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Characterization of the degradation performance of the sulfamethazine antibiotic by photo-Fenton process

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ABSTRACT

The present study provides results describing the degradation performance of the Sulfamethazine (SMT) antibiotic via photo-Fenton treatment. Experiments were carried out using 1 L solution samples of SMT (50 mg L^{-1}) under different conditions. HPLC results reveal that both Fenton and photo-Fenton reactions were able to completely remove SMT antibiotic from the studied samples in less than 2 min treatment. Half-life times and kinetic parameters (assuming a pseudo-first-order kinetics at reaction initial stage, far from the equilibrium) for SMT degradation were determined and discussed. Hence, appropriate Fenton reagent loads are given to attain different targets proposed. TOC and HPLC data also revealed the presence of reaction intermediates; thus toxicity assays were performed regarding bacterial growth rate. The toxicity of an SMT solution was shown to increase during its degradation by means of photo-Fenton reactions.

1. Introduction

Advanced Oxidation Processes (AOPs) have demonstrated to be effective for contamination remediation of wastewater involving pharmaceutically active compounds (Kaniou et al., 2005; Palominos et al., 2008; Raja et al., 2005).

The recent concern about the degradation of pharmaceutical compounds, which have been consumed for many decades, is due to their presence in several public water systems (Kaniou et al., 2005). Several studies (Ternes, 1998; Heberer, 2002) already alert of pharmaceuticals not being removed during sewage treatment. These substances are found in surface waters that could be possible drinking water sources (Zwiener and Frimmel, 2000). Moreover, current drinking water standards do not even require testing for any of the pharmaceutical compounds being prescribed (Chatzitakis et al., 2008).

Specifically, active antibiotics alter the normal water flora and allow the development of antibiotic-resistant bacteria (Palominos et al., 2008). Many of these substances have environmental and health effects still not clearly defined and reported. In particular, there is no reliable information on their long-term effect on humans (Zwiener and Frimmel, 2000). Thus, specific treatments are required to guarantee their elimination from wastewaters.

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Pharmaceuticals, usually designed for oral intake, are as a rule resistant to hydrolysis suggesting the mechanism of direct and indirect photolysis as a primary pathway for their abiotic transformation (Andreozzi et al., 2003). Thus, the removal of these contaminants with conventional methods may not be effective. Conversely, pre or post AOP treatments usually result in an interesting alternative to efficiently remediate the contamination of water produced by the presence of antibiotics.

Among AOPs, treatment with Fenton reagents and a light source results in a low-cost process for wastewater treatment (Pérez et al., 2002a). Since the photo-Fenton reaction requires a wavelength close to 400 nm, and mixtures of Fe(III) and H_2O_2 have shown absorption up to 580 nm (Sun and Pignatello, 1993; Pignatello et al., 1999; Krutzler and Bauer, 1999), the photo-Fenton process can also run under solar irradiation (Safarzadeh-Amiri et al., 1996; Pérez et al., 2002b; Bandala et al., 2008). Driven under solar light, the treatment using Cobalt/peroxynonosulphate has also been investigated and shown to have enhanced degradation rate (Bandala et al., 2007).

The generally accepted Fenton reaction mechanism considers that hydroxyl radicals $^{\bullet}OH$ are produced by interaction of H_2O_2 with ferrous salts (Equation (1)). Additionally, the so-called Fenton-like reactions (Equations (2) and (3)) regenerate Fe(II) and support the Fenton process (Pignatello, 1992).

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\bullet}OH + OH^-,\ k = 53\text{–}76\ L\,mol^{-1}\,s^{-1} \quad (1)$$

$$Fe(III) + H_2O_2 \rightarrow Fe(II) + HO_2^{\bullet} + H^+,\ k = 1\text{–}2 \times 10^{-2}\ L\,mol^{-1}\,s^{-1} \quad (2)$$

$$Fe(III) + HO_2^{\bullet} \rightarrow Fe(II) + O_2 + H^+,\ k = 0.33\text{–}2.1 \times 10^{6}\ L\,mol^{-1}\,s^{-1} \quad (3)$$

The degradation rate of the Fenton reaction may be increased by the iron photo-reduction (Equation (4)) that produces new $^{\bullet}OH$ radicals and regenerates Fe(II) ions, which in turn can further react with H_2O_2 (Kušić et al., 2006).

$$FeOH^{2+} + h\nu \rightarrow Fe(II) + {}^{\bullet}OH,\ k = 3.3 \times 10^{-6}\ L\,mol^{-1}\,s^{-1} \quad (4)$$

The irradiation of Fe(III) + H_2O_2 enhances the reaction rate of oxidant production through the involvement of high valence Fe intermediates (Pignatello et al., 1999; Bossmann et al., 1998). The absorption of visible light by the complex formed by Fe(III) and H_2O_2 seems to cause the formation of such high valence Fe-based oxidants.

