Photocatalytic removal of fluoroquinolones and their antimicrobial activity from water matrices at trace levels: a comparison of commercial TiO$_2$ catalysts

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ABSTRACT

In this study, a solution containing the fluoroquinolones (FQs) ciprofloxacin, lomefloxacin, and ofloxacin (antimicrobial agents) was subjected to photocatalytic oxidation under UVA irradiation, employing the commercial titanium dioxide as catalyst. On-line solid phase extraction coupled to ultra-high-performance liquid chromatography–mass spectroscopy was used to pre-concentrate and quantify the analytes. The process provided an almost 95% degradation efficiency for all the FQs. The TiO$_2$ PC500 (100% anatase) was more efficient than TiO$_2$ P25 (80% anatase) for FQs degradation. The matrix effect on the efficiency of the process was evaluated by ultrapure water – UW, simulated water – SW, bottled water – BW, and public drinking tap water – TW. Simulated water showed lower interference, compared to drinking water and bottled mineral water, due to the lower concentrations of hydroxyl radical scavengers. The assessment of the residual antimicrobial activity in the solution, when using 50 mg L$^{-1}$ PC500 or 100 mg L$^{-1}$ P25, showed reductions of biological activity (after 120 min of reaction) of 92.4% and 95.4% for Escherichia coli, and 78.1% and 84.2% for Bacillus subtilis, respectively. It shows that the photocatalytic oxidation process was able to not only degrade the FQs but also deactivate its biological activity in the resultant solution.

Key words | anatase, antimicrobial activity, ciprofloxacin, lomefloxacin, ofloxacin, UVA/TiO$_2$

INTRODUCTION

Fluoroquinolones (FQs) are a class of synthetic antimicrobials that shows broad-spectrum activity against Gram-positive and Gram-negative bacteria (Rusu et al. 2015; Guo et al. 2016). FQs are a major antimicrobial administered to treat infections caused by bacteria in humans. The World Health Organization highlights the FQs as of critical importance for human medicine. In addition, the use of these antimicrobials in concentrated animal feeding operations is a practice widely adopted for prophylactic or therapeutic purposes. After administration, these compounds can be excreted in feces and urine and released into the environment matrices (Liu et al. 2014). Little is known about the acute and chronic effects of these substances in the environment (Van Doorslaer et al. 2011; Leal et al. 2012). Among the FQs, those that are most frequently found in aqueous matrices are ciprofloxacin, norfloxacin, and ofloxacin, up to 44.0 µg L$^{-1}$, 17.0 µg L$^{-1}$, and 35.5 µg L$^{-1}$, respectively (Prieto et al. 2011; González-Pleiter et al. 2013; Rodrigues-Silva et al. 2014).

Due to the complexity of environmental samples and the low concentrations of FQs in water matrices, a step employing solid phase extraction (SPE) is required to pre-concentrate the analytes prior to quantification. The sorbents commonly used in off-line SPE cartridges are HLB (Peres et al. 2015; Oliveira et al. 2015) and C$_{18}$ (Silva et al. 2011). These off-line procedures are time consuming and require substantial sample handling. In addition, the use of SPE cartridges increases analytical costs. Due to the difficulties of using SPE, some degradation studies are normally carried out at concentrations in the mg L$^{-1}$ range (Michael et al. 2013; Zhang et al. 2015; Bhatia et al. 2016), which do not reflect the concentrations at which these emerging pollutants are found in environmental matrices (i.e. µg L$^{-1}$ to ng L$^{-1}$) (Peres et al. 2015). The on-line SPE method requires...
lower volumes of solvents and offers shorter analysis times, reducing sample handling and analysis costs and improving analytical reproducibility (Tetzner et al. 2016).

Among the advanced oxidation processes, based on the formation of the hydroxyl radical (HO·), heterogeneous photocatalysis has been shown to be effective for the purification of aqueous matrices contaminated with emerging pollutants (Malato et al. 2009; Monteiro et al. 2015b). The oxidants generated by this process are a non-selective oxidant with the ability to mineralize organic compounds due to its reduction potential (E°d = 2.80 V/SHE), which is higher than those of conventional oxidants. TiO2 photocatalyst has been extensively employed due to its wide photosensitivity bandwidth and suitability for use with UV radiation (Malato et al. 2009). Equations (1)–(6) show the reactions involved in the formation of radicals in photocatalytic processes using TiO2 (Malato et al. 2009).

