

Oxolinic acid photo-oxidation using immobilized TiO₂

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Abstract

This work studied the photocatalysed oxidation of the antibiotic oxolinic acid (OA) in an annular reactor operated with immobilized TiO₂ on sintered glass cylinders (SGC). Experiments were carried out in 1 l solution of OA (18 mg l⁻¹) at pH 9 with oxygen bubbling. Irradiation was performed with black light (36 W). The reaction was monitored by COD, TOC and average oxidation state (AOS) calculations. The antibacterial activity of intermediates was followed using the inhibition halo technique on *Escherichia coli* cultures. The initial antibiotic concentration decreases in one order of magnitude after 60 min irradiation, and was completely eliminated at 100 min reaction. The TOC was reduced in 54% and the AOS reach values around +3 indicating the formation of low molecular weight carboxylic acids. The oxidation reaction fit well with the Langmuir–Hinshelwood kinetic model indicating the dependence of reaction rate with initial adsorption step. The antibacterial activity of the solution decreases with antibiotic removal, demonstrating that intermediates do not present antibiotic activity.

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1. Introduction

The increasing consumption of antibiotics by humans and their excessive application in food production could transform the native flora of soils and waters into sources of antibiotic-resistant bacteria. Considering that isolated ecological communities do not exist, the high possibility that this resistance be transferred to nearby humans poses a serious risk for public health [1].

Oxolinic acid (OA) is a widely used antibiotic in the industrial aquaculture and poultry and bovine production. It has been monitored in agriculture soils, lake sediments, and in aquatic environments near fishing farms [2]. Antibacterial activity half-life of the OA in marine sediments surpasses 150 days [3]. Additionally, the quinolone structure of the OA has been reported as potentially genotoxic, which could provoke harmful effects on the environment [4].

Due to their antibacterial activity, waters containing antibiotics commonly used in animal and human health (antibiotics

included) are refractory to natural biological degradation or conventional wastewater treatments. The great concern is that waters contaminated with antibiotics can reach waters used for human consumption or municipal water treatment facilities [5,6]. Several alternatives to destroy these kinds of compounds have been considered in recent studies in the literature. These include reverse osmosis, adsorption on activated carbons or advanced oxidation technologies, such as Fenton reaction, ozonation and peroxidation combined with UV light [7–9].

Heterogeneous photocatalysis using a semiconductor (mainly TiO₂) have earned an important place among the advanced oxidation technologies due to the stability of the catalyst in the entire pH range, the absence of residuals after the treatment, and the possible use of solar light as light source. This heterogeneous system has been recently proposed as an alternative treatment for antibiotics, such as tetracycline and flumequine, where mechanistic aspects as well as the use of immobilized catalyst were discussed [10–12]. The photocatalysis is initiated with the formation of charge-carrier pairs (e⁻, h⁺) resulting from photon absorption by the catalyst. The photon must have an energy equal or larger than the catalyst's band gap (3.2 eV, λ < 385 nm). After that, the charge-carriers

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can migrate to the catalyst's surface, recombining or reacting with adsorbed species on the surface. Detailed aspects regarding the mechanism have been amply discussed in the literature [13–15]. In summary, it has been proposed that conduction band electrons can reduce molecular oxygen, forming superoxide anion. Simultaneously, holes oxidize adsorbed molecules of hydroxide anions or water molecules generating hydroxyl radicals. Furthermore, these holes are able to directly oxidize the organic matter by electron abstraction. In spite of the great number of papers published on photocatalysis, there are still some uncertainties in relation with the primary mechanisms involved [16]. A first source of debate comes from whether delocalized (free), photogenerated valence band holes (h_f^+) react directly with the organic substrate via a direct transfer (DT) mechanism [17], or rather photo-oxidation occurs via an indirect transfer (IT) mechanism with intervention of photogenerated OH^\bullet radicals (surface-trapped holes, h_s^+) [18].

