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Abstract

A combined process coupling an electro-Fenton and a biological degradation was investigated in order to mineralize synthetic and industrial pharmaceutical effluent containing trimethoprim, a bacteriostatic antibiotic. Electro-Fenton degradation of trimethoprim was optimized by means of a Doehlert experimental design, showing that 0.69 mM Fe^{2+}, 466 mA and 30 min electrolysis time were optimal, leading to total trimethoprim removal, while mineralization remained limited, 12% for 30 min electrolysis times. The aromatic and aliphatic by-products were identified and a plausible degradation pathway was proposed. Biodegradability was improved, since the BOD_5/COD ratio increased from 0.11 initially to 0.32 and 0.52 after 30 and 60 min electrolysis times respectively, confirmed by activated sludge culture, 47 and 59% mineralization of the byproducts from electrolysis.

The relevance of the proposed combined process was then confirmed on an industrial pharmaceutical effluent. Its electrolysis under the above conditions showed an almost total removal of trimethoprim after 180 min of electrolysis, while TOC removal remained low, 14 and 16% for 180 and 300 min reaction times, respectively. Overall removal yields of the industrial effluent during the combined process were therefore 80 and 89% for 180 and 300 minutes of effluent pretreatment followed by 15 days activated sludge culture, respectively.

Keywords: Trimethoprim; Combined processes; Electro-Fenton; Activated sludge culture; Pharmaceutical effluents; Mineralization.

1. Introduction

In these last years, antibiotics were considered to be an emerging environmental problem due to their continuous entry and persistence in the aquatic ecosystem [1]. This pollution proceeds from human excretion after drug administration and passes through wastewater treatment

plants (WWTPs). Comparatively, veterinary antibiotics do not undergo WWTPs treatment and may directly enter surface water. Animal manure, dispersed in the fields as fertilizer, can contaminate soil and consequently surface and groundwater [2]. Moreover, waste effluents from manufacture can also be considered as significant points of contamination [3].

Most conventional treatments applied in WWTPs were unsuccessful in the removal of antibiotics [4–6]. Consequently, the accumulation and persistence of these compounds in the environment can threat aquatic and terrestrial ecosystems [1]. Moreover, antibiotics residues in water are also suspected to be responsible for the production of resistant microorganisms, causing serious problems of public health, namely difficulties in treating pathologies and imbalance of microbial ecosystems [7]. Therefore, efficient and economical methods must be developed as an urgent need to remove these pollutants.

Advanced oxidation processes (AOPs) are potentially powerful methods; they are based on the generation of hydroxyl radicals ($^{\bullet}OH$) which are very reactive and non-selective oxidizing agents, leading to the degradation of organic pollutants by hydrogen atom abstraction reaction (Eq. (1)), electron transfer (Eq. (2)), or electrophilic addition to π systems (Eq. (3)) [8–12]. These processes involve chemical, photochemical or electro-chemical techniques such as Fenton, UV/ or H_2O_2/ozonation, photo-Fenton, heterogeneous photocatalysis and electro-Fenton [11–15].

$$RH + {}^{\bullet}OH \rightarrow R^{\bullet} + H_2O \quad (1)$$

$$RX + {}^{\bullet}OH \rightarrow RX^{\bullet+} + OH^{-} \quad (2)$$

$$ArX + {}^{\bullet}OH \rightarrow OHArX^{\bullet} \quad (3)$$

Nevertheless, AOP used for complete mineralization can be expensive, and hence its combination with a biological treatment can significantly reduce operating costs [16]. This combination is especially suitable when microorganisms present in activated sludge are not able to metabolize the parent compound; the AOP, applied as a pre-treatment step, may

enhance the overall biodegradability by transforming this parent compound into easily biodegradable and less toxic intermediates, preventing cellular lysis [1,17,18].

The present work intends to perform three objectives. The first one is the use of Doehlert methodology design to optimize the electro-Fenton operating conditions for the removal of trimethoprim (TMP) i.e. its degradation into organic by-products; the second aim is to combine electro-Fenton process and biological treatment for the mineralization of TMP i.e. its transformation into water, carbon dioxide and inorganic ions; and the last one is the validation of the combined process through the remediation of an industrial pharmaceutical effluent.

Electro-Fenton is an electrochemical advanced oxidation process based on the continuous generation of H_2O_2 in an acidic medium through the electrochemical reduction of O_2 at the cathode (Eq. (4)). The generated H_2O_2 reacts with the added Fe^{2+} ions to produce hydroxyl radicals ($^{\bullet}OH$) and Fe^{3+} ions via the Fenton's reaction (Eq. (5)), which is favored by the catalytic action of the Fe^{3+}/Fe^{2+} system, mainly from the regeneration of Fe^{2+} by the cathodic reduction of Fe^{3+} (Eq. (6)) [8,11,13,19]. At the anode, oxygen is formed by the oxidation of water (Eq. (7)). Moreover, the method and the involved reactor are easy to handle and to use.

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2 \qquad (E^\circ = 0.69\ \text{V, Standard Hydrogen Electrode: SHE}) \qquad (4)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH \qquad \text{(Fenton's reaction)} \qquad (5)$$

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \qquad (E^\circ = 0.77\ \text{V, SHE}) \qquad (6)$$

$$2\ H_2O \rightarrow O_2 + 4e^- + 4H^+ \qquad (7)$$

As mentioned above, the chosen target compound is trimethoprim ($C_{14}H_{18}N_4O_3$, 290.32 g mol^{-1}, CAS 738-70-5), a bacteriostatic antibiotic commonly prescribed in combination with sulfamethoxazole for the treatment of infectious diseases in humans. It is also widely used in veterinary medicine, for prevention and treatment of infections and as a growth promoter. The removal of TMP in wastewater treatment plants has been reported to be less than 10% [20]. Therefore, the TMP (water solubility of 400 mg L^{-1} at 25°C) has been

detected in many environmental monitoring studies in the concentration range of μg L^{-1} (0.1 to 5 μg L^{-1}) in WWTP effluents [21].

2. Materials and Methods

2.1. Chemicals

Trimethoprim (98%) was purchased from Sigma Aldrich (Saint Quentin Fallavier, France). $FeSO_4.7H_2O$ (purity 99%) and Na_2SO_4 (purity 99%), used as a catalyst source and inert supporting electrolyte respectively, were provided from Acros Organics (Thermo Fisher Scientific, Illkirch, France). Acetonitrile (purity 99.9%) (HPLC grade) was also obtained from Sigma Aldrich. The initial pH of the solutions was adjusted using analytical grade sulfuric acid from Acros. All solutions were prepared in ultra-pure water and all the other chemicals used for analysis were purchased from Acros Organics and Sigma Aldrich.

