentration increased. These results suggest that the by-products formed during photocatalysis are less toxic to *P. aeruginosa* than tetracycline.

Some results have led researchers to suggest that toxicity can increase during the oxidation process due to the combined effect of all the by-products in solution. Jiao et al. [34] studied the photolysis of tetracycline and evaluated the toxicity of the photolysis compounds. They used luminescent bacteria to assess the toxicity of photoproducts and their results revealed that the toxicity increased with irradiation. The same authors also studied the photolysis of oxytetracycline and the toxic effect of the photoproducts, the results again showed an increase in toxicity with irradiation [35]. López-Peñalver et al. [36] also found an increase in toxicity during the oxidation of tetracycline using UV and UV/ H_2O_2 . In contrast, our results demonstrate a

decrease in toxicity toward *P. aeruginosa* during the photocatalytic process. Reyes et al. [27] also found that tetracycline oxidation by-products did not present antibacterial activity against *Staphylococcus aureus* like tetracycline. These different results suggest that the toxicity of by-products depends on the oxidation process due to the possible different reaction mechanisms involved, and also on the organism used in the toxicity test. Clearly, the toxicity of by-products has to be studied for each process.

3.3. Biodegradability study

The biodegradability of tetracycline and the products formed during the photocatalysis was evaluated by the Sturm test. The concentration of CO_2 in reactor 2 was 56 mg/L, higher than that advocated in the standard (40 mg/L) but, as the inoculum is the same in all the reactors and this value is taken into account in the calculation of the biodegradation of the tested compounds, the bias is counterbalanced. In Fig. 3, the percentage of sodium benzoate biodegraded as a function of time is given. After 15 days, sodium benzoate was 60% mineralized, thus validating the Sturm test. At the end of the test, this value had reached 70%. Total mineralization

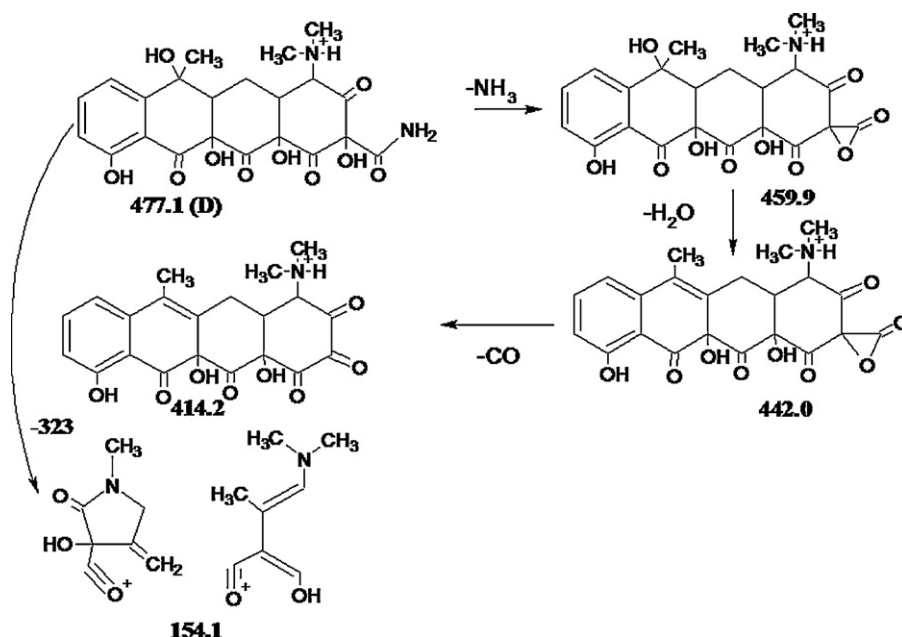


Fig. 8. Fragmentation pattern of compounds with an m/z value of 477.1.

(100%) cannot be achieved due to the use of a part of the substrate for cellular biosynthesis.

Tetracycline biodegradability was evaluated in reactor 4. The CO₂ produced in this reactor corresponded to that issuing from biodegradation in addition to that from the endogenous respiration of bacteria (estimated in reactor 2) and the CO₂ produced by the abiotic reaction (estimated in reactor 1). At the end of the Sturm test, the concentrations of CO₂ were 66 mg/L in reactor 1, 59 mg/L in reactor 2 and only 96 mg/L in reactor 4. As the sum of the CO₂ in reactors 1 and 2 is higher than that in reactor 4, it was concluded that only the endogenous respiration of bacteria and the abiotic process had led to the production of CO₂ in reactor 4. Thus, no tetracycline biodegradation was measured. This observation is in agreement with the results obtained by Gartesier et al. [37], Kim et al. [38] and Prado et al. [39].

