EMERGING MICROPOLLUTANT OXIDATION DURING DISINFECTION PROCESSES USING UV-C, UV-C/H_2O_2, UV-A/TiO_2 AND UV-A/TiO_2/H_2O_2.

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Abstract

Regeneration of wastewater treatment plant effluents constitutes a solution to increase the availability of water resources in arid regions. Water reuse legislation imposes an exhaustive control of the microbiological quality of water in the operation of disinfection tertiary treatments. Additionally, recent reports have paid increasing attention on emerging micropollutants with potential biological effects even at trace level concentration. This work focuses on the evaluation of several photochemical technologies as disinfection processes with the aim of simultaneously achieving bacterial inactivation and oxidation of pharmaceuticals as examples of emerging micropollutants typically present in water and widely studied in the literature. UV-C-based processes show a high efficiency to inactivate bacteria. However, the bacterial damages are reversible and only when using H_2O_2, bacteria reproduction is affected. Moreover, a complete elimination of pharmaceutical compounds was not achieved at the end of the inactivation process. In contrast, UV-A/TiO_2 required a longer irradiation time to inactivate bacteria but pharmaceuticals were completely removed along the process. In addition, its oxidation mechanism, based on hydroxyl radicals (•OH), leads to irreversible bacterial damages, not requiring of chemicals to avoid bacteria regrowth. For UV-A/TiO_2/H_2O_2 process, the addition of H_2O_2 improved *E. coli* inactivation since the cell wall weakening, due to •OH attacks, allowed H_2O_2 to diffuse into the bacteria. However, a total elimination of the pharmaceuticals was not achieved during the inactivation process.

Keywords: Pharmaceuticals; disinfection; *Escherichia coli*; photocatalysis; immobilized TiO_2; wastewater effluents.

1. Introduction

The world's population is growing by about 80 million people a year, implying an increase in freshwater demand of about 64 billion cubic meters a year (Unesco, 2009). Growing demand for water in the domestic, industrial, and agricultural sectors has led to an increase in water shortages, which can be a serious problem in the future. At the same time climate change may reduce water availability at some locations such as Spain, the most arid country of the European Union and the one that devotes most water resources to irrigation. Thus, a solution to increase the water availability for several uses can be the reuse of sewage treatment plants effluents, which obliges to carry out a more exhaustive control in the operation of tertiary treatments. Above all, attention must be paid to microbiological parameters such as *Escherichia coli*, microorganism which has traditionally been used as faecal contamination indicator and whose concentration level usually conditions the possible applications of the regenerated water. Therefore, water reuse is unavoidable linked to the use of disinfection processes.

Traditional disinfection processes such as chlorination and ozonation are widely accepted to produce chlorinated and brominated disinfection by products (DBP's) with potential carcinogenic effects on mammals. In addition, micropollutants have recently been detected at trace level concentrations not only in wastewater treatment plant effluents but also in drinking water. Their reported negative effects make it necessary to remove them especially for reuse purposes (Ikehata et al., 2006). Therefore, the use of alternative disinfection technologies for simultaneously removing traces of these emerging micropollutants has to be explored.

Advanced Oxidation Processes (AOPs), based on the generation of highly reactive hydroxyl radicals for the unselective oxidation of chemical pollutants and inactivation of microorganisms can be an adequate alternative.

UV-C alone (without H_2O_2 addition) has been used to a large extent for the oxidation of several chemicals (Legrini et al., 1993; Tuhkanen, 2004). This process is not usually considered as an AOP since it is based on the direct photolysis of organic compounds which are able to absorb UV-C photons (wavelength of 254 nm). When hydrogen peroxide is added, the process is enhanced since the photolytic dissociation of hydrogen peroxide with radiation of 254 nm yields hydroxyl radicals responsible for an additional oxidation pathway. The use of UV-C radiation for disinfection processes dates back to 1916 (U.S. Environmental Protection Agency, 1996). The germicidal effect is based on the absorption of these high-energy UV-C photons by the DNA, inhibiting its replication and consequently the microorganism reproduction (Ravanat et al., 2001). However, these damages can be reversible due to the self-repairing mechanism of the microorganism cells. In contrast, UV-C/H_2O_2 has not been used to such a large extent for disinfection applications. Despite early publications concerning the role of UV-C radiation and H_2O_2 for inactivation purposes were reported (Hartman and Eisenstark, 1978; Sundström et al., 1992), and the disinfectant properties of hydrogen peroxide together with its additional production of hydroxyl radicals are already accepted (Yasar et al., 2007), it remains unclear if the hydrogen peroxide has positive or negative effects on the disinfection processes (Labas et al., 2009).

Regarding the oxidation mechanism of TiO_2 photocatalysis, it is based on the activation of a semiconductor material upon UV-A radiation, eventually producing hydroxyl radicals. The addition of H_2O_2 also improves the process since it reduces the recombination of the hole/electron pair and reacts with the conduction band electron (Legrini et al., 1993) and the superoxide radical anion to yield additional hydroxyl radicals (Haber-Weiss reaction) (Tuhkanen, 2004). However, its addition at high concentrations has also been reported not to improve the photocatalytic process since the oxidation of H_2O_2 by the photogenerated holes lead to a decrease in •OH production (Wang and Hong, 1999). Since the first work of Matsunaga et al. (1985), several publications have been reported for bacteria inactivation with

heterogeneous photocatalysis, showing very successful results (Malato et al., 2009). The mechanism of microorganism inactivation is more complex since a significant amount of hydroxyl radical attacks are required to cause the bacterial lysis, leading to irreversible damages (Malato et al., 2009). Once again, although it is expected that the presence of H_2O_2 favors the process, there are controversial results in the literature when microrganisms are involved (Hartman and Eisenstark, 1978; Bayliss and Waites, 1980; Lanao et al., 2010).

A notably increase in recent literature regarding these technologies to remove micropollutants such as pharmaceuticals in wastewater effluents has taken place. They seem to be appropriate for this application as shown by the successful results reported by several authors (Ikehata et al., 2006; Klavarioti et al., 2009). However, very few approaches have been done to their application to disinfection processes which might achieve the simultaneous inactivation of microorganisms and oxidation of micropollutants (Buffle et al., 2006; Gerrity et al., 2011).