\[
\begin{align*}
\text{TiO}_2 + h\nu &\rightarrow e^- + h^+ \\
\text{O}_2 + e^- &\rightarrow \text{O}_2^- \\
\text{H}_2\text{O} + h^+ &\rightarrow \text{HO}^+ + \text{H}^+ \\
\text{O}_2^- + \text{H}^+ &\leftrightarrow \text{HO}_2^- \\
2\text{HO}_2^- &\rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \\
\text{H}_2\text{O}_2 + \text{O}_2^- &\rightarrow \text{HO}^+ + \text{OH}^- + \text{O}_2 \\
\text{H}_2\text{O}_2 + e^- &\rightarrow \text{HO}^+ + \text{OH}^- 
\end{align*}
\]

Several commercial photocatalytic TiO2 powders are available on the market, such as P25 (Evonik) and PC500 (Cristal). These products differ in terms of their composition, particle size, and crystalline phases (anatase and rutile). P25 is the TiO2 powder most commonly employed in photocatalytic studies (Klauson et al. 2010; Wang et al. 2015; Salma et al. 2016), although a few works have evaluated PC500 (100% anatase on the particle surface) (Egerton et al. 2011). The P25 material consists of 80% anatase (the most active TiO2 crystalline phase) and 20% rutile. Anatase has a band gap of 3.50 eV and its high activity is due to the prolonged lifetime of charge carriers and spatial charge separation, while rutile has a smaller band gap of 3.00 eV and a shorter charge lifetime (Kaplan et al. 2016). Consequently, the use of commercial PC500 could be promising for the photocatalytic oxidation (PCO) of pollutants such as FQs.

Another issue is the lack of information concerning the residual antimicrobial activities of FQ solutions subjected to advanced oxidation processes was reported by Escher et al. (2011), González-Pleiter et al. (2015), Wammer et al. (2013) and He et al. (2015). This highlights the need for evaluation of the residual antimicrobial activities of FQs towards the microorganisms targeted for elimination (i.e. Gram-positive and Gram-negative bacteria).

The objective of this study was to investigate the use of UV/TiO2 process for the degradation of the FQs ciprofloxacin (CIP), lomefloxacin (LOM) and ofloxacin (OFL) at concentration levels where they are detected in the environment. A method employing on-line SPE coupled to ultra-high-performance liquid chromatography–mass spectroscopy (UHPLC–MS/MS) was validated and used to monitor FQs at ppt levels. Two commercial TiO2 catalysts were evaluated, P25 and PC500. The influences of the compound concentration, catalyst load, and dissolved oxygen concentration were investigated, as tested in different matrices such as ultrapure water, simulated water, bottled water, and tap water. Finally, to evaluate the impact of the discharge of treated effluents by the proposed processes in the environment the residual antimicrobial activities of the solutions were evaluated for both Gram-positive and Gram-negative bacteria.

