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Degradation and inactivation of tetracycline by TiO_2 photocatalysis

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Abstract

To compare tetracycline abatement efficiency, tetracycline solutions were irradiated in aqueous suspensions of TiO_2 with three different light sources: a UV lamp, a solarium device and a UV-A lamp. Negligible degradation was observed when irradiations were performed in absence of TiO_2. In contrast, rapid tetracycline degradation was observed in the presence of 0.5 g l^{-1} of TiO_2. Close to 50% of its initial concentration was eliminated after 10, 20 and 120 min when the irradiation source used was a UV lamp, a solarium device and a UV-A lamp, respectively. Significant mineralization was also obtained when the UV lamp and solarium were used for photocatalysis. The antibacterial activity of selected microorganisms was drastically inhibited when exposed to tetracycline solutions treated by the photocatalyst over short irradiation periods.

Keywords: Antibiotic; Bacterial activity; Photocatalysis; Tetracycline; TiO_2

1. Introduction

The intensive use of pharmaceuticals, although beneficial for preserving human health and in food production, results in their undesirable accumulation in different environmental compartments as a secondary effect. Several kinds of drugs, such as antibiotics, hormones, preservatives and anesthetics, have been identified in surface water, groundwater, sewage water, and drinking water [1–8].

The principal sources of antibiotics and other drugs in the environment are from pharmaceutical industry, intensive farming and human excretion residues [9]. Antibiotics from the tetracycline family have been extensively used in human and veterinary medicine to treat and prevent bacterial infections. Their use in salmon production has been reported [10], and it has been established that their excessive accumulation can produce arthropathy, nephropathy, central nervous system alterations, spermatogenesis anomalies, possible mutagenecity and photosensitivity in human beings [11]. Important amounts of these antibiotics have been found in animal tissues and in wastewater [12]. Antibiotics can also have a direct effect on the environment by disrupting ecosystem equilibrium [5]. Natural bacteria exposed to residual antibiotics could modify their genetic information developing higher antibiotic resistance and resulting in multi-resistant strains of microorganisms [1].

Due to their antibacterial nature, antibiotic residues or contaminated waters cannot be effectively eliminated by traditional biological methods [11,13]. On the other hand, advanced oxidation processes (AOPs) have proved to be a suitable alternative for rapid degradation of recalcitrant and non-biodegradable compounds in water [14–16]. In particular, TiO_2 photocatalysis has been successfully used in the degradation of several kinds of organic compounds such as azo-dyes, pesticides, aromatics and herbicides [17–21].

In this article, TiO_2 photocatalysis has been used to degrade tetracycline (TC) as a model antibiotic compound. The effect of different luminous sources has been tested as well as the effect of TiO_2 loading. The degradation processes were monitored by measuring tetracycline disappearance, pH, and total organic carbon evolution. To determine the photocatalysis effect on antibacterial activity, treated solutions were inoculated with a *Staphylococcus aureus* strain.

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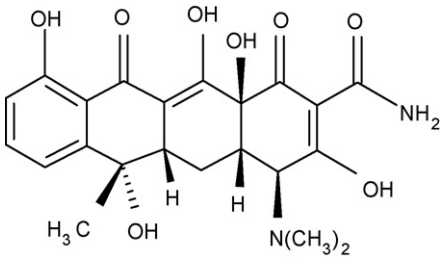

Fig. 1. Structure of the antibiotic tetracycline.

2. Experimental

2.1. Materials

Titania P-25 (surface area 50 $m^2\,g^{-1}$) was obtained from Degussa. Tetracycline 95% (TC, $C_{22}H_{24}N_2O_8{\cdot}HCl$) was purchased from Sigma and used without further purification. The tetracycline structure is depicted in Fig. 1. Stock tetracycline solutions (7.3×10^{-5} mol l^{-1}) were prepared in deionized water and kept in darkness at −4 °C until use.

