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Sulfamethoxazole abatement by means of ozonation

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Abstract

Sulfamethoxazole (SMX) is a bacteriostatic antibiotic largely used for diverse types of illness. Its widely use in humans and even in animals releases unmetabolized and active metabolites that have a strong potential in terms of effect in organisms. In this work, 200 mg L^{-1} solution of sulfamethoxazole was treated by ozonation at different pH. Results showed that ozonation was proved to be an efficient method to degrade sulfamethoxazole. After 15 min of ozonation (corresponding dose = 0.4 g of ozone L^{-1}), the complete antibiotic abatement was almost achieved with just 10% of mineralization. The biodegradability and toxicity of the ozonation intermediates were also studied. A biodegradability enhancement (increment of BOD_5/COD ratio) from 0 to 0.28 was observed after 60 min of ozonation. The acute toxicity of the intermediates was followed by the Microtox® test and the toxicity profile showed a slight acute toxicity increment in the first stage of ozonation. The pH variation had an important role in the TOC and COD removal, promoting their growth with the increment of alkalinity. The second order kinetic constants for the ozonation of the SMX in an order of magnitude of 10^5 L mol^{-1} s^{-1} were also determined for pH 5 and 7.

Keywords: Pharmaceuticals; Antibiotic; Ozonation; Sulfamethoxazole; Biodegradability; Kinetic

1. Introduction

Nowadays, it could be observed a recent concern growth about the emerging contaminants presence in different types of water [1,2]. Among the pollutants of the lists, various pharmaceuticals can be found. Some classes of antibiotics and hormones have been detected in surface water and sewage wastewater treatment plants (WWTP) effluents [3–5]. Antibiotics deserves a special concern due to their widely use and even for the specific effect like bacterial resistance caused by the human contamination via environmental exposure [6]. Their presence in the environment is directly related with the resistance to antibiotic showed by bacteria in natural media [7]. The pollutants removal by biological treatment is correlated with characteristics like toxicity, biodegradability and inhibition. Thus, the presence of an antibiotic in a WWTP could even disturb the removal of the rest of organic matter content. In addition, the lack of appropriated detections methods of pharmaceuticals and their metabolites is also a problem for a suitable treatment [8].

Sulfamethoxazole (SMX) in combination with trimethoprim is largely used to treat respiratory diseases like pneumonia. Coccidiosis, diarrhea, gastroenteritis are well known illnesses that can also be treated with sulfamethoxazole. A large number of animals are also treated by a combination of drugs containing sulfamethoxazole, generating residues by the excreting of unmetabolized or active metabolites [9,10]. Concerned about this, many countries have establishing 1 mg kg^{-1} the maximum residue levels for this compound in food products [11]. Ozone is a strong oxidant that undergoes self-decomposition in water to release hydroxyl free radical that has a stronger oxidizing capability than ozone [12]. Therefore, ozonation is an efficient method capable to degrade several xenobiotic compounds and transforms refractory natural organic matter to biodegradable form, i.e. biodegradable dissolved organic carbon [13–16]. Ozonation has been demonstrated to be successfully used to treat water containing antibiotics [17] and also as a pretreatment step to improve the biodegradability of wastewater containing them [18].

The aim of this work was to study the degradation of 200 mg L^{-1} SMX aqueous solutions by means of ozonation. TOC, BOD_5, COD, UV_{254} absorbance and SMX concentration analysis were carried out in order to follow its abatement. BOD_5/COD ratio was used as biodegradability indicator. The

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acute toxicity of intermediates was followed by means of Microtox® test according to the standard procedure and a second-order kinetic constant for the SMX ozonation at pH 5 and 7 was calculated.

2. Materials and methods

Ozonation experiments were carried out in a 1.2-L reactor with a continuous supply of O_3 (2.04 g h^{-1}). Ozone was generated by means of a Sander Labor Ozonizator (Germany) using oxygen as a feeding gas. The gaseous outlet from the reactor was led to a killer, where the remaining ozone was destroyed by means of a reaction with KI. SMX solutions containing 200 mg L^{-1} were charged into the reactor. A scheme and description of the experimental device may be found elsewhere [19]. For experiments carried out at fixed pH, SMX solutions were buffered by the addition of adequate quantities of Na_2HPO_4, H_3PO_4 and KH_2PO_4. To avoid the interference of the radical pathway on the SMX ozonation, some runs were performed in presence of *t*-butanol. Ozonation experiments lasted 1 h, and during this period of time samples were withdrawn from the reactor and quickly analyzed.