The present study addresses the application of the Fenton and photo-Fenton reactions, for the degradation of sulfamethazine (SMT). This antibiotic belongs to the group of heterocyclic sulfonamides and it is widely used as antibacterial drug in medicine and veterinary medicine.

Design of experiments (DOE) was used to plan the monitoring of the degradation attained (in terms of a lumped parameter such as total organic carbon, TOC) as well as the evolution of hydrogen peroxide.

2. Material and methods

Analytical grade SMT was purchased from Sigma–Aldrich and was diluted to 1.88×10^{-4} M (50 mg L^{-1}). Analytical grade hydrogen peroxide and heptahydrated ferrous sulfate were purchased from Panreac and Sigma–Aldrich, respectively, and were used as received. The rest of the chemicals used were, at least, of reagent grade. Solutions were prepared with deionized water obtained from a Millipore Milli-Q system.

Experiments were conducted in a thermostatic cylindrical 1500 mL Pyrex cell. The reaction mixture inside the cell, consisting of 1000 mL of SMT solution and the given amount of Fenton reagent, was continuously stirred with a magnetic bar. Temperature was maintained at 18–19 °C. SMT solution pH is 3 after adding heptahydrated ferrous sulfate and hydrogen peroxide. Temperature and pH were measured online to ensure they kept constant along all the reaction time.

The experiments were carried out with a sunlight lamp (Ultra-Vitalux®, Osram, 300 W). The Ultra-Vitalux®, sunlight lamp consists of a quartz burner and a tungsten filament that are blended in such a way that the radiation emitted is similar to natural sun radiation. The spectral radiation presents wavelengths close to 400 nm (appropriate for photo-Fenton reaction) and up to 580 nm (useful for the Fenton-like reaction). The accumulated irradiance energy in the UVA region (315–400 nm) after one-hour assay was 49 kJ L^{-1} and 11 kJ L^{-1} in the UVB region (280–315 nm). The reactor was irradiated from the top and there were 21 cm between the lamp and the liquid surface.

Total organic carbon (TOC) of samples was determined with a Shimadzu 5000 TOC analyzer. The concentration of the hydrogen peroxide was determined spectrophotometrically after reaction with ammonium metavanadate (Nogueira et al., 2005).

The HPLC-UV/DAD method was used to determine SMT concentration, using an Agilent 1100 series chromatographic system (Darmstadt, Germany) equipped with an online mobile phase degasser, a quaternary pump, an auto-sampler with Peltier sample thermostat, a column oven and a UV-diode array detector. The chromatographic conditions and data analysis were performed using the Agilent Chem-Station (rev.B.01.03) software. The chromatographic conditions were a 5 μm 0.46 × 25 cm C18 Spherisorb ODS analytical column (Waters, Milford, USA), maintained at 15 °C, and the diode array detector set at 270 nm. Samples, injected by the auto-sampler, were eluted by a 55/45 water/acetonitrile mixture (milli-Q grade and J.T. Baker ultragradient HPLC grade, respectively) flowing at 1.5 mL min^{-1}. The retention time of SMT under these conditions was 2.2 min.

A duplicate four level calibration curve (range 5–50 mg L^{-1}) was used for the SMT quantification. The calibration curve was performed from working solutions prepared from standard SMT and injected in the chromatographic system. A linear-regression analysis (ANOVA with a statistical significance of 0.05), including a lack-of-fit (LOF) test (statistical significance of 0.1), was performed in order to assess the linearity. The value of the determination coefficient (r^2) was 0.9999, and the p-value for ANOVA and LOF test was <0.05 and 0.758, respectively. The intraday and interday coefficients of

variation, as well as the accuracy (percent error of the spiked concentration), were found to be less than 10%.

The toxic effect of SMT and their degradation products was evaluated by traditional bacterial growth. Two common representative bacteria *Escherichia coli* (gram-negative bacteria) and *Staphylococcus aureus* (gram-positive bacteria) were grown in tryptic soy broth (TSB) (Merck, Germany). The assays were carried out in a 96 well microtiter plates containing 50 μL of a bacterial suspension per well (0.2 O.D.550 nm unit) with the addition of 50 μL of the different samples and 100 μL of TSB medium (final volume of 200 μL). The plates were incubated at 37 °C and the optical density (O.D.550 nm) was measured using a microtiter reader plate (TECAN® Genios, Spain) during 24 h at 1 h intervals.