**MATERIAL AND METHODS**

**Chemicals and microorganisms**

CIP (99%, C17H16FN5O2, 331.4 g mol\(^{-1}\)), OFL (99%, C17H19F2N3O5, 361.4 g mol\(^{-1}\)), LOM (98%, C17H19F2N3O5, 351.4 g mol\(^{-1}\)), and gentamicin (GAT, 98%, C19H22FN3O4, 375.42 g mol\(^{-1}\)) were purchased from Sigma-Aldrich (USA). Hydrogen peroxide (30% w/w) was from Synth (Brazil). Mueller-Hinton agar and Mueller-Hinton culture broth were supplied by Himedia (India). Ultrapure water, 18.2 MΩ cm, was obtained from a Direct-Q system (Millipore). Bottled water (Bonafont) was used as acquired and had the following composition: [C\(_{\text{HCO}_3}\)\(^{-}\) = 29.23 mg L\(^{-1}\), C\(_{\text{K}^+}\) = 1.93 mg L\(^{-1}\), C\(_{\text{Na}^+}\) = 3.12 mg L\(^{-1}\), C\(_{\text{NO}_3}\)\(^{-}\) = 0.85 mg L\(^{-1}\), C\(_{\text{Cl}^-}\) = 0.15 mg L\(^{-1}\), C\(_{\text{SO}_4}\)\(^{2-}\) = 0.09 mg L\(^{-1}\), C\(_{\text{F}^-}\) = 0.08 mg L\(^{-1}\), pH = 7.6]. Tap water was supplied from SANASA (C\(_{\text{CaCO}_3}\) = 124 mg L\(^{-1}\), C\(_{\text{PO}_4}\)\(^{3-}\) = 0.25 mg L\(^{-1}\), C\(_{\text{NO}_3}\)\(^{-}\) = 1.29 mg L\(^{-1}\), C\(_{\text{NO}_2}\)\(^{-}\) = 0.1 mg L\(^{-1}\), C\(_{\text{NH}_2}\text{Cl}\)\(^{-}\) = 2.0 mg L\(^{-1}\), C\(_{\text{Na}^+}\) = 15 mg L\(^{-1}\), C\(_{\text{SO}_4}\)\(^{2-}\) = 8.3 mg L\(^{-1}\), C\(_{\text{F}^-}\) = 0.6 mg L\(^{-1}\), C\(_{\text{total solids}}\) = 156 mg L\(^{-1}\), pH = 7 (SANASA 2018)). Simulated water was prepared according to the standard USEPA microcosm formula and consisted of ultrapure water fortified as follows: C\(_{\text{NaNO}_3}\) = 42.5 mg L\(^{-1}\),
The FQ degradation studies were carried out using a batch reactor \( (h = 59 \text{ cm}, d_{\text{inner}} = 10 \text{ cm}, V_{\text{net}} = 1.0 \text{ L}) \) that was jacketed to maintain the solution temperature stable \((25 \pm 1 \degree \text{C})\) during the reaction. As previously reported by Caianelo \textit{et al.} (2017), a quartz tube with a closed base \((h = 32.5 \text{ cm}, d_{\text{outer}} = 2.5 \text{ cm})\), positioned concentrically to the reactor, was used as a support for the UV lamps \((\text{UVC}_{254} \text{ nm} \text{ and UVA}_{365} \text{ nm}, \text{both 8 W})\). The photonic flux emitted by the UVA lamp was measured with a Parker chemical actinometer \((\text{Chevremont et al. 2012; Moreira et al. 2012})\). Iron concentrations were determined according to ISO 6685 \((\text{ISO 1982})\). The absorbance of the samples at 510 nm was measured with a spectrophotometer \((\text{Model DR4000, Hach})\).

FQ adsorption tests were performed over 30 min, in the absence of radiation. A solution containing \(100 \mu \text{g L}^{-1}\) of each compound was maintained under stirring in the presence of different loadings of \(\text{TiO}_{2} \) \((10\text{–}200 \text{ mg L}^{-1})\). Therefore, the degradation assays were performed after the equilibrium in the adsorption was observed \((\text{i.e. 10 min})\).

### Analytical methods

#### On-line SPE-UHPLC-MS/MS

The on-line SPE-UHPLC-MS/MS system \((\text{Waters})\) was described in our previous work \((\text{Tetzner et al. 2016})\). The system consisted of an autosampler, a column manager to control the flow direction of the mobile phase and activate the valves used in the on-line SPE process, a quaternary solvent manager pump to load the sample into the SPE sorbent column, and a binary solvent manager pump to elute the analytes and convey them to the analytical column \((\text{C18 BEH Waters})\). A triple quadrupole detector \((\text{Xevo TQD Zspray})\) was used, with an electrospray ionization interface operating in the positive mode at a capillary voltage of \(1.0 \text{ kV} \), desolvation temperature of \(600 \degree \text{C}\), and desolvation gas flow rate of \(800 \text{ L h}^{-1}\). Argon was used as the collision-induced dissociation gas. Quantitation was performed in the selective reaction monitoring mode, and GAT was used as an internal standard.

The MS/MS detection parameters for the three analytes and the internal standard were \((\text{precursor} \ (m/z) / \text{cone} \ (V)), \text{quantitation ion} \ (m/z) / \text{collision energy} \ (V), \text{confirmation ion} \ (m/z) / \text{collision energy} \ (V))\): \(332/20, 314/22, 288/16 \) \((\text{CIP})\); \(362/20, 318/20, 261/30 \) \((\text{OFL})\); \(352/39, 308/26, 265/16 \) \((\text{LOM})\); and \(376/42, 289/28, 332/19 \) \((\text{GAT})\). An Acquity UPLC BEH \(\text{C}_{18} \ (2.1 \times 50 \text{ mm} \times 1.7 \mu \text{m})\) was used as analytical column at \(40 \degree \text{C}\).