The reactants used in this study were supplied by Sigma–Aldrich (Germany). Total organic carbon (TOC) was analyzed by means of a Shimadzu 5055 TOC analyzer. The concentration of the SMX was quantified by means of a high performance liquid chromatography (HPLC) supplied by Waters Corporation (Massachusetts, USA). The column used was a TR-016059 supplied by Tecknokroma S. Coop. C. Ltd. (Barcelona, Spain) with a length of 250 mm and an inner diameter of 4.6 mm. The mobile phase used was a mixture of acetonitrile and millipore water at a 40:60 volume ratio, acidified at pH 3 by the addition of phosphoric acid. The wavelength used in the UV detector was 270 nm. To determine the mass of the intermediates during ozonation, an Agilent 6890 HPLC coupled with a Delta Plus Finnigan MAT Mass Spectrophotometer was used. To follow the biodegradability of the samples, the biological oxygen demand (BOD_5) (by means of an Oxitop system, Standard method, 5210 D) and the chemical oxygen demand (COD) (Standard method, 1250 D) analysis were carried out. The ratio BOD_5/COD has been chosen as biodegradability indicator. The acute toxicity test was carried out in the Microtox® M500 toxicity analyzer according to the standard procedure for the basic test (Azur Environmental, Delaware, USA).

3. Results and discussion

3.1. Sulfamethoxazole abatement, biodegradability and toxicity of intermediates

Fig. 1 shows the ozonation profile of a 200 mg L^{-1} SMX solution at free pH during 1 h of reaction. According to the degradation profile, an ozone dosage of 0.4 g L^{-1} (15 min of reaction) was enough to achieve almost a complete SMX abatement (up to 98.6%). After this period of time, the antibiotic removal rate becomes lower due to the low SMX concentration available in the medium to react with the ozone. From 20 min of ozonation

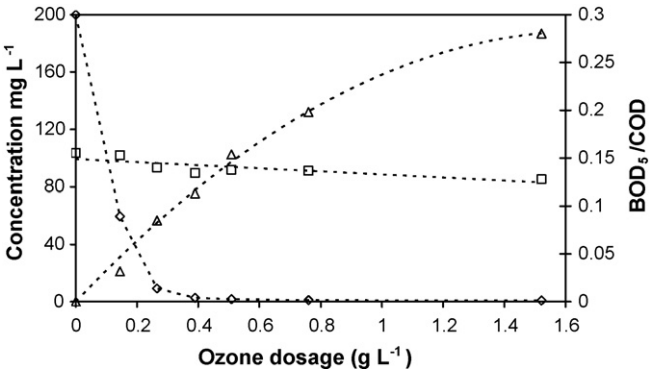

Fig. 1. Ozonation of sulfamethoxazole. (◊) SMX concentration; (□) TOC concentration; (△) ratio BOD_5/COD. 20 °C and pH no adjusted.

(0.5 g L^{-1} of ozone dose), the SMX concentration was lower than 1 mg L^{-1}. Taking into account the moles of ozone needed to remove 95.5% of the SMX present in the solution (10 min of ozonation = 0.26 g L^{-1} of O_3 dose), a stoichiometric coefficient of approximately 2 moles O_3/mol SMX was calculated. At the end of the ozonation time (60 min) only an 18% of TOC was removed, indicating that a high ozone dosage should be used for achieving a complete mineralization in a reasonable reaction time. This fact supposes that ozonation presents a high efficiency to remove SMX but not to mineralize the by-products produced along the reaction. One viable alternative to reduce the ozonation costs would be the combination with biological treatment. Thus, to verify the biodegradability of intermediates in the course of ozonation, the ratio BOD_5/COD at different ozonation times was followed. The analysis carried out all along the run showed that after 60 min of ozonation (1.5 g L^{-1} of ozone dose) the biodegradability increases up to values near 0.3 indicating a good conversion of the antibiotic to biodegradable products.

In order to assess the acute toxicity in natural media caused by intermediates produced during the SMX ozonation, Microtox® test was carried out. Microtox® is a test that measures the inhibition of the light emission of bioluminescent bacteria (*Vibrio fischeri*) caused for the presence of toxic compounds. The obtained data was used to calculate the EC_{50}, which is the percentage of sample dilution (v/v) that causes a 50% reduction in bacteria bioluminescence [20,21]. In Fig. 2, the variation of the EC_{50} with time for the ozonation of a SMX solution is pre-