The design of experiments (DOE) was used to arrange the measurement of the degree of organic degradation attained (TOC) as well as the SMT elimination levels. Central composite design with star points was been adopted for determining the influence of Fenton reagent loads (Fe(II), H_2O_2) in the SMT antibiotic degradation. Certainly, other experimental conditions, mainly other wavelengths and net irradiative fluxes, also have an effect on the degradation performance. However, the inclusion of these conditions as variables in the DOE would increase the demand of experimental data. The present research takes these conditions as pre-established experimental settings aimed at addressing the study of the influence of chemical reactants in an affordable way.

Two variable levels (low and high) were considered, as well as two variables corresponding to iron and hydrogen peroxide concentrations, which were varied in the ranges 20–60 mg L^{-1} and 300–900 mg L^{-1} respectively. Three central points for statistical validity and star points at −1.41 to +1.41 were also taken into account.

3. Results and discussion

3.1. Preliminary study

The work by Kaniou et al. (2005) addresses photocatalytic degradation of 500 mL of 50 mg L^{-1} SMT solution in distilled water, and reports the occurrence of quantitative degradation after 240 min. Hence, different tests in this work were performed using samples of double volume SMT solution (1000 mL) and the same 50 mg L^{-1} SMT concentration (TOC = 30 ± 2 mg L^{-1}). Temperature was kept at 18–19 °C. In contrast to the results obtained from photocatalysis, the preliminary experiments performed in this work using the photo-Fenton treatment showed that the total elimination of the SMT of the sample may be achieved in less than 5 min.

Table 1 – Blank assays.

Assays	H_2O_2 (mg L^{-1})	Fe(II) (mg L^{-1})	Light
B1	600		No
B2		40	No
B3			Yes
B4	600		Yes
B5		40	Yes

Fig. 1 shows the evolution of SMT, TOC and H_2O_2 concentration along the photo-Fenton treatment investigated in this work. The Fenton reagent loads were: 600 mg L^{-1} of hydrogen peroxide and 40 mg L^{-1} of Fe(II). These reagent loads were selected in order to test a Fenton reagent ratio between 5:1 and 20:1 (concentration of H_2O_2 to concentration of Fe(II)), which is recommended as a threshold value in the literature (Herney Ramirez et al., 2005).

Fig. 1 also shows that, while SMT elimination is complete in around five minutes, 1 h reaction time does not ensure the total organic carbon (TOC) removal. Thus, the residual TOC may be attributed to the organic intermediates produced, and it is important to study their implication in the system.

The same figure shows that the hydrogen peroxide decrease is especially fast for short reaction times. The H_2O_2 concentration was also determined to ensure that it would not interfere with the toxicity tests performed.

Several blank experiments were performed (see Table 1) aimed at establishing that the treatment effectiveness is due to Fenton reagent enhanced with the appropriate light radiation. In Fig. 2, TOC evolution was plotted as a function of reaction time, under different experimental conditions. In all the cases the initial conditions were 50 mg L^{-1} SMT and Temperature = 18–19 °C.

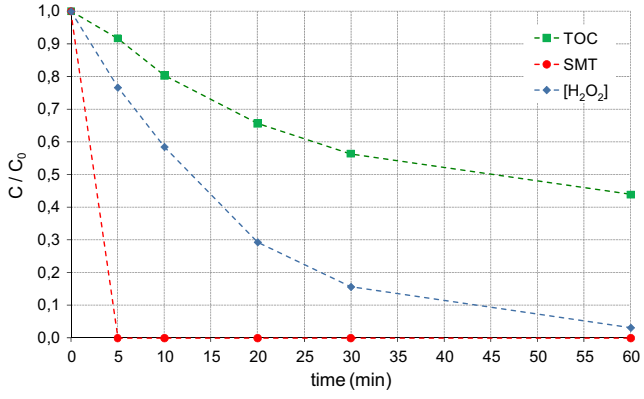

Fig. 1 – Sulfamethazine, TOC and H_2O_2 evolution. Fenton reagent loads: [H_2O_2] = 600 mg L^{-1} and [Fe(II)] = 40 mg L^{-1}.