#### Method validation

The on-line SPE procedure was optimized by evaluating the effects of different sorbents, sample volumes, loading, washing, and elution solvents, in order to achieve the maximum area signal with minimum matrix effect. After optimization of all on-line SPE parameters, the method was validated. For all the analytes, the analytical curves presented linearity \((r)\) higher than 0.99 for the concentration range evaluated \((1\text{–}160 \mu \text{g L}^{-1})\). The limits of quantification, determined using a signal-to-noise ratio of 10, were \(0.5 \mu \text{g L}^{-1}\) for all FQs. The intra-day precision of the method was \(0.2\text{–}1.1\%\) and the inter-day precision was \(3.7\text{–}7.4\%\), obtained for sample analysis in quintuplicate. The accuracy was in the range from \(83.3\) to \(119.0\%\). For the loading step, the mobile phase composed of \(95:5 \) \(\text{v/v} \) \((\text{water:methanol})\) with \(0.1\% \) of formic acid was used and no analyte losses were observed. For all analytes, the best ratio for elution was \(50:36:14 \) \((\text{v/v/v}) \) \((\text{water:methanol:acetonitrile})\) with the additive \(0.1\% \) formic acid. This on-line SPE system enabled quantification of a sample in \(1.9 \) min \((0.5 \text{ min for extraction of the analytes and 1.4 min for the analysis})\). The Oasis HLB column \((2.1 \times 30 \text{ mm} \times 10 \mu \text{m})\) at \(30 \degree \text{C}\) was therefore selected based on its common use in off-line SPE.

The configuration of the SPE-UHPLC-MS/MS system allows a volume of \(50 \mu \text{L}\) per injection in the SPE system, with no loss of analyte in the SPE column, representing pre-concentration factors of \(20\)-fold.
Antimicrobial activity

Antimicrobial activity was monitored using Escherichia coli K12 and Bacillus subtilis bacteria as test organisms, according to the methodology described by Dodd et al. (2009) and Caianelo et al. (2017). Briefly, 100 μL of potassium phosphate buffer were added in all the wells of a 96-well plate, except in the first well of each row. These wells received 300 μL aliquots of the solutions subjected to the degradation processes. A serial dilution was then performed by transferring 200 μL from the first well to the second, homogenizing, and repeating the procedure until the penultimate (12th) well, which remained with buffer only. Finally, all the wells were inoculated with 100 μL of bacterial suspension culture. The plate was sealed and stored at a controlled temperature of 37 °C for 8 h, followed by measurement of the absorbance at 620 nm.

RESULTS AND DISCUSSION

Photolysis

The degradation of the three FQs by photolysis was evaluated using a UVA lamp (Figure 1), with photonic flux (F) of 0.54 J s⁻¹. The highest degradation rate was obtained for LOM, where 85% of its initial concentration was degraded after 3.9 kJ of UVA irradiation (i.e. 120 min of assay). This process achieves the maximum degradation of 56% and 60% respectively for OFL and CIP, after 120 min of UVA irradiation. The UVA radiation represents around 90% of the solar UV radiation, and due to that, in terms of energy, the use of a UVA lamp in the photoreactor might be a more reliable approach for a future project of a pilot scale photoreactor employing both sunlight and UVA lamps (that enable the reactor to still work in periods without solar radiation).

Comparison of the efficiency of OFL degradation by photolysis obtained in this study with the values reported in the literature indicated that photolysis is not a highly efficient process for degradation of this compound in aqueous solution, as also found by Peres et al. (2015) and Oliveira et al. (2015), who observed greater LOM degradation by photolysis using a longer irradiation time. Photolysis was also evaluated by Salma et al. (2016) for degradation of CIP from aqueous solution; the efficiency was increased to over 85% after 50.1 kJ of UVA irradiation (i.e. 120 min of irradiation).

Degradation by photocatalysis

Adsorption equilibrium was reached after 10 min of contact between FQ solution and the catalyst; so the photocatalysis assays were carried out after this equilibrium time. For the highest concentration used (200 mg L⁻¹), the maximum adsorptions of CIP, LOM, and OFL on the TiO₂ surfaces were 11, 19, and 21% (P25), and 18, 25, and 13% (PC500), respectively.