The aim of this work is to carry out a comparative evaluation of the efficiency of several photochemical technologies (UV-C, UV-C/H_2O_2, UV-A/TiO_2 and UV-A/TiO_2/H_2O_2) as disinfection processes of secondary wastewater treatment plant effluents, focusing not only on the bacterial inactivation results but also on the possibility of achieving the simultaneous oxidation of emerging micropollutants such as pharmaceuticals with purposes for water regeneration. The use of a fixed-bed photocatalytic reactor using immobilized TiO_2 to avoid the necessity of removal of the TiO_2 particles after the treatment has been also tested in UV-A/TiO_2 and UV-A/TiO_2/H_2O_2 processes. Long-term microbiological quality of the treated water has also been studied to make the storage of regenerated water possible before further use.

2. Materials and methods

2.1. Experimental set-up and conditions.

Experiments have been carried out in an experimental set-up consisting of an annular photoreactor (15 cm long, 3 cm inner diameter and 5 cm outer diameter) operating in a closed recirculating circuit with a reservoir tank. Illumination was performed by a tubular lamp placed in the reactor axis. Two different lamps have been used: i) an Osram HNS 6W lamp, which provides an UV-C irradiation power of 1.63 W (115.6 W m^{-2}) with an emission peak centered at 254 nm. Experiments at a lower UV-C irradiation power corresponding to 0.11 W (7.80 W m^{-2}) have been carried out placing a neutral filter between the lamp and the reactor wall, and ii) a Philips TL 6W black light lamp, which provides an UV-A radiation power of 0.91 W (64.5 W m^{-2}) with a maximum emission peak centered at 365 nm. The UV-C and UV-A incident photon flow, determined by ferrioxalate actinometry, correspond to 3.5 x 10^{-6} and 2.4 x 10^{-7} Einstein s^{-1} for UV-C radiation (1.63 and 0.11 W, respectively), and 2.8 x 10^{-6} Einstein s^{-1} for UV-A radiation. Degussa P25 TiO_2 was used as photocatalyst, both in suspension at a concentration of 0.1 g L^{-1} and immobilized on a fixed-bed reactor using 6 x 6 mm glass Raschig rings as support. The amount of photocatalyst which maximizes the UV-A photons absorbed was previously optimized for both photoreactors. Duplicated experiments have been carried out to estimate the experimental reproducibility. The relative error of the calculated kinetic constants was below 25%, reasonable for microbiological and trace micropollutants. More details about the reactor and the immobilization procedure can be found elsewhere (van Grieken et al., 2009).

Deionized water (18.2 MΩ cm) and simulated municipal wastewaters were used as model waters for the experiments. Simulated municipal wastewaters consist of a mixture of salts (K_2HPO_4, NaCl, $CaCl_2$, and $MgSO_4$) and organic matter (meat peptone, beef extract and

urea) diluted to a total organic carbon value of 15 mg L^{-1}, similar to effluents of a secondary wastewater treatment plant (SWTP) (Kositzi et al., 2004).

2.2. Bacterial analysis.

Escherichia coli was selected as model microorganism due to its wide use as faecal contamination indicator. *E. coli* K12 strain was provided by the Colección Española de Cultivos Tipo (CECT 4624, corresponding to ATCC 23631). The reactions were followed by analyzing the concentration of viable bacteria along the reaction through a standard serial dilution procedure and growing samples up to 1 mL of on agar plates. The bacterial detection limit reached by this protocol corresponds to 1 CFU mL^{-1}. Experiments were carried out using an initial bacterial concentration of 10^3 CFU mL^{-1}, typical value of bacterial concentration found in secondary wastewater treatment plant effluents. More details about the bacterial procedure can be found elsewhere (van Grieken et al., 2009).

2.3. Pharmaceutical analysis.

Some pharmaceuticals corresponding to different families of drugs have been selected as model micropollutants for the experiments: i) 4-acetamidoantipyrine (4-AAA) (Alfa-Aesar, CAS 83-15-8, $C_{13}H_{15}N_3O_2$, antipyretic and non-steroidal anti-inflammatory drug, NSAID); ii) hydrochlorothiazide (HCTZ) (Sigma-Aldrich, CAS 58-93-5, $C_7H_8ClN_3O_4S_2$, cardiovascular drug); iii) sulfamethoxazole (SMX) (Fluka, CAS 723-46-6, $C_{10}H_{11}N_3O_3S$, antibiotic); and iv) diclofenac sodium salt (DCF) (Sigma-Aldrich, CAS 15307-79-6, $C_{14}H_{10}Cl_2NNaO_2$, NSAID). Experiments have been carried out using mixtures with an initial concentration of 20 μg L^{-1} of each compound, being analyzed the elimination of the initial molecules. A Varian 325 LC-MS/MS triple quadrupole mass spectrometer, equipped with a vortex electrospray ionization interface (vESI) and a Pursuit XRs Ultra 2.8 C18 100 x 2.0 mm column was used for the quantification of the concentration of the pharmaceuticals along the reaction. Mobile phase

consisted of buffered water with formic acid and its ammonium salt (pH = 2.7, mobile phase A) and methanol (mobile phase B) with a gradient program as follows: 0 min 95 % A; 0.10 min 70 % A; 5 min 55 % A; 9 min 5 % A; 11 min 95 % A kept for 6 min and a flow rate of 250 μL min^{-1}. The API capillary potentials used for the ionization of each compound were i) 30 V for 4-AAA; ii) -128 V for HCTZ; and iii) 40 V for SMX and DCF. The precursor → product multiple reaction monitoring (MRM) transitions together with the corresponding Q2 collision energy (in brackets) used for quantification and qualification of each pharmaceutical were i) 246.0 → 228.0 (12 V) and 246.0 → 83.0 (22 V) for 4-AAA; ii) 295.7 → 268.5 (18.5 V) and 295.7 → 204.5 (22.5 V) for HCTZ; iii) 254.0 → 92.0 (24 V) and 254.0 → 156 (16 V) for SMX; and iv) 296.0 → 214.0 (32 V) and 296.0 → 250.0 (12 V) for DCF.

2.4. H_2O_2 analysis.

An initial concentration of hydrogen peroxide of 100 mg L^{-1} was used in UV-C/H_2O_2 and UV-A/TiO_2/H_2O_2 experiments. This value is higher than the stoichiometric amount of H_2O_2 required for the total mineralization of the organic matter present in the SWTP effluents, which corresponds to a theoretical value 85 mg L^{-1}. This value has been estimated by multiplying the TOC content of 15 mg L^{-1} by the stoichiometric amount of H_2O_2 required for the total oxidation of C to H_2O and CO_2 (5.67 $mg_{H_2O_2}\,mg_C^{-1}$). Thereby, it is guaranteed that the amount of H_2O_2 is not limiting for the oxidation of the parent pharmaceutical compounds since their mineralization only requires a stoichiometric amount of H_2O_2 of 0.3 mg L^{-1} (estimated also from the stoichiometric equation for total oxidation of each pharmaceutical to CO_2 and H_2O). Hydrogen peroxide concentration was followed colorimetrically at 410 nm after adding titanium sulfate (Eisenberg, 1943).