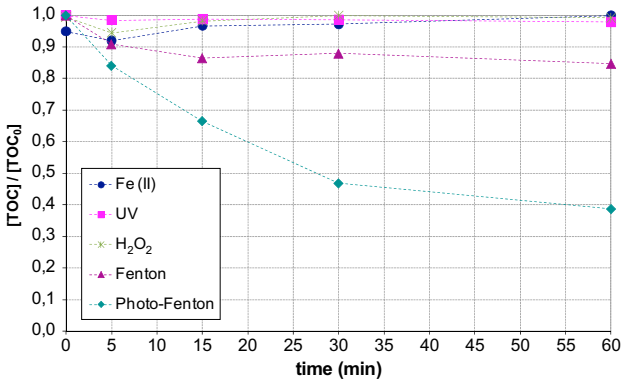

Fig. 2 – TOC evolution as function of reaction time for different experimental conditions.

Table 2 – Factorial experimental design of SMT treated by Fenton and Photo-Fenton reactions.

Assays	Codified values		Variable levels		% TOC reduction after	
	$[H_2O_2]$	[Fe(II)]	H_2O_2 (mg L^{-1})	Fe(II) (mg L^{-1})	30 min	60 min
A	−1	−1	300	20	18	41
B	1	−1	900	20	26	47
C	−1	1	300	60	44	51
D	1	1	900	60	31	45
E	0	0	600	40	44	56
F	0	0	600	40	44	56
G	0	0	600	40	42	55
H	−1.41	0	176	40	38	45
I	1.41	0	1024	40	45	56
J	0	−1.41	600	12	0,4	12
K	0	1.41	600	68	44	54

When the specific experiment requires Fenton reactive, the loads used were 600 mg L^{-1} of H_2O_2, 40 mg L^{-1} of Fe(II) and the appropriate light radiation to enhance Fenton reactive system. Clearly, Fig. 2 shows that Fenton reaction without light or just photolysis is less efficient than the photo-Fenton process.

Subsequently, it is necessary to study the appropriate experimental conditions for the photo-Fenton treatment to reduce the concentration of the SMT antibiotic and its byproducts.

3.2. *Experimental design*

Photo-assisted Fenton degradation technology is a multifactor system. For this reason, the characterization of the system requires taking into account not only single-factor effects, but also cross-effects.

Experimental design methodologies are conceived to identify the factors affecting a multivariate process. For this case, these factors are the amount of Fenton reagents determining the SMT or/and TOC removal.

In order to evaluate the effect of the Fenton reagent concentration on the SMT antibiotic degradation, a factorial experimental design (2^2) was applied. Variables studied were the iron and hydrogen peroxide concentrations, varying in the ranges 20–60 mg L^{-1} and 300–900 mg L^{-1} respectively. The different operating conditions are summarized in Table 2.

Hydrogen peroxide required for total SMT mineralization is given by the following reaction stoichiometry:

$$C_{12}H_{14}N_4O_2S + 19H_2O_2 \rightarrow 12CO_2\uparrow + SO_4^{2-} + 4NO_3^- + 52H^+ \quad (5)$$

Given 50 mg L^{-1} of SMT, a minimum of 121.45 mg L^{-1} of hydrogen peroxide is required. Thus, all the experiments in the design ensure hydrogen peroxide loads beyond the limiting stoichiometric value. For all the planned photo-Fenton assays acidity conditions remained at pH = 3.0 ± 0.5 after the rapid pH drop observed at the very first reaction stages (less than 2 min).

3.3. *Characterization of TOC reduction performance*

Results show that the selection of the correct Fenton reagents load is an important aspect that should be considered during the treatment process. However, this decision should be made regarding the goal of the process. Two targets are considered:

- Minimum reaction time to attain 50% TOC reduction (Half-life time)
- Minimum Fenton reagents load to ensure the highest initial degradation rate

3.3.1. *TOC half-life time*

A first look to Table 3 allows grouping the results into two sets: the experimental conditions that need more than one-hour reaction time to reduce to one half the initial TOC of the sample and those reaching the same degradation after less than 50 min of reaction. This second set of experiments may be considered to lead to similar results in terms of the reaction time goal.

Clearly, more experimental data is required to solve the trade-off between this goal and the associated cost in terms of Fenton reagent load. However, these initial results for the SMT degradation indicate that suitable Fenton loads may be found around: 600 mg L^{-1} of H_2O_2 concentration and 40 mg L^{-1} of Fe^{2+} concentration.

