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Sulfamethoxazole abatement by photo-Fenton Toxicity, inhibition and biodegradability assessment of intermediates

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Abstract

The objective of this work was to study the abatement of 200 mg L^{-1} sulfamethoxazole (SMX) solution by means of photo-Fenton process. Biodegradability of the treated solutions was followed by the ratio biochemical oxygen demand at five days/chemical oxygen demand (BOD_5/COD) and toxicity by Microtox® and inhibition tests. Experiments with different initial concentration of H_2O_2 were carried out. The initial amount of Fe^{2+} and pH of the solution were set at 10 mg L^{-1} and 2.8 respectively. The temperature of the reactor was kept constant in all the experiments (25 ± 0.8 °C). Photo-Fenton process is thought to be a successful treatment step to improve the biodegradability of wastewater containing SMX. The complete antibiotic removal was achieved for a H_2O_2 dose over 300 mg L^{-1}. Biodegradability (BOD_5/COD) rose from zero (SMX solution) to values higher than 0.3 (treated solutions). Toxicity and inhibition tests pointed out in the same direction: oxidized intermediates for initial H_2O_2 dose over 300 mg L^{-1} showed no toxicity effects on pure bacteria and no inhibition on activated sludge activity.

Keywords: Photo-Fenton; Sulfamethoxazole; Antibiotic; Biodegradability; Toxicity; Inhibition

1. Introduction

In the beginning of the 21st century, the lack of water is one of the biggest concerns. The shortage of fresh pure water affects more than 25% of the world population and according to the World Health Organization (WHO), every year, 2.2 millions people die because of this reason [1]. Additionally, and especially in developed countries, large amounts of water are being polluted due to industrial activity and domestic use. Restoring the quality of wastewater is essential in order to avoid further pollution of the environment and also, to allow the reuse of this water, decreasing thus the fresh water consumption. Reflecting a new environmental conscience, the European Directive 2000/60/CE pointed out the necessity of a progressive reduction of pollutants in water effluents. Antibiotics are one of the numerous recalcitrant pollutants present in aqueous medium that might not be treatable in the biological step of the sewage treatment plants, because of their antibacterial nature [2]. Furthermore, the presence of antibiotics in wastewaters has increased in the last years and their abatement will be a challenge in the near future. Among all the emerging organic pollutants, antibiotics are those, which have become of increasing concern, due to the possible appearance of resistant bacterial cultures as a consequence of their extensive use.

Advanced oxidation processes (AOP) have proved to be highly effective for the removal of most of the pollutants in wastewaters [3]. Photo-Fenton reaction is also well-known in the literature. It is an efficient method for wastewater and soil treatment [4,5]. Photo-Fenton is known to be able to improve the efficiency of dark Fenton reagent by means of the interaction of radiation with Fenton's reagent [6]. Hydroxyl radicals are produced by the decomposition of hydrogen peroxide when reacting with ferrous ions in presence of UV light, which contributes in an additional pathway to the generation of free radicals, increasing the concentration of hydroxyl radicals [7]. It should be pointed that the recognition of the $^{\bullet}OH$ radical as the active intermediate is not yet universal, and even doubts as to its very existence in the system have been raised [8].

Only a few works dealing with the treatment of water containing sulfamethoxazole (SMX) have been reported in the literature [9–13]. However, no studies aiming its treatment using photo-Fenton process have been found. The aim of the present

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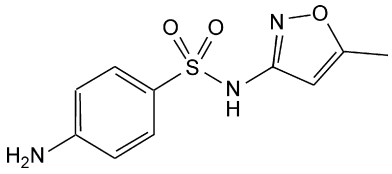

Fig. 1. Chemical structure of SMX.

work is to study the degradation of an antibiotic, the SMX ($C_{10}H_{11}N_3O_3S$) (see Fig. 1), by means of photo-Fenton process, with the further intention of decreasing the toxicity and improving the biodegradability of the resultant waters.

2. Materials and methods

2.1. Experimental device and procedure

The photochemical reactor was a Pyrex-jacketed thermostatic 2 L vessel (inner diameter 11 cm; height 23 cm) (Fig. 2), equipped with three black-light blue lamps (length 30 cm), placed in its centre (Philips TL 8W-08 FAM), with nominal power of 8 W each, which emitted radiation between 350 and 400 nm, with a maximum at 365 nm. The vessel was covered with aluminum foil to avoid personal damage. The photon flow arriving in the system was measured before and after the experimental set by means of actinometries [14]. It changed from 6.85 to 5.67 μ Einstein s^{-1}. The temperature was kept at 25 ± 0.8 °C with a thermostatic bath (Haake C-40) and good mixing was provided using a magnetic stirrer.

