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TiO_2 photocatalysis applied to the degradation and antimicrobial activity removal of oxacillin: Evaluation of matrix components, experimental parameters, degradation pathways and identification of organics by-products

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ABSTRACT

The TiO_2 photocatalytic degradation of oxacillin (OXA) in synthetic and natural waters was studied. The matrix effects, in terms of antibiotic and antimicrobial activity removal, were evaluated in the presence of iron ions, natural mineral water and additives contained in commercial formulations of the antibiotic. A slight improvement in degradation was observed in the presence of iron ions. On the other hand, the presence of excipients in a commercial formulation or inorganic ions in natural mineral water slightly inhibited the efficiency of the system. An experimental design using pH, catalyst load and light intensity as variables was also evaluated. The best performances were achieved at natural pH (~6.0) using 2.0 g L^{-1} of TiO_2 with 150 W of applied power. The evaluation of OXA concentration indicated that the photodegradation process showed a Langmiur–Hinshewoold kinetic model. The extent of the process was evaluated following the evolution of chemical oxygen demand (COD) and dissolved organic carbon (DOC). Total removal of both, the antibiotic and its antimicrobial activity, was achieved after 120 min; while 100% of mineralization was observed within 480 min of treatment. Finally, five by-products were identified, the degradation routes were elucidated and a schema of the antibiotic degradation was proposed.

1. Introduction

Active pharmaceutical compounds are designed to intervene in the health–disease process and fulfill a biological function in living organisms. Therefore, they are considered essential to preserve public health and quality of life. However, like many foods and supplements that are consumed by humans and animals, pharmaceuticals are not completely absorbed or metabolized in the body and are eventually excreted into the environment [1–3]. Although little is known of the potential effects of pharmaceuticals in the environment, the problems of microbial resistance, allergenicity and endocrine disruption are of greatest concern to the scientific community [4–7].

Antibiotics are one of the groups of pharmacologically active substances, which have caused adverse environmental effects. Among them, resistance to antibiotics by microorganisms is a serious and growing problem in contemporary medicine, to the point of being considered one of the most eminent risks to public health of the 21st century. β-lactam antibiotics, which include penicillin derivatives, cephalosporins, carbapenems, monobactams, carbacephems, and β-lactamase inhibitors, are examples of antimicrobials that generate widespread bacteria resistance.

Oxacillin (OXA) an isoxazolyl penicillin, is a semisynthetic pharmaceutic member of the group of β-lactam antibiotics, which are resistant to acidic conditions and penicillinase. OXA is widely used in clinic for the treatment of infections caused by aerobic gram-positive cocci [8–10].

A high percentage of the antibiotic OXA is then excreted unchanged into domestic wastewater. Eventually, OXA ends up in wastewater treatment plants (WWTPs); and therefore, it is

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released into the environment. Indeed, OXA was found at the concentration of 10 ng L^{-1} in surface waters [11]. Once in the environment OXA spreads its bacterial resistance, which leads to problems in the therapeutic effectiveness of this antibiotic. In fact, the proliferation of methicillin-resistant *Staphylococcus aureus* (MRSA) and borderline oxacillin-resistant *S. aureus* (BORSA) has been reported [12]. As a consequence, efficient treatment systems that eliminate the antibiotic and the antimicrobial activity of the treated water need to be developed urgently.

The treatment of water contaminated by antibiotics using conventional technologies is not easy. The usual complex mixture of chemicals in the effluents impedes the recovery of the organics. Furthermore, the low calorific power of the waste preclude the use of incineration, and the non-biodegradable character of antibiotics exclude the use of classical biological treatments [13]. Therefore, advanced oxidation processes (AOPs) would appear to be a very promising solution for the environmental problems generated by the discharge of these effluents [13].

Heterogeneous TiO_2 photocatalysis is an AOP that has earned an important place among the new emerging technologies for the degradation of compounds such as antibiotics. The photocatalytic process is initiated when a semiconductor absorb photons with an energy equal or superior to their band gap ($\lambda \leq 380$ nm), promoting electrons from the valence band to the conduction band (Eq. (1)), and thus producing an electron–hole pair [14–16].

$$\text{Semiconductor} + h_{\nu} \rightarrow e^{-}_{CB} + h^{+}_{VB}$$

Pollutant elimination during TiO_2 photocatalytic oxidation, mainly ascribed to strong oxidative reactions at the hole (h^{+}_{VB}), is due to the semiconductor high oxidative power (E° = +2.7 V vs NHE) [17]. Thus, the vacancies generated in the valence band can be trapped on the catalyst surface, which promote the splitting of adsorbed water molecules or hydroxide anions to produce hydroxyl radicals ($^{\bullet}$OH) (Eqs. (2) and (3)). These hydroxyl radicals are considered responsible for the oxidation of organic matter due to their high oxidation capability (E° = +2.8 V vs NHE) [15].

$$h^{+}_{VB} + H_2O_{(adsorbed)} \rightarrow H^{+} + {}^{\bullet}HO \tag{2}$$

$$h^{+}_{VB} + OH^{-}_{(adsorbed)} \rightarrow {}^{\bullet}HO \tag{3}$$

The holes can directly oxidize the adsorbed organic matter (Eq. (4)).

$$h^{+}_{VB} + \text{organic matter (OM)} \rightarrow OM^{+\bullet} \rightarrow \text{oxidized products} \tag{4}$$

Given its high efficiency, TiO_2 photocatalysis has been amply described as one of the most promising advanced oxidation technologies for eliminating contaminants in low concentration using artificial or natural light. In spite of this, to our knowledgement, the application of the TiO_2 photocatalysis for the removal of oxacillin has not been reported.

In this study, the application of TiO_2 photocatalysis for the treatment and removal of OXA in waters is investigated. Special focus is given to evaluate the effect of the water matrix composition: ions in natural mineral water and excipients in a commercial product of the antibiotic. Additionally, the influence of the initial pH and antibiotic concentration are evaluated. The effects of the most relevant operational parameters (TiO_2 loading and light intensity), the extend of degradation in terms of the chemical oxygen demand (COD) and dissolved organic carbon (DOC), and the changes in antimicrobial activity are also investigated. Finally, the degradation routes are elucidated, the primary organic by-products are identified and a degradation schema for this antibiotic is depicted.