2.2. Characterization of the pharmaceutical effluent

The effluent used in this study was supplied by a pharmaceutical industry located in north Tunisia, collected in a container, closed and stored in obscurity at 4°C. This effluent contained a high TMP concentration of 3.56 g L^{-1} and was characterized by a conductivity of 4.36 mS cm^{-1}, a COD of 438.50 g L^{-1} and a TOC of 125.40 g L^{-1}. Before treatment, the effluent was diluted to obtain an initial TMP concentration of 0.2 mM, in order to conduct the treatment in the same conditions as the synthetic solution.

2.3. Analytical determinations

2.3.1. High Performance Liquid Chromatography (HPLC)

The evolution of trimethoprim concentrations was monitored by HPLC using a Waters 996 system equipped with Waters 996 PDA (Photodiode Array Detector) and Waters 600LCD

Pump. The separation was achieved on a Waters C_{18}, (5 µm; 4.6 × 250 mm) reversed-phase column. The eluent consisted of a mixture of acetonitrile/ultra-pure water (20/80 v/v) buffered at pH 3 with phosphoric acid and 0.01 M Na_2HPO_4, delivered at a flow rate of 1 mL min^{-1}. Detection of trimethoprim was carried out at 204 nm.

2.3.2. Chemical Oxygen Demand (COD) measurements

Chemical Oxygen Demand (COD) was measured by means of Nanocolor® tests CSB 160 and 1500 from Macherey-Nagel (Düren, Germany). The amount of oxygen required for the oxidation of the organic and mineral matter at 148°C for 2 h was quantified after oxidation with $K_2Cr_2O_7$ at acidic pH [22].

2.3.3. Total Organic Carbon (TOC) measurements

The solutions were filtered on Sartorius Stedim Minisart 0.40 µm GF prefilters (Goettingen, Germany). TOC was measured by means of a TOC-$V_{CPH/CPN}$ Total Organic Analyzer Shimadzu. Organic carbon compounds were combusted and converted to CO_2, which was detected and measured by a non-dispersive infrared detector (NDIR). Reproducible TOC values were always obtained using the standard NPOC (Non Purgeable Organic Carbon) method. For each sample, each measurement was triplicated [16].

2.3.4. Activated Sludge Preparation

Activated Sludge used in this study was collected from a local wastewater treatment plant (Rennes Beaurade, Bretagne, France). It was washed several times by successions of centrifugation, supernatant withdrawing and resuspension of pellet in water, in order to remove any residual carbon or mineral nutrients.

2.3.5. Biological Oxygen Demand (BOD_5) measurements

BOD_5 measurements were carried out in Oxitop IS6 (WTW, Alès, France). The following mineral basis was used for all experiments (g L^{-1}): $MgSO_4.7H_2O$, 22.50; $CaCl_2$, 27.50; $FeCl_3$, 0.15; NH_4Cl, 2.00; Na_2HPO_4, 6.80; KH_2PO_4, 2.80. Washed activated sludge was added in order to have 0.05 g L^{-1} initial concentration of dry matter. The BOD_5 value was initially estimated based on the COD value experimentally measured, BOD_5 = COD/1.46. The range of expected BOD_5 measurement was then deduced and hence led to the volumes of sample, of activated sludge solution and nitrification inhibitor (10 mg L^{-1} solution of N-allylthiourea) which have to be added to the shake flask of the Oxitop apparatus. Similar protocol was applied for the control sample except that it was replaced by a solution of easily biodegradable compounds, namely glutamic acid (150 mg L^{-1}) and glucose (150 mg L^{-1}). Before use, KOH was added to achieve neutral pH (7.0 ± 0.2). Similar protocol was also considered for the blank solution, for which the sample was replaced by water to have a negligible BOD_5 value.

2.3.6. Ultra-pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS)

Aromatic by-products of trimethoprim were separated using a Waters (Acquity UPLC) liquid chromatographic system (Milford, USA). 5 µL of samples were injected into an Acquity BEH C_{18} column (2.1 mm × 100 mm, 1.7 µm) thermostated at 45°C. Isocratic elution was performed with a mixture of acetonitrile/ultra-pure water (30/70 v/v) (with the addition of 0.1% formic acid when needed), delivered at a flow rate of 400 µL min^{-1}. A Quattro Premier triple-quadrupole mass spectrometer (Manchester, UK) equipped with an electrospray ionization source (ESI) was used for by-products detection. MS/MS detection was performed in negative or positive mode with a capillary voltage of ±3 KV. The desolvation gas flow (N_2) was set to 750 L h^{-1}. The source temperature and desolvation gas temperature were

120°C and 350°C, respectively. The analytical device was controlled by Micromass Masslynx 4.1 software.

2.3.7. Ion chromatography (IC)

Generated carboxylic acids were identified by ion chromatography (Dionex DX120), coupled to a conductivity detector, using an anion-exchange column Dionex AS19 (4 × 250 mm) equipped with a Dionex AG19 guard column (4 × 50 mm) coupled to a ASRS suppressor. The eluent gradient was generated using the Dionex ECG-KOH Elu GenII cartridge; it was as follows: 0-10 min: 10 mM isocratic; 10-25 min: gradient from 10 to 45 mM; 25-35 min: 45 mM isocratic with a flow rate of 1 mL min^{-1}.

2.4. Experimental Procedure

2.4.1. Electro-Fenton process

The degradation of the organic matter by the electro-Fenton process was carried out in a 1 L undivided cylindrical glass cell equipped with two electrodes. The dimensions of the carbon felt piece placed on the inner wall of the cell (Le Carbone Lorraine RVG 4000 – Mersen, Paris La Défense, France), which was used as the cathode, were 260 mm × 80 mm. Its specific area, measured by the BET method was 0.7 m^2 g^{-1}, its thickness was 12 mm, its density was 0.088 g cm^{-3} and its carbon yield was 99.9%. The anode was a cylindrical platinum electrode (50 mm × 20 mm) located in the center of the electrochemical reactor to have a good potential distribution. Prior to electrolysis, compressed air was bubbled for 10 min through the solution at a flow rate of 450 cm^3 min^{-1} to saturate the aqueous solution with oxygen.

The pH of the solutions was adjusted to 3 by sulfuric acid (H_2SO_4). A catalytic quantity of $FeSO_4.7H_2O$ was introduced into the cell just before the beginning of the electrolysis. The

electrodes were connected to a DC power supply (Metrix, model AX 322, Chauvin Arnoux Group, Paris, France) operating in galvanostatic mode to control the current intensity. The ionic strength was maintained constant by the addition of 0.05 M Na_2SO_4. The electrolytic solution was in circulation with the help of a peristaltic pump (flow rate of 2 L min^{-1}). The temperature was maintained at 18°C and the initial synthetic solution or the industrial pharmaceutical effluent was diluted to achieve an initial trimethoprim concentration of 0.2 mM.