In Section 3.1, the formation of by-products during the photocatalysis of tetracycline was proved. The Sturm test was carried out on by-products to see if their biodegradability was higher in comparison with the parent compound (tetracycline). By-products were tested in reactors 5 and 6, where the concentrations of CO₂ at the end of the Sturm test were 77 mg/L and 78 mg/L, respectively. For these values, the CO₂ issuing from the endogenous respiration of bacteria and from the abiotic reaction must also be taken into account. However, each of the CO₂ values obtained in reactors 5 and 6 is lower than the sum of the CO₂ values in reactors 1 and 2. Therefore, it can be concluded that, like tetracycline, the by-products obtained are not biodegradable. This result is in contrast with other findings. Previous researchers have shown that applying AOPs can enhance biodegradability; the by-products are more oxidized and they can easily be degraded biologically [16,24]. Reyes et al. [27] evaluated the increase in biodegradability during the photocatalysis of tetracycline. The TC biodegradability was assessed by the ratio BOD/COD with BOD corresponding to the biochemical oxygen demand and COD to the chemical oxygen demand. They observed an increase in the BOD/COD ratio, which indicated the ability of photocatalysis to improve biodegradability. The main explanations for this difference between their conclusions and ours can be attributed to the different methods used to assess the biodegradability.

3.4. By-product identification

The test described above showed that the by-products of tetracycline were not biodegradable. To understand better the persistence of the resulting mixture of by-products, it was necessary to extend our investigation by identifying the major by-products of tetracycline formed during photodegradation.

Aliquots taken during the photocatalytic process of tetracycline were analyzed by LC-MS/MS. The main parent ions (m/z) obtained in a full Q1 MS analysis are summarized below. Collision energies were varied and the product ions with the highest sensitivity were selected for the detection and confirmation of the respective parent ion. The results obtained are summarized in Table 2.

One of the by-products 2 and one of the by-products 3 have already been identified as oxidation products of tetracycline whose structure is presented in Fig. 4. Khan et al. as well as Damalzio et al. [40,41] found one compound with an m/z of 461.1 and one with an m/z of 477.1 during the ozonation of tetracycline. One other compound with an m/z of 461.1, resulting from the radiolysis of TC, was found by Jeong et al. [42]. It was formed by the addition of a hydroxyl group on the phenol group of tetracycline.

All of these compounds previously found by other researchers could be formed during the photocatalysis of tetracycline. As observed in Table 2, four main m/z values (431.1, 461.1, 477.1 and 459.1) were detected in samples after the photocatalysis of tetracycline.

The parent ion with an m/z of 461.1 was monitored using product ions with an m/z of 426.1 and 443.0. The LC-MS/MS chromatograms obtained after 80 min of treatment (data not shown) present many peaks. The presence of different product ions with the same m/z value suggests that several compounds with an m/z value of 461.1 were formed during the photocatalytic treatment.

There is a difference of 16 units of mass between the parent ion of tetracycline and that of the by-products 2. This could come from the addition of a hydroxyl group on tetracycline. For convenience, different structures of ions with the m/z value of 461.1 are noted A, B and C. Tetracycline presents several reactive sites; double bond, ketone group, amino group and keto-enol group. OH[•] can