2. Material and methods

2.1. Reagents

Oxacillin sodium purchased from Sigma–Aldrich had a 95% of purity and was used without further purification. OXA sterile powder for injection, from laboratory Blaskov Ltd., was used to evaluate the effect of excipients in a real commercial product of the antibiotic. Titanium dioxide Evonik P-25 was used for all experiments. Sulfuric acid and sodium hydroxide were provided by Merck and used for pH adjustment. The sample solution was prepared using deionized water, which was obtained by passing water through a Pureline deionization system. Milli-Q water,

Table 1
Experimental results of the applied factorial design for oxacillin degradation by TiO_2 photocatalysis.

Block	[TiO_2] (g L^{-1})	Light intensity (W)	pH	Degradation percentage observed	Degradation percentage estimated
1	2	150	9	61.52	64.99
1	1.025	90	6	53.8	39.33
1	2	30	9	33.24	41.70
1	0.05	30	9	14.68	13.67
1	0.05	150	3	20.38	36.96
1	0.05	30	3	7.18	13.67
1	0.05	150	9	47.71	36.96
1	2	150	3	64.82	64.99
1	2	30	3	37.31	41.70
1	1.025	90	6	52.71	39.33
2	2	150	9	62.83	65.04
2	1.025	90	6	56.33	39.38
2	2	30	9	34.26	41.75
2	0.05	30	9	16.48	13.72
2	0.05	150	3	18.42	37.02
2	0.05	30	3	9.64	13.72
2	0.05	150	9	36.99	37.02
2	2	150	3	64.09	65.04
2	2	30	3	37.62	41.75
2	1.025	90	6	57.22	39.38

acetonitrile and methanol (Merck, HPLC grade) were used for the preparation of the HPLC mobile phase. 2-Propanol provided by Merck was used to evaluate the effect of the presence of organics in the degradation efficiency. Characterized natural mineral water was employed to investigate the antibiotic elimination in natural waters.

2.2. Photocatalytic reactor

Photocatalytic oxidation experiments were carried out using 100 mL of tested solution placed in beakers with constant stirring. An aluminum closed reactor, containing 5 Phillips cylindrical black lamps (maximum emission at 365 nm), which supplied between 30 and 150 W of power, was used as a reactor. The reactor temperature was controlled using mechanical ventilation. Solutions containing the pollutant were placed into the vessel reactor and the system was conditioned in darkness until the adsorption-desorption equilibrium was established. During degradation, samples were taken at regular time intervals and filtered through cellulose nitrate filters of 0.45 μm in diameter and analyzed by HPLC–UV–VIS.

2.3. Experimental design

A two-level full factorial design (2^3) with two replicate center points and multivariate analysis (response surface methodology) was used in order to find the optimal conditions of the process. The replicate center points serve to evaluate the experimental error and the curvature of the evolution of a response factor, that is whether or not the evolution of the response factor is linear within the experimental range studied. Using the experimental design the effects of several parameters were evaluated. Three variables were analyzed: pH in the range of 3–9, TiO_2 concentration (0.05–2.0 g L^{-1}) and light intensity (30–150 W). The response factor was the degradation percentage of the antibiotic after 20 min of irradiation. The test concentration of OXA was 203 μM. The statistical analysis of the data was performed using the software STATGRAPHICS CENTURION XVI v16.0.07. The most favorable conditions, in the range of the evaluated variables, was used to evaluate the reaction kinetic, the matrix effects, the degradation pathway, the reduction or loss of antimicrobial activity and the organic by-products. All experiments were carried out at least in duplicated to confirm reproducibility and average results were reported.

2.4. Chemicals analysis

Sampling was carried out at specific time intervals using 1.0 mL of sample to perform the chromatographic readings. 1.0 mL of sample was also used to take COD, antimicrobial activity, and DOC readings.

The evolution of OXA concentration was monitored using a Waters® liquid chromatograph equipped with a 486 absorbance (UV–vis) detector set at 225 nm. Isocratic separation was performed at 25 °C in a 250 × 4.6 mm ID RP18 5 μm reverse phase HPLC column (Merck LiChrospher®). Optimum separation occurred using a mixture of a phosphate buffer: acetonitrile: methanol (64:27:9 v/v), operating at a flow-rate of 0.6 mL min^{-1} and using 20 μL as the injection volume. Under work conditions, the accuracy of the method, in terms of the recovery (percent), was always higher than 93%, while uncertainties were found to be lower than 3.5%.

The chemical oxygen demand (COD) was determined using the methodology established in the Standard Methods for the Examination of Water and Wastewater (Standard Methods 5220), in accordance with the closed reflux titrimetric method. To quantify the COD, the optical density required to measure the change of color of the dichromate solution was determined at