A 200 mg L^{-1} SMX (Sigma, >99%) aqueous solution (total organic carbon, TOC = 94.5 mg C L^{-1} and COD = 290 mg O_2 L^{-1}) was fed into the reactor. Then, 10 mg L^{-1} of Fe^{2+} were added in form of $FeSO_4 \cdot 7H_2O$ (Panreac PA). The pH was adjusted to 2.8 with H_2SO_4 aqueous solution. Finally, the necessary amount of H_2O_2 (Panreac PRS, 30%, w/v) was introduced under vigorous magnetic stirring and the UV lamps were switched on simultaneously. The system was let to react until all the H_2O_2 was consumed.

Samples were withdrawn periodically from the reactor to monitor the H_2O_2 consumption using Quantofix® test sticks (Macherey–Nagel) and were quenched with sodium hydrogen sulphite (Panreac QP, 40%, w/v) or with the same volume of methanol (Panreac PAI) (to avoid further reactions) in order to use these samples for total organic carbon and HPLC analysis

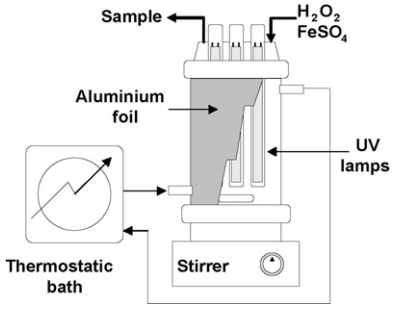

Fig. 2. Scheme of the device where the photo-Fenton reactions were carried out.

respectively. The resulting effluent was kept to carry out chemical oxygen demand (COD), biochemical oxygen demand at five days (BOD_5), Microtox® and inhibition test analysis. TOC was measured by means of a Shimadzu TOC-VCSN TOC analyzer. The concentration of the antibiotic was quantified by means of a HPLC supplied by Waters Corporation (configuration described elsewhere [15]; wavelength used: 270.5 nm). To analyze the COD, the Standard Methods 5220D procedures were followed [16]. For the evaluation of the BOD_5, the WTW OxiTop® measuring system (Weilheim, Germany) thermostated at 20 °C was used. The measure was done following the Standard Methods 5210D procedures [16].

2.2. Microtox® test

Acute toxicity of the SMX initial solution and the photo-Fenton final effluents was measured by Microtox® toxicity test, using *Vibrio fischeri* strains. Analysis was conducted according to the standard Microtox® test procedures recommended by the manufacturer (Azur Environmental, Delaware, USA). Toxicity is expressed as EC_{50}value, the concentration of sample that causes a 50% reduction in light emission. In this paper, the $EC_{50,15\ min}$ value, that is after 15 min of contact, was used to trace the change in toxicity due to the photo-Fenton treatment of SMX. The pH value of all test samples was neutralized at seven before toxicity was measured. A color correction procedure was incorporated to the highly colored photo-Fenton samples, according to the manufacturer procedure. The EC_{50} values used in this study are expressed as percentage (% v/v) of the initial sample. In addition, EC_{50} values were transformed to toxicity units (TU). TU = 100/EC_{50}. The conversion of EC_{50} values to TU brings out the lowest EC_{50} values.

2.3. Activated sludge inhibition test

Activated sludge inhibition tests were conducted in accordance with the test procedure described in OECD method 209. All experiments were run at constant temperature of 20 ± 2 °C. The activated sludge was obtained from the domestic wastewater treatment plant of Gavà (Barcelona). The biomass used in the test was fed with synthetic sewage as described in OECD. The 500-mL test samples were aerated for 3 h containing proper amounts of activated sludge. (Total suspended solids = 1.2 g L^{-1}.) Susceptibility of each batch of activated sludge was checked determining the EC_{50} value of 3,5-dicholophenol, and its acceptable range was defined between 5 and 30 mg L^{-1}. The test result was considered acceptable when the deviation between the oxygen-consumption rates of the blanks was lower than 15%.