The photocatalytic process can proceed using the catalyst in suspension or immobilized on inert surfaces. The use of a catalyst coated on porous materials avoids the catalyst-recovery step after the reaction, resulting in energy and cost savings [19]. To develop an approach to apply this technology, several immobilization methodologies have been proposed and new reactors have been designed. Immobilization techniques, such as dip coating, spray coating, sputtering, sol–gel-related methods and electrophoretic deposition have been investigated [19,20]. Moreover, different support materials, including glass beads, glass tubes, fiberglass, quartz, stainless steel, aluminum, activated carbon, and silica have been assayed [19,20].

In the present work, a sinterized glass bed was used as support for titania Degussa P-25. The support material, in the form of cylinders, was placed surrounding a black lamp in a reactor setup described in a recent report [11]. Oxolinic acid was used as a target compound, following its oxidation profile by spectrophotometric analyses, COD and TOC determinations and average oxidation state calculations. The antibacterial activity was determined applying the inhibition halo methodology on *Escherichia coli* cultures.

2. Experimental

2.1. General procedure of photocatalytic reactions

The laboratory-scale annular reactor shown in Fig. 1 was used in all the photocatalytic experiments. The reactor's lamp was completely encircled in its internal length (75 cm) by TiO_2 -impregnated SGC. Altogether, 13 cylinders were placed around the lamp with a total of 8.97 g of titania distributed more or less uniformly on the total of the cylinders. A black light lamp (360 nm, 36 W) was used in the experiments. The solution of oxolinic acid ($7 \times 10^{-5} \text{ mol l}^{-1}$, 18 ppm), adjusted to pH 9, was impelled through the reactor by a peristaltic pump operated at 155 ml min^{-1} . The pH of the solution was chosen due to the low solubility of the antibiotic at neutral or acidic pH. The solution was recirculated in the dark during 30 min before the lamp was turned on. All the photocatalytic experiments were performed with direct oxygen bubbling in the solution and under magnetic stirring in the vessel outside of the reactor. Samples of irradiated solutions were taken at different intervals for analysis and bacteriological measurements.

2.2. Materials and methods

Titanium dioxide Degussa P-25 with a surface area $50 \text{ m}^2 \text{ g}^{-1}$ (size $\sim 20\text{--}30 \text{ nm}$) was used as provided. Oxolinic acid was purchased from Sigma and used without any further purification. The oxolinic acid degradation was monitored at 341 nm by spectrophotometric analysis using a Shimadzu UV-1603 instrument. TOC analyses were carried out in a Shimadzu 5000 A instrument. The chemical oxygen demand was determined using a commercial kit (HACH) in a Digital Reactor Block 200 (HACH).

To determine the antibacterial activity of irradiated OA samples, tripticase agar plates were seeded with a 10^5 CFU ml^{-1} suspension of *E. coli* (ATCC 6317). The plates were loaded, in small holes made in the agar, with $100 \mu\text{l}$ of irradiated samples and incubated for 24 h at 37°C . The inhibition halo formed around the spot was measured in mm and compared to the calibration curve made with the pure solutions of antibiotic and

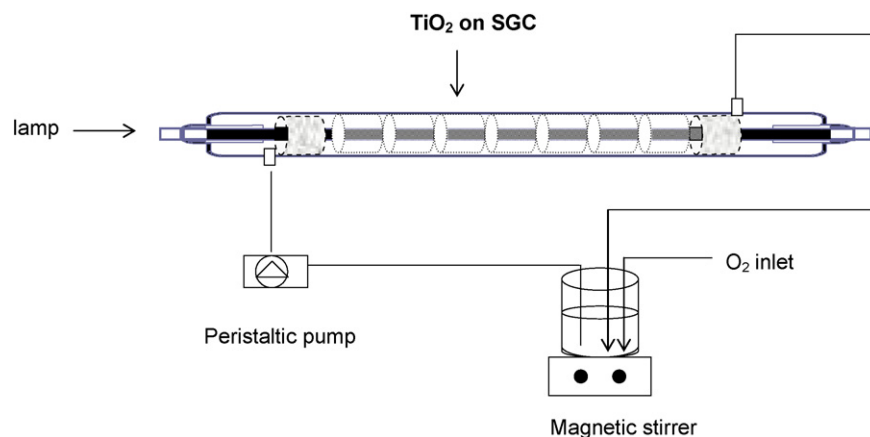


Fig. 1. Schematic representation of the experimental setup for photocatalytic reactions.

the respective microorganism. The general procedure has been previously described [12].