2.2. Photochemical reaction

In a typical experiment, 750 ml of 7.3×10^{-5} mol l^{-1} (40 mg l^{-1}) of TC were irradiated in the presence of TiO_2 suspension, in a 1-l open reactor, featuring top illumination and vigorous magnetic stirring, for a 2-h period. Oxygen gas was continuously bubbled through at 200 ml min^{-1} during illumination. TiO_2 was loaded at two different concentrations: 0.5 and 1 g l^{-1}. As a comparison, three light sources were used: a 125 W Philip HPLN lamp (UV, $\lambda > 254$ nm), a commercial Solarium device commonly used for cosmetic purposes, Philips HB31 arranged with 6 W × 20 W lamps (solarium, $\lambda = 300$–400 nm), and a 160 W Philips black light MLW lamp (black light, $\lambda = 365$ nm). The TC adsorption on TiO_2 was measured by stirring the suspensions prior to each irradiation during 30 min in darkness.

2.3. Chemical analysis

Samples were taken at different intervals, using a 10 ml plastic syringe and then filtered in a 0.45 μm Millipore disk. Absorbance measurements were performed in a Shimadzu UV 1603 spectrophotometer. The maximum absorbance at 365 nm was monitored during the course of the reaction and compared with a calibration curve. The tetracycline concentration was also determined by HPLC using a Merck-Hitachi instrument equipped with a LicroCART 250-4 RP-18 column. Acetonitrile/water/methanol (30/55/15) was used as eluent. The tetracycline absorbance was recorded at 356 nm. Total organic carbon was measured in a Shimadzu 5000 TOC analyzer.

2.4. Antibacterial activity of treated solutions

Microbiologic assays with irradiated samples were carried out by the modified methodology published by San Martín et al. [22]. *S. aureus* ATCC 6538 P was used as an assay microorganism. Tripticase agar plates were loaded with a 10^5 CFU ml^{-1} suspension of *S. aureus*. Small holes made in the agar were inoculated with 100 μl of irradiated TC samples. After incubation for 24 h at 37 °C, the inhibition halo diameters were measured in mm. To make a standard curve, controls were performed with TC at different concentrations in the range of 10–100 μg l^{-1}.

3. Results and discussion

3.1. Tetracycline degradation with suspended TiO_2

As seen in Fig. 2, a very low tetracycline degradation was observed when it was illuminated with the most energetic lamp ($\lambda > 254$ nm) in absence of TiO_2, indicating that it is also photochemically resistant. The adsorption equilibrium was achieved after 15 min of contact, and no further TC adsorption on TiO_2 was observed after 30 min. In photocatalytic experiments in presence of 0.5 g l^{-1} TiO_2, a maximum degradation was obtained under UV Lamp illumination. To get 50% degradation, approximately 10, 20 and 120 min irradiation were necessary for UV, solarium and black light lamps, respectively. No significant differences were observed between the use of 1 g l^{-1} of TiO_2 and 0.5 g l^{-1} TiO_2 (data not shown) in the photocatalytic reactions.

Table 1 presents the pseudo-first order constants calculated from Fig. 2 for each lamp. The pseudo-first order constants corresponding to solarium and UV lamps are seven and 13 times higher than for black light, respectively. In addition, a negligible rate was observed for direct photolysis. These values correlate well with each lamp's measured intensities at two different wave-

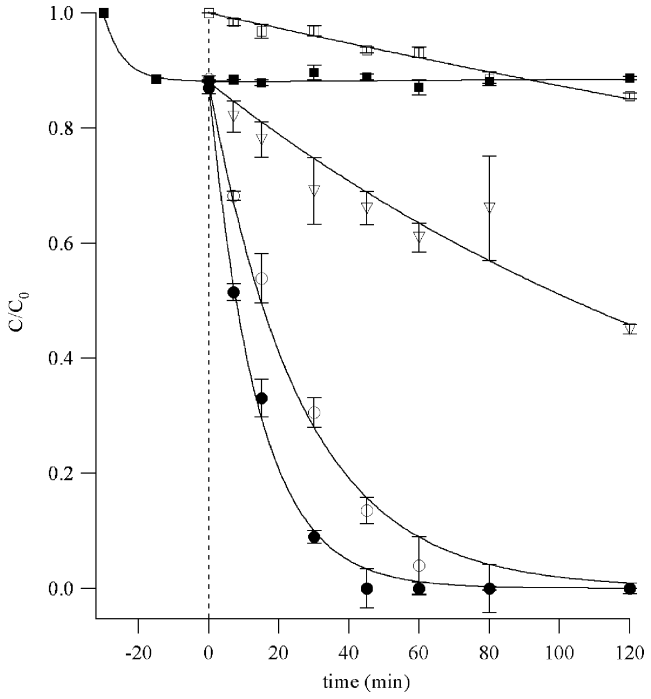

Fig. 2. Degradation profile of tetracycline irradiated by different lamp sources. [TC] = 7.3×10^{-5} mol l^{-1}, [TiO_2] = 0.5 g l^{-1}. (■) Adsorption; (□) UV irradiation in absence of catalyst; (▽) black light + TiO_2; (◯) solarium + TiO_2; (●) UV + TiO_2.