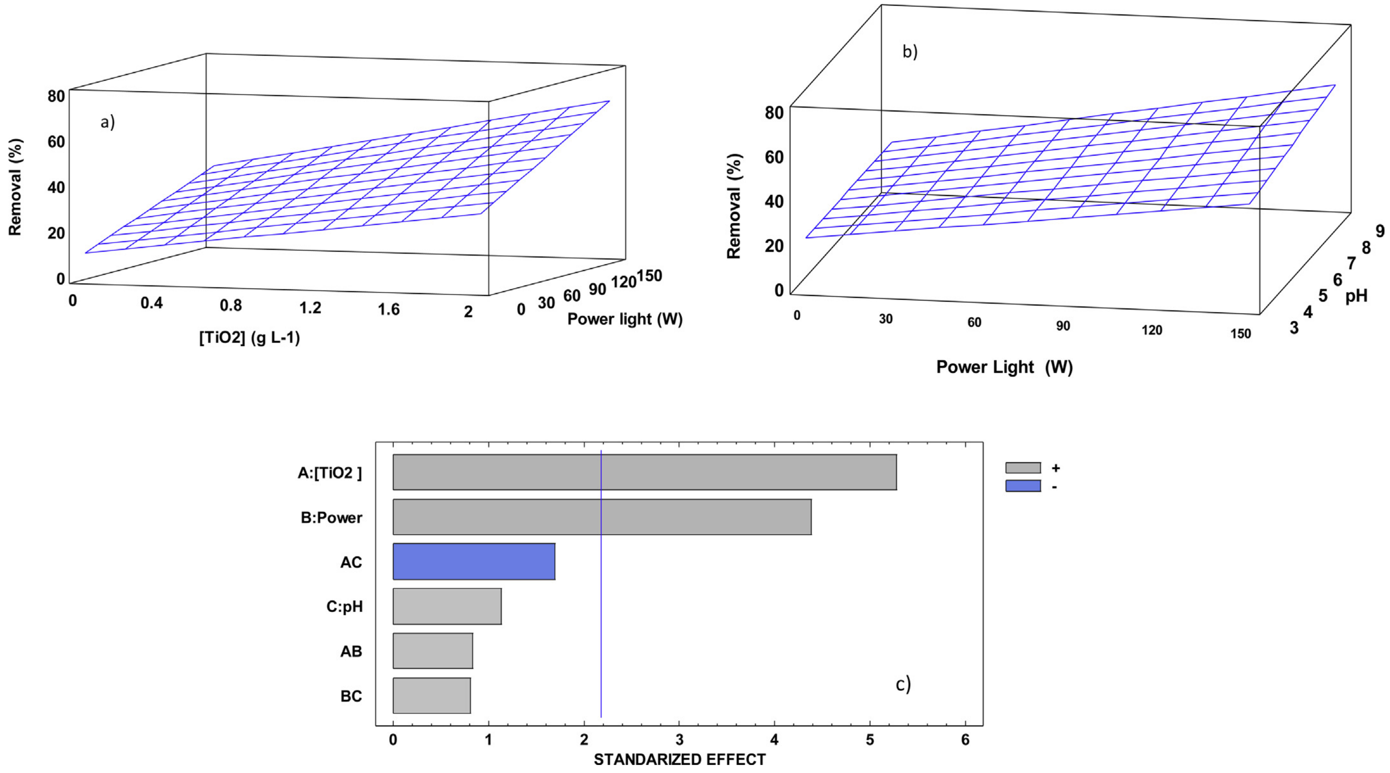

Fig. 1. Optimization of TiO_2 load, power light and pH for photocatalytic degradation of oxacillin (203 μmol L^{-1}) at natural pH after 20 min of irradiation. (a) Response surface for TiO_2 load vs power light. (b) Response surface for power light vs. pH. (c) Pareto diagram plot. All figures were obtained from the software STATGRAPHICS CENTURION XVI v16.0.07 according to the experimental data presented in Table 1.

445 nm with a Spectronic Genesys 2.0 spectrophotometer. Meanwhile, the dissolved organic carbon (DOC) was analyzed using a Shimadzu TOC 5000A analyzer in accordance with the methodology reported previously [18]. Uncertainties were found to be lower than 5 and 7% for figures reporting DOC and COD evolution, respectively.

2.5. Biological analysis

To determine the antibacterial activity of treated OXA samples the disk diffusion method was used. In this method Mueller–Hilton agar (DIFCO®) plates were seeded with a 10^5 CFU mL^{-1} suspension of *S. aureus* (ATCC 6538). The plates were loaded with 100 μL of irradiated samples and incubated for 24 h at 37 °C. The inhibition halo formed around the holes was measured (in mm) to determine the decrease in potency of the antibiotic during the treatment.

2.6. Identification of degradation by-products

Degradation intermediates for oxacillin were extracted from treated solutions by solid-phase extraction, using Strata X® cartridges to improve intermediate detectability. Cartridges were conditioned with 2 mL of methanol and 2 mL of water, and loaded with a 50 mL aliquot of the sample. The sorbent was washed with 2 mL of Milli-Q® water and then eluted with two aliquots of 2 mL of 2% formic acid in water. The by-products generated were monitored by an ORBITRAPS mass spectrometer (Thermo Scientific®) connected to a HPLC Ultimate 3000 system (Thermo Dionex®) equipped with a 250-mm reverse-phase C18 analytical column that had a 5-μm particle size (LiChrospher, RP-18, Merck®). The mobile phase was a mixture of acetonitrile acidified with 0.1% formic acid (A/F) and water acidified with 0.1% formic acid (W/F) at a flow rate of 0.4 mL min^{-1}. A linear gradient progressed from 10% A/F (initial conditions) to 100% A/F in 50 min, and then remained stable at 100% A/F for 5 min. The injection volume was 20 μL. This HPLC system was connected to an ORBITRAPS mass spectrometer (Thermo Scientific®) equipped with an electrospray interface, which was operated under the following conditions: spray voltage 3.5 KV, sheath gas flow 40, auxiliary gas flow 10, capillary temperature 320 °C, heater temperature 300 °C, S-lens RF level 55 and a positive polarity. Data were processed with the Thermo Xcalibur Instrument software.

3. Results and discussion

3.1. OXA adsorption onto the photocatalyst

The adsorption of the pollutant onto the catalyst surface can be a determining step in the heterogeneous photocatalytic process. Therefore, the adsorption behavior of OXA onto the catalyst in dark conditions was evaluated. The results indicated that the adsorption–desorption equilibrium was reached after 30 min (Fig. S1, see Supplementary data). Tests conducted at different pH values indicate that at pH 3.0, natural pH (6.0) and pH 9.0 adsorptions were approximately 5%, 30% and 1% respectively (Fig. S1, see Supplementary data). The implications and reasons for these different adsorptions will be discussed later.

3.2. Evaluation and optimization of operative degradation parameters during the TiO_2 photocatalytic degradation of oxacillin

3.2.1. Evaluation and optimization of TiO_2 loading, light intensity and initial pH

During the photocatalytic degradation process, the efficiency of the system is related to experimental parameters such as light irradiation intensity, the number of catalyst particles and pH. Typically, the rate of the photocatalytic degradation increases with an increase in light intensity and catalyst concentration until it reaches a limiting value [19], which is particular to each organic compound and photocatalytical system. Regarding the pH, it has been generally observed that best degradation occurs at pH values where there is a higher adsorption of the pollutant onto the catalyst surface [20,21]. However, in some cases, the pollutant adsorption can also be detrimental. Consequently, to maximize the efficiency of the process an evaluation and optimization of the experimental parameters should be always carried out.