3. Results and Discussion

3.1. UV-C.

Experiments at low UV-C irradiation power (Figure 1) have shown hardly differences in irradiation time required to reach a complete *E. coli* inactivation for deionized water and SWTP effluents. It suggests that the inactivation mechanism, based on the direct absorption of UV-C photons by the DNA, in agreement with Ravanat et al. (2001) and Chen et al. (2009) is not sensitive to the chemical composition of water. The negligible absorption of radiation of SWTP effluent at 254 nm (not shown) and the very similar values of pseudo-first order kinetic constants of bacterial inactivation for deionized water (0.44 min^{-1}) and SWTP effluents (0.46 min^{-1}) supports this fact (Table 1).

Figure 1 also shows that if the irradiation time to reach a complete bacterial inactivation (of ca. 15 min) is considered, the UV-C dose required to totally inactivate bacteria was comparable to those reported by other groups (Labas et al., 2009). Although *E. coli* inactivation is fast, even after 45 more minutes of UV-C radiation after reaching an apparent complete inactivation, values of initial viable bacteria concentration were reached after 24 h in dark. Bacterial regrowth has previously been reported by Yasar et al. (2007), and Shang et al. (2009) suggesting that the mechanism of UV-C inactivation does not lead to irreversible damages on the microorganisms. Actually, Trombert et al. (2007) also reported an increase in viable bacterial concentration of 3-log after UV-C irradiation and 24 h in dark. Several repairing enzymatic mechanisms have been reported to favor the bacterial regrowth (Coohill and Sagripanti, 2008; Shang et al., 2009) reversing the DNA damages produced upon absorption of UV-C photons. And even, inactivation-reactivation curves have been reported (Nebot et al., 2007), showing a regrowth phase as function of availability of nutrients and kind of bacteria.

Concerning the pharmaceuticals, Figure 1 inset shows that their photolytic degradation has been really slow as compared to the inactivation of bacteria. It confirms that bacterial inactivation and pharmaceutical degradation are not based on the same photolytic mechanism. The strong germicidal power of UV-C radiation observed for bacterial inactivation is based on the generation of very specific damages in the bacterial DNA what inhibits its duplication and consequently bacterial reproduction. Those damages, reported to be produced by the formation of thymine dimmers (Ravanat et al., 2001), can be reverted due to lack of cell wall lysis, which leads to irreversible damages.

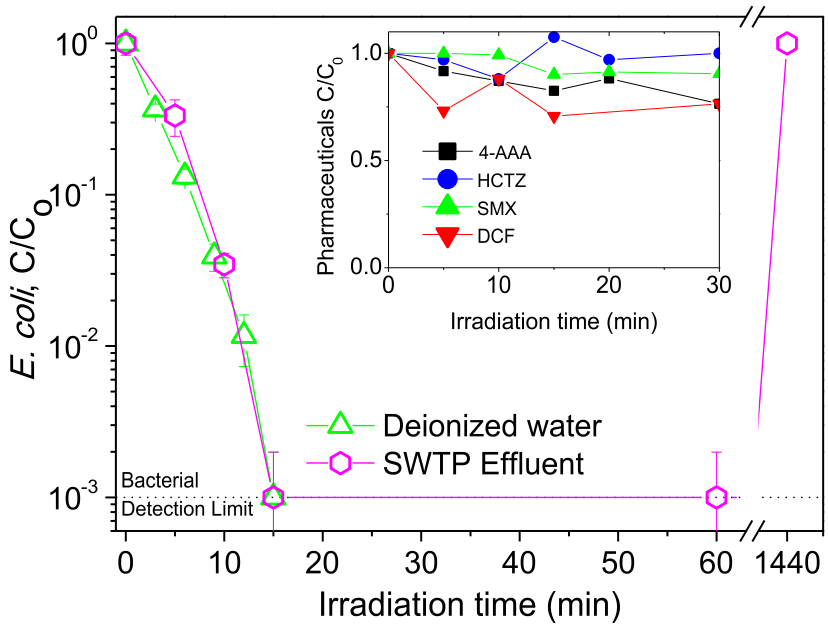

Figure 1. Simultaneous *E. coli* inactivation and pharmaceutical photolytic degradation (inset) under UV-C radiation for deionized water and SWTP effluents. UV-C irradiation power: 0.11 W. Lamp switched off after 60 minutes of irradiation. Pharmaceuticals: 4-acetamidoantipyrine, 4-AAA; hydrochlorothiazide, HCTZ; sulfamethoxazole, SMX; diclofenac sodium salt, DCF.

Table 1 also shows the values of pseudo-first order kinetic constants (*k*) of the simultaneous inactivation of *E. coli* and oxidation of pharmaceuticals in a SWTP effluent under UV-C radiation at a higher irradiation power. As expected, an increase in the irradiation power of the UV-C lamp has led to an increase in the values of *k* for bacteria and pharmaceuticals, leading to a decrease in the irradiation time required to reach both, a complete *E. coli* inactivation and pharmaceutical oxidation (figure not shown). With regard to pharmaceuticals, they have needed a longer irradiation time or a higher UV-C dose to be oxidized than that required for reaching a complete bacterial inactivation (figure not shown), which is in agreement with previous findings from Gagnon et al. (2008) and Dotson et al. (2010). In this case, the direct absorption of UV-C photons seems to lead to the photolytic degradation of all the pharmaceuticals. Although all the pharmaceuticals are able to absorb at 254 nm (absorption spectra not shown), the total oxidation of HCTZ showed a lower value of *k* and consequently required of a longer irradiation time as compared to 4-AAA, SMX and DCF, since its absorption at 254 nm is lower than that of the other compounds. Therefore, the pharmaceutical structure seems to notably influence the sensitivity to the oxidation process, in agreement with previous reports (Kim et al., 2009). Thus, although the irradiation time required to completely inactivating the bacteria has been really low, it does not guarantee a complete photolytic degradation of pharmaceuticals and other possible emerging pollutants present in the water.