Table 1
Pseudo-first order constant for TC degradation under different light sources

Lamp	K_1 ($\times 10^3$ s^{-1}) (±S.D.)
UV only	1.30 (±0.06)
Black light + TiO_2	5.53 (±0.53)
Solarium + TiO_2	39.3 (±0.003)
UV + TiO_2	71.6 (±0.004)

[TC] = 7.3×10^{-5} mol l^{-1}; [TiO_2] = 0.5 g l^{-1}.

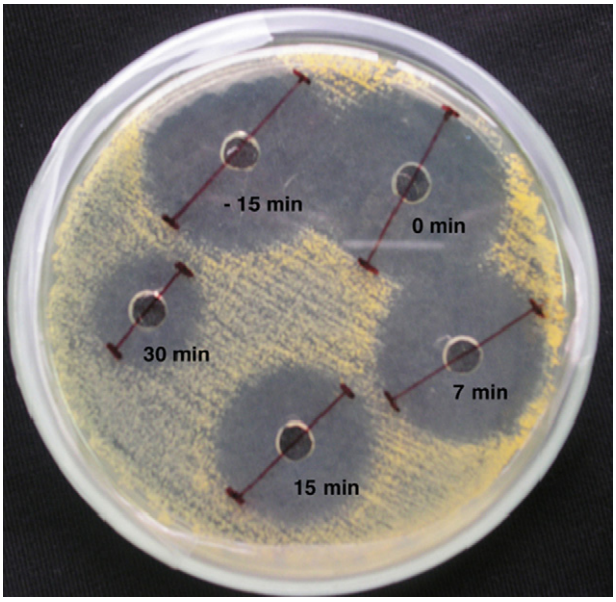

Fig. 3. *S. aureus* inhibition halo for different times of irradiation under solarium lamp in the presence of TiO_2. [TiO_2] = 0.5 g l^{-1}; [TC] = 7.3×10^{-5} mol l^{-1}.

lengths, 254 and 360 nm, as shown in Table 2. It can be observed that the black light lamp has the lowest irradiance at 360 nm, while the solarium lamp presents higher irradiance values centered in the 300–400 nm range. The UV lamp has emissions in the UV and UV-A regions, enhancing the photocatalytic reaction.

3.2. Effect of the TiO_2 photocatalysis on the antibacterial activity

Experiments with selected bacteria (*S. aureus*) were performed to determine the evolution of antibacterial activity during photocatalysis. Assays were performed on bacteria-inoculated agar plates. The effect on biological activity for the irradiated samples was determined by measuring the inhibition halo formed around the microdrop seeded on the agar plate. Fig. 3 presents typical results, where the inhibition halo of TC solutions