2.4.2. Biological treatment

Culture media were prepared in duplicate in 500 mL serum bottles containing 200 mL of synthetic trimethoprim solution or pharmaceutical effluent, beforehand electrolyzed. Minerals were spiked in the medium as highly concentrated solutions to reach the following initial composition (mg L^{-1}): Na_2HPO_4, 334; K_2HPO_4, 208; KH_2PO_4, 85; $CaCl_2$, 27.4; $MgSO_4.7H_2O$, 22.6; NH_4Cl, 2; $FeCl_3.6H_2O$, 0.26; and the initial pH was adjusted to 7. Activated sludge was added in order to have 1 g L^{-1} initial concentration of dry matter. Cultures were agitated at 350 rpm and kept at room temperature (25°C). Samples (5 mL) were taken regularly, filtered through 0.45 μm-syringe filters and injected for TOC measurements.

2.5. Doehlert experimental design

Doehlert experimental design [23] was used to determine the optimal operating conditions for the degradation of trimethoprim. The influence of three factors: current intensity (U_1), initial Fe^{2+} concentration (U_2) and electrolysis time (U_3) were studied. The analyzed response (Y) was the removal rate of trimethoprim. The Doehlert matrix consists of N experiments with $N = K^2 + K + 1$, where K is the number of variables. For K = 3, the matrix comprised 13 experiments which were uniformly distributed within the space of the coded variables (X_i). The number of replicates in the central point of the design was fixed at 3 (experiments 13-15)

in order to obtain an estimation of the experimental error. The transformation of natural variables (U_i) into coded variables (X_i) was made according to the following equation [24]:

$$X_i = \left[\frac{U_i - U_{i\,(0)}}{\Delta U_i}\right] \alpha_i \quad (8)$$

Where $U_{i\,(0)}$ is the value of U_i at the center of the study domain, ΔU_i is the variation step and α_i is the maximum coded value of X_i: $\alpha_1 = 1$; $\alpha_2 = \sqrt{3} / 2$; $\alpha_3 = \sqrt{6} / 3$.

$$U_{i\,(0)} = \frac{\text{upper limit of } U_i + \text{lower limit of } U_i}{2} \quad (9)$$

$$\Delta U_i = \frac{\text{upper limit of } U_i - \text{lower limit of } U_i}{2} \quad (10)$$

For the Doehlert experimental design construction, the retained domain for each variable ($300 < U_1/\text{mA} < 500$; $0.050 < U_2/\text{mM} < 1.000$ and $6 < U_3/\text{min} < 30$) was determined after preliminary tests (data not shown).

The experimental response associated to the Doehlert matrix is represented by a quadratic polynomial model:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (11)$$

Where Y is the experimental response, b_0 is a constant of the model, b_i is the estimation of the main effects of the factor i, b_{ii} is the estimation of the second order effects and b_{ij} is the estimation of the interactions between factor i and factor j.

The calculation of coefficients is carried out through the least squares method by means of

$$B = (X^T X)^{-1} X^T Y \quad (12)$$

Where B is the vector of estimates of the coefficients, X is the model matrix, X^T is the transposed model matrix and Y is the vector of the measured response.

The statistical significance of the model was checked using the variance analysis (ANOVA). The relationship between the response and the experimental variables was graphically illustrated by plotting the three-dimensional response surface and the two dimensional isoresponse curves. NEMRODW Software [25] was used for data calculation and treatment.

3. Results and Discussion

3.1. Determination of the optimal conditions for the removal of TMP

The performances of the electro-Fenton process depend on various operating parameters, like current intensity, initial ferrous ions concentration and electrolysis time [26]. The optimal conditions of these variables, for the removal of trimethoprim, have been investigated by the use of Doehlert matrix. The experimental design is represented in Table 1.

The obtained responses (TMP removal (%)) were used to compute the polynomial model coefficients, leading to propose the following model equation:

$$Y = 98.9 + 2.7\ X_1 + 1.5\ X_2 + 13.2\ X_3 - 2.2\ X_1^2 - 3.4\ X_2^2 - 13.2\ X_3^2 + 0.8\ X_1X_2 + 0.7\ X_1X_3 - 2.6\ X_2X_3 \quad (13)$$

The variance analysis for the fitted model indicated that the model was statistically meaningful (P-value < 0.01) (Table 2). Moreover, the correlation coefficients (R^2 = 0.98 and $R^2_{Adjusted}$ = 0.95) were high, implying that in the studied domain more than 95% of the response variability was explained by the second-order polynomial predicted equation (Eq. (13)). Consequently, one can conclude that the response Y is adequately described by the polynomial model, and so the generated equation can be used to predict the Y-values in the studied domain.

Furthermore, the isoresponse curves of TMP removal and the corresponding three-dimensional representations can be seen in Fig. 1. The graphic analysis of these figures showed that increasing electrolysis time enhanced the removal of trimethoprim. Indeed, a higher rate of trimethoprim removal could be reached when the reaction time would vary from 20 to 30 minutes (Fig. 1a-d).

It can also be seen that TMP removal rate was improved when the initial Fe^{2+} concentration was located between 0.64 and 0.74 mM (Fig. 1c and d), most likely due to a higher production of hydroxyl radicals in the presence of more Fe^{2+} ions in the electro-Fenton reaction [27].

However, the use of high ferrous ions concentration ($[Fe^{2+}]_0$ > 0.74 mM) led to a decrease of the removal efficiency, probably due to the consumption of $^{\bullet}OH$ by the excessive ferrous ions according to equation (14), decreasing the quantity of this radical and hence inhibiting the degradation reaction [8,27].