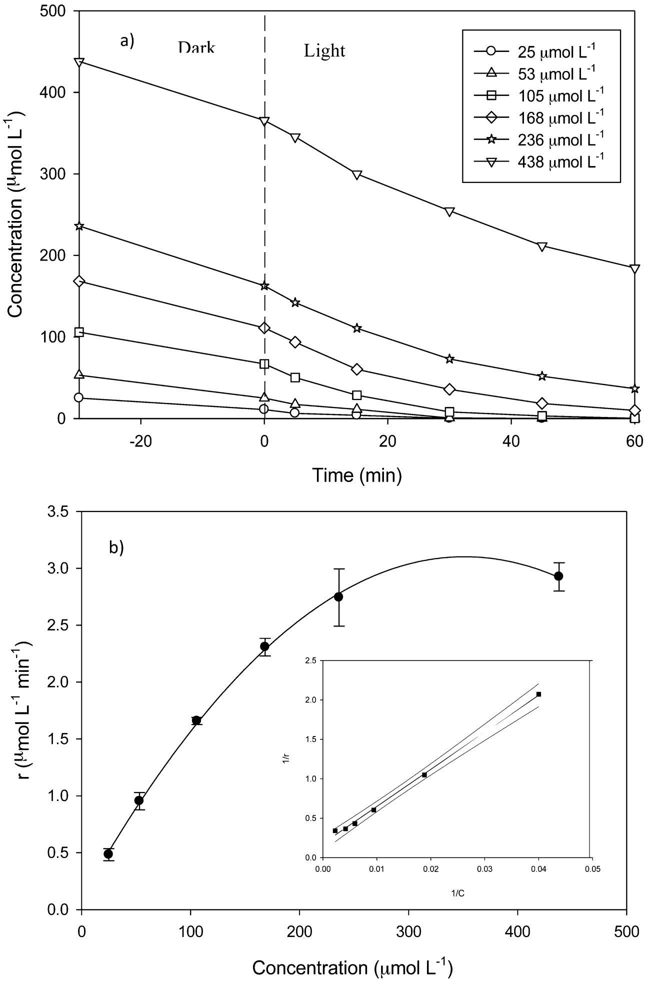

Fig. 2. (a) Effect of the antibiotic concentration during oxacillin degradation by UV/TiO_2. 2.0 g L^{-1} of TiO_2, 150 W and natural pH. (b) Initial oxacillin degradation rate (*r*) as a function of their initial concentrations. Inset: linear regression 1/*C* vs 1/*r*. Experiments were carried out at least in duplicated.

Table 1 shows the factorial array used to analyze the influence of TiO_2 concentration, pH and light intensity when subjected to photocatalytical degradation of OXA. The response variable was the degradation percentage after 20 min of photocatalytical action. As can be seen in Table 1, the catalyst load and the light intensity are the most important factors influencing the process. For a better interpretation of the results, Fig. 1a and b shows the response surfaces for OXA degradation; while Fig. 1c depicts the Pareto chart. The response surface figure (Fig. 1a and b) shows a significant improvement in the degradation percentage as the applied power increased from 30 to 150 W. This is because an increase in light intensity generates more emitted photons, thereby forming more electron–holes. These can generate a higher number of hydroxyl radicals or electronic vacancies where direct oxidation of the pollutant can occur [22]. Higher catalyst concentrations also improved the efficiency of the process (Table 1 and Fig. 1a), which can be explained by a greater availability of active sites on the catalyst surface. However, TiO_2 concentrations higher than 2 g L^{-1} were not taken into account in this study to avoid having an excessive catalyst load that would have to be recovered at the end of the treatment.

As can be seen in the Pareto diagram (Fig. 1c), the light intensity and the catalyst concentration significantly influenced the efficiency of OXA degradation. On the contrary, according to Fig. 1b and the Pareto diagram (Fig. 1c), the initial pH of the solution (3–9) did not significantly affect the removal percentage of the antibiotic.

To interpret the effect of initial pH on the process, the stability of OXA at the different tested pH values was investigated. The results (Fig. S2, see Supplementary data) indicated that in the working conditions, no change on the initial antibiotic concentration was observed. Therefore, changes to both, TiO_2 (Point of zero charge, pzc = 6.30 for P25 Evonik) [20] and the pollutant structure (pK_a 2.8 OXA) [23], must be considered. At natural and basic pH, the OXA carboxylic group is deprotonated, giving to the antibiotic a negative charge. Meanwhile in acidic media (pH 3.0), according to the pK_a of the substrate, a significant fraction of OXA is in its neutral and uncharged form. On the other hand, in acidic media (pH 3.0) the surface TiO_2 is positively charged, while in basic media (pH 9.0) it is negatively charged. At natural pH the TiO_2 is very close to its pzc value; and thus it has practically the same number of negative and positive sites on its surface [20,24]. Therefore, when using the basic pH, repulsion occurs between the OXA structure and the catalyst, and consequently a negligible adsorption (~1%) of the pollutant is observed (Fig. S1, see Supplementary data). At the more acidic pH (3.0), electrostatic attraction forces between the antibiotic and the catalyst is not predominant, because at this pH value the substance is close to their pK_a value. Under such conditions, only the ionized form of such molecule interacts with the positively charged catalyst, which may explain the low adsorption observed (~5%) (Fig. S1, see Supplementary data). Furthermore, at natural pH (~6.0) electrostatic attractive forces between the antibiotic and the catalyst can be important. In fact, even if under such pH values the catalyst is close to its isoelectric point and the pollutant is negatively charged, the high concentration of catalyst molecules in relation to the pollutant concentration makes the adsorption of a considerable fraction of this compound possible. This explains why better adsorption of OXA (~30% of the antibiotic) is observed at this pH value (Fig. S1, see Supplementary data). The higher adsorption at natural pH suggests that at this pH pollutant adsorption can also contribute to the destruction of the antibiotic. On the contrary, at basic or acid pH, where an insignificant absorption was observed, free OH radicals seem to be the main factor responsible for OXA degradation. However, whether or not the adsorption of the antibiotic on the catalyst surface takes place, the degradation rate of the substrate is similar and as a result, the effect of pH on the photocatalytical system was not significant (Fig. 1b and c). Therefore, from a practical point of view, natural pH is the most suitable pH condition to perform the OXA degradation by TiO_2 photocatalysis.