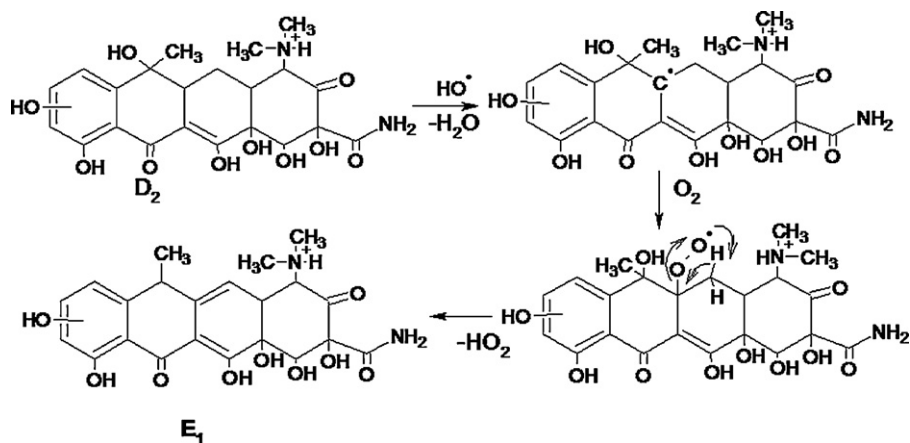
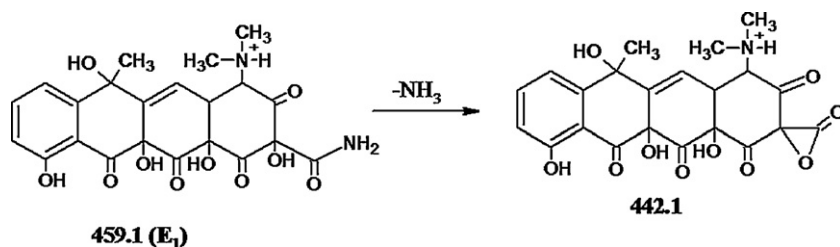
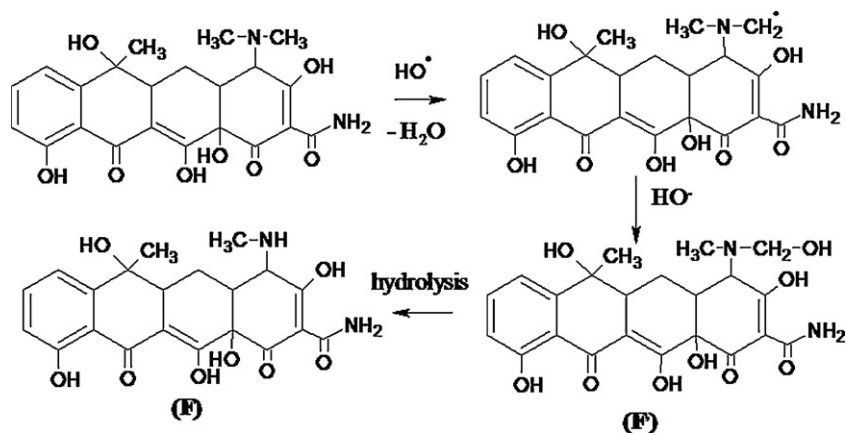
Fig. 9. Formation mechanism of compound E₁.Fig. 10. Fragmentation pattern of compounds with $m/z = 459.1$.

Fig. 11. Formation mechanism of compound F.

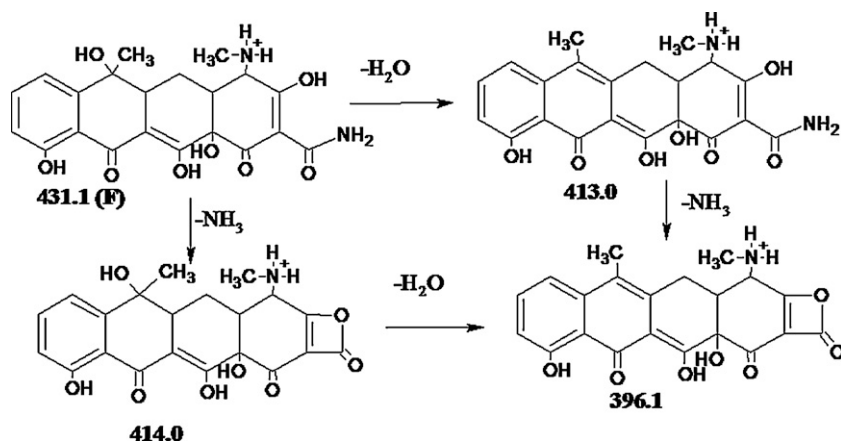


Fig. 12. Fragmentation pattern of compound F.