$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^{-} \quad (14)$$

Moreover, it can be noted that an increase of the applied current intensity would increase the removal efficiency; the maximum TMP removal rate was obtained when the current intensity was ranging between 455 and 477 mA (Fig. 1e and f). This trend can be explained by the excess production of hydrogen peroxide and an increased regeneration rate of Fe^{2+} that would promote hydroxyl radical production [28]. Besides, at a higher current intensity (I > 477 mA) the TMP removal rate decreased; this behavior can be attributed to the 4e- reduction of O_2 leading to the formation of H_2O (Eq. (15)), which inhibits H_2O_2 formation reaction (Eq. (4)) [29]. In addition, at current values higher than 477 mA, the hydrogen gas evolution, at the cathode (Eq. (16)), compete with the formation of H_2O_2 [30]. This situation reduces the production of hydroxyl radicals and consequently, decreases the removal efficiency of trimethoprim.

$$O_2 + 4e^{-} + 4H^{+} \rightarrow 2H_2O \quad (15)$$

$$2H_2O + 2e^{-} \rightarrow H_2 + 2OH^{-} \quad (16)$$

3.2. Electro-Fenton pretreatment of TMP

The removal of trimethoprim was carried out in the optimal operating conditions deduced from the Doehlert matrix, namely $[Fe^{2+}]_0$ = 0.69 mM and I = 466 mA, leading to a rapid and total TMP removal within 30 min pretreatment (Fig. 2a). Contrarily, mineralization and oxidation yields remained low, 12 and 21% for 30 and 60 min electrolysis times for the former (Fig. 2a), and 29 and 56% from an initial amount of 86 mg L^{-1} O_2 for the latter after 30 and 60 min pretreatment respectively (Fig. 2b). This suggested the formation of organic

intermediate products, as confirmed at the examination of the HPLC profiles (data not shown), showing that the disappearance of TMP was accompanied by the formation of several intermediates products. This behavior is coherent with the fast destruction of TMP and TOC decrease and can be explained by the oxidation of generated products by hydroxyl radicals [31].

3.3. Improvement of the biodegradability

In order to follow the evolution of the biodegradability profile during the electro-Fenton pretreatment, the samples were analyzed for BOD_5 and COD, showing a ratio of 0.11 for the target molecule confirming its recalcitrance and the need for its oxidation prior to a biological treatment (Fig.2b), while the BOD_5/COD ratio increased to 0.32 and 0.52 after 30 and 60 min electrolysis times, namely above the limit of biodegradability (0.4 [32]) after 60 min pretreatment. From this and owing to the total TMP removal after only 30 min electrolysis, in addition to TMP removal part of by-products was also oxidized after 60 min pretreatment.

3.4. Identification of intermediate products and pathway of TMP degradation

The electro-Fenton degradation of 0.2 mM trimethoprim aqueous solution containing 0.69 mM ferrous ions and 50 mM Na_2SO_4 at pH 3 and 466 mA constant current led to the formation of aromatic by-products and short-chain carboxylic acids. The identification of the main aromatic by-products was carried out by UPLC-MS/MS; it was based on the analysis of the Total Ion Chromatogram (TIC) and the corresponding mass spectra. The proposed structures were also determined according to the structure of the parent compound and the molecular weight (MW) of the by-products. Among the degradation products, six compounds were confirmed by comparing their retention time and their molecular weight with those of commercially available standard compounds (Table 3); their evolution during electrolysis was

presented in Fig. 3. Besides, generated carboxylic acids were identified by ion chromatography and their retention times were compared with standard compounds (Table 3). The obtained results allowed suggesting a degradation pathway for the electro-Fenton oxidation of trimethoprim (Fig. 4). As can be seen, the cleavage of the central methylene group of TMP, at the beginning of electrolysis, was accompanied by the formation of 1,2,3-trimethoxybenzene (MW 168) and 2,4-diamino-5-pyrimidinemethanol (MW 140) which has already been reported during the TiO_2 photocatalysis of TMP [33]. The compound MW 168 was further transformed to 3,4,5-trimethoxyphenol (MW 184) through hydroxylation reaction. In addition, the compound MW 140 was converted into 2,4-diaminopyrimidine-5-carbaldehyde (MW 138) which was presumably further oxidized by $^{\bullet}OH$ to give 2,4-diaminopyrimidine-5-carboxylic acid (MW 154). These latter by-products have been proven by earlier studies [21,33]. Furthermore, the hydroxylation of the 2,4-diaminopyrimidine portion of TMP yielded the phenolic product 6-hydroxytrimethoprim (MW 306) which has also been identified during the $^{\bullet}OH$ oxidation of TMP [20]. 2,4-diamino-6-hydroxypyrimidine (MW 126) and (3,4,5-trimethoxyphenyl)methanol (MW 198) were formed during the cleavage of the central methylene group of MW 306 by the attack of $^{\bullet}OH$. The latter compound (MW 198) has already been reported during the electrochemical degradation of TMP at boron-doped diamond electrode [34]. Moreover, we presume that 2,6-diaminopyrimidine-4,5-diol (MW 142) was the result of hydroxylation of the compound MW 126. In addition, the product (MW 198) was attacked by $^{\bullet}OH$ to form the 3,4,5-trimethoxybenzaldehyde (MW 196). The latter degradation product, which has been proven by earlier study [33], can also undergo oxidation leading to 3,4,5-trimethoxybenzoic acid (MW 212).

The observation of Fig. 2a and Fig. 3 shows that the disappearance of TMP and the formation of intermediate compounds took place simultaneously. As can be seen, the major aromatic by-

products were 2,4-diaminopyrimidine-5-carbaldehyde and 3,4,5-trimethoxybenzaldehyde, their formations were very fast, they reached their maximum concentrations (196.5 and 91 µg L^{-1} respectively) at 5 min and completely disappeared within 40 and 50 min, respectively. Whereas, 3,4,5-trimethoxyphenol and 2,4-diamino-6-hydroxypyrimidine reached their maximal accumulation values (lower than 2 µg L^{-1}) after 10 min of treatment and then their concentrations decreased until complete disappearance. It was found that the concentration of 1,2,3-trimethoxybenzene remained at small steady-state values during the electrolysis. On the other hand, the 3,4,5-trimethoxybenzoic acid concentration increased rapidly attaining a maximum concentration of 4 µg L^{-1} at 5 min of electrolysis and then followed a slow degradation kinetic. Finally, all aromatic by-products would be further oxidized by •OH through ring cleavage reactions into short-chain aliphatic carboxylic acids, such as acetic acid (MW 60), glyoxylic acid (MW 74), oxalic acid (MW 90) and succinic acid (MW 118). The time-course evolution of some carboxylic acids was followed; the obtained results showed that glyoxylic and succinic acids appeared during the first minutes of treatment, reaching concentrations of 11.5 and 2 mg L^{-1} after 60 min of electrolysis. According to Haidar *et al*. [35], the action of hydroxyl radicals on the aromatic intermediates is easier than on the carboxylic acids, which have a good resistivity and are hardly oxidizable by •OH.