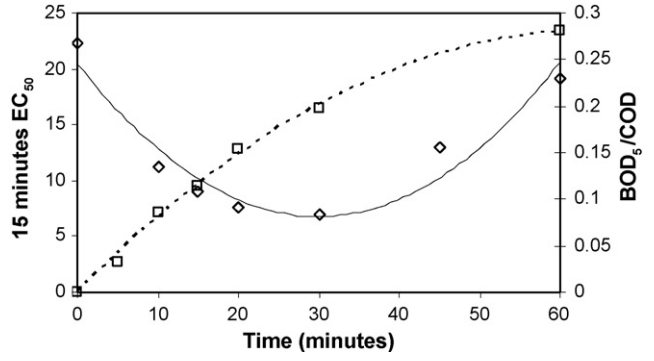

Fig. 2. Biodegradability and toxicity evolution for the SMX ozonation. (□) Ratio BOD_5/COD; (◊) 15 min EC_{50}. 20 °C and pH no adjusted.

sented and compared with the biodegradability profile. Since lower EC_{50} value implies more inhibition to the bacteria [22], the toxicity profile showed that in the first 30 min of reaction the ozonation of SMX induces the formation of intermediates with higher acute toxicity than the SMX untreated solution. After this point, the formation of secondary intermediates promotes an EC_{50} increment to values near the initial value. However, the biodegradability indicator increases constantly along the ozonation time. Thus, it could be stated that the ozonation of SMX produces more biodegradable by-products that will facilitate the organic matter biodegradation in a wastewater treatment plant. Nevertheless, short ozonation times could induce an increment of the acute toxicity for some organisms. The discrepancy between the biodegradability indicator and the acute toxicity profile could reside in the sensibility of the microorganisms used in the cited tests. While the bacteria used in the microtox basic test (*V. fischeri*) are sensitive marine bacteria, the seed used to carry out the BOD tests are a community of microorganisms, which are supposed to be much more adaptable and resistant to different substrates. This fact along with the difference in the contact time used in these tests, 15 min for microtox and 5 days for the BOD could let to different bacteria responses in terms of inhibition.

3.2. Understanding the ozone reaction with sulfamethoxazole

It is well known that compounds presenting in their structures double bonds and aromatic rings readily react with ozone [23]. In Fig. 3, the molecular structure of the sulfamethoxazole is presented with its dissociation pathway ($pK_{a1} = 1.8$; $pK_{a2} = 5.57$) [24]. Observing the structure of the SMX, the possible reaction centers that are more susceptible to ozone electrophilic attack can be deduced. The amino group seems to be the primary site where ozone attack can be expected. However, the pH of the medium has an important role on the mechanism of reaction. At pH lower than the pK_{a1} value (1.8) the nitrogen of the amino group is in a protonated form, which is less susceptible to ozone attack. On the other hand, at pH > 3.8 the non-protonated amine is the predominant species. When the pH reaches values higher than the second pK_a, the dissociation of the hydrogen present in the sulfonamide group promotes a slight increment of the SMX reactivity.

In order to better understand the reaction mechanism of the SMX ozonation, the by-products identification by means of a HPLC-MS was attempted. From the obtained chromatograms, four mass peaks were detected (I = 283 *m/z*, II = 271 *m/z*, III = 227 *m/z* and IV = 196 *m/z*). The mass peaks 271 (+18) and the 283 (+30) could indicate a possible hydroxylation of the aromatic amino group and/or aromatic ring. The structure of the product ions with a mass of 227 (−26) *m/z* and 196 (−57) *m/z* could not be identified with only the use of HPLC-MS. However, it is possible that these fragments are originated by the cleavage of the aromatic ring. The final products of SMX ozonation that are supposed to be compounds like organic acids, aldehydes and ketones with low molecular weight could not be identified by the used method.

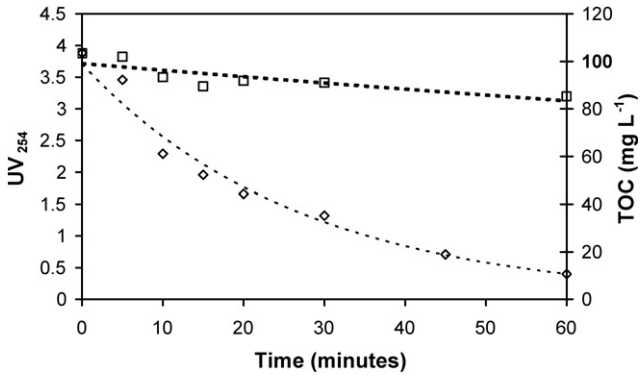

Fig. 4. UV_{254} absorbance (◊) and TOC (□) vs. time for the ozonation of SMX. 20 °C and pH no adjusted.