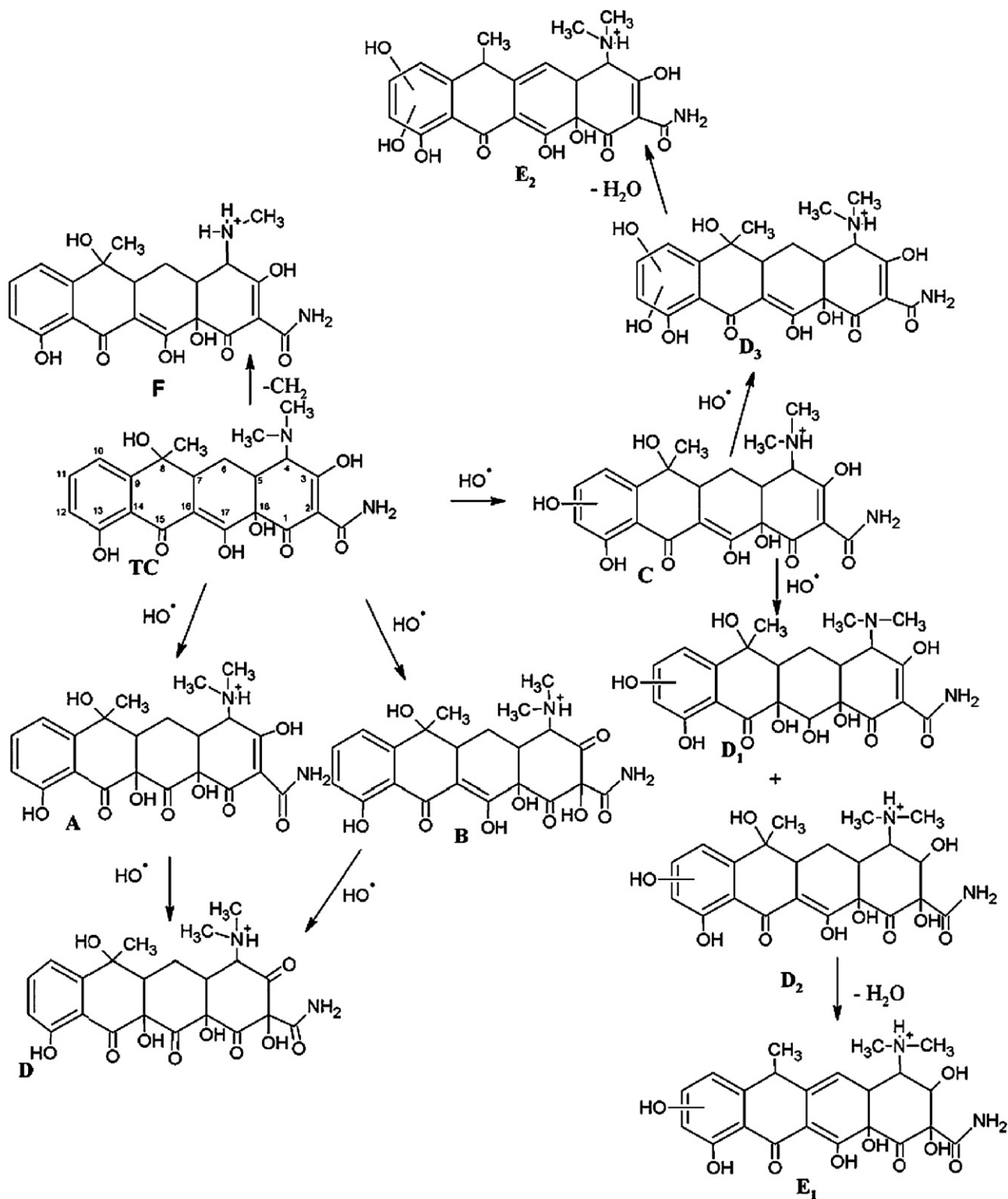


Fig. 13. Reaction pathway of tetracycline photocatalysis.

react with all of these sites. Because of the keto-enol group, site 16 is very reactive thus OH^\bullet could react with it and lead to compound A. Site 2 is also reactive but, because of the amide group, it is less reactive than 16. If OH^\bullet reacts first with C₂, it leads to the formation of compound B. The addition of OH^\bullet to the aromatic ring leads to compound C. The proposed formation mechanism of these compounds is presented in Fig. 5.

In order to justify the proposed structure for the ions with an m/z of 461.1, a fragmentation was carried out. The fragment ions obtained and the fragmentation pattern are given in Fig. 6.

Vartanian et al. [43] studied the fragmentation pattern of tetracycline. They proposed some structures for the ions with m/z values of 98 and 154, which were used to propose the structures below. The fragmentation pattern obtained for ions with an m/z of 461.1

justifies the structures proposed for them (the example given is for compound A, but it is the same for compounds B and C).