According to the response surface diagrams (Fig. 1a and b), the most favorable conditions for the degradation of OXA in aqueous TiO_2 suspensions are: natural pH (6.0), 2.0 g L^{-1} of TiO_2 and a light power of 150 W.

These conditions can also be presented in a polynomial expression (Eq. (5)); where Y (%), represents the degradation percentage of the antibiotics:

$$Y\,(\%) = 7.15944 + 14.3724 \times [\mathrm{TiO_2}] + 0.194115 \times \mathrm{Power} \qquad (5)$$

The expression shows the coefficients of the reduced model calculated by multiple regression analysis, which directly relates the response factor with the influential variables. Table 1 presents both the experimental values and the results calculated with the mathematical model. As seen in Table 1, the estimated values are similar to the experimental values in most of the experiments. Therefore, as observed by other treatment systems and pollutants [25,26], the reduced model can satisfactorily describe the TiO_2 photocatalytical process for the degradation of the antibiotic OXA.

3.2.2. Effect of the initial concentration

To investigate the influence of the initial concentration of the studied penicillin during the photocatalytical treatment, different solutions of OXA, in a concentration range of two orders of magnitude (from 25 μmol L^{-1} to 438 μmol L^{-1}) at a fixed TiO_2 concentration (2.0 g L^{-1}) and light intensity (150 W) were tested (Fig. 2a). The evolution of the curves seems to indicate that the

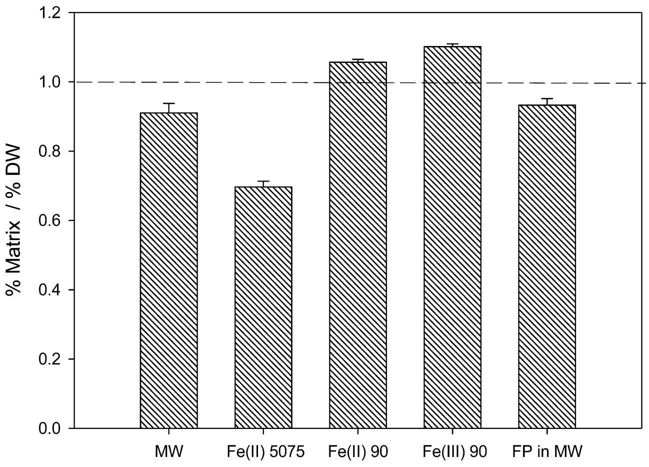

Fig. 3. Relative oxacillin degradation percent by TiO_2 photocatalysis in presence of different matrices after 60 min of irradiation and 2.0 g L^{-1} of TiO_2, 150 W and natural pH. %DW stands for the degradation percent in deionized water and %Matrix represent the degradation percent in presence of: mineral natural water (MW), mineral natural water containing a commercial product of the antibiotic (FP in MW), Fe^{2+} 5075 μmol L^{-1} (Fe(II) 5075), Fe^{2+} 90 μmol L^{-1} (Fe(II) 90) and Fe^{3+} 90 μmol L^{-1} (Fe(III) 90). Experiments were carried out at least in duplicated.

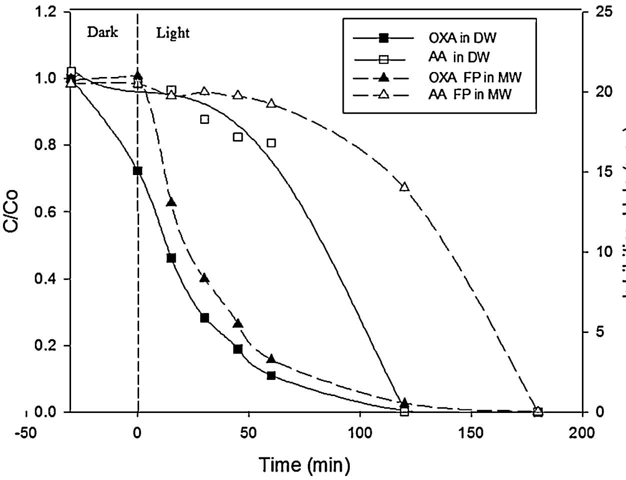

Fig. 4. TiO_2 photocatalytic degradation of oxacillin (OXA) and antimicrobial activity removal (AA), in deionized water (DW) and in presence of mineral natural water containing a commercial product of the antibiotic (FP in MW). Test conditions were the same as those in Fig. 3. Experiments were carried out at least in duplicated.

reaction fits a pseudo-first order kinetics law. However, Fig. 2b clearly shows that the degradation rate does not increase linearly with the concentration of the pollutant, as seen with first order kinetics. Instead, particularly at relatively high pollutant concentration, the slopes of the curves gradually decrease. This behavior can be better represented in terms of a Langmuir–Hinshelwood (LH) kinetic model. To obtain the kinetic parameters from the LH model, linearization can be performed (Eq. (6)).

$$\frac{1}{r}=\frac{1}{Kk}\frac{1}{C}+\frac{1}{k} \tag{6}$$

In Eq. (6), r represents the initial degradation rate for OXA (μmol L^{-1} min^{-1}), C is the initial concentration of the pollutant (μmol L^{-1}), k is the apparent rate constant and K is the adsorption–desorption equilibrium constant. The calculated parameters are: $k = 5.62$ μmol L^{-1} min^{-1} and $K = 3.78 \times 10^{-3}$ μmol^{-1} L; with a small average percentage error (APE = 5.6%). It should be noted, that the values of the constants obtained in the development of this work are very similar to those obtained by Dimitrakopoulou et al. when working with amoxicillin, another antibiotic of the penicillin class [27]. As can also be seen in the inset of Fig. 2b, the experimental data shows a good fit to the LH model (R^2 is 0.9979).