3.5. Biological treatment

Owing to biodegradability improvement, activated sludge cultures of pretreated solutions of TMP were carried out in duplicates for 20 days (Fig. 5). 37 and 41% mineralization were obtained after 4 and 6 days of culture for the solutions pretreated during 30 and 60 minutes, respectively (Fig. 5). This behavior can be attributed to a readily assimilation of some of the degradation products by microorganisms [36]. Low mineralization rates can be then observed

beyond this time until reaching 47 and 59% on the 20th day for the solutions electrolyzed during 30 and 60 minutes, respectively (Fig. 5). From this, the level of mineralization observed during activated sludge culture increased with the pretreatment time. It is noteworthy that in the absence of pretreatment, no noticeable TMP biodegradation was observed (Fig. 5), in agreement with its low BOD_5/COD ratio, 0.11 (Fig. 2b).

It should be noted that possible biosorption of TMP or the degradation products from the pretreatment was checked and was found to be not significant (data not shown).

Moreira *et al*. [37], have studied the mineralization of 20.0 mg L^{-1} of TMP by electro-Fenton process, with a 10 cm² BDD anode and a 10 cm² carbon PTFE air diffusion cathode. The treatment was performed using a low Fe^{2+} dose of 2.0 mg L^{-1}, a low current density of 5 mA·cm² and a pH of 3.5; it has led to a 14% mineralization after 180 min reaction.

3.6. Application to an industrial pharmaceutical effluent

The pretreatment of an industrial pharmaceutical effluent was carried out in the same operating conditions as for the synthetic solution (Fig. 6). As shown in Fig. 6a, continuous TMP removal was observed during the oxidation of the industrial effluent until reaching 98% after 180 minutes. The rate of TMP degradation in the industrial effluent was therefore slower than in the synthetic solution; this behavior may be due to the competitive consumption of hydroxyl radicals by other organic compounds contained in the real effluent, since TMP accounted for only 6% of the organic content of the industrial effluent (Tables 1 and 2). A low level of mineralization was concomitantly observed, 14 and 16% for 180 and 300 minutes electrolysis times (Fig. 6a), as well as a relatively low level of oxidation, 18 and 20% from an initial amount of 8770 mg L^{-1} O_2 after 180 and 300 minutes of pretreatment respectively (Fig. 6b). These results can be explained by the generation of intermediate compounds simultaneously with the degradation of the target compound, as confirmed at the examination

of the HPLC profiles (data not shown). The low BOD_5/COD ratio (0.14 – Fig. 6b) of the industrial effluent confirmed the need for a pretreatment prior to a biological treatment; while after electrolysis, the solutions became biodegradable since the BOD_5/COD ratios increased to 0.45 and 0.47 for 180 and 300 minutes pretreatment (Fig. 6b). The corresponding biological treatments were therefore carried out and are displayed in Fig. 7. The mineralization yields observed were close to 70% after about 10 days of culture for the effluent pretreated during 180 minutes and final mineralization yields were 76 and 87% for 180 and 300 minutes of effluent pretreatment, respectively (Fig. 7). Contrarily, in the absence of pretreatment, the rate and the yield of mineralization were significantly lower, since only 15% TOC removal was observed after 8 days of culture and about 50% at the end of culture (15^{th} day). Overall removal yields of the industrial effluent during the combined process were therefore 80 and 89% after 180 and 300 minutes of effluent pretreatment, respectively.

It should also be noted that as for the synthetic solution possible biosorption of the organic content of the industrial effluent or the degradation products from the pretreatment was checked and was found to be negligible (data not shown).

The higher levels of mineralization observed for the industrial effluent if compared to the synthetic solution should be underlined. Indeed, TMP contributed for only 6% of the carbon content of the industrial effluent, and hence at least part of this organic content was also oxidized during pretreatment and mineralized during biological treatment. Indeed, in the absence of pretreatment, no TMP degradation was observed (Fig. 5), while significant mineralization was observed at the end of the biological treatment of the industrial effluent (Fig. 7). Furthermore, for 30 and 180 minutes pretreatment of the synthetic solution and the industrial effluent, namely the time needed for a total TMP removal (Figs.2a and 6a), important mineralization was observed for the industrial effluent, 70% after about 10 days

biological treatment (Fig. 7), while only 47% after 20 days culture on the synthetic solution (Fig. 5).

4. Conclusions

Doehlert experimental design was used to determine the optimal operating conditions for the degradation of trimethoprim by the electro-Fenton process. The obtained results showed a total TMP removal after an electrolysis time of 30 min, for an initial ferrous ions concentration of 0.69 mM and using a current intensity of 466 mA. The degradation of TMP was accompanied by the formation of aromatic by-products and short-chain carboxylic acids. Based on the identified intermediates, a plausible degradation pathway was proposed.

Trimethoprim pretreatment was carried out in the optimal operating conditions deduced from the Doehlert matrix, leading to low levels of mineralization, 12 and 21% after 30 and 60 min electrolysis times respectively. Simultaneously, an improvement of the biodegradability was noted, (0.32 and 0.52 from an initial BOD_5/COD ratio of 0.11). Therefore, biological treatments were performed during 20 days and showed that the level of mineralization increased with the electrolysis time, from 47 to 59% for 30 and 60 min pretreatment times.

In order to confirm the relevance of the proposed combined process, an industrial pharmaceutical effluent was electrolyzed under the same conditions, showing an almost total TMP removal (98%), while the mineralization yields remained low, 14 and 16% after 180 and 300 minutes electrolysis times, respectively. Moreover, the BOD_5/COD ratio increased from 0.14 to 0.45 and 0.47 proving the enhancement of biodegradability of the pretreated effluent. The corresponding biological treatments led to 76 and 87% of mineralization after 15 days. Overall removal yields of the industrial effluent during the combined process were therefore 80 and 89% for 180 and 300 minutes of effluent pretreatment, respectively. An increase of the

pretreatment time entailed therefore a better biodegradability resulting in a higher mineralization of the electrolyzed solution during the subsequent biological culture.

References

[1] Homem V, Santos L. Degradation and removal methods of antibiotics from aqueous matrices – A review. J Environ Manage 2011;92:2304–47.

[2] Sharma VK. Oxidative transformations of environmental pharmaceuticals by Cl2, ClO2, O3, and Fe(VI): Kinetics assessment. Chemosphere 2008;73:1379–86.

[3] Mompelat S, Le Bot B, Thomas O. Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water. Environ Int 2009;35:803–14.

[4] Adams C, Wang Y, Loftin K, Meyer M. Removal of Antibiotics from Surface and Distilled Water in Conventional Water Treatment Processes. J Environ Eng 2002;128:253–60.

[5] Gobel A, Mcardell C, Joss A, Siegrist H, Giger W. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. Sci Total Environ 2007;372:361–71.

[6] Vieno NM, Härkki H, Tuhkanen T, Kronberg L. Occurrence of Pharmaceuticals in River Water and Their Elimination in a Pilot-Scale Drinking Water Treatment Plant. Environ Sci Technol 2007;41:5077–84.