In relative terms, this suggests the following approximate ratios:

SMT (mg L^{-1}):Fe^{2+} (mg L^{-1}) = 1.1:1

H_2O_2 (mg L^{-1}):SMT (mg L^{-1}) = 13:1

H_2O_2 (mg L^{-1}):Fe^{2+} (mg L^{-1}) = 15:1

Table 3 – Reaction time necessary to reduce a half of the initial TOC.

Time (min) to ensure 50% TOC abatement					
Fe(II) (mg L^{-1})	H_2O_2 (mg L^{-1})				
	176	300	600	900	1024
12			>60		
20		>60		>60	
40	>60		45		43
60		50		>60	
68			46		

Wide Fenton reagent load ratios are reported elsewhere regarding the degradation of different substances. Some experimental works provide values from 5:1 to 20:1, (Herney Ramirez et al., 2005), while other papers present values in the range of 10:1 to 200:1 (Gernjak et al., 2006). The diverse natures of contaminants produce these varied values. However, specialized literature indicates a limiting iron concentration (around 10 mg L^{-1}) to guarantee the degradation process. This work shows that for SMT the condition 12 mg L^{-1} of Fe^{2+} concentration results less efficient than other conditions with higher iron loads. Nevertheless, it has been shown that an excess of iron may also decrease the effectiveness of the photo-Fenton process (Pérez-Moya et al., 2007) as well as that of the Fenton process (Burbano et al., 2008).

3.3.2. TOC degradation rate

A semi-empirical model is next proposed for characterizing the performance of the Fenton treatment under consideration. The degradation of the SMT contaminant measured through the concentration of total organic carbon, [TOC] is monitored from its initial value, $[TOC]_0$, at $t = 0$. The degradation rate (d[TOC]/dt) is related to the varying concentrations of the SMT and derived organic intermediates (I_1, I_2, … I_n), the Fenton reactants, iron and hydrogen peroxide, as well as operating conditions such as irradiation, pH, temperature, etc… (eq. (6)).

$$\frac{d[TOC]}{dt} = f_1([SMT], [I_1], [I_2], \ldots [I_n], [Fe^{2+}], [H_2O_2], h\nu, \ldots, pH, T, \ldots) \quad (6)$$

However, a usual simplifying approach is to describe the TOC evolution as a first-order kinetics, far from the equilibrium, for which the rate constant is a function of the initial values of these factors. Thus, this kind of degradation models discards the influence of all intermediates in addition to the influence of all fixed factors in the experiments (eq. (7)). Hence, given fixed operating conditions (irradiation, pH, temperature, etc…) and a fixed initial value for the SMT concentration, the kinetic rate may be regarded as a function of the initial concentrations of iron and hydrogen peroxide (eq. (8)).

$$\frac{d[TOC]}{dt} = -k \cdot [TOC] \quad (7)$$

being

$$k = f_2([Fe^{2+}]_0, [H_2O_2]_0) \quad (8)$$

It is assumed to follow a pseudo-first-order kinetics regarding only the H_2O_2 disappearance (which is totally related with the TOC decreasing). Table 4 summarizes the constant k values, which are providing an idea of the initial degradation rate related to the specified experimental condition.

For the studied reaction span, Fenton reagent load ratios (H_2O_2 (mg L^{-1}):Fe^{2+} (mg L^{-1})) lower than 5 lead to higher k values (0.0419 min^{-1}, 0.0462 min^{-1}), which implies faster TOC reduction during the first 30 min reaction. Remarkably, the selected experimental condition (H_2O_2 (mg L^{-1}):Fe^{2+} (mg L^{-1}) = 15:1), which achieves 50% TOC abatement in 45 min, presents quite lower value for the corresponding first-order rate constant, $k = 0.0253$ min^{-1}.

Since more effective conditions may be possible, regarding both target 1 and 2, a new experiment was performed by decreasing the Fenton reagent load, so that the initial TOC abatement rate was increased and a 50% TOC abatement was guaranteed in less than 50 min.

Table 4 – Constant k related with the initial degradation velocity.

k (first-order reaction rate constant, min^{-1})					
Fe(II) (mg L^{-1})	H_2O_2 (mg L^{-1})				
	176	300	600	900	1024
12			0.0117		
20		0.0068		0.0250	
40	0.0419		0.0253		0.0270
60		0.0462		0.0223	
68			0.0384		

Compared to the previous ones, the new assay (Assay L: 600 mg L^{-1} of H_2O_2 concentration, 50 mg L^{-1} of Fe^{2+} concentration, H_2O_2 (mg L^{-1}):Fe^{2+} (mg L^{-1}) = 12:1) improved both targets the highest degradation rate ($k = 0.0460$ min^{-1}) and the time to attain 50% TOC abatement, which was less than 30 min (Fig. 3).