Evaluating the linearity coefficients from the reactions, it can be assumed that the degradation data fit well in a pseudo-first order model. Figure 2 shows the degradation constant k (min⁻¹) for each condition studied. The calculation of the constant k shows that the degradation of the FQs follows the same profile. The degradation constant k increases with increasing TiO₂ loading in the suspension up to 100 mg L⁻¹ for P25 and up to 50 mg L⁻¹ for PC500. Subsequent increase in TiO₂ loading promotes a decrease in the value of constant k, possibly due to the decrease in the area available for radiation absorption due to high catalyst loading. The number of active sites increases as the catalyst load increases in the suspension, but there is a concentration limit, where the light is blocked by the catalyst particles themselves, decreasing the photocatalytic activity (Bhatia et al. 2016). Using TiO₂ P25, a slight decrease in the constant k was observed even when high catalyst concentrations were used (e.g. CIP: k₁₀₀ mg/L = 0.041 min⁻¹ and k₂₀₀ mg/L = 0.032 min⁻¹). For TiO₂ PC500 a different behavior was observed: concentrations above 50 mg L⁻¹ of TiO₂ in the suspension promoted the inhibition of the photocatalytic process by reducing the constant k from 0.046 min⁻¹ to 0.030 min⁻¹ and 0.020 min⁻¹ for CIP depletion, when using 50, 100 and 200 mg L⁻¹ of TiO₂, respectively.

For CIP and OFL using any of the TiO₂ P25 loading concentrations evaluated (i.e. 15 to 200 mg L⁻¹), the PCO
process was more efficient than photolysis, with gains of 50% and 43% of degradation for CIP and OFL, respectively. Similar behavior was observed when the PC500 catalyst was used. For LOM, the gains with the photocatalytic process were only observed when the TiO2 concentrations were higher than 50 mg L\(^{-1}\) for P25 and 25 mg L\(^{-1}\) for PC500. TiO2-mediated photocatalysis resulted in substantial degradation of the three FQs, with almost complete depletion after 120 min of irradiation. The same behavior was observed by Bhatia et al. (2014) and Peres et al. (2018). For PC500, the highest degradation was obtained using a 50 mg L\(^{-1}\) (the best condition) catalyst loading, presenting greater than 94% removal of the FQs in 120 min of irradiation.

After evaluating the optimum TiO2 load in the suspension, the initial antimicrobial concentration was varied, from 25 to 400 μgL\(^{-1}\), to evaluate the maximum FQs load to be converted by the TiO2 charge in the suspension. The assays were carried out using the optimum conditions for degradation of 100 μgL\(^{-1}\) FQs in aqueous solution, when using 50 and 100 mg L\(^{-1}\) TiO2 load for PC500 and P25, respectively. The efficiencies of PCO of the FQs were affected by its initial concentration (C\(_0\)). An increase in the FQ concentration from 100 to 400 μgL\(^{-1}\) resulted in decreases in conversion of 48.3% (CIP), 30.3% (LOM), and 70.3% (OFL). No significant change in process efficiency was observed in assays employing FQ concentrations below 100 μgL\(^{-1}\). As shown in Figure 2(c), the degradation profile of the FQs employing the TiO2 P25 is similar, achieving the highest \(k\) constant when the FQs concentration in the solution was 50 μgL\(^{-1}\), and increasing the antimicrobial concentration to 200 μgL\(^{-1}\) or further, the \(k\) constant was below 0.01 min\(^{-1}\). Assays employing the TiO2 PC500 showed that the solution could be fortified with higher concentration of antimicrobial agents without losing degradation rate (Figure 2(d)), because the PC500 surface (350 m\(^2\) g\(^{-1}\)) is 100% anatase phase and its superficial area is higher if compared with the 80% anatase P25 (35–65 m\(^2\) g\(^{-1}\)). Monteiro et al. (2015a) and Taranto et al. (2009) highlight that, even considering that the smaller crystal size of PC500 may foresee higher density in surface defects reducing the electron–hole recombination, which ultimately results in lower photocatalytic efficiencies, the larger area of PC500 nanoparticles in comparison to P25 promotes higher

Figure 2 | Kinetics of pseudo-first order reaction for photocatalytic degradation by varying the catalyst loading: (a) P25 (100 mg L\(^{-1}\)) and (b) PC500 (50 mg L\(^{-1}\)); (c) and (d) reaction kinetics for photocatalytic degradation varying the concentration of the drugs (25 μgL\(^{-1}\) to 400 μgL\(^{-1}\)) respectively for P25 (100 mg L\(^{-1}\)) and PC500 (50 mg L\(^{-1}\)). Ciprofloxacin (●) and ofloxacin (▲).

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adsorption capacity to this material, and it is directly related to the superior activity of PC500. Thus, the maximum efficiency of FQs conversion was reached when using 100 μg L⁻¹ of each FQ and 50 mg L⁻¹ of PC500 or 100 mg L⁻¹ of P25 under UVA irradiation.