3. Results and discussion

3.1. Photocatalytic degradation of OA

Fig. 2 shows the absorption spectra of the oxolinic acid under illumination in the presence of TiO₂-impregnated sintered glass cylinders with Degussa P-25. Before the lamp was connected the solution was in contact with the cylinders during 30 min in dark in order to attain the adsorption equilibrium. The maximum adsorption was rapidly achieved reaching around 60% of the initial antibiotic concentration after 30 min of contact. It is clearly shown that the antibiotics rapidly modify their chromophoric groups, decreasing the absorption intensity at 265, 328 and 341 nm indicating structural modification of the antibiotic. The effect can be completely attributed to a catalytic effect because no alteration in absorption was observed in absence of the catalyst, even for large irradiation periods. The degradation profile of oxolinic acid in the reactor with immobilized catalyst is shown in Fig. 3. Around 60% of degradation was observed after 20 min irradiation with black light (36 W). The complete removal of the antibiotic was reached at 120 min irradiation. A very similar profile was obtained for the chemical oxygen demand (COD) in the course of the reaction. It is indicative that the oxidation of the antibiotic is very fast, generating after large periods of irradiation, stable intermediates. For comparison, the reaction was performed with titania in suspension (1 g l⁻¹) using the same reactor but without the rings impregnated with the catalyst. The reaction's performance increased with more than 90% antibiotic abatement reached after 20 min reaction, getting total abatement after 40 min irradiation. In a recent publication, similar results were obtained with flumequine using the same reactor [11].

The TOC profile shown in Fig. 4 indicates that intermediates refractory to mineralization are formed in the course of the

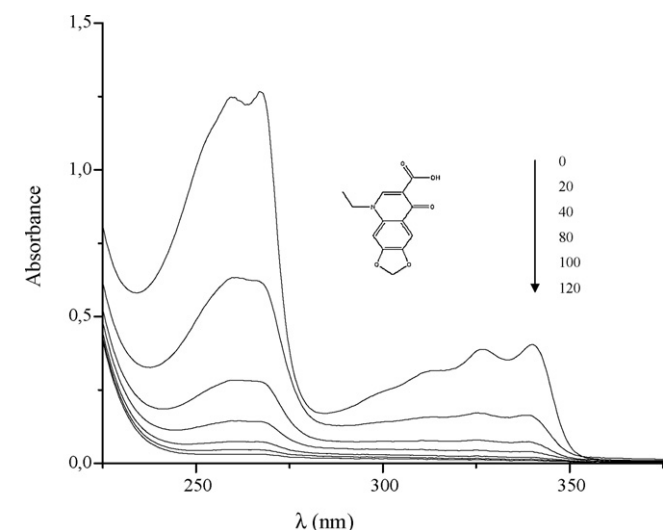


Fig. 2. Changes in the absorption spectra of the antibiotic in the course of the reaction. The structure of oxolinic acid is included.