Although other conditions may be investigated, experimental design shows that the most favorable conditions are around these values. Iron loads around 68 mg L^{-1} already produce lower initial degradation rates ($k = 0.0384$ min^{-1}).

The chemical analysis indicates some suitable Fenton reagent loads. However, the choice of appropriate reaction conditions requires contemplating biological issues (Herney Ramirez et al., 2005). Some toxicity tests were accordingly performed, whose results are presented in the last section of the paper.

3.3.3. SMT elimination – HPLC results

Despite the possible degradation intermediates causing non-null TOC, Fig. 3 demonstrates that the photo-Fenton reaction is successful to attain the complete degradation of SMT. On the other hand, the planned assay in Fig. 3 does not provide evidence regarding partial SMT degradation. This is why additional assays were decided at lower reaction times.

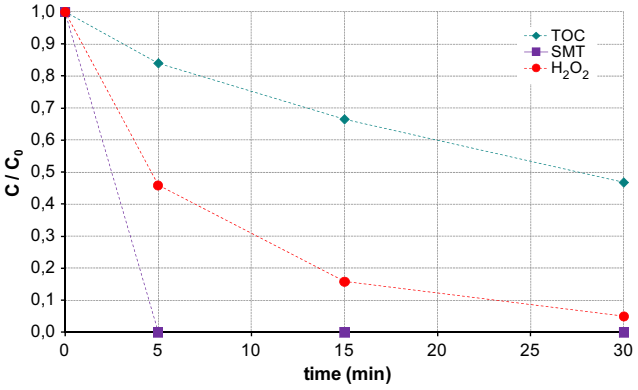

Fig. 3 – Sulfamethazine, TOC and H_2O_2 evolution. Photo-Fenton process, Assay L: 600 mg L^{-1} of H_2O_2 concentration, 50 mg L^{-1} of Fe^{2+} concentration, (H_2O_2 (mg L^{-1}):Fe^{2+} (mg L^{-1}) = 12:1).

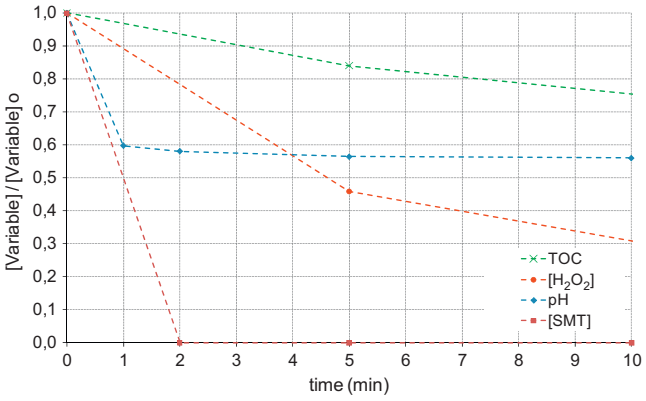

Fig. 4 – pH monitoring during the first 10 min for assay L, 600 mg L^{-1} of H_2O_2 concentration, 50 mg L^{-1} of Fe^{2+} concentration, (H_2O_2 (mg L^{-1}):Fe^{2+} (mg L^{-1}) = 12:1). Initial pH = 5.37, after 1 min pH = 3.21.

Hence, total SMT removal was also obtained after only 2 min (shorter sampling times were considered unpractical as well as inaccurate). The corresponding HLPC results for the photo-Fenton treatment, Assay L, at reaction times zero and two minutes evidence complete degradation of SMT. Similar result was obtained in the Fenton assay (same Fenton reagents without light irradiation).

Among these conditions leading to SMT removal after 2 min reaction time, it seems advisable to highlight the option requiring less resource consumption, that is the Fenton reaction without light and with minimum hydrogen peroxide and iron.

These results show again that SMT degradation is much more efficient by means of Fenton and photo-Fenton reactions than using photocatalysis, which needs 60 min treatment to abate the SMT in an equivalent sample (Kaniou et al., 2005).

HPLC analyses also evidence the presence of diverse byproducts along the degradation span.

Further studies and identification of the intermediates are required for elucidating the reaction pathways for each treatment. The determination of the reaction pathways is beyond both the aim of the paper and the limits of the experimental method, mostly the limitation of HLPC detection method to identify intermediate products. However, a possible reaction pathway is likely to start with the desulphurization of the SMT. On one hand, sulfonamide photosensitivity is well known and it has been established that the sulfonamide bond is relatively susceptible to photolytic cleavage (D'Souza and Day, 1968). Photo-degradation intermediates have been studied and aniline has been reported among them (Weiss et al., 1980).