Few studies report the photonic efficiency (ξ), which makes the proper comparison between the results obtained in this study and those described in the specialized literature difficult. The reaction quantum yield, or photonic efficiency, describes the number of transformed antimicrobial molecules (antimicrobial) relative to the number of photons, at a given wavelength, incident on the photoreactor (ξ = r (mol s⁻¹)/rate of incident photons (Einstein s⁻¹) x 100%) (Serpone 1997; da Costa Filho et al. 2017). The ξ values were 3.91, 3.44 and 3.81% respectively for CIP, LOM and OFL when applied in the photoreactor 100 mg_P25 TiO2 L⁻¹ (best condition) and 3.70, 3.07 and 3.30% respectively for CIP, LOM and OFL when applied in the photoreactor 50 mg_PC500 TiO2 L⁻¹ (best condition). The photonic flux values were similar to those published by Marinho et al. (2017). It relays to an illuminated catalyst surface area per unit volume inside the reactor of 22.5 m² m⁻³, corresponding to 7.9 μg m⁻² s⁻¹.

Real water samples (i.e. bottled water, tap water and simulated water) were fortified with CIP (100 μg L⁻¹), LOM (100 μg L⁻¹) and OFL (100 μg L⁻¹) and subjected to degradation by UV/TiO₂. The best operation conditions for each commercial TiO₂ was used. The results for CIP degradation are shown in Figure 3, where it is possible to observe that as the complexity of the matrix increased (i.e. ultrapure water < simulated water < bottled water < tap water; related to water samples characterization, presented in the ‘Chemicals and microorganisms’ section) the process efficiency decreased. The same behavior was observed for both commercial P25 and PC500, regardless the FQs studied (Supplementary Material Figure S1, available with the online version of this paper). It is also possible to suggest that the salts and minerals present in real water samples acted as hydroxyl radical scavengers, which resulted in the reduction of the FQs photocatalytic oxidation. The presence of high concentration of (bi)carbonate ions, chloride, nitrite, nitrate, sulfite, and sulfide in these water samples act as hydroxyl radical scavengers. Iron, chloride, nitrite, nitrate, phosphate, and total organic carbon absorb radiation and reduce its penetration through the solution (Khan et al. 2015) and reduce the photocatalytic oxidation of the antimicrobial agents. It is worth emphasizing that the tap water had higher contents of organic matter and chlorine compared to the other water samples and the degradation results were as low as UVA photolysis assays performed in ultrapure water.

Spina-Cruz et al. (2018) and Caianelo et al. (2017) reported that the water matrix significantly influences antimicrobial degradation by advanced oxidation processes, concluding that the higher the concentration of radical scavengers in the matrix, the lower will be the efficiency of the advanced oxidation processes employed. In addition, Malato et al. (2009) reported that turbidity impedes further penetration of light in the reactor, avoiding the catalyst activation.

**Influence of pH on fluoroquinolone degradation**

The zero charges of the TiO₂ vary between 4.5 and 7, and above and below these values the catalyst is negatively or positively charged (Malato et al. 2009), because those reactions at pH 4 and 9 are not favorable for FQs adsorption on the catalysts surface, since there is electronic repulsion between the charges of the compound and the catalyst (Peres et al. 2015). As shown in Figure 4, the highest
adsorption for CIP was observed at pH 6 (CIP and 100 mg L\(^{-1}\) of P25: 9% at pH 4, 15% at pH 6 and 10% at pH 9; CIP and 50 mg L\(^{-1}\) of PC500: 10% at pH 4, 14% at pH 6 and 11% at pH 9), PC500 achieved the same adsorption rate as P25 with half of the catalyst load due to its higher superficial area.

The pH of the solution can influence the PCO efficiency due to changes in the interaction between the surface of the catalyst (where the radicals are formed, and the reduction reactions take place) and the target compound. Under acidic conditions (pH <4), the TiO\(_2\) surface is positively charged, while under basic conditions (pH >8) it is negatively charged. At pH between 4 and 8, uncharged TiO\(_2\) species predominate in the suspension (Peres et al. 2015). The photocatalytic study was performed under three different pH conditions: acidic (pH 4), basic (pH 9), and at the natural pH of the FQs solution (pH ~6). The pH values of the solutions (pH 4 and 9) were selected based on the characteristics of real effluents, and to observe the influence of the charge condition of the catalyst and the molecules (Kimura et al. 1999; Almeida et al. 2004; Bilal et al. 2016; Budiman & Wu 2016).