3.3. Study of OXA degradation in presence of different matrix composition

The presence of ions, organic matter, biological materials and xenobiotics can affect the processes of degradation, reducing or increasing the speed of degradation, either by interaction or by competition with the substances of interest. To enhance our understanding of these phenomena, it is important to test the performance of the aforementioned technology in samples of natural waters containing the pollutant. It is also important to evaluate the effect of different species that may be present during OXA degradation by TiO_2 photo-catalysis. Therefore, mineral spring water containing 4.930 mmol L^{-1} HCO_3^-, 1.160 mmol L^{-1} Mg^{2+}, 0.137 mmol L^{-1} NO_3^-, 0.043 mmol L^{-1} SO_4^{2-}, 0.020 mmol L^{-1} K^+, 1.220 mmol L^{-1} Ca^{2+}, 0.252 mmol L^{-1} Na^+ and 0.068 mmol L^{-1} Cl^- was used as an example of a natural water sample. Moreover, the effect of the presence of Fe^{2+} ions and the degradation of the pharmaceutical, in a commercial formulation, were investigated. The Fe^{2+} ion was chosen to be studied given its widespread presence in a lot variety of water sources. The results shown that in the case of mineral water, the degradation is slightly affected. Fig. 3 shows that the percentage of degradation decreases by approximately 6% after 60 min of reaction compared to degradation in deionized water. Interestingly, a reduction in the adsorption was also observed in natural water. In fact during the equilibration time, in the natural water the adsorption decreases from ~30% to 1%, which probably leads to the slight reduction observed in the percentage of degradation. The reduction in the percentage of degradation may be due to the presence of inorganic anions in water, such as phosphate, sulfate, nitrate, bicarbonate and chloride, which are known scavengers of hydroxyl radicals. Additionally, bicarbonate, the ion present in the highest concentration, can easily adsorb onto the catalyst and avoid the pollutant-catalyst interaction, thereby negatively affecting the process. The lower adsorption of the pollutants in the presence of this water matrix supports the previous hypothesis [28,29].

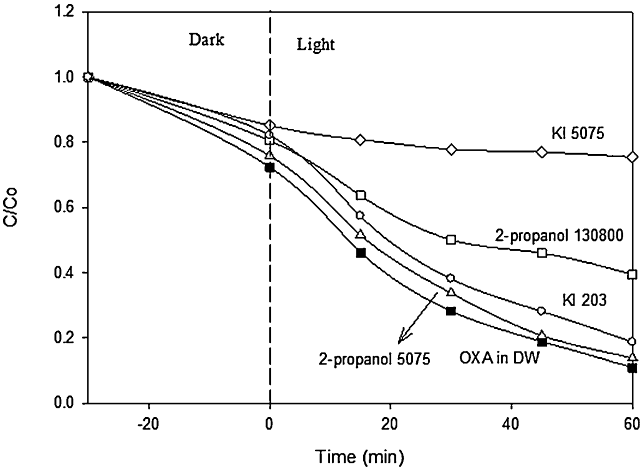

Fig. 5. TiO_2 photocatalytic degradation of oxacillin in presence of some additives: 2-propanol at 5075 μmol L^{-1} and 130800 μmol L^{-1}, and KI at 203 μmol L^{-1} and 5075 μmol L^{-1}. Test conditions were the same as those in Fig. 3. Experiments were carried out at least in duplicated.

When iron is present in the reaction medium, interesting phenomena occur during the degradation of the substance. When the concentrations of Fe^{2+} and Fe^{3+} are below the concentrations of the substance used, the degradation of the antibiotic is slightly enhanced (Fig. 3). In water solutions and due to the presence of dissolved oxygen, Fe^{2+} ions easily undergoes into Fe^{3+} ions. In presence of UV light, the generated ferric ion is able to produce extra hydroxyl radicals [18,30], which improves OXA degradation. The higher improve in the OXA degradation due to Fe^{3+}, is in agreement with this hypothesis. Additionally, the electron scavenging effect of iron ions may prevent the recombination of electrons and holes, which favors the formation of $^{\bullet}OH$ and $O_2^{\bullet-}$ on the TiO_2 surface [28]. However, when the concentration of Fe^{2+} is 25 times higher (5075 μmol L^{-1}) than the concentration of OXA, the percentage of degradation is significantly reduced (Fig. 3). This can be attributed to the suppression of $^{\bullet}OH$ radicals by Fe^{2+}, as the conduction band electrons are trapped by the adsorbed metal ions [28]. Such behavior is in accordance with the reduction in OXA adsorption during equilibration in the dark in the presence of Fe^{2+} (Fig. S3, see Supplementary data).

On the other hand, in commercial formulations OXA is found mixed with a number of excipients or inert substances so that it can be supplied as a medicine. To further prove the potential of the technology, a commercial finished product (FP), OXA sterile powder for injection, was treated by TiO_2 photocatalysis. The results shown in Fig. 3 indicate that the excipients in the finished product only cause a slight inhibition (~5%) in the OXA degradation.

The aforementioned results highlight the ability of the technology to remove the antibiotic even in the presence of excipients in commercial products or ions in natural waters.

3.4. Evaluation of the loss of antimicrobial activity

One of the most important aspects to be taken into account in the treatment of antibiotics is the residual antimicrobial activity (potency) of the substance and the by-products produced in the course of treatment. Thus, the susceptibility of *S. aureus* (ATCC 6538) strains was used to evaluate the antimicrobial activity of the treated solutions during the application of the photocatalytical process. *S. aureus* was chosen because of its good response at low antibiotic concentrations using the inhibition halo methodology [31]. In this analysis, the smaller inhibition halo around the microdrop spread on the agar plate and inoculated with the bacteria suggests the antimicrobial activity is less important.

Fig. 4 indicates that the inhibition halo of the treated solution is completely eliminated by photocatalytical action. Interestingly, the elimination of antimicrobial activity is consistent with the elimination of the antibiotic. The concomitant elimination of the antimicrobial activity with the pollutant suggests that the initial