3.2. UV-C/H_2O_2.

In comparison with the results shown in Table 1, the addition of H_2O_2 under low UV-C irradiation power seems to have improved the inactivation process in a SWTP effluent since the values of *k* obtained for bacterial inactivation have increased when adding H_2O_2. These results of simultaneous inactivation of *E. coli* and oxidation of pharmaceuticals at low UV-C irradiation power in the presence of H_2O_2 are depicted in Figure 2. Although values of *k* have

been increased, the irradiation time required for a complete *E. coli* inactivation has hardly been affected, leading to a slight decrease. Consequently, the main inactivation mechanism seems to be UV-C radiation, in agreement with Mamane et al. (2007). This group reported that *E. coli* inactivation based on hydroxyl radicals produced due to H_2O_2 photolysis is hardly noticeable compared to that of UV-C absorption. In contrast, Bayliss and Waites (1980), Standard et al. (1983), and Sundström et al. (1992), reported a more significant enhancement in bacteria inactivation after the addition of hydrogen peroxide, whereas Hartman and Eisenstark (1978), and Rajala-Mustronen and Heinomen-Tanski (1995) did not observe any effect of H_2O_2 on bacteria inactivation. Labas et al. (2009) have even reported a detrimental effect on bacteria inactivation due to the addition of H_2O_2 based on the competition for the absorption of photons at 254 nm, leading to a parallel hydroxyl radical based mechanism with lower efficiency. This detrimental effect, not observed in the present study, would probably appear at much higher concentration of H_2O_2 in which the competition for photon absorption would be significantly higher.

The measured consumption rate of H_2O_2 during the process was of 0.18 mg H_2O_2 L^{-1} min^{-1}, with a total consumption of only 11 % of the initial concentration of H_2O_2 after the 60 min of irradiation. This fact suggests that the slight enhancement in bacteria inactivation observed when adding H_2O_2 as compared to only UV-C may not necessarily be due to the formation of hydroxyl radicals but to a direct disinfectant effect of H_2O_2.

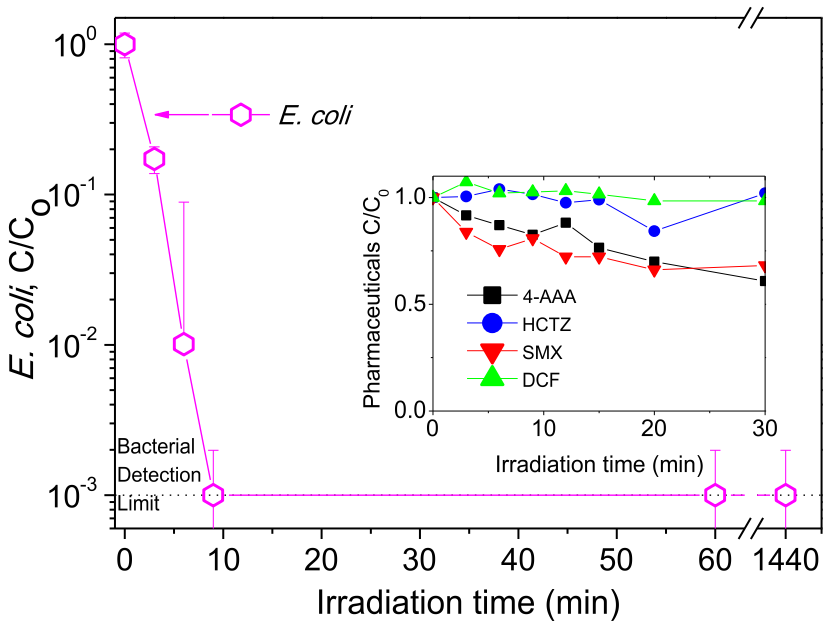

Figure 2. Simultaneous *E. coli* inactivation and pharmaceutical oxidation (inset) under UV-C radiation in the presence of H_2O_2 in a SWTP effluent. UV-C irradiation power: 0.11 W. Lamp switched off after 60 minutes of irradiation. Pharmaceuticals: 4-acetamidoantipyrine, 4-AAA; hydrochlorothiazide, HCTZ; sulfamethoxazole, SMX; diclofenac sodium salt, DCF.

Bacterial regrowth was not observed after 24 h in dark (Figure 2) confirming the irreversibility of the process reported by other authors (Yasar et al., 2007; Shang et al., 2009) when adding H_2O_2. It points out to the oxidizing properties of H_2O_2 as responsible for the irreversible damages in bacteria. The consumption of hydrogen peroxide during the 24 h in the dark was negligible, what means that a high concentration of H_2O_2 remains in the water at the end of the treatment. In addition, it must be noticed that reference dark experiments in presence of H_2O_2 (not shown) show a negligible *E. coli* inactivation suggesting that only the presence of H_2O_2 in water is not enough to lead to bacterial inactivation. Thus, these results might point out to the fact that the H_2O_2 that remains in the water after the process has a bacteriostatic effect, not inactivating bacteria but preventing bacterial reproduction when bacteria have been previously damaged. Shang et al. (2009) have also pointed out the effect of H_2O_2 repressing bacterial repair and reactivation although the mechanism is not clear.

Bacterial damages on the cell wall that would increase the permeability and consequently would allow the diffusion of H_2O_2 inside the cell are dismissed since the photolysis of H_2O_2 hardly occurs, which would have led to the formation of •OH, responsible for these cell wall damages. Thus, the presence of H_2O_2 in the water at the end of the process would be required to avoid the reactivation of bacteria, as the UV-C process responsible for bacteria inactivation does not produce irreversible damages.

Therefore, UV/H_2O_2 processes avoid the limitation of UV-C treatment, allowing the storage of water without bacteriological quality problems. The effect seems not to be based on the hydroxyl radical generation but on the bacteriostatic effect of the H_2O_2 in the water after the treatment, being the direct UV-C absorption the main mechanism responsible for bacteria inactivation. However, since high concentrations of H_2O_2 could remain in the treated water, its application would be limited due to the necessity of a post-treatment for removing the excess of H_2O_2.

