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# 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.



## 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, $TiO_2$ 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® $TiO_2$ P25, $S_{BET}$ 50 $m^2/g$) 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 $TiO_2$ 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. 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 μm, 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 × 3.9 mm, I.D. 4 μm, 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 × 2.1 mm I.D., 3 μm, 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 ($N_2$) 40 psi, auxiliary gas ($N_2$) 15 psi, spray

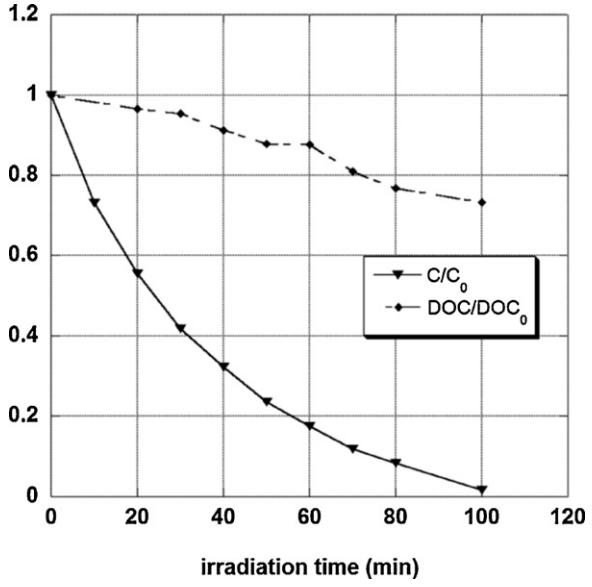


**Fig. 1.** TC and DOC concentration during the photocatalytic treatment ($C_0$ = 67 mg/L, $DOC_0$ = 40 mg/L, [$TiO_2$] = 30 mg/L).

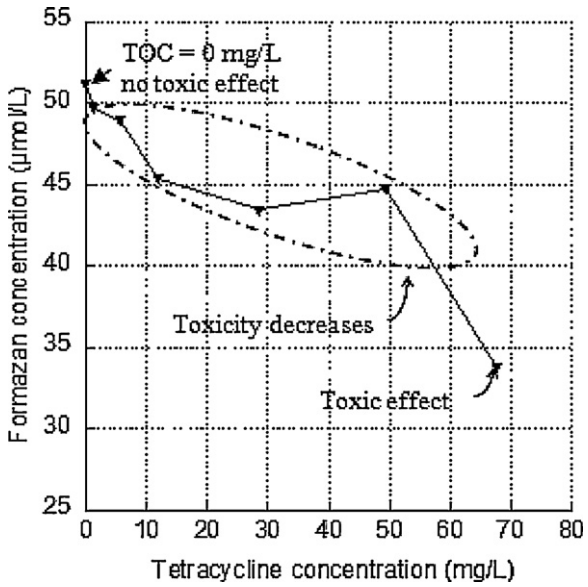


**Fig. 2.** Effect of tetracycline remaining in solution and by-products on formazan formation.