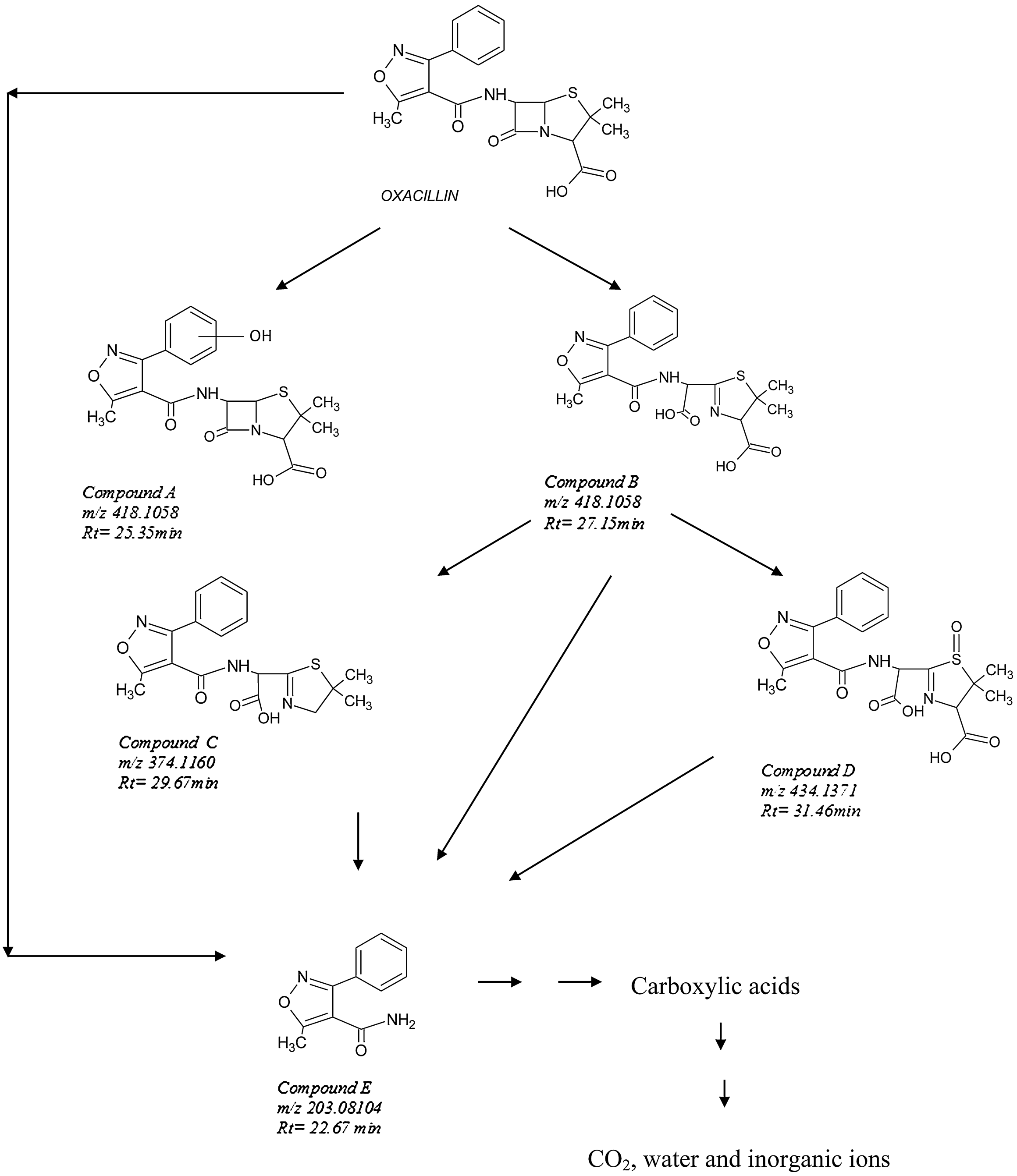

Fig. 6. Main reaction pathways during the TiO_2 photocatalytical degradation of the antibiotic oxacillin.

breaking up of the compound takes place in the β-lactam ring. Finally, the action of light alone is not able to reduce either the pollutant concentration or the antimicrobial activity (data not shown). For a better interpretation, the UV emission spectrum of the lamp and the OXA UV absorption spectrum were obtained (Fig. S4, see Supplementary data). As seen, the fact that there were no band matches indicates that the OXA cannot absorb light radiation from the lamp. Accordingly, direct photolysis of the antibiotic is not plausible.

On the other hand, Fig. 4 also shows the joining effects of excipients in a commercial formulation of the antibiotic with the effects of matrix components in natural water on the removal of antimicrobial activity. From the figure it can be noted that ions in the mineral water and excipients of the commercial product increase and delay the degradation time of the OXA and therefore also retard the loss of antimicrobial activity. In fact, 180 min of photocatalytic treatment, instead of 120 min under experimental conditions, is required to eliminate the pollutant and its antimicrobial activity. However, even in as such complex matrix, the TiO_2 photocatalytical system resulted effective to eliminate the antibiotic and its antimicrobial activity acting against *S. aureus.*

3.5. Determination of OXA photocatalytic degradation route

The application of AOPs for organic pollutant degradation can be carried out by different degradation pathways, which involve variations on both the degradation rate and the by-products generated. Thus, organic chemicals in TiO_2 photocatalysis can be oxidized by three different mechanisms: direct photolysis, oxidation at the photo-generated holes or hydroxyl radical attack.

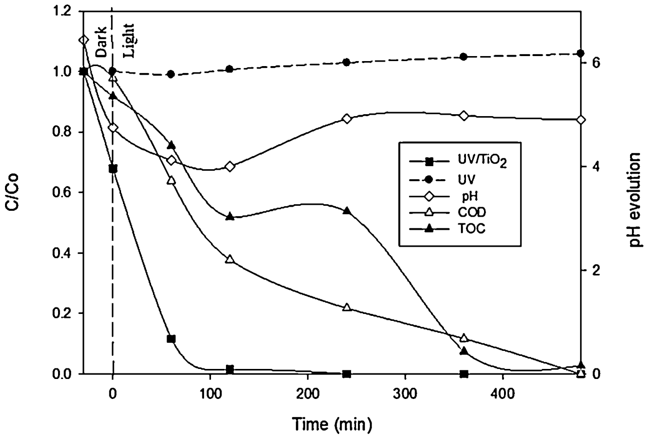

Fig. 7. Evolution of oxacillin, COD, TOC and pH during OXA degradation by TiO_2 photocatalysis and photolysis (UV). Test conditions were the same as those in Fig. 3. Experiments were carried out at least in duplicated.

In the previous section it was shown that, under the working conditions, direct photolysis is not plausible.