[7] Bailón-Pérez MI, García-Campaña AM, Cruces-Blanco C, del Olmo Iruela M. Trace determination of β-lactam antibiotics in environmental aqueous samples using off-line and on-line preconcentration in capillary electrophoresis. J Chromatogr A 2008;1185:273–80.

[8] Brillas E, Sirés I, Oturan MA. Electro-Fenton Process and Related Electrochemical Technologies Based on Fenton's Reaction Chemistry. Chem Rev 2009;109:6570–631.

[9] Ghoneim MM, El-Desoky HS, Zidan NM. Electro-Fenton oxidation of Sunset Yellow FCF azo-dye in aqueous solutions. Desalination 2011;274:22–30.

[10] Oturan MA. An ecologically effective water treatment technique using electrochemically generated hydroxyl radicals for in situ destruction of organic pollutants: Application to herbicide 2,4-D. J Appl Electrochem 2000;30:475–82.

[11] Oturan MA, Oturan N, Edelahi MC, Podvorica FI, Kacemi KE. Oxidative degradation of herbicide diuron in aqueous medium by Fenton's reaction based advanced oxidation processes. Chem Eng J 2011;171:127–35.

[12] Özcan A, Şahin Y, Oturan MA. Complete removal of the insecticide azinphos-methyl from water by the electro-Fenton method – A kinetic and mechanistic study. Water Res 2013;47:1470–9.

[13] Loaiza-Ambuludi S, Panizza M, Oturan N, Özcan A, Oturan MA. Electro-Fenton degradation of anti-inflammatory drug ibuprofen in hydroorganic medium. J Electroanal Chem 2013;702:31–6.

[14] Shu H, Chang M. Decolorization effects of six azo dyes by O, UV/O and UV/HO processes. Dyes Pigments 2005;65:25–31.

[15] Yang H, An T, Li G, Song W, Cooper WJ, Luo H, et al. Photocatalytic degradation kinetics and mechanism of environmental pharmaceuticals in aqueous suspension of TiO2: A case of β-blockers. J Hazard Mater 2010;179:834–9.

[16] Oller I, Malato S, Sánchez-Pérez JA. Combination of Advanced Oxidation Processes and biological treatments for wastewater decontamination—A review. Sci Total Environ 2011;409:4141–66.

[17] Ballesteros Martín MM, Sánchez Pérez JA, García Sánchez JL, Casas López JL, Malato Rodríguez S. Effect of pesticide concentration on the degradation process by combined solar photo-Fenton and biological treatment. Water Res 2009;43:3838–48.

[18] Mansour D, Fourcade F, Huguet S, Soutrel I, Bellakhal N, Dachraoui M, et al. Improvement of the activated sludge treatment by its combination with electro Fenton for the mineralization of sulfamethazine. Int Biodeterior Biodegrad 2014;88:29–36.

[19] Garcia-Segura S, Garrido JA, Rodríguez RM, Cabot PL, Centellas F, Arias C, et al. Mineralization of flumequine in acidic medium by electro-Fenton and photoelectro-Fenton processes. Water Res 2012;46:2067–76.

[20] Luo X, Zheng Z, Greaves J, Cooper WJ, Song W. Trimethoprim: Kinetic and mechanistic considerations in photochemical environmental fate and AOP treatment. Water Res 2012;46:1327–36.

[21] Michael I, Hapeshi E, Osorio V, Perez S, Petrovic M, Zapata A, et al. Solar photocatalytic treatment of trimethoprim in four environmental matrices at a pilot scale: Transformation products and ecotoxicity evaluation. Sci Total Environ 2012;430:167–73.

[22] Fourcade F, Yahiat S, Elandaloussi K, Brosillon S, Amrane A. Relevance of Photocatalysis prior to Biological Treatment of Organic Pollutants - Selection Criteria. Chem Eng Technol 2012;35:238–46.

[23] Doehlert DH. Uniform Shell Designs. J R Stat Soc Ser C Appl Stat 1970;19:231–9.

[24] Hammami S, Ouejhani A, Bellakhal N, Dachraoui M. Application of Doehlert matrix to determine the optimal conditions of electrochemical treatment of tannery effluents. J Hazard Mater 2009;163:251–8.

[25] Mathieu D, Nony J, Phan-Tan-Luu R. New Efficient Methodology for Research using Optimal Design (NEMRODW) Software, LPRAI, Univ. Aix-Marseille III, France 2000.

[26] Mansour D, Fourcade F, Bellakhal N, Dachraoui M, Hauchard D, Amrane A. Biodegradability Improvement of Sulfamethazine Solutions by Means of an electro-Fenton Process. Water Air Soil Pollut 2012;223:2023–34.

[27] Panizza M, Cerisola G. Electro-Fenton degradation of synthetic dyes. Water Res 2009;43:339–44.

[28] Atmaca E. Treatment of landfill leachate by using electro-Fenton method. J Hazard Mater 2009;163:109–14.

[29] Özcan A, Şahin Y, Koparal AS, Oturan MA. Degradation of picloram by the electro-Fenton process. J Hazard Mater 2008;153:718–27.

[30] Masomboon N, Ratanatamskul C, Lu M-C. Chemical oxidation of 2,6-dimethylaniline by electrochemically generated Fenton's reagent. J Hazard Mater 2010;176:92–8.

[31] Hammami S, Bellakhal N, Oturan N, Oturan MA, Dachraoui M. Degradation of Acid Orange 7 by electrochemically generated •OH radicals in acidic aqueous medium using a boron-doped diamond or platinum anode: A mechanistic study. Chemosphere 2008;73:678–84.

[32] Salles NA, Fourcade F, Geneste F, Floner D, Amrane A. Relevance of an electrochemical process prior to a biological treatment for the removal of an organophosphorous pesticide, phosmet. J Hazard Mater 2010;181:617–23.

[33] Sirtori C, Agüera A, Gernjak W, Malato S. Effect of water-matrix composition on Trimethoprim solar photodegradation kinetics and pathways. Water Res 2010;44:2735–44.

[34] De Amorim KP, Romualdo LL, Andrade LS. Electrochemical degradation of sulfamethoxazole and trimethoprim at boron-doped diamond electrode: Performance, kinetics and reaction pathway. Sep Purif Technol 2013;120:319–27.

[35] Haidar M, Dirany A, Sirés I, Oturan N, Oturan MA. Electrochemical degradation of the antibiotic sulfachloropyridazine by hydroxyl radicals generated at a BDD anode. Chemosphere 2013;91:1304–9.