On the other hand, recent studies addressing the photo-Fenton degradation of sulfamethoxazole (a sulfonamide antibiotic similar to SMT) also propose the release of the sulfonile group as a first pathway step (González et al., 2008). Finally, mechanisms for SO_2 removal from sulfonamides have been proposed from experimental and theoretical results (Wang et al., 2003; Hu et al., 2008).

A final point on the feasibility of such a fast step is related to SO_2 elimination, which accordingly would cause a fast release of H^+ and a fast change of the initial pH (Kaniou et al., 2005). In this sense, the pH data from the assays monitoring provides an additional indication of the consistency of this hypothesis (Fig. 4).

3.3.4. *Bacterial toxicity evaluation*

Results show that SMT may be degraded into different intermediates in a very short time. However, the SMT elimination does not guarantee the toxicity abatement of the resulting wastewater, especially if TOC remains after the treatment. Thus, toxicity studies were carried out to assess the biodegradability given by these intermediates to the resulting wastewaters.

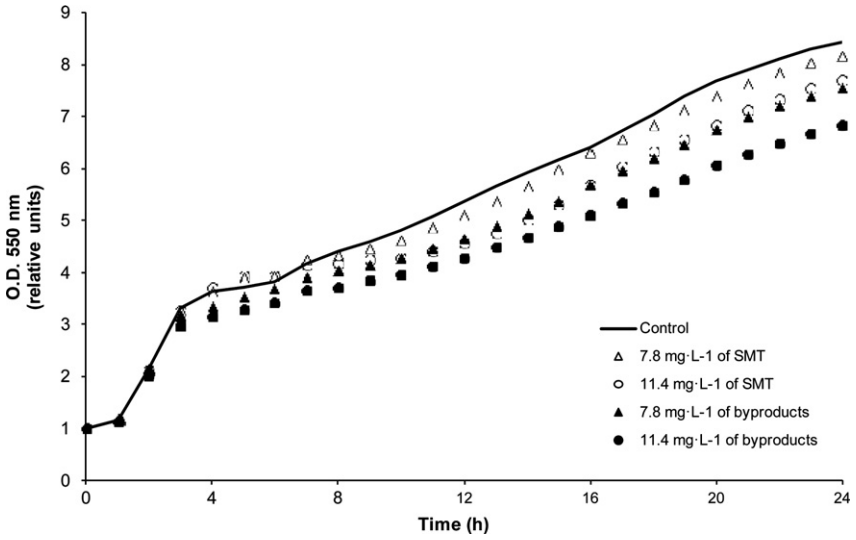

Fig. 5 – *E. coli* bacteria's growth relative to the control. Keys for SMT concentration measured as TOC: △ and ○. Keys for byproducts measured as TOC after SMT complete elimination by Photo-Fenton process (Assay L, 75 and 30 min) ▲ and ●. Assays were performed by triplicate and produced a standard error for each point less than 0.01 O.D. (optical density unit), which is not represented.

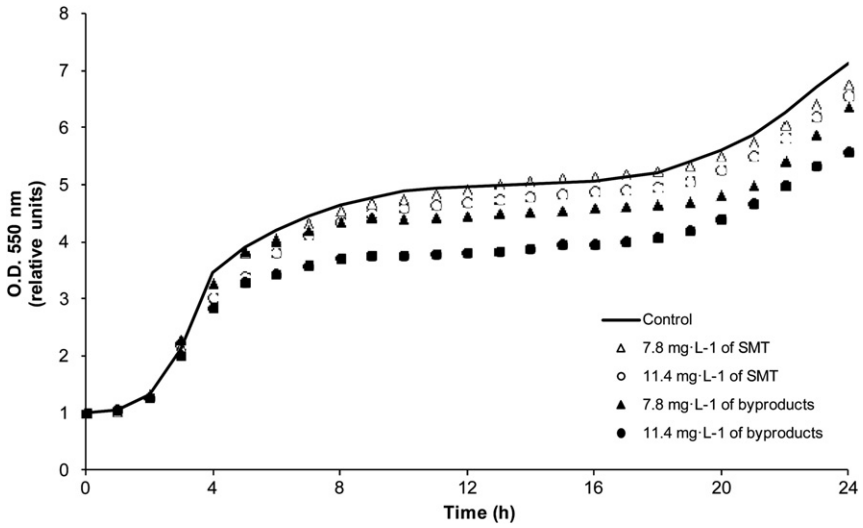

Fig. 6 – *S. aureus* bacteria's growth relative to the control. Keys for SMT concentration measured as TOC: △ and ○. Keys for byproducts measured as TOC after SMT complete elimination by Photo-Fenton process (Assay L, 75 and 30 min) ▲ and ●. Assays were performed by triplicate and produced a standard error for each point less than 0.01 O.D. (optical density unit), which is not represented.