The decrease in dissolved oxygen concentration in the blank synthetic wastewater and in different dilutions of raw and treated SMX effluent samples was monitored from 6.5 to 2.5 mg L^{-1}, or over a period of 15 min using a Crison Oxi 330i model oxygen meter. During the measurement, the sludge was kept suspended with a magnetic stirrer. Oxygen uptake rates (OUR), expressed in mg L^{-1} h^{-1} were calculated on the basis of the linear part of the decreasing dissolved oxygen concentration curves versus time. Inhibition percentage of OUR, i.e., I_{OUR}, for every tested

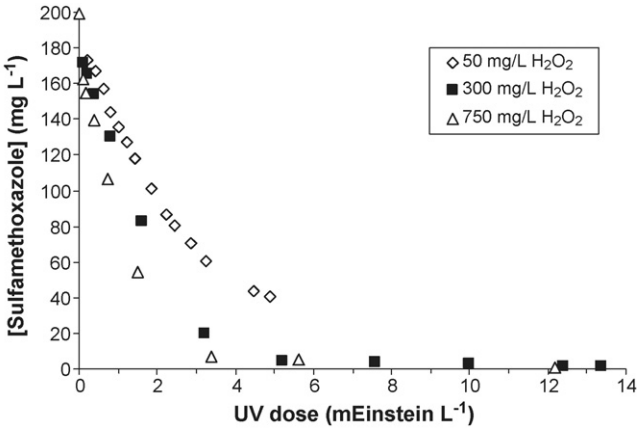

Fig. 3. SMX degradation for three different initial H_2O_2 concentration used in photo-Fenton experiments; $[SMX]_0 = 200\ mg\ L^{-1}$, $[Fe^{2+}]_0 = 10\ mg\ L^{-1}$, pH_0 2.8, $T = 25\ ^\circ C$.

sample dilution was calculated using the Eq. (1):

$$I_{OUR}(\%) = \left[1 - \frac{2R_S}{R_{c1} + R_{c2}}\right] \times 100 \quad (1)$$

where R_S is the oxygen uptake rate in the sample effluent mixture and R_{c1} and R_{c2} are the oxygen uptake rates in the blank samples.

3. Results and discussion

3.1. Photo-Fenton results

Photo-Fenton reactions at different concentrations of H_2O_2 were carried out to investigate the influence of hydrogen peroxide in the degradation of a $200\ mg\ L^{-1}$ SMX solution. TOC and SMX concentration were monitored for each concentration of H_2O_2. The accumulated photons entering the system were chosen instead of time to study the evolution of the experiments because a decrease in the photon flow of the lamps was observed along the period of time that the experiments were carried out. Three of these experiments have been chosen to show the antibiotic degradation and the TOC evolution. They are shown in Figs. 3 and 4, respectively.

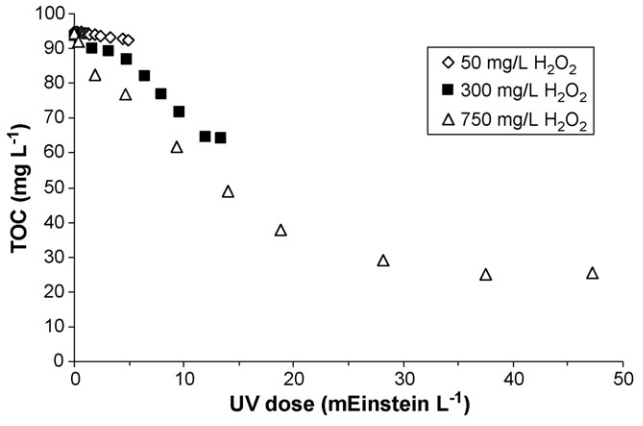

Fig. 4. TOC monitoring for three different initial H_2O_2 concentration experiments; $[SMX]_0 = 200\ mg\ L^{-1}$, $[Fe^{2+}]_0 = 10\ mg\ L^{-1}$, pH_0 2.8, $T = 25\ ^\circ C$.

It should be pointed out that the total abatement of SMX was not reached when working with a low, initial H_2O_2 concentration, as shown in Fig. 3. The final antibiotic concentration was below $5\ mg\ L^{-1}$ when working with more than $100\ mg\ L^{-1}$ and below $1\ mg\ L^{-1}$ when more than $300\ mg\ L^{-1}$ of H_2O_2 were used.

There are nearly no TOC variations when carrying out the reaction with $50\ mg\ L^{-1}$ H_2O_2 concentration but an increase in the H_2O_2 dose results in higher decreases in the TOC content of the treated solutions. In order to evaluate the effects of the hydrogen peroxide amount used, final (when all the H_2O_2 is consumed), TOC and COD abatements have been calculated and plotted in Fig. 5 for all the runs carried out.