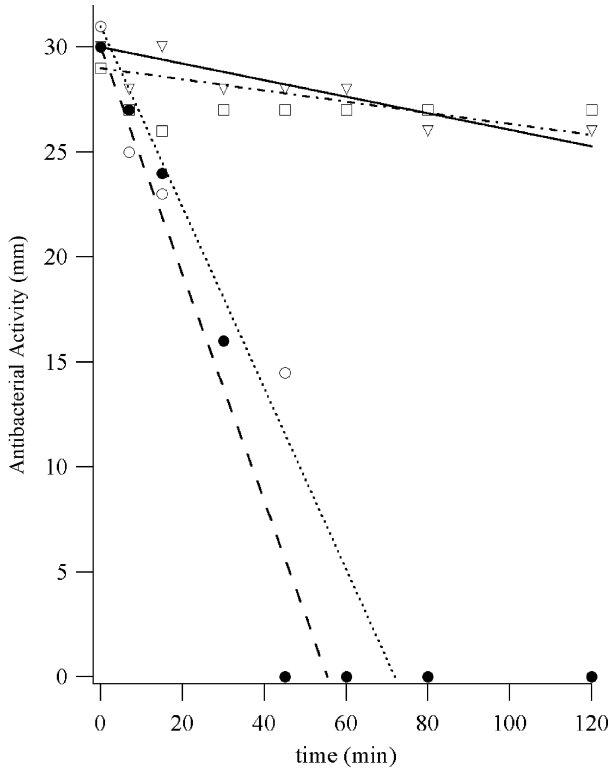

Fig. 4. Effect of the light source on the antibacterial activity of irradiated TC solutions. [TiO_2] = 0.5 g l^{-1}; [TC] = 7.3×10^{-5} mol l^{-1}. (□) UV irradiation in absence of catalyst; (▽) black light + TiO_2; (◯) solarium + TiO_2; (●) UV + TiO_2.

was found to decrease with irradiation time, indicating a loss of antibacterial activity as photocatalytic treatment progresses.

As seen in Fig. 4, similar results in terms of antibacterial activity (AA) decay were obtained for solarium and UV lamps: total deactivation was reached after 55 and 70 min, respectively. In contrast, only a 15% reduction in the inhibition halo was found after 120 min for treatment with the black light source. As seen in Fig. 2, most TC was removed after 55 and 70 min treatment using solarium and UV lamps, respectively. The fact that no antibacterial activity was detected after such treatments indicates that the TC degradation by-products of photocatalysis do not present antibacterial properties.

As shown in Fig. 5, partial mineralization occurs during TC photocatalytic reactions. Indeed, extensive TOC depletion was observed under UV and solarium light sources, reaching 90% and 75% removal after 120 min, respectively. On the other hand, only 12% mineralization was achieved after 120 min irradiation with black light. Negligible mineralization was observed during illumination with UV lamp. Similar results were obtained when 1 g l^{-1} of TiO_2 was used instead of 0.5 g l^{-1}.

Table 2
Wavelength emission and measured intensities of the three different lamps

Lamp	Nominal power (W)	Irradiance at 254 nm (μW cm^{-2})	Irradiance at 360 nm (μW cm^{-2})
Philips HPLN (>254 nm)	125	32	1210
Solarium (300–400 nm)	120	–	1980
Black light (365 nm)	160	–	59

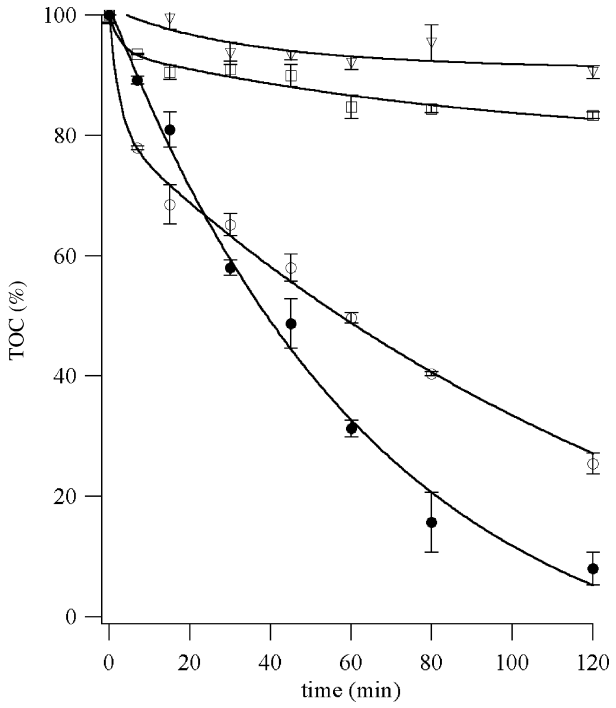

Fig. 5. TOC decrease profiles with the different lamps in the presence of TiO_2. UV lamp (●); solarium (◯); black light (□); UV in absence of catalyst (▽). $[TiO_2] = 0.5\,g\,l^{-1}$; $[TC] = 7.3 \times 10^{-5}\,mol\,l^{-1}$.