Two bacteria, *E. coli* (gram-negative bacteria) and *S. aureus* (gram-positive bacteria), were selected to evaluate the antibiotic capacity of the samples.

Preliminary tests were undertaken to assess the effect of hydrogen peroxide on bacteria's growth, demonstrating to be negligible below 40 mg L^{-1}. Therefore, toxicity assays did not require removing residual hydrogen peroxide (e.g. adding a scavenger) since its concentration for all the samples was below this threshold.

When cultured in the partially degraded media, the growth of both bacteria proved to be lower than that showed when cultured in the initial SMT medium. Detailed data is provided in Figs. 5 and 6 corresponding to the sample obtained from the latest assay L, at 30 and 75 min.

At 30 min, assay L produced a sample that was diluted for the toxicity test, and the TOC concentration of the resulting sample was measured to be 11.4 mg L^{-1}. Since SMT was known to have been completely removed from the sample, it may be designed as 11.4 mg L^{-1} of byproducts concentration. Accordingly, a reference of 11.4 mg L^{-1} of SMT concentration medium was prepared. The *E. coli* growth was lower in the former than in the latter (Fig. 5), both obviously lower than the growth obtained for the control medium. The same was determined for the growth of *S. aureus* (Fig. 6). Therefore, it is established that toxicity of the partially degraded wastewater obtained after 30 min treatment (11.4 mg L^{-1} byproducts concentration) is higher than that of the original SMT solution.

At 75 min, further oxidation is obtained, but relative results are found to be equivalent for the corresponding situation (7.8 mg L^{-1} of byproducts concentration). In absolute terms, however, the toxicity of this new situation is lower than that obtained at 30 min. This conclusion is drawn for both *E. coli* and *S. aureus* (Figs. 5 and 6 respectively). Therefore, the photo-Fenton treatment of SMT is demonstrated to follow a pathway including intermediates whose capacity for inhibiting bacterial growth is higher than that given by the parent SMT.

Similar conclusions arise from results on sulfonamide toxicity reported elsewhere: prolonged SMT exposition to UV has been shown to generate byproducts that decrease the growth rate of *Daphnia magna* (Jung et al., 2008).

4. Conclusions

The Fenton photo-assisted treatment of sulfamethazine (SMT) has been studied. Experimental data has been obtained for a series of conditions arranged under a design of experiments scheme. The assays planned have been monitored for a 60 min reaction span and practical operational conclusions have been obtained regarding the efficient degradation of the SMT problem sample (1 L, 1.88×10^{-4} M).

For characterizing the degradation rate, a first-order kinetic approach for TOC has been adopted. The corresponding kinetic constant and TOC half-life time has been determined for all the assays planned. Hence, the most suitable Fenton reagents load to achieve minimum TOC half-life time and maximum TOC degradation rate has been determined as 600 mg L^{-1} of H_2O_2 concentration and 50 mg L^{-1} of Fe^{2+} concentration.

These concentration values imply the following ratios:

$$\text{SMT (mg L}^{-1}\text{):Fe}^{2+}\text{ (mg L}^{-1}\text{)} = 0.9{:}1$$

$$\text{H}_2\text{O}_2\text{ (mg L}^{-1}\text{):SMT (mg L}^{-1}\text{)} = 13{:}1$$

$$\text{H}_2\text{O}_2\text{ (mg L}^{-1}\text{):Fe}^{2+}\text{ (mg L}^{-1}\text{)} = 12{:}1$$

HPLC analyses have revealed that both Fenton and photo-Fenton reactions were able to completely remove the SMT antibiotic from the studied samples in less than 2 min

treatment. For longer reaction times, TOC and HPLC analyses also reveal the presence of organic reaction intermediates, for which some hypothesis have been formulated according to all the experimental data recorded. Finally, the toxicity of some of the samples has been assessed through the measurement of their effect on the growth rate of two kinds of bacteria (*E. coli* and *S. aureus*). It has been established that the toxicity of an SMT solution undergoing a photo-Fenton treatment increases during its first reaction stages.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.watres.2010.01.032.

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