The TOC abatement achieved at the end of the reactions ranges from 2.4 to 79.9% depending on the hydrogen peroxide doses (from 50 to $1000\ mg\ L^{-1}$). In Fig. 5 it can be observed that an increase in the hydrogen peroxide dose results in an increase in the TOC removal attained after the reaction. However, two different tendencies are observed in this plot. Regarding the first one, a sharp increase in the TOC removal is observed when increasing the H_2O_2 up to $550\ mg\ L^{-1}$. Secondly, only a slight increase in the TOC removal is obtained when increasing the peroxide from 550 to $1000\ mg\ L^{-1}$.

With respect to COD, the removal achieved at the end of the reactions increase from 12 to 92.5% depending on the hydrogen peroxide doses (from 50 to $1000\ mg\ L^{-1}$). The same tendency as in TOC abatement is observed in the case of COD removal. Both the acute and the slight increases appear in Fig. 5. The change in the slope seems to be around the $550\ mg\ L^{-1}$ initial peroxide concentration.

In both cases, (TOC and COD abatements) the change in the tendency could be explained taking into consideration the different compounds or intermediates formed during the reaction. A higher dose of hydrogen peroxide provokes a higher removal of the target compound and the early intermediates. These compounds are characterized by being easily abated by the hydroxyl radical. However, this tendency stops when low molecular weight acids are formed. These substances are usually the last step before a complete mineralization is achieved.

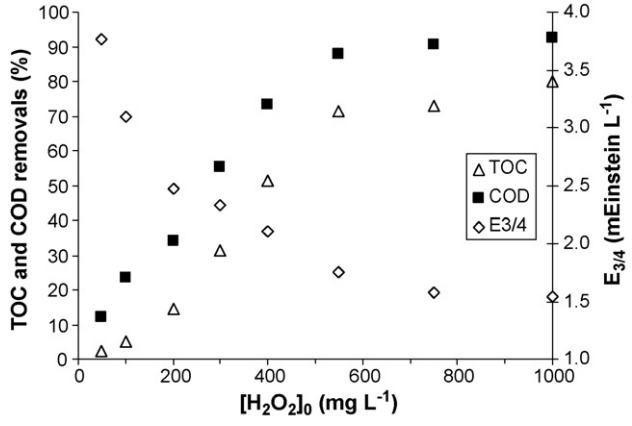

Fig. 5. Final TOC and COD abatements and "accumulated energy 3/4 ($E_{3/4}$)" for the different initial H_2O_2 concentrations; $[SMX]_0 = 200\ mg\ L^{-1}$, $[Fe^{2+}]_0 = 10\ mg\ L^{-1}$, pH_0 2.8, $T = 25\ ^\circ C$.

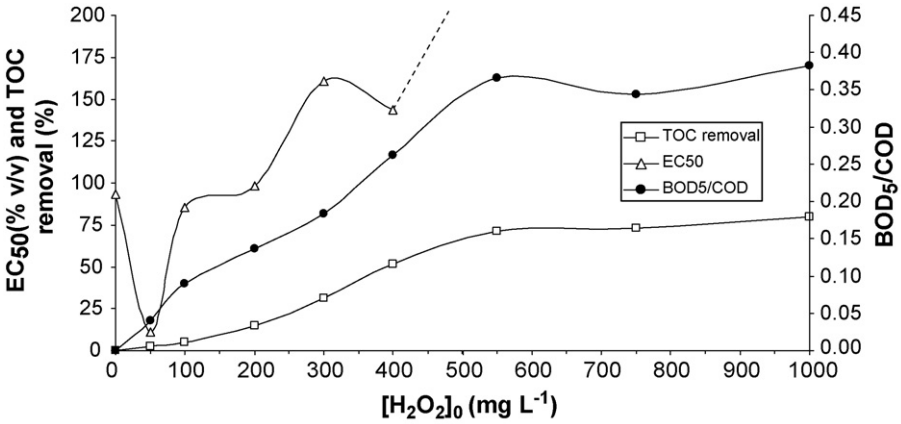

Fig. 6. Final TOC removals, EC_{50} and BOD_5/COD ratios vs. H_2O_2 dose. Initial conditions in photo-Fenton experiments: $[SMX]_0 = 200\ mg\ L^{-1}$, $[Fe^{2+}]_0 = 10\ mg\ L^{-1}$, pH_0 2.8, $T = 25\ ^\circ C$.