Assays performed with titanium dioxide P25 and carried out at pH 4 and 9 showed lower photocatalytic efficiency than those the assays performed at pH of the natural FQs solutions (i.e. pH around 6), for the three compounds studied (Figure 4). A decrease in the degradation profile of 13.6 and 31.6% was observed in assays at pH 4 and 9, respectively, when compared to the CIP degradation efficiency achieved at pH 6. CIP has two main pK\(_a\) (acid dissociation constant at logarithmic scale) values (5.9 and 8.2). At pH 4, 98.3% of the molecules are in the protonated form and can be repelled by the positively charged TiO\(_2\) surface, which diminishes the adsorption due to Van der Waals interactions, resulting in lower degradation. For the same reason, CIP degradation was lower at pH 9, at which 95% of the CIP molecules are deprotonated and the TiO\(_2\) molecules are negatively charged. The pK\(_a\) values for LOM (5.6 and 8.7) and OFL (6.5 and 8.1) determined the degradation behaviors of these FQs, which were similar to those of CIP in the photocatalytic assays with P25. At pH 4, the photocatalytic degradation of OFL was most influenced by the repelling charges effect. In the experiments with 100% anatase TiO\(_2\) (PC500), the photocatalysis was positively affected by the alteration of suspension pH, probably due to lower electron–hole recombination and the higher concentration of anatase than in the experiments with P25 (80% anatase). Increased FQ degradation was observed when the photocatalytic assays were performed under basic conditions; as an example, CIP degradation was improved by 45%, compared to the results obtained at pH 6.

**Effect of aeration**

It is well known that the presence of oxygen is essential to improve the efficiency of pollutant removal from water by applying photocatalytic processes (Malato et al. 2009). Dissolved oxygen available in water often serves as the electron acceptor in the photocatalytic process and promotes a greater generation of hydroxyl radicals and other
oxidants, such as superoxide, hydroperoxyl radicals, and hydrogen peroxide (Nogueira & Jardim 1998), according to Equations (1)–(6).

The concentration of dissolved oxygen was increased by bubbling air through the suspensions. The working solutions contained approximately 1.5 mg L$^{-1}$ of dissolved oxygen, which became saturated at around 8 mg L$^{-1}$ after 10 min of aeration. It is important to observe that aeration by itself did not promote FQ degradation. This was shown in experiments carried out after 15 min of aeration, prior to irradiation. When the dissolved oxygen concentration was increased, depletion of the FQs also increased, independent of the TiO$_2$ used (P25 or PC500).

Reactions using P25 and 1.5 mg L$^{-1}$ of dissolved oxygen resulted in FQ degradation exceeding 90% after 120 min of reaction, while increase of the dissolved oxygen to 8 mg L$^{-1}$ resulted in similar degradation of the FQs after 60 min of reaction. Using a shorter degradation time, it is therefore possible to increase the performance of the process and the volume of effluent treated in a given time.

### Degradation products formation

The study of the degradation products for each FQ was done in ultrapure water, and isolated solutions of CIP, OFL, and LOM were individually prepared and subjected to degradation at the best photocatalytic conditions for each catalyst (i.e. using 50 mg L$^{-1}$ PC500 or 100 mg L$^{-1}$ P25). The main degradation products were identified by the comparison between the full MS-scan spectra of the FQ solution (i.e. $t = 0$ min) and the sample subjected to degradation. No degradation product signals were observed (that exceeded three times the noise signal) in the assays performed with PC500. Moreover, fewer degradation products were identified when assays were performed with P25. It is important to report that the lower number of the degradation products could be related to the high PCO of the FQ.

The major intermediates identified during the PCO of CIP were the compounds with $m/z$ 305 and 362, and for PCO of OFL, the compounds with $m/z$ 249 and 348. Two major compounds with $m/z$ 301 and 363 were identified during PCO of LOM. All these identified intermediates were previously reported in the literature by An et al. (2010), Hubicka et al. (2013), Liu et al. (2012), Paul et al. (2010) and Yahya et al. (2014). The identified degradation products were formed after primarily successive attacks on the piperazine ring of the studied FQs (Supplementary Material Table S1, available with the online version of this paper). In addition, due to the attacks in the piperazine ring of the FQs (responsible for antimicrobial activity against Gram-positive bacteria), it is possible to propose that these intermediates present lower antimicrobial activity than the parent compound (Peres et al. 2015; Caianelo et al. 2017).