[36] Fontmorin J-M, Fourcade F, Geneste F, Floner D, Huguet S, Amrane A. Combined process for 2,4-Dichlorophenoxyacetic acid treatment—Coupling of an electrochemical system with a biological treatment. Biochem Eng J 2013;70:17–22.

[37] Moreira FC, Garcia-Segura S, Boaventura RAR, Brillas E, Vilar VJP. Degradation of the antibiotic trimethoprim by electrochemical advanced oxidation processes using a carbon-PTFE air-diffusion cathode and a boron-doped diamond or platinum anode. Appl Catal B Environ 2014;160-161:492–505.

Table 1 – Doehlert matrix experiments and experimental results.

Experiment Number	Coded variables			Real variables			Results
	X_1	X_2	X_3	Current intensity: U_1 (mA)	Fe^{2+} concentration: U_2 (mM)	Electrolysis time: U_3 (min)	Y (%)
1	1	0	0	500	0.525	18	100
2	-1	0	0	300	0.525	18	93
3	$\frac{1}{2}$	$\frac{\sqrt{3}}{2}$	0	450	1.000	18	100
4	$-\frac{1}{2}$	$-\frac{\sqrt{3}}{2}$	0	350	0.050	18	92
5	$\frac{1}{2}$	$-\frac{\sqrt{3}}{2}$	0	450	0.050	18	97
6	$-\frac{1}{2}$	$\frac{\sqrt{3}}{2}$	0	350	1.000	18	94
7	$\frac{1}{2}$	$\frac{\sqrt{3}}{6}$	$\frac{\sqrt{6}}{3}$	450	0.683	30	100
8	$-\frac{1}{2}$	$-\frac{\sqrt{3}}{6}$	$-\frac{\sqrt{6}}{3}$	350	0.367	6	78
9	$\frac{1}{2}$	$-\frac{\sqrt{3}}{6}$	$-\frac{\sqrt{6}}{3}$	450	0.367	6	76
10	0	$\frac{\sqrt{3}}{3}$	$-\frac{\sqrt{6}}{3}$	400	0.841	6	80
11	$-\frac{1}{2}$	$\frac{\sqrt{3}}{6}$	$\frac{\sqrt{6}}{3}$	350	0.683	30	100
12	0	$-\frac{\sqrt{3}}{3}$	$\frac{\sqrt{6}}{3}$	400	0.209	30	100
13	0	0	0	400	0.525	18	99
14	0	0	0	400	0.525	18	99
15	0	0	0	400	0.525	18	99

Table 2 – ANOVA result for TMP removal (%) under electro-Fenton treatment.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F-ratio	P-value
Regression	991.773	9	110.197	28.721	0.00088
Residual	19.184	5	3.8368		
Total	1010.957	14			

$R^2 = 0.98$; $R^2_{Adjusted} = 0.95$.

Table 3 – Chromatographic identification of the products generated during the electro-Fenton degradation of trimethoprim.

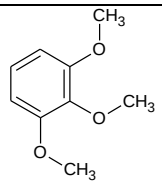
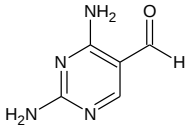
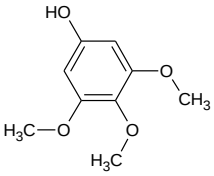
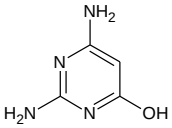
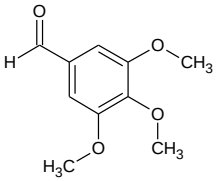
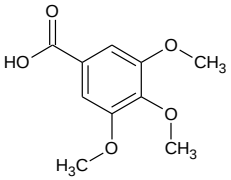
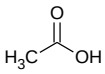
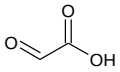
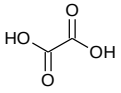
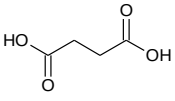
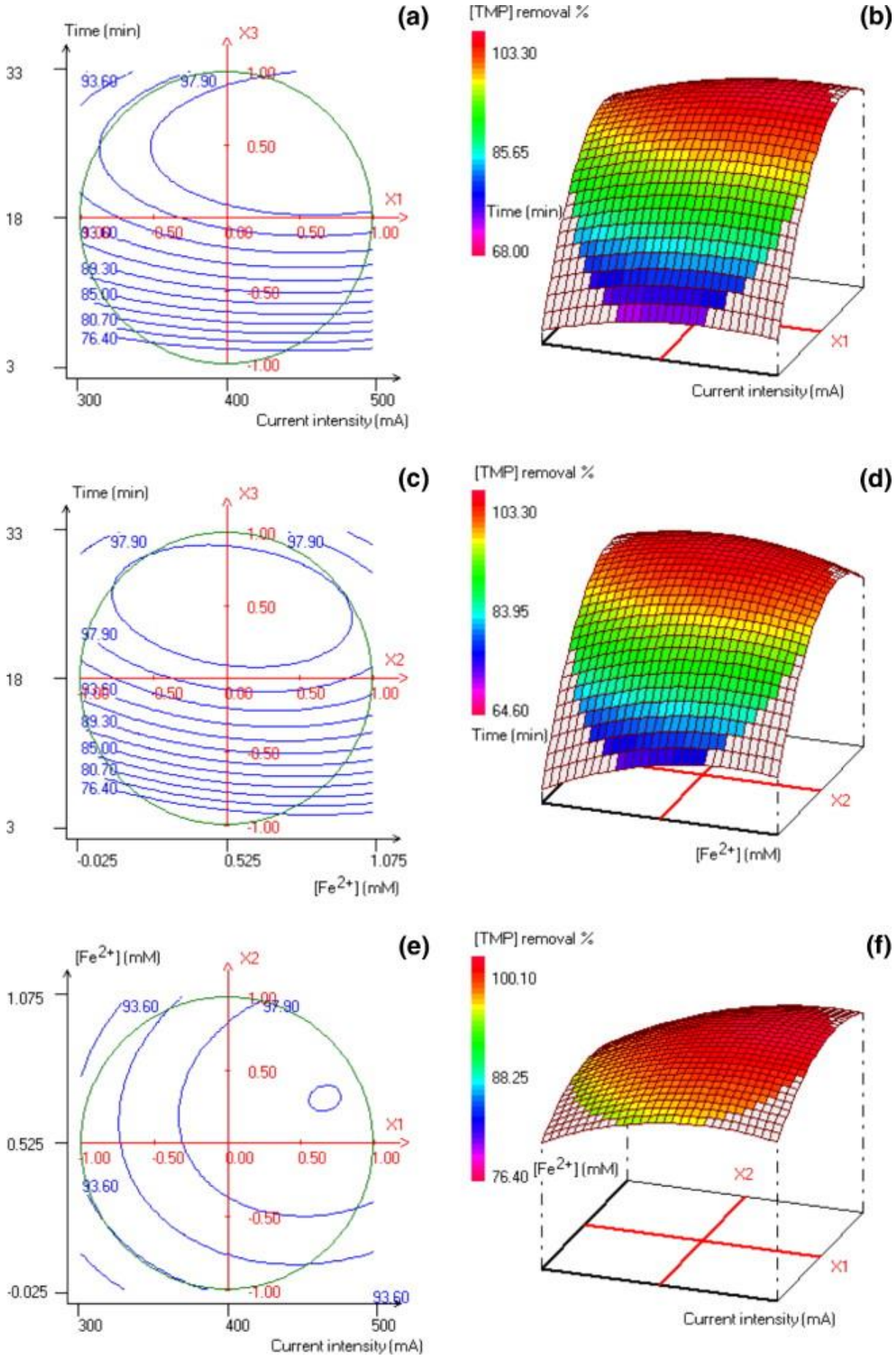
Compound	Chromatography	Chemical structure	Retention time (min)	ESI mode	Precursor ion (m/z)	Product ions (m/z)
1,2,3-trimethoxybenzene (MW 168)	UPLC-MS/MS		1.98	Positive	169	154 138
2,4-diaminopyrimidine-5-carbaldehyde (MW 138)	UPLC-MS/MS		0.69	Positive	139	97 69
3,4,5-trimethoxyphenol (MW 184)	UPLC-MS/MS		0.91	Negative	183	168 153
2,4-diamino-6-hydroxypyrimidine (MW 126)	UPLC-MS/MS		0.58	Positive	127	85 68 60
3,4,5-trimethoxybenzaldehyde (MW 196)	UPLC-MS/MS		1.63	Positive	197	169 138
3,4,5-trimethoxybenzoic acid (MW 212)	UPLC-MS/MS		0.72	Negative	211	167 152
Acetic acid (MW 60)	IC		4.2			
Glyoxylic acid (MW 74)	IC		4.5			
Oxalic acid (MW 90)	IC		5.4			
Succinic acid (MW 118)	IC		14.6			