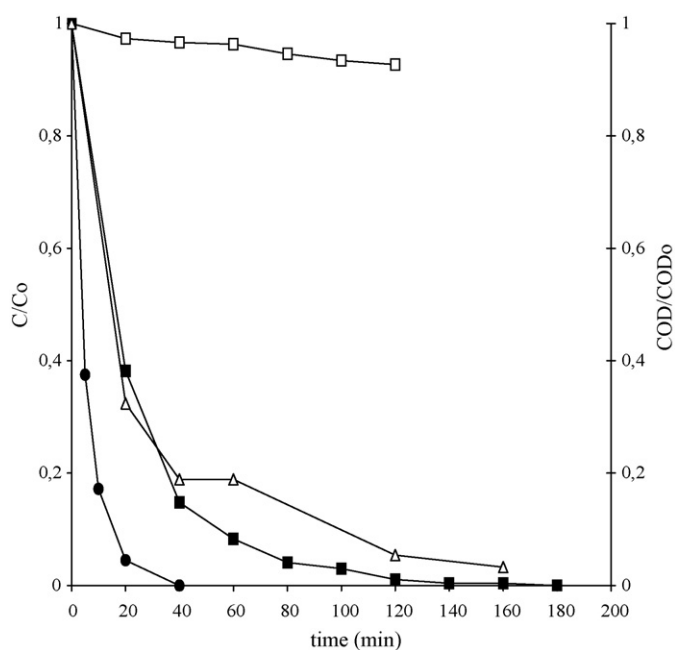


Fig. 3. Degradation profile of 18 ppm antibiotic solution. (□) photolysis in absence of catalyst; (■) impregnated TiO₂ on sintered glass cylinders + black light; (●) suspended TiO₂ (1 g l⁻¹) + black light; (Δ) COD removal by TiO₂-impregnated photocatalysis. Initial COD value 26 mg O₂ l⁻¹.

reaction. The mineralization became constant around 50% after 100 min irradiation in the reactor with the titania-impregnated sintered glass cylinder. At that time, the antibiotic was almost completely abated. Although the intermediates were not identified, their nature was monitored by the average oxidation state (AOS) changes during the photocatalytic process. The AOS was calculated according to Eq. (1), where TOC and COD are

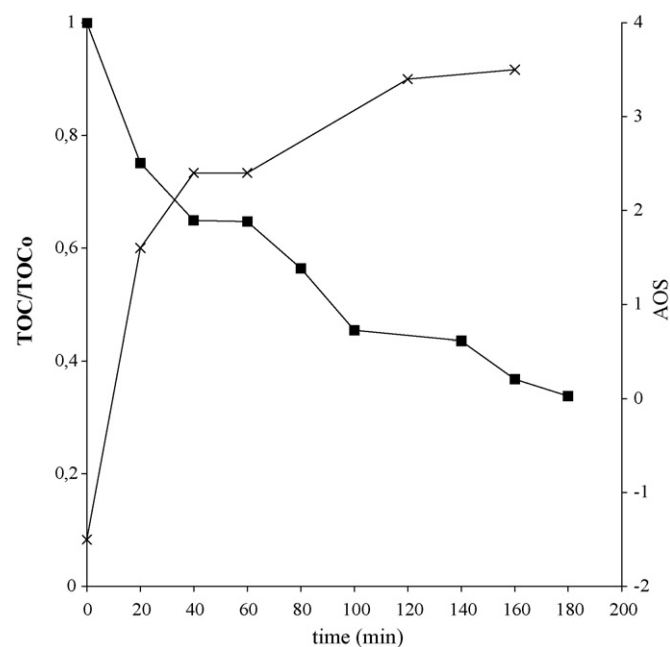


Fig. 4. Course of the TOC removal (■) and average oxidation state evolution (×), during the photocatalysis of the antibiotic in the reactor operated with immobilised TiO₂. Initial TOC value 10.9 mg C l⁻¹.

expressed in mol l^{-1} of C and O_2 , respectively [21].

$$\text{AOS} = \frac{4(\text{TOC} - \text{COD})}{\text{TOC}} \quad (1)$$

The maximum value for AOS (+4) corresponds to the most oxidized state for C (CO_2) and the lower value (−4) represents the most reduced state (CH_4). Calculated values for several organic compounds were reported by Stumm and Morgan [22]. For instance, the assigned AOS values for benzene (−1), formaldehyde (0), acetic acid (0), formic acid (+2) and oxalic acid (+3), and other substances are listed. Fig. 4 presents the AOS values calculated for long-lived OA intermediates. The AOS increase from the initial value around −1.5 for OA to values over +2 after 40 min irradiation. A second increase is observed after 120 min irradiation, reaching values between +3 and +4, when the antibiotic was completely abated. These AOS correspond to low molecular weight carboxylic acids [22], which are known as refractory for mineralization [23]. These results are in agreement with the low mineralization observed at large irradiation times.