### Antimicrobial activity

Bacterial responses to the treated and untreated ($t = 0$ min and control) samples were quantified in terms of growth inhibition. Gram-positive and Gram-negative microorganisms were exposed to the serially diluted samples, and the EC$_{50}$ values were calculated from the dose-response curves. As the antimicrobial activity decreased, less dilution of the solution was needed to obtain 50% growth inhibition (EC$_{50}$). In other words, the solution needed to be more concentrated in order to be effective against the microorganisms. The potency equivalent (PEQ) values represent a correlation between the dilution factor needed to reach the EC$_{50}$ of the untreated solution (DF$_{EC50}$ control solution) and the dilution required to reach 50% growth inhibition of a sample subjected to a degradation process (DF$_{EC50}$ treated solution) (PEQ = DF$_{EC50}$ treated solution/DF$_{EC50}$ control solution).

The antimicrobial activity of the solution decreased during the period of the reaction, indicating that as the FQs were degraded, the biological activity of the solution against Gram-positive and Gram-negative microorganisms decreased (Figure 5). As the antimicrobial activity decreased, the dose-response curves moved in the direction of log dilution equal to 0 (Figure 5(b)–5(d)). No antimicrobial activity was observed when the reaction was carried out with UVA/P25 (100 mg L$^{-1}$ TiO$_2$ and 120 min of irradiation) (Figure 5(a)); hence, there was no inhibition of microorganism growth, when an undiluted solution was used.

The results (Figure 2(a) and 2(b)) showed that the suspension with 50 mg L$^{-1}$ of PC500 provided the best FQ degradation, and that the suspension with P25 required double the catalyst mass to achieve similar degradation efficiency. The use of 50 mg L$^{-1}$ PC500 caused almost complete FQ degradation after 120 min of irradiation. However, almost total antimicrobial activity removal was only achieved in the reactions with 100 mg L$^{-1}$ PC500, with removal values of 93 and 97% for the Gram-positive and Gram-negative bacteria, respectively (data not shown). The use of 100 mg L$^{-1}$ P25 decreased the solution antimicrobial activity in a shorter reaction time, compared to the reactions carried out with 100 mg L$^{-1}$ PC500. It is likely that the degradation products generated by the photocatalytic reactions with PC500 possessed the active sites responsible for the antimicrobial activity. The removal of the antimicrobial
activity was slower for the reactions with PC500 than with P25. Using the P25 catalyst, these bonds of the FQs molecules were probably removed, resulting in diminished antimicrobial activity.

CONCLUSIONS

An on-line method using SPE coupled to UHPLC-MS/MS was validated and found to be suitable for monitoring the photocatalytic degradation of FQs. The UVA/TiO$_2$ was more efficient for degradation of the three FQs, compared to UVA alone. Over 94% degradation of the FQs was obtained using 100 mg L$^{-1}$ TiO$_2$ P25 or 50 mg L$^{-1}$ TiO$_2$ PC500 and 75 min of reaction. Similar degradation was achieved for the two catalysts when the loading of P25 was twice that of PC500. PC500 presented high degradation rate due to higher superficial area and high content of TiO$_2$ anatase phase. However, the P25 catalyst was more effective in reducing the antimicrobial activity of the solution, compared to the 100% anatase catalyst (PC500). Varying the composition of the sample matrix, it was observed that the concentration of the salts and minerals acted as hydroxyl radical scavengers, affecting the efficiency of the process. Degradation of the FQs was accompanied by decreased biological activity of the solutions against Gram-positive and Gram-negative microorganisms.

Finally, it can be concluded that the photocatalytic process provides effective removal of FQs. The technique is suitable for treatment of water containing this class of antimicrobials, reducing the concentrations of the pollutants and eliminating the antimicrobial activity, if the sample does not present high concentration of organic matter, chloride components and high concentration of (bi)carbonate.
ACKNOWLEDGEMENTS

Venancio and Rodrigues-Silva contributed equally to this work. Financial support was provided by FAPESP (2014/06064-3, 2014/06201-0, 2013/09543-7). Caio Rodrigues-Silva acknowledges FAPESP (09/2014–04/2018: 2014/16622-3) and CNPq (10/2018:154061/2018-2) for providing a post-PhD scholarship. Maniero (2013/07817-2) acknowledges FAPESP for providing the post-PhD scholarships, and Venancio acknowledges CAPES for providing an MSc scholarship.

CONFLICT OF INTEREST

There is no conflict of interest.

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First received 28 June 2018; accepted in revised form 10 October 2018. Available online 17 October 2018.