Moreover, they are well-known for being refractory to oxidation substances. This fact could explain why similar results for the TOC and COD removals are attained at the highest concentrations of hydrogen peroxide [3].

The necessary UV dose to abate the 75% of the antibiotic has been calculated to check the improvement in the antibiotic degradation rate when the initial concentration of H_2O_2 is increased. The UV dose (mEinstein L^{-1}) needed to reach this antibiotic removal has been named "accumulated energy 3/4 ($E_{3/4}$)". In Fig. 5, the influence of the initial hydrogen peroxide dose on the $E_{3/4}$ can be observed.

As expected, the necessary energy to reach a 75% of SMX removal is lower when the initial dose of H_2O_2 becomes higher. Antibiotic degradation rate improves when the H_2O_2 dose is increased up to approximately $600\ mg\ L^{-1}$, which would mean that until this concentration, an excess of hydroxyl radicals is not present in the reaction medium. On the other hand, it can be observed that at higher initial values of hydrogen peroxide, the $E_{3/4}$ remains almost constant, which would indicate that the reaction medium is saturated with hydroxyl radicals. These results highlight the fact that the degradation rate of the target compound cannot be significantly improved even when the hydrogen peroxide concentration is increased up from $600\ mg\ L^{-1}$.

3.2. Biodegradability, toxicity and inhibition tests

The characteristics of oxidized intermediates after photo-Fenton treatment from the biodegradability and toxicity points of view were evaluated. The results will allow considering the integration of a photo-Fenton pretreatment and a final biological TOC removal as a possible optimal strategy to eliminate SMX in industrial wastewater. The BOD_5 for the different photo-Fenton treatment effluents was tested and the BOD_5/COD ratios are plotted in Fig. 6. The BOD_5 of $200\ mg\ L^{-1}$ SMX solution was zero, meaning that the antibiotic is not readily biodegradable. The effluents from the photo-Fenton process increase their biodegradability indicator with increasing H_2O_2 dose, which is indicative of improved biodegradability due to an enhancement in the proportion of organic matter (the intermediates) able to biodegradation. From a treatment with more than $400\ mg\ L^{-1}$ of H_2O_2 on, the biodegradability ratio was greater than 0.25, representing an easily biodegradable effluent. On the other hand, effluents with lower biodegradability ratio fit with some presence of SMX in the solution (up to $300\ mg\ L^{-1}$ H_2O_2 dose).

Fig. 6 also includes the TOC removal observed with the treatment conditions tested. The TOC removal profile reveals that rather destructive treatment is required to ensure an appreciable biodegradability (over 50% TOC abatement for a BOD_5/COD ratios higher than 0.3).

The acute toxicity of SMX and changes in acute toxicity of photo-Fenton effluents were evaluated by the Microtox® bioassay. The marine photo bacteria *Vibrio fischeri* has been used to determine the influence of toxic substances on pure bacteria. EC_{50} after 15 min of exposition measured by the Microtox® is presented in Fig. 6.

From the figure it can be observed that acute toxicity of SMX is relatively low ($EC_{50} = 93.43\%$). This result is in accordance to EC_{50} data available in specialized literature for SMX and others antibiotics [17,18]. Some authors point out that acute and even usual chronic toxicity tests are relatively short in comparison with the life cycle of organisms. They neglect the accumulative nature of some antibiotics and possible accumulation of their toxic effects [19,20].

Acute toxicity response from Microtox® changes considerably when analysing the photo-Fenton products. A significant increase in toxicity was observed for the waters resulting from treatment using a $50\ mg\ L^{-1}$ initial H_2O_2 concentration. It seems that some different intermediate with a higher toxicity is generated and present at higher concentration in the reaction medium when the photo-Fenton reaction is carried out with this low concentration of H_2O_2 (see representation of TU (toxicity units = $100/EC_{50}$) in Fig. 7).

This would indicate that the photo-Fenton oxidized SMX intermediates increase the toxicity when using low H_2O_2 concentration (around $50\ mg\ L^{-1}$). From this point on, acute toxicity of raw solution decreases appreciably after the treatment with photo-Fenton, and the detoxification effect increases with the H_2O_2 dose. For treatments with H_2O_2 dose over $500\ mg\ L^{-1}$, EC_{50} were higher than 200%, which would mean that bacteria were insensitive to antibiotic photo-Fenton intermediates.