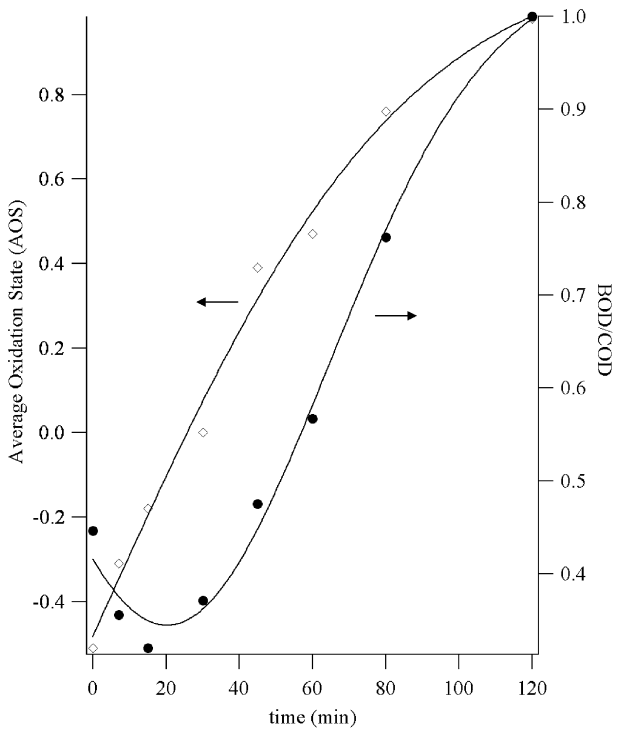

Fig. 7. Course of average oxidation state (AOS) (□) and biodegradability (BOD/COD) (●), during photocatalysis of tetracycline.

Even though TC was rapidly degraded, complete mineralization was only reached after a long irradiation period with the solarium device (Fig. 6). In contrast, COD decreases rapidly attaining the 50% of their initial value in 40 min, while only 20% of TOC was removed. Complete mineralization (Eq. (1)), will be obtained only after 2 h of irradiation

$$C_{22}H_{24}N_2O_8 + \frac{45}{2}O_2 \xrightarrow{TiO_2/h\nu} 22CO_2 + 2NH_3 + 9H_2O \quad (1)$$

The initial BOD corresponds to less than a half of the initial COD, highlighting TC's low biodegradability. TC's biodegradability, defined as the BOD/COD ratio, increased from 0.45 to 0.85 after 120 min of solarium irradiation (Fig. 7). To monitor the chemical changes occurring in the TC intermediates, the average oxidation state (AOS) was calculated during the course of the reaction. AOS is a valuable parameter that can be used to estimate the oxidation degree of a complex solution, consisting in the initial component (TC) and its oxidation products in the case being studied. AOS is calculated using Eq. (2), where TOC and COD are expressed in mM of C and O_2, respectively. AOS takes values between +4 for (CO_2, the most oxidized state of C) and −4 for CH_4 (the most reduced state of C).

$$AOS = \frac{4\,(TOC - COD)}{TOC} \quad (2)$$

From Fig. 7, it is observed that AOS increased as a function of treatment time, almost attaining a plateau after approximately 80 min. As mentioned earlier, TC was completely decomposed by photocatalysis at around 60 min under solarium irradiation (Fig. 2). These results suggest that more oxidized organic intermediates are formed at the beginning of the treatment and that subsequently the chemical nature of most did not vary substantially, even if the treatment was prolonged. A similar behavior was obtained with different radiation source devices, also reaching a good BOD/COD ratio when AOS attained the plateau.

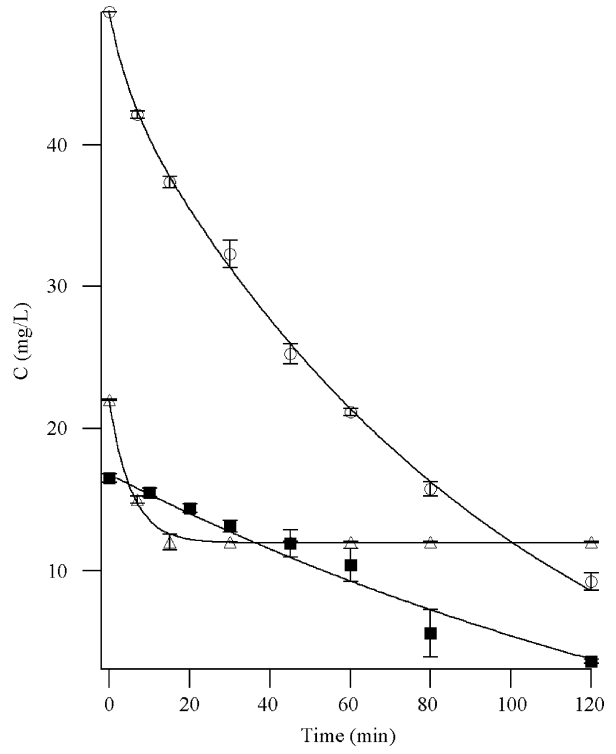

Fig. 6. Evolution of TOC (■), BOD (△) and COD (◯) under illumination with the solarium device.