A second hydroxylation of tetracycline leads to the compounds with an m/z value of 477.1. The evolution of these compounds during the photocatalytic process was monitored by using product ions with m/z values of 442 and 459.9. The LC–MS/MS chromatograms after 80 min present also many peaks (data not shown). It can be concluded that, like compounds with a mass value of 460.1, there are several compounds with a mass value of 476.1 formed during photocatalysis.

Parent ions with an m/z value of 477.1 are noted D, D₁, D₂ and D₃. D could be obtained after the addition of •OH to C₂ on A or to C₁₆ on B. D₁ and D₂ could be formed after the addition of •OH to compound C on C₁₆ or on C₂. D₃ may be formed after the addition of •OH on the aromatic rings of C. The proposed formation mechanism is given in Fig. 7.

The fragmentation obtained for the parent ions with an m/z value of 477.1 and the fragmentation pattern are presented in Fig. 8. This fragmentation pattern justifies the structures proposed for the ions with an m/z of 477.1. The example is given for D, but the fragmentation pattern is the same for the others.

Compounds at m/z = 459.1 may come from the abstraction by •OH of one H from C₇ of compounds D₂ or D₃. A tertiary carbon, stabilized by the delocalization of the double bond, is formed. This tertiary carbon is attacked by O₂ to produce a peroxide radical. Then, the hydroperoxyl radical is eliminated and compounds at m/z = 459.1, noted E₁ and E₂, are obtained [42]. The proposed reaction mechanism for E₁ is shown in Fig. 9; it is the same for E₂ which come from the H-abstraction reaction on B and D₃, respectively.

One other mechanism of formation of compounds at m/z = 459.1 could be the oxidation of compound C to form a quinone [42], but this hypothesis is less probable. The fragmentation of ions with an m/z of 459.1 was done and the fragmentation pattern is presented in Fig. 10. If the quinone group was formed, fragmentation would result in a loss of an H₂O molecule but this loss is not observed. So it can be supposed that compounds with an m/z of 459.1 are predominantly formed by the H-abstraction reaction with •OH. The fragmentation pattern justifies this affirmation.

The compound with the mass value of 430.1 results from the N-dealkylation of tetracycline. In the oxidation process, the N-dealkylation of compounds with amino groups often occurs because of the low N–C bond energy [34,35,44,45]. In the presence of a hydroxyl radical, the first step of the N-dealkylation reaction is H-abstraction; a radical ion is formed and a rebound of the hydroxyl group leads to the intermediate noted F'. Then, F' is hydrolyzed and the compound with an m/z value of 431.1 is obtained (Fig. 11) [46]. The fragment ions obtained and the fragmentation pattern (Fig. 12) justifies the proposed structure for the compound with an m/z value of 431.1.

As has been observed, the structures proposed for tetracycline by-products are quite similar to that of tetracycline. Therefore, it can be concluded that, during the photocatalytic degradation in our study, the aromatic ring of tetracycline was not opened. The main reaction mechanism was the addition of the •OH radical on the reactive sites of tetracycline. Based on the main by-products that have been identified using an LC–MS/MS analysis and according to data in the literature, a reaction pathway for the photocatalysis of tetracycline is proposed and presented in Fig. 13.

4. Conclusion

The results of the toxicity test carried out on *Pseudomonas* showed a decrease in toxicity during tetracycline photocatalysis. At the end of the Sturm test, no CO₂ coming from the biodegradation was produced. These results indicate that the by-products of

tetracycline are less toxic to *Pseudomonas* than tetracycline and are not biodegradable. The structures of these by-products were studied using LC–ESI(+)-MS/MS. Combining the results obtained with a review of the literature, the structures of some of these by-products are proposed. It appears that, under the photocatalytic conditions used in our study, the aromatic ring of TC is not opened and thus the structure of the by-products is not so different from that of tetracycline. The main reaction mechanism occurring during photocatalysis is the addition of the •OH radical onto the reactive site of tetracycline.

The main conclusion of this study is that, under our experimental conditions, just 90 min is not sufficient to generate biodegradable by-products. Further studies must be done in order to optimize the experimental conditions, like irradiation time, loading of TiO₂ and pH of the solution, for the best degradation. Increasing irradiation time might generate more oxidized by-products with the aromatic ring opened, which are more biodegradable.

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