Table 1 shows the values of kinetic constants obtained in the simultaneous inactivation of *E. coli* and oxidation of pharmaceuticals in a SWTP effluent for a UV-C/ H_2O_2 process at a higher irradiation power. Despite the H_2O_2 consumption rate was of 0.54 mg H_2O_2 L^{-1} min^{-1}, no significant differences in values of *k* for *E. coli* inactivation are observed between the use of UV-C radiation and UV-C/H_2O_2, confirming that the germicidal effect of UV-C absorption by the bacterial DNA is the main inactivation mechanism. This conclusion was also supported by additional experiments (not shown) of bacterial inactivation by UV-C/TiO_2 and UV-C/TiO_2/H_2O_2 processes. The UV-C power irradiation used for those processes corresponded to 0.11 W, and Degussa P25 TiO_2 and H_2O_2 concentration corresponded to 0.1 g L^{-1} and 100 mg L^{-1} respectively. In both cases, the addition of TiO_2 led to an increase in irradiation time required to totally inactivate bacteria. The presence of TiO_2 reduced the direct absorption of UV-C photons by bacteria, being this negative effect no counteracted by the formation of hydroxyl radical and other reactive oxygen species through a photocatalytic mechanism.

The addition of H_2O_2 did not seem to significantly accelerate the degradation of the pharmaceuticals either according to values of *k* except for HCTZ, the compound with lower absorption at 254 nm and consequently more susceptible of improvement on its degradation rate by a hydroxyl radical mechanism. In any case, the irradiation time required for the removal of all the pharmaceutical remains to be four times higher than that required for the inactivation of *E. coli* below the experimental detection limit (figure not depicted).

TABLE 1

3.3. UV-A/TiO_2.

In a previous work, Pablos et al. (2012) reported the simultaneous photocatalytic inactivation of bacteria and oxidation of pharmaceuticals at high concentration levels. Despite the tested concentrations were 2-3 orders of magnitude higher above the values typically found in wastewater treatment plant effluents used in the present study, significant conclusions were obtained from the non-photocatalytic reference experiments that can directly be extrapolated. No significant dark adsorption of any pharmaceutical was observed either onto TiO_2 in suspension or immobilized TiO_2. In contrast, *E. coli* seemed to be quite sensitive to simultaneous osmotic and mechanical stress, leading to a 2-log decay in the viable concentration of *E. coli* when using TiO_2 in suspension in the dark in deionized water. Photolytic experiments showed no degradation of any pharmaceutical either, but again *E. coli* seemed to be quite sensitive to the simultaneous irradiation and osmotic stress in deionized water. No effect was observed on the *E. coli* inactivation rate due to the presence of high concentrations of the studied pharmaceuticals, discarding possible toxic effects. The details of these experiments and their exhaustive discussion can be found elsewhere (Pablos et al., 2012).

Figure 3 shows the results of the photocatalytic oxidation of pharmaceuticals and inactivation of *E. coli* using TiO_2 in suspension, in deionized as well as in a SWTP effluent. It is clear that the inactivation of *E. coli* using UV-A/TiO_2 required a longer irradiation time that using UV-C. Moreover the composition of the water has played a significant role in the efficiency of the process, increasing the irradiation time required for the bacterial inactivation and pharmaceutical oxidation up to 3- and 5-fold, respectively. It agrees with the values of kinetic constant for bacterial inactivation and pharmaceutical oxidation (Table 2) which were notably reduced in a SWTP effluent compared to those in deionized water. In contrast, the UV-A/TiO_2 treatment showed two significant advantages: i) the complete elimination of the pharmaceuticals was achieved in the required effective disinfection time; and, ii) no bacterial regrowth was observed after 24 hours of dark storage of the water.

The use of TiO_2 immobilized in a fixed-bed reactor (Figure 4) led to an increase in the irradiation time required for both, the bacterial inactivation and the pharmaceuticals oxidation in deionized water compared to that of the slurry reactor. It agrees with a reduction as general trend in the values of *k* for both reactors in deionized water, shown in Table 2. However, in agreement with previous results (Pablos et al., 2011), the efficiency of this immobilized system for bacterial inactivation seems to be less negatively affected by the chemical composition of water. The irradiation times required to reach a complete bacterial inactivation and pharmaceutical oxidation in a SWTP effluent when using immobilized TiO_2 are similar compared to those of the TiO_2 in suspension. In fact, the values of *k* for bacterial inactivation and pharmaceutical oxidation were significantly higher in deionized water for the slurry reactor compared to those of the fixed-bed reactor (Table 2). In contrast, the differences in values of *k* in a SWTP effluent between both reactors were not so high. The values of pseudo-first order kinetic constants (*k*) of pharmaceutical oxidation in a SWTP effluent corresponded to 0.0074, 0.0108 and 0.0081 min^{-1} for 4-AAA, HCTZ and SMX respectively when using TiO_2 in suspension. Higher values are observed in TiO_2 immobilized, corresponding to 0.0146

(4-AAA), 0.0177 (HCTZ) and 0.0184 min^{-1} (SMX). Both systems (slurry and fixed-bed reactor) also led to similar bacterial kinetic constants in a SWTP effluent (3.67 ± 0.86 and 2.92 ± 0.20 x 10^4 CFU L^{-1} min^{-1} respectively), calculated according to an inactivation mechanism based on sequential •OH attacks on the cell membrane (Marugán et al., 2008). This comparable efficiency observed for both photocatalytic systems was discussed in a previous work (Pablos et al., 2011) and attributed to the bacteria-catalyst interaction since the bacteria-TiO_2 contact is the main difference between both reactors.

In any case, UV-A/TiO_2 processes seemed to be more sensitive to the presence of ions and organic matter in the water than the UV-C disinfection processes due to the photocatalytic bacterial inactivation mechanism based on hydroxyl radicals, scavenged by these species in water, in comparison with UV-C disinfection based on direct DNA damages. In contrast, the mechanism based on hydroxyl radical seemed to lead to irreversible damages in the microorganisms, allowing a long-term storage of the treated water, and guaranteeing that a complete removal of the pharmaceuticals can be simultaneously achieved.

Summarising, the UV-A/TiO_2 process has been less efficient in terms of time of irradiation required to totally inactivate bacteria and degrade pharmaceuticals as compared to UV-C radiation. However, a UV-A/TiO_2 process seems to be more efficient in terms of allowing the use of the disinfection process for additionally degrading pharmaceuticals and guaranteeing the absence of viable bacterial regrowth for longer.

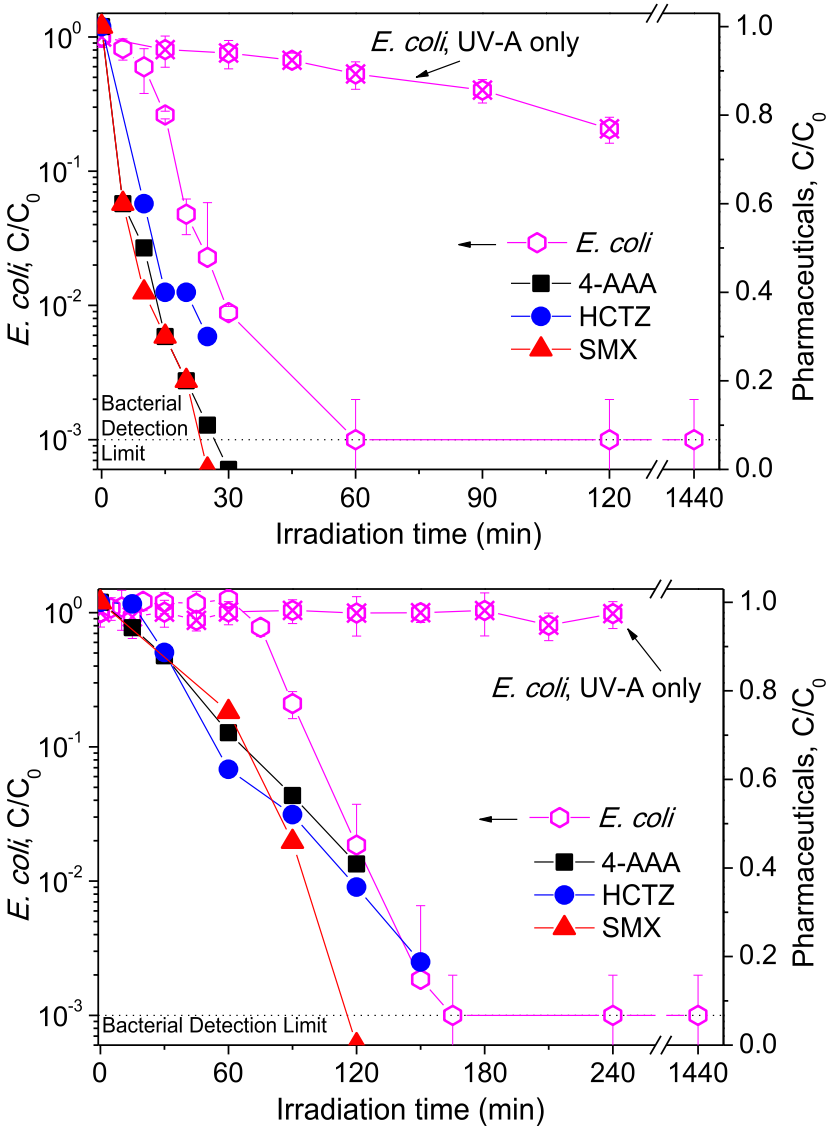

Figure 3. Simultaneous photocatalytic inactivation of *E. coli* and oxidation of pharmaceuticals with TiO_2 in suspension in deionized water (top) and a SWTP effluent (bottom). Pharmaceuticals: 4-acetamidoantipyrine, 4-AAA; hydrochlorothiazide, HCTZ; sulfamethoxazole, SMX.

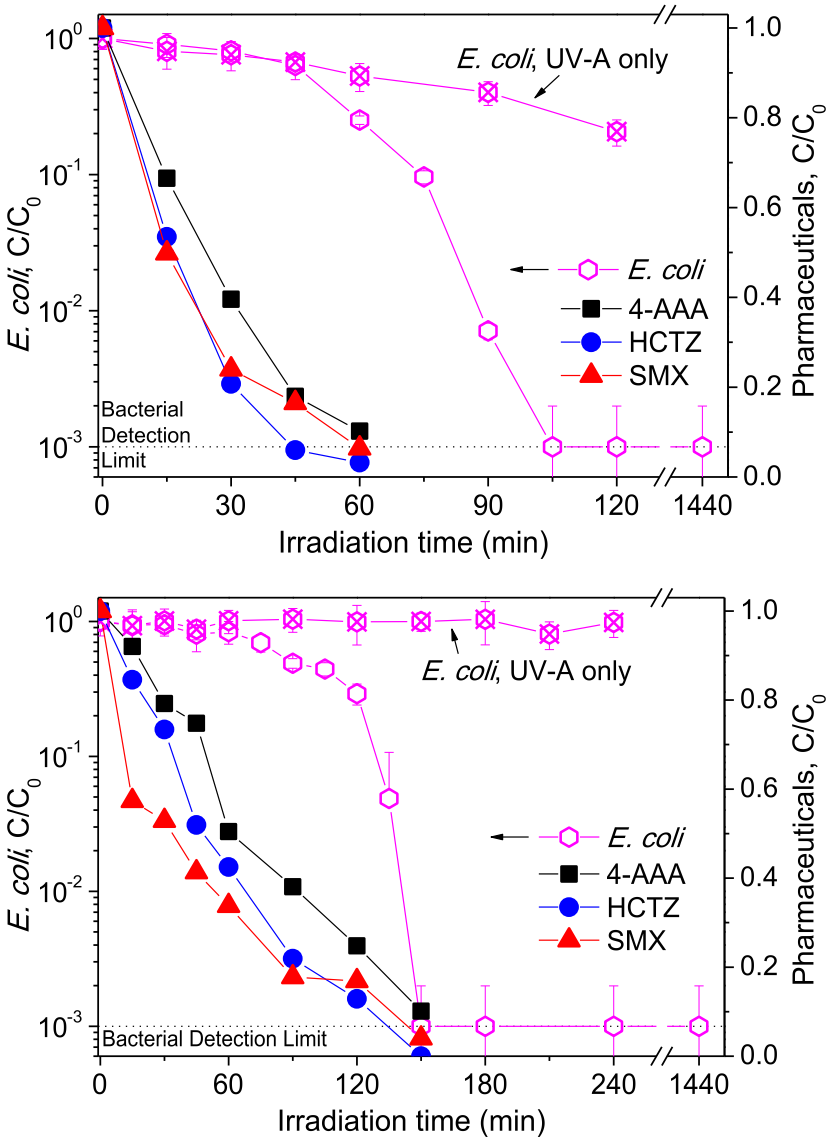

Figure 4. Simultaneous photocatalytic inactivation of *E. coli* and oxidation of pharmaceuticals with TiO_2 immobilized in a fixed-bed reactor in deionized water (top) and a SWTP effluent (bottom). Pharmaceuticals: 4-acetamidoantipyrine, 4-AAA; hydrochlorothiazide, HCTZ; sulfamethoxazole, SMX.

3.4. UV-A/TiO_2/H_2O_2.

Reference experiments of *E. coli* inactivation in the presence of H_2O_2 (not shown) indicated that a significant effect in the decrease of viable bacteria was only observed in deionized water for both, in the dark and under irradiation with UV-A. In contrast, a negligible effect was observed under both conditions in a SWTP effluent, suggesting that the simultaneous osmotic stress is required to weaken the bacterial cell, decreasing their resistance to oxidative stress by increasing the permeability of the membrane to H_2O_2. This absence of disinfectant effect of H_2O_2 in SWTP effluents is in agreement with other authors (Rincón and Pulgarín, 2004; Mamame et al., 2007; Paleologou et al., 2007). Concerning the oxidation of the pharmaceuticals, no significant effect of H_2O_2 addition was observed either in the dark or under UV-A irradiation for both, deionized water and SWTP effluent. Consumption of H_2O_2 was not observed either in photolytic and adsorption in dark reference experiments.

Figure 5 shows the UV-A/TiO_2/H_2O_2 efficiency in the simultaneous inactivation of *E. coli* and oxidation of pharmaceuticals. The H_2O_2 consumption rate corresponded to 0.31 mg H_2O_2 L^{-1} min^{-1}. With regard to pharmaceuticals, all of them are successfully oxidized by the combination of photocatalysis with hydrogen peroxide in SWTP effluents for both photocatalytic reactor configurations. The values of pseudo-first order kinetic constants of pharmaceutical oxidation in a SWTP effluent shown in Table 2 corresponded to 0.0119, 0.0176 and 0.0123 min^{-1} for 4-AAA, HCTZ and SMX respectively when using TiO_2 in suspension, and 0.0172, 0.0297 and 0.0227 min^{-1} when using immobilized TiO_2. It is worth noticing that the fixed-bed reactor required a similar irradiation time compared to that of the slurry reactor to reach a complete oxidation of the parent pharmaceuticals. Although the rate of oxidation of pharmaceutical compounds slightly increased by adding H_2O_2, this fact did not involve a remarkable reduction in irradiation time as compared to the UV-A/TiO_2 treatment. Therefore, the reduction in charge carrier recombination and the increase in the number of

hydroxyl radicals produced by the presence of H_2O_2, as well as its higher efficiency as electron acceptor than O_2 did not seem to notably enhance the oxidation of pharmaceuticals compared to that corresponding to UV-A/TiO_2. Even, if considering the possibility of the direct oxidation of H_2O_2 by holes, it leads to species with a lower oxidizing power such as HO_2^- as compared to •OH, which might be responsible for not obtaining an increase in the efficiency of the process (Wang and Hong, 1999; Paleologou et al., 2007). In contrast to UV-C/H_2O_2, where the addition of H_2O_2 only led to the oxidation of pharmaceuticals not absorbing at 254 nm, the photocatalytic oxidation of all pharmaceuticals was improved by adding H_2O_2. However, the oxidation process is still slower for UV-A/TiO_2/H_2O_2 as compared to UV-C/H_2O_2, which agrees with the higher values of *k* for pharmaceutical oxidation obtained for high UV-C (1.63 W)/H_2O_2 (Table 1) in comparison with those of UV-A/TiO_2/H_2O_2 (Table 2).

On the contrary, *E. coli* inactivation is really improved by addition of H_2O_2 in comparison with the UV-A/TiO_2 process, since the irradiation time required is notably reduced to 45 min. It agrees with the values of *k* for photocatalytic bacterial inactivation in presence of H_2O_2 which noticeably increased when adding H_2O_2 (UV-A/TiO_2/H_2O_2) in comparison with those without H_2O_2 (UV-A/TiO_2) for both reactors (Table 2). These results are in agreement with those observed by several authors (Hartman and Eisenstark, 1978; Rincón and Pulgarín, 2004; Paleologou et al., 2007; Lanao et al., 2010) although Bayliss and Waites (1980) reported that there is no enhancing effect on *E. coli* inactivation due to the presence of hydrogen peroxide since *E. coli* catalase enzymatic activity destroys H_2O_2 before its diffusion into the cell. Since a great difference in the efficiency achieved by the addition of H_2O_2 has been observed between pharmaceuticals and bacteria, it suggests that *E. coli* inactivation should not only be due to an increase in the hydroxyl radical generation rate. The attacks of the hydroxyl radicals to the cell wall might increase the permeability of H_2O_2 inside

the cell, allowing a direct inactivation mechanism without equivalent in the case of pharmaceuticals oxidation. To confirm this hypothesis, two parallel experiments of bacteria partial inactivation by UV-A/TiO_2 were carried out, either adding or not H_2O_2 after switching off the lamp. When hydrogen peroxide was present, it was observed that viable concentration of bacteria decreased up to the detection limit for 15 min in dark. In contrast, if there was no addition of H_2O_2, viable concentration of bacteria remained constant, confirming that the increase in the permeability of *E. coli* cell wall took place during the photocatalytic treatment, allowing the diffusion of H_2O_2 inside the cell and leading to the enhancement of *E. coli* inactivation.

Therefore, the addition of H_2O_2 significantly reduced the irradiation time required for bacteria inactivation but not for the removal of the pharmaceuticals, whose elimination can not be guaranteed at the end of the disinfection process.

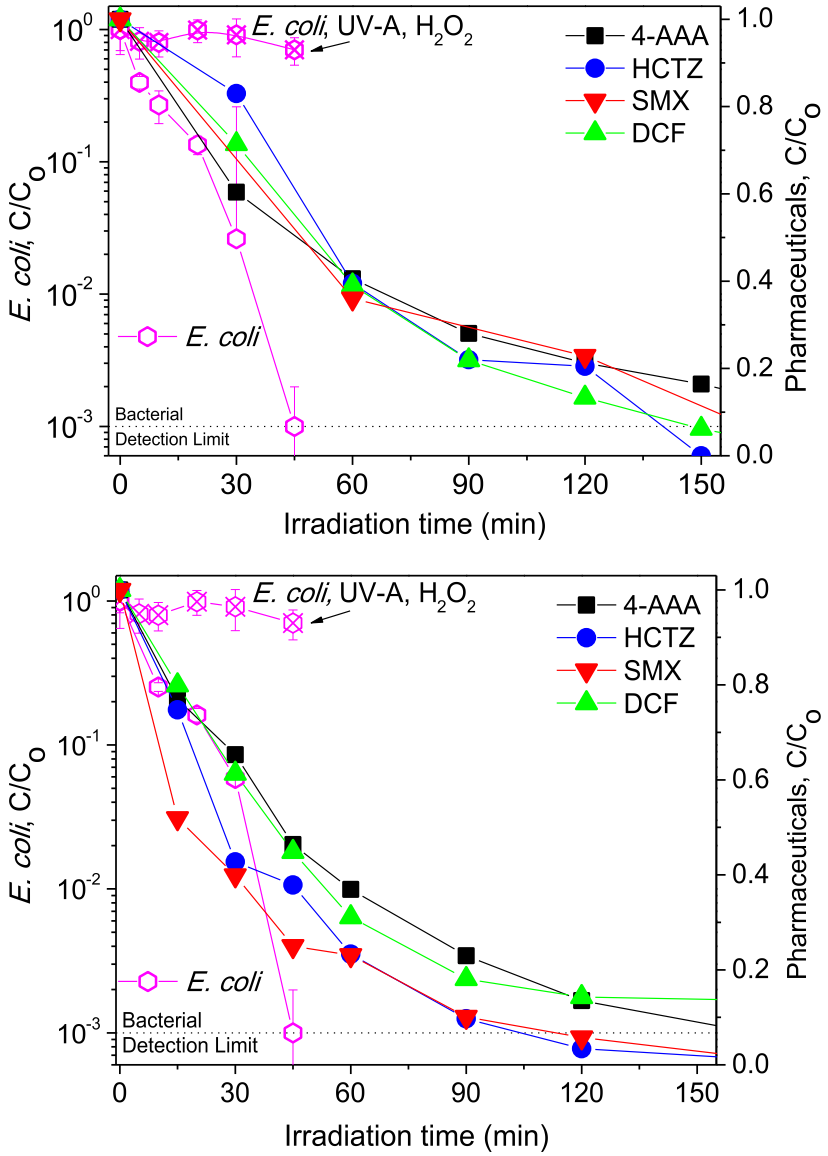

Figure 5. Simultaneous photocatalytic inactivation of *E. coli* and oxidation of pharmaceuticals in a SWTP effluent in the presence of H_2O_2 with TiO_2 in suspension (top) and with TiO_2 immobilized in a fixed-bed reactor (bottom). Pharmaceuticals: 4-acetamidoantipyrine, 4-AAA; hydrochlorothiazide, HCTZ; sulfamethoxazole, SMX; diclofenac sodium salt, DCF.

4. Conclusions

In conclusion, for comparable power consumption, UV-C/H_2O_2 seems to be the most adequate technology for water disinfection and reuse for industrial applications, in which a faster process would show significant advantages. However, it have to overcome disadvantages related with cost such as UV-C and H_2O_2 availability, the need of monitoring both, microbiological an chemical quality of the water during the process, and removal of H_2O_2 before the final use of the water. The optimization of the initial concentration of H_2O_2 is required to set up this technology as a commercial process. In contrast, since the UV-A/TiO_2 process shows a lower photonic efficiency as compared to UV-C/H_2O_2, its use would be preferable for applications in which a high availability of natural UV-A solar radiation is guaranteed. In addition, the UV-A/TiO_2 process avoids the consumption of electricity and H_2O_2, and the removal of pharmaceuticals and possibly other micropollutants could be assured along the disinfection process, without the need of monitoring. It also allows a long-term storage of the water without the necessity of adding chemicals. These factors become important when the production of drinking water is addressed to rural areas of developing countries as well as if tertiary water treatments are required for water regeneration purposes.

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Table 1. Simultaneous *E. coli* inactivation and pharmaceutical oxidation under UV-C radiation and UV-C/H_2O_2 in deionized water and a SWTP effluent. UV-C irradiation power: 0.11 and 1.63 W.

Pollutant	Kinetic constant, k (min^{-1})				
	UV-C (0.11 W)		UV-C (1.63 W)	UV-C/H_2O_2 (0.11 W)	UV-C/H_2O_2 (1.63 W)
	Deionized water	SWTP Effluent	SWTP Effluent	SWTP Effluent	SWTP Effluent
E. coli[1]	0.440	0.460	2.30	0.758	2.30
4-AAA[1]	0.0105	0.0076	0.498	0.0041	0.675
HCTZ[1]	0.0014	0.0002	0.068	0.0018	0.303
SMX[1]	0.0158	0.0047	0.376	0.0068	0.570
DCF[1]	0.0068	0.0025	0.447	0.0018	0.552

[1]Pseudo-first order kinetic constant (*E. coli*; 4-acetamidoantipyrine, 4-AAA; hydrochlorothiazide, HCTZ; sulfamethoxazole, SMX; diclofenac sodium salt, DCF).

Table 2. Simultaneous photocatalytic inactivation of *E. coli* and oxidation of pharmaceuticals in deionizaed water and a SWTP effluent with TiO_2 in suspension and with TiO_2 immobilized in a fixed-bed reactor (UV-A/TiO_2) and in the presence of H_2O_2 (UV-A/TiO_2/H_2O_2).

Pollutant	Kinetic constant, k (CFU L^{-1} min^{-1} x 10^4; min^{-1})					
	UV-A/TiO_2 (Slurry reactor)		UV-A/TiO_2 (Fixed-bed reactor)		UV-A/TiO_2/H_2O_2 (Slurry reactor)	UV-A/TiO_2/H_2O_2 (Fixed-bed reactor)
	Deionized water	SWTP Effluent	Deionized water	SWTP Effluent	SWTP Effluent	SWTP Effluent
E. coli[1]	12.76	3.67	4.65	2.92	11.73	9.85
4-AAA[2]	0.0875	0.0074	0.0392	0.0167	0.0119	0.0146
HCTZ[2]	0.0476	0.0108	0.0604	0.0177	0.0149	0.0297
SMX[2]	0.0782	0.0081	0.0441	0.0178	0.0107	0.0222
DCF[2]	–	–	–	–	0.0216	0.0123

1Bacterial inactivation kinetic constant based on sequential •OH attacks on the membrane (Marugán et al., 2008).

[2]Pseudo-first order kinetic constant (4-acetamidoantipyrine, 4-AAA; hydrochlorothiazide, HCTZ; sulfamethoxazole, SMX; diclofenac sodium salt, DCF).