In order to follow qualitatively the content of aromatic intermediates along the reaction, the UV_{254} absorbance, which representing the aromatic content of wastewater [25] was determined. In Fig. 4, the UV_{254} absorbance is plotted against time along with the TOC for a run carried out with a 200 mg L^{-1} SMX solution without pH adjustment. The decrease of absorbance with time indicates that ozonation reduces considerable the quantity of aromatic intermediates in the medium, achieving values higher than 80% of UV_{254} absorbance removal. However, it is evident that only a low percentage of TOC was removed at the end of 60 min of reaction (18%). These results show that at the end of ozonation time a high quantity of organic compounds was still present. Nevertheless, those compounds have a small aromatic character. An important fact to remark is that since the aromatic content is directly related to the presence of toxic and/or non-biodegradable compounds, its reduction is in agreement with the increment of biodegradability previously shown.

3.3. pH and temperature influence

To study the pH influence on the ozonation of SMX, runs with 1 L of 200 mg L^{-1} SMX solution buffered with adequate quantities of Na_2HPO_4 and K_2HPO_4 in presence and absence of hydroxyl radical scavenger *t*-butanol were carried out. Table 1 presents the data recorded during the runs performed. TOC, COD and UV_{254} absorbance removals are the values at the end

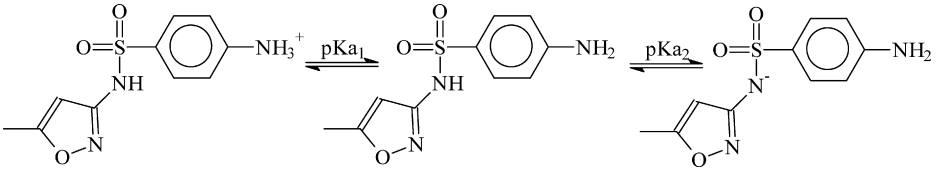

Fig. 3. Structure of sulfamethoxazole and its dissociation.

Table 1
Data recorded after 60 min of SMX ozonation at different pH

pH	*t*-Butanol	TOC removal (%)	COD removal (%)	UV_{254} removal (%)
3	–	10	48	78
7	–	25	63	86
11	–	31	79	84
3	+	–	–	79
7	+	–	–	79
11	+	–	–	82
Free	–	18	40	81

of 60 min of ozonation (1.5 g h^{-1} of O_3 dosage). The runs carried out in absence of *t*-butanol showed similar trend of TOC and COD removals, increasing with the pH. This fact can be explained for the formation of hydroxyl radicals, which are more powerful than ozone to oxidize a wide range of organic and inorganic compounds and besides react in a non-selectively way [26]. Experiments in presence of *t*-butanol had TOC and COD measurements disturbed by the presence of carbons proceeding from the *t*-butanol. Both experiments in absence and in presence of *t*-butanol achieved a high UV_{254} removal, although no major changes with the increment of the pH were observed.

Ozonation at different temperatures (10, 20 and 30 °C) in absence of *t*-butanol and without pH adjustment were also carried out. Nevertheless, the temperature variation did not show any changes in the degradation behavior (results not shown).

3.4. Determination of the kinetic constant

To calculate the kinetic constant of the SMX ozonation, buffered solutions containing both fumaric acid (FA) and SMX in concentration of 0.5 mmoles L^{-1} were ozonated and their concentrations were followed with time. In Fig. 5, an example of a run is presented. The runs were carried out at pH 5 and 7 in presence of *t*-butanol and each run was performed three times to ensure reproducibility. The method applied is based on the comparison between the degradation rate of SMX and that of a reference compound, which in this case was the fumaric acid.

Considering that the kinetic of both SMX and FA follow the equations:

$$\frac{d[SMX]}{dt} = -k_{SMX} \cdot [O_3] \cdot [SMX] \quad (1)$$

$$\frac{d[FA]}{dt} = -k_{FA} \cdot [O_3] \cdot [FA] \quad (2)$$

Dividing Eq. (1) by Eq. (2) and solving the resulting integration, Eq. (3) is obtained:

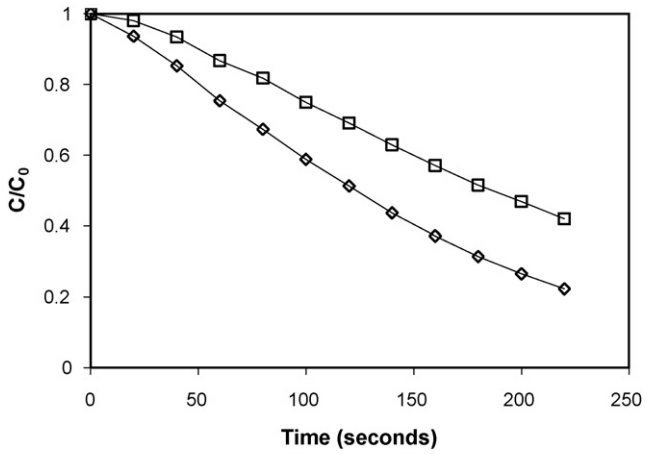

Fig. 5. Ozonation of SMX 0.5 mmol L^{-1} + FA 0.5 mmol L^{-1}. 20 °C, I = 0.1 M, pH 5. (□) FA; (◊) SMX.