3.2. Effect of the initial oxolinic acid concentration in the photocatalytic reaction

Several reports have established that the heterogeneous photo-oxidation rate fit well to the classic Langmuir–Hinshelwood (L–H) mechanism assuming the existence of an adsorption/desorption equilibrium under dark or illumination conditions [10,24,25]. The L–H mechanism considers that the initial rate (r_0) depends on the substrate's initial concentration (C_0 , Eq. (2)). In this equation, k_{LH} represents the apparent L–H rate, K_{L} the adsorption/desorption equilibrium constant, which is independent of the photon flux, and C_0 is the initial concentration in equilibrium [16].

$$r_0 = \frac{k_{\text{LH}}K_{\text{L}}C_0}{1 + K_{\text{L}}C_0} \quad (2)$$

Even though several opinions are against the use of the L–H mechanistic model because not all the assumptions are considered, its use is still recommended for its simplicity and the ability to well fit experimental results in heterogeneous photocatalysis [25].

Using the data from photocatalytic experiments with different initial OA concentrations, the values of k_{LH} and K_{L} can be calculated using the linearized equation by plotting $1/r_0$ versus $1/C_0$. The calculated values are presented in Table 1. The experimental results fit well with the kinetic model and the obtained values for constants are concordant with reported values for reaction

Table 1
L–H constants calculated from linearized equation of L–H kinetic model

Photocatalytic system	k_{LH} ($\text{mol l}^{-1} \text{min}^{-1}$)	K_{L} (l mol^{-1})
TiO_2 immobilised ^a	3.2×10^{-6}	1.2×10^4
TiO_2 suspension ^b (1 g l^{-1})	12×10^{-6}	3.5×10^4

^a $y = 26.14x + 313,878$; $R^2 = 0.9941$.

^b $y = 2.44x + 84,423$; $R^2 = 0.9985$.

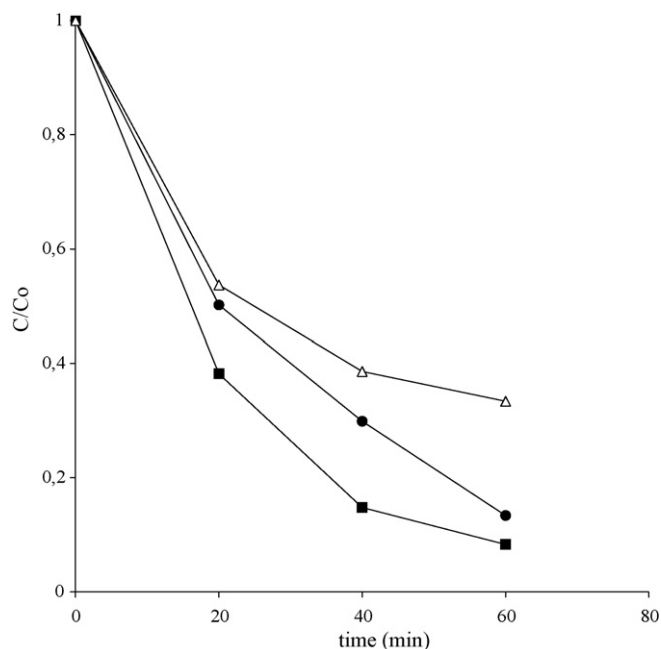


Fig. 5. Effect of the number of sintered glass cylinders impregnated with TiO_2 on degradation of oxolinic acid. (Δ) 2 cylinders; (\bullet) 10 cylinders; (\blacksquare) 13 cylinders.