To investigate whether the other two pathways are followed in the degradation of OXA by TiO_2 photocatalysis, •OH radical scavengers and substances that act on the valence band holes of the catalyst can be tested. Alcohols, such as 2-propanol, have commonly been used to estimate the oxidation mechanism in photocatalytic processes due to their powerful •OH radical scavenger effect at the solution bulk [31,32]. In it turn, the iodide ion is an excellent probe specie in TiO_2 photocatalysis, as it reacts with valence band holes and adsorbed •OH radicals [32].

The results presented in Fig. 5 show that a concentration of 2-propanol 25 times higher than the antibiotic produces a slight reduction (~3%) in the removal rate of the pollutant. On the other hand, a concentration 645 times higher than the pollutant causes ~30% of inhibition. This indicates that hydroxyl radicals at the solution bulk may contribute to the degradation of these molecules. However, it is not the main mechanism involved given the moderate reduction in the extent of OXA removal. Assays in the presence of KI were also conducted to study the involvement of the catalyst surface in the pollutant degradation. When a KI concentration 25 times higher than the concentration of the substance was employed, ~75% inhibition occurred (Fig. 5). When an equimolar KI concentration was employed, a 13% reduction was observed in the degradation rate of OXA. Interestingly, in all of aforementioned cases, the reduction in the degradation rate is associated with a reduction in the adsorption of the pollutant to the catalyst surface. Consequently, the degradation of OXA by heterogeneous photocatalysis seems to occur mostly at the catalyst surface via two routes: radical attack and photo-Kolbe mechanism.

3.6. Identification of OXA by-products

During the photocatalytic degradation of OXA, using TiO_2 as the photocatalyst, various organic intermediates were produced. Five OXA by-products were identified by HPLC-MS: 6-({[3-(4-hydroxyphenyl)-5-methylisoxazol-4-yl]carbonyl}amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (compound A), 2-(carboxy{[(5-methyl-3-phenylisoxazol-4-yl) carbonyl]amino}methyl)-5,5-dimethyl-4,5-dihydro-1,3-thiazole-4-carboxylic acid (compound B), (5,5-dimethyl-4,5-dihydro-1,3-thiazol-2-yl) {[(5-methyl-3-phenyl-1,2-oxazol-4-yl) carbonyl]amino}acetic acid (compound C), 2-(carboxy{[(5-methyl-3-phenylisoxazol-4-yl) carbonyl]amino}methyl)-5,5-dimethyl-4,5-dihydro-1,3-thiazole-4-carboxylic acid 1-oxide (compound D) and 5-methyl-3-phenylisoxazole-4-carboxamide (compound E). The chromatogram and mass spectrum of the identified compounds is shown in Fig. S5 (see Supplementary data). Most of the by-products were produced half-way through the irradiation and, as time progressed, they gradually disappeared. The results indicated that the photocatalytical degradation of OXA began with the hydroxylation on the aromatic ring to produce compound A and with the breaking up of the β-lactam ring to produce compound B. Such as suggested in the previous section, both of initial routes are carried at the catalyst surface by attack of the adsorbed hydroxyl radicals. The signal at m/z 160, in the mass spectra of compounds A and B (m/z 160), support the presence of the penicillin nucleus in both intermediates [33]. Decarboxylation of oxacillin penicilloic acid (compound B), via photo-Kolbe reaction, produces compound C. The reduced sulfur moiety of compound B can also be oxidized by the hydroxyl radical to produce a sulfoxide group (Compound D). Secondary amine group are also susceptible to hydroxyl radical attack. Therefore, compound E can be formed from hydroxyl radical attack on oxacillin and compounds B–D. Finally, successive attack of hydroxyl radicals would conduct to the formation of carboxylic acids and then to the complete mineralization of the initial pollutant into CO_2, water and inorganic ions. A schema depicting the main degradation pathways of OXA is shown in Fig. 6.

3.7. Evaluation of the extend of oxidation and mineralization during the photocatalytical treatment

The evolution of the OXA concentration, COD, and DOC in individual solutions containing the antibiotic was determined to comparatively evaluate pollutant elimination, oxidation and mineralization of the antibiotic, respectively. The experiments were performed at natural pH (~6.0), 2.0 $g\,L^{-1}$ of TiO_2 and 150 W of light intensity. Fig. 7 reveals that the initial OXA concentration decrease. Total removal of the antibiotic is reached after 120 min of reaction. In turn, COD evolution indicates that the antibiotic is transformed into more oxidized compounds. This is consistent with the by-products identified previously. Interestingly, the pH decrease, to ~3.5–4.0 after two hours of treatment (Fig. 7), suggests that these by-products are subsequently transformed into carboxylic acids. Additionally, as shown by the DOC evolution, successive photocatalytical action is able to further degrade the initial pollutant into CO_2, water and inorganic ions. In fact, after 480 min of treatment both COD and DOC are practically eliminated. As can also be observed, with the elimination of these by-products the solution pH progressively increases.

Recently Magureanu et al. [34] evaluated the use of non-thermal plasma to remove OXA in water, which is up to now, the only AOPs reported to deal with this antibiotic. TiO_2 photocatalysis have the advantage over non-thermal plasma by using ambient sunlight as energy source and being environmental friendly technology. Additionally, as indicated by Malato et al. [14], TiO_2 is a cheap photo-stable catalyst, and the process may run at ambient temperature and pressure. In fact, in principle, the process involves a mild catalyst working under mild conditions with mild oxidants. The aforementioned characteristics of the TiO_2 photocatalytic system could promote its use for real applications and the feasibility of the process has been already demonstrated at large scale [14–15,35].

4. Conclusions

The results of this work show that the TiO_2 photocatalysis system has the potential to eliminate the environmentally damaging effects of β-lactam antibiotics such as OXA. High light intensity and catalyst loading increased the efficiency of the process. Experiments at natural pH (6.0) showed that the system followed Langmuir–Hinshelwood kinetics. The identification of OXA by-products and assays in the presence of probe substances showed that OXA degradation occurred via a photo-Kolbe mechanism with some participation of hydroxyl radical attack. Under the working conditions, the system showed to be able to remove the pollutant and the antimicrobial activity acting against *S. aureus*, transforming OXA into CO_2, water and inorganic ions. It has the additional advantage that excipients in a commercial preparation of the antibiotic, as well as inorganic species present in natural waters, do not significantly affect the efficiency of the system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jphotochem.2015.06.021.

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