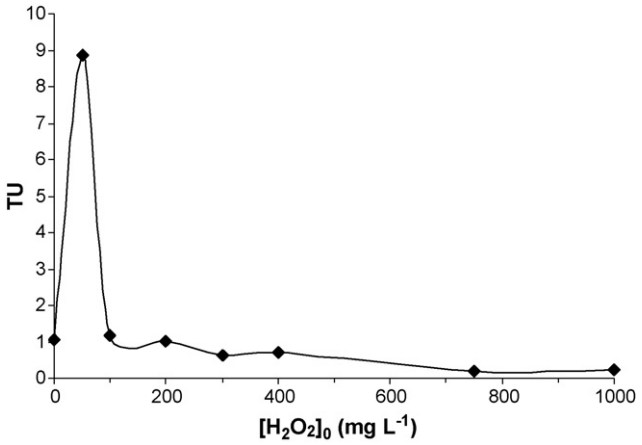

Fig. 7. TU values (toxicity units = 100/EC_{50}) for the different initial H_2O_2 doses.

Table 1
Oxygen uptake rates obtained for different SMX concentrations during the inhibition test performance

[SMX] (mg L^{-1})	OUR (mg O_2 L^{-1} h^{-1})
0 ($Blank_{average}$)	89.6
113.6	66.6
56.8	74.4
28.4	89.5
14.2	89.4

Among the oxidative treatment conditions, photo-Fenton with H_2O_2 dose over 300 mg L^{-1} appeared to have enough positive effect on SMX toxicity. These results would be in accordance with BOD_5/COD ratio.

In summary, the earliest intermediates of oxidized SMX might produce the risk of acute toxicity if these toxic intermediates are not further oxidized.

Based on the results of the OUR test, the percentage of inhibition for SMX is shown in Table 1 and Fig. 8. The results revealed that the highest percentage of inhibition was 25.64% for a SMX concentration of 113 mg L^{-1}. Percentage of inhibition decreased with SMX dilution and no inhibition was observed

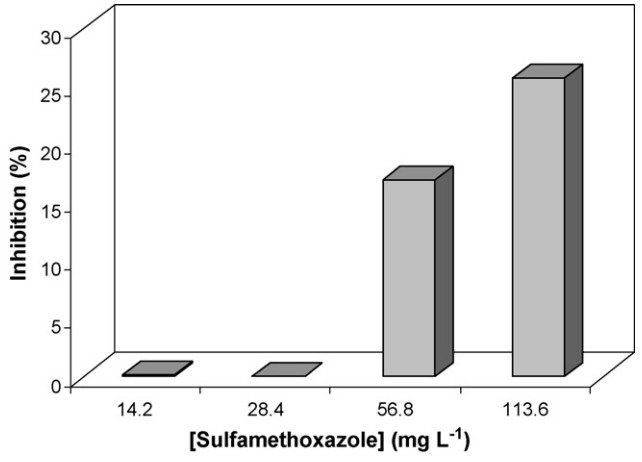

Fig. 8. Percentages of inhibition for different SMX concentrations observed in activated sludge inhibition test.

for concentration lower than 28.4 mg L^{-1}. On the other hand, no inhibition effect was observed for the oxidized intermediates when working with 100, 330 and 550 mg L^{-1} of H_2O_2 dose. These results would indicate that photo-Fenton oxidized intermediates from SMX solutions would not inhibit the biological activity of a sewage treatment plant when integrating approaches were applied.

4. Conclusions

Photo-Fenton process is thought to be a successful treatment for both the abatement and the improvement of the biodegradability of wastewater containing SMX. The complete antibiotic removal was achieved for H_2O_2 dose over 300 mg L^{-1} ($[Fe^{2+}] = 10$ mg L^{-1}). On the other hand, BOD_5/COD ratio increased with increasing H_2O_2 dose, obtaining biodegradabilities over 0.25 for 400 mg L^{-1} H_2O_2. Toxicity test by means of Microtox® and inhibition test revealed that the earliest intermediates of oxidized SMX for low H_2O_2 dose might produce the risk of acute toxicity. A treatment photo-Fenton process with a H_2O_2 concentration over 400 mg L^{-1} will produce an oxidized intermediates with enough acute detoxicity and no inhibition effects on sewage sludge as well as a BOD_5/COD higher than 0.25. These conclusions would allow us to suggest to integrate the technology of photo-Fenton process and biological treatment as an optimal strategy for antibiotic removal in industrial wastewater.

Acknowledgements

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