Figure captions


(a)
Time (min)
X3
33
18
3
93.60
97.90
1.00
0.50
X1
-1.00
-0.50
0.00
0.50
1.00
89.30
85.00
80.70
76.40
-0.50
-1.00
300
400
500
Current intensity (mA)
(b)
[TMP] removal %
103.30
85.65
68.00
Time (min)
X1
Current intensity (mA)
(c)
Time (min)
X3
33
18
3
97.90
1.00
0.50
X2
-0.025
0.525
1.075
[Fe2+] (mM)
(d)
[TMP] removal %
103.30
83.95
64.60
Time (min)
X2
[Fe2+] (mM)
(e)
[Fe2+] (mM)
X2
1.075
0.525
-0.025
93.60
97.90
X1
300
400
500
Current intensity (mA)
(f)
[TMP] removal %
100.10
88.25
76.40
[Fe2+] (mM)
X2
X1
Current intensity (mA)

1

Figure 1. (a) Contour plots of TMP removal versus the electrolysis time (min) and the current intensity (mA); (b) corresponding three-dimensional plot; (c) contour plots of TMP removal versus the electrolysis time (min) and the initial Fe^{2+} concentration (mM); (d) corresponding three-dimensional plot; (e) contour plots of TMP removal versus the initial Fe^{2+} concentration (mM) and the current intensity (mA); (f) corresponding three-dimensional plot. Results obtained from the Doehlert matrix (Table 1).

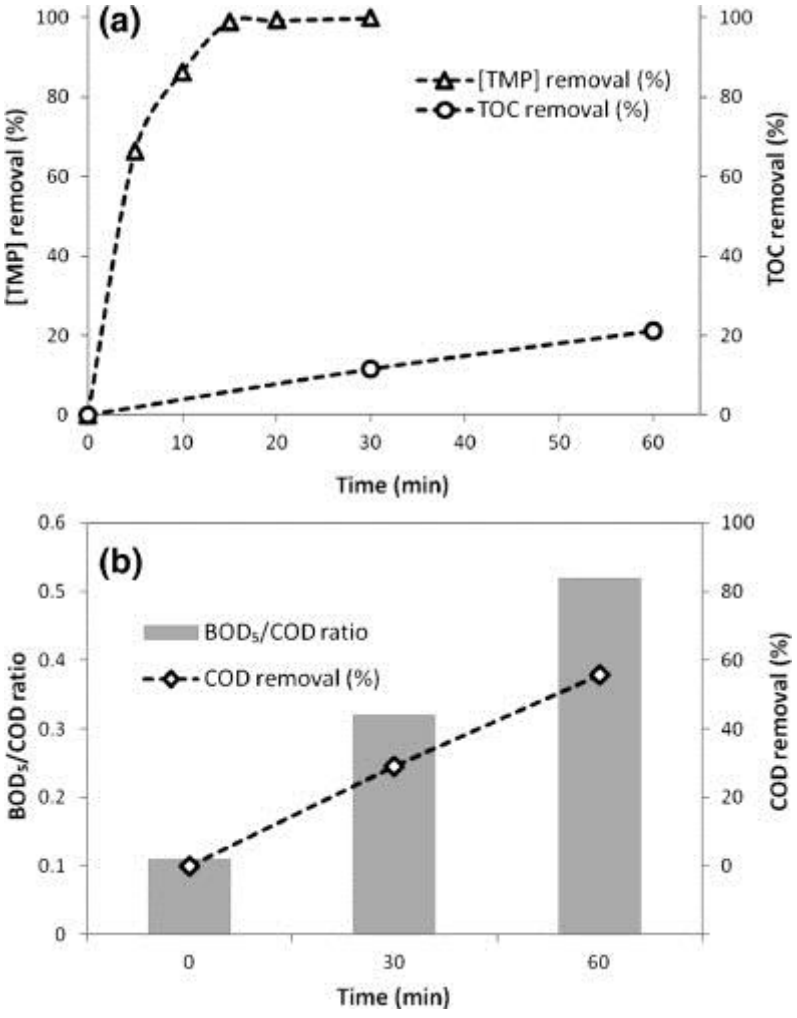


Figure 2. (a) Time evolution of TMP and TOC removals; (b) BOD_5/COD ratio and COD removal. Experimental conditions: $[TMP]_0$ = 0.2 mM, $[Fe^{2+}]_0$ = 0.69 mM, $[Na_2SO_4]$ = 50 mM, pH = 3, T = 18°C, I = 466 mA, V= 1 L.