systems that uses the catalyst immobilized [11] or in suspension [26]. Higher adsorption involves an increase in the reaction rate. The correspondence with the model indicates that adsorption is the essential step of the oxidation reaction. It is interesting to observe that adsorption constant (K_{L}) for the immobilized system is around three times lower than the system in suspension, considering the amount of titania in the immobilized system is higher (around 13 g in total) [11]. Thus, in addition to the known fact that titania coating provokes reduction on the surface, an important amount could be inaccessible due to catalyst sintering on the glass.

To determine the effect of the number of glass rings in the annular reactor, experiments were performed with different numbers of cylinders. Fig. 5 shows that when the number of rings increases, the photocatalytic activity is linearly increased ($R^2 = 0.995$). The extent of OA oxidation after 60 min irradiation reach values of 67, 87 and 92% for 2, 10 and 13 cylinders, respectively.

3.3. Microbiologic assays on irradiated antibiotic solutions

To determine the antibacterial activity (AA) of the OA-oxidized intermediates, the bacterial response to these compounds was determined during the course of the reaction. Experiments were conducted on agar plates inoculated with *E. coli* (ATCC 6317). To quantify the antibacterial activity of the irradiated samples, the inhibition halo formed around the microdrop of the sample seeded on the plate cultured with the bacteria was measured and reported in mm. The larger the halo formed, the greater the antibacterial activity. A consistent correlation between the photocatalytic degradation of the antibiotic and the decrease in the AA was observed (Fig. 6). The reac-

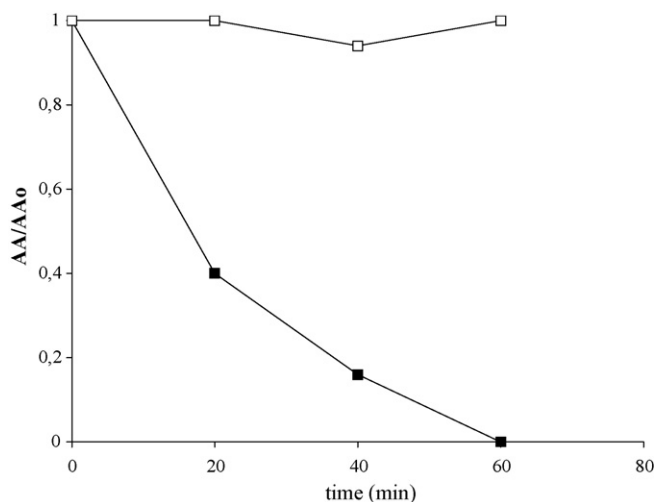


Fig. 6. Antibacterial activity evolution of the irradiated solution in the presence of immobilized TiO₂ (■) and black light (□).

tion in absence of TiO₂ indicate that antibacterial activity was not altered by the photolytic process. After 20 min irradiation, the antibacterial activity was reduced in 60%, the same abatement rate of OA (see Fig. 3). After 60 min irradiation, when the OA residual is less than 10% of the initial concentration, no activity was found. With this result it can be reliably concluded that the oxidation intermediates formed during photocatalysis do not show antibiotic activity. This information indicates that the photocatalytic treatment can be coupled to a biologic treatment system as proposed for other substances [27].

4. Conclusions

The conclusions based on the experimental results can be summarized as follows:

1. The annular photocatalytic reactor operated with TiO₂ coated on sintered glass cylinder is efficient in to remove the antibiotic oxolinic acid from aqueous solution. Over 90% abatement was reached after 60 min irradiation with black light.
2. The extent of OA oxidation is proportional to the number of titania-impregnated glass cylinders.
3. The adsorption step was demonstrated to be a crucial step in the oxidation reaction in both suspension and immobilized catalyst systems.
4. None of the OA oxidation intermediates possesses antibacterial activity.

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