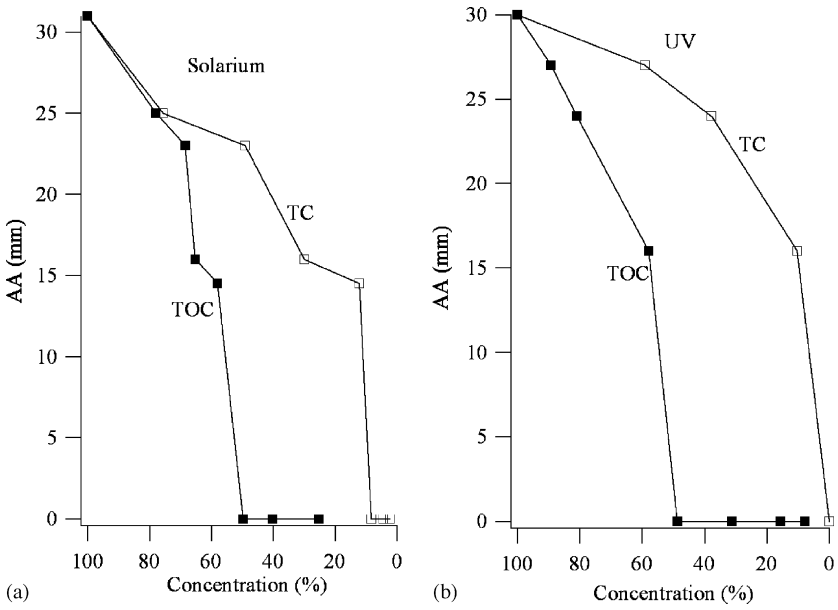

Fig. 8. Antibacterial activity and their relation with TOC (■) and tetracycline concentration (□) under solarium (a) and UV (b) irradiation.

Formation of more oxidized intermediates is an indirect indication of the treatment's ability to improve biodegradability. When AOS is stabilized, the purpose of the chemical treatment is to finish the mineralization of organic contaminants. AOS provides indirect information on the solution's biodegradability and correlates very well with the BOD/COD ratio, with the advantage that BOD determination is not necessary. Consequently, it has been proposed as a suitable parameter to determine if an advanced oxidation process treatment increases the biodegradability of the treated contaminant [23,24].

To determine the antibacterial activity of the residual TC together with the oxidation products remaining in solution during the photocatalytic process, the antibacterial activity was compared with TC concentration and TOC. Fig. 8 shows that antibacterial activity was maintained until TC was almost completely degraded. For example, the remaining 10% of the initial TC concentration is able to produce the inhibition of 50% of the initial bacterial activity after irradiations with both solarium and UV lamps (Fig. 8(a) and (b)). On the other hand, it is important to note that the intermediate products of the photocatalytic process do not present any antibacterial activity. When 50% of the TOC is still in solution, antibacterial activity disappears completely. As shown in Fig. 5, TOC reached 50% of the initial value when TC had been completely degraded (at around 60 min, see Fig. 2). Both illumination sources presented the same behavior, suggesting that the generated intermediates are very similar. In conclusion, the degradation of the antibiotic tetracycline in solution is accompanied by its inactivation against bacteria, indicating that the oxidation products dissolved do not present bactericidal or bacteriostatic activities.

4. Conclusions

The tetracycline structure was effectively degraded by TiO_2 photocatalysis using different kinds of light sources and small amounts of catalyst. Greater oxidation was observed when the UV lamp and solarium were used. Partial mineralization and a slight pH increases was also observed. The later could be explained by the N-atom mineralization forming basic compounds.

The most remarkable conclusion is that tetracycline oxidation by-products do not present antibacterial activity against *S. aureus*. Total inactivation of the antibiotic was reached after around 1-h irradiation using UV and solarium lamps. Therefore, TiO_2-based photocatalysis emerges as a feasible way to inactivate antibiotics, as a pretreatment prior to further biological treatments.

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