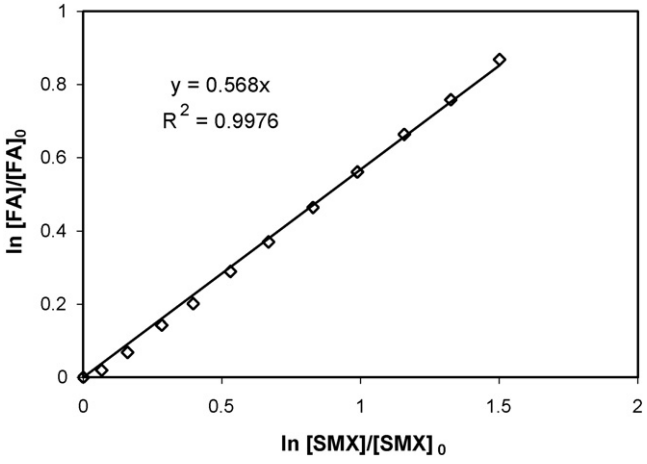

Fig. 6. Neperian logarithm of the normalized concentration of FA and SMX. 20 °C, I = 0.1 M, pH 5.

$$\ln \frac{[FA]}{[FA]_0} = -\frac{k_{FA}}{k_{SMX}} \ln \frac{[SMX]}{[SMX]_0} \quad (3)$$

To calculate the ratio k_{FA}/k_{SMX} the neperian logarithm of the normalized concentration of both compounds was plotted thus obtaining a straight line (Fig. 6). As the k_{FA} for the studied pH values are known from the literature [27,28], the k_{SMX} can be calculated.

In Table 2, calculated values of k_{SMX} are presented along with the values of the ratio k_{FA}/k_{SMX} recorded from the experimental data and the k_{FA} found in literature.

A slight increase of the kinetic constants values was observed when the pH passes from 5.0 to 7.0 can be attributed to the dissociation degree of the SMX molecule. At pH 5 the sulfonamide

Table 2
Calculated values of k_{SMX} (L mol^{-1} s^{-1})

pH	Ratio K_{FA}/k_{SMX}	K_{FA}—Hoignè and Bader [27]	K_{FA}—Benbelkacem et al. [28]	k_{SMX}
5.0	0.57(±0.005)	1×10^5	–	1.8×10^5
7.0	0.34(±0.005)	–	1.5×10^5	4.4×10^5

T = 25 °C, I = 0.1 M.

group is in a protonated form witch consequently may reduces the reactivity of the molecule. At pH values higher about two unites than the pK_{a2} (5.57) practically all the SMX molecules are present in a complete dissociated form, what gives higher reactivity to the structure.

4. Conclusions

Ozonation was proved to be a suitable method to remove sulfamethoxazole antibiotic in water. However, it is stated that a high ozone dosage would be necessary to achieve the complete mineralization of the intermediates. An increment of the biodegradability indicator (BOD_5/COD) from 0 up to 0.28 after 60 min of ozonation was observed. Regarding the acute toxicity, the trend of the EC_{50} values along the ozonation indicates that the SMX ozonation favor, in the early stages of ozonation (first 30 min), the formation of intermediates with higher acute toxicity than the SMX untreated solution. However, at longer ozonation times the acute toxicity increase to a vicinity of an initial value. Therefore, from the overall results it could be stated that the combination of ozonation with conventional biological treatment would be a suitable option to mineralize water contaminated with sulfamethoxazole, although ozonation pretreatment would not decrease its acute toxicity to natural media.

The ozone attack on the SMX molecule probably occurs first on the amino group and aromatic ring, giving by-products with mass equals to 283, 271, 227 and 196 *m*/*z*. However, their structures could not be determined only with the use of the HPLC-MS. In absence of *t*-butanol, TOC and COD removals increased with the pH. However, the aromaticity of the samples did not undergo important variation with pH increment. Temperature variation in the range between 10 and 30 °C had no significant effect on SMX degradation. The kinetic constants calculated for the SMX are in an order of magnitude of 10^5 L mol^{-1} s^{-1}. Furthermore, a slight increment of the constant is observed when the pH passes from 5 to 7.

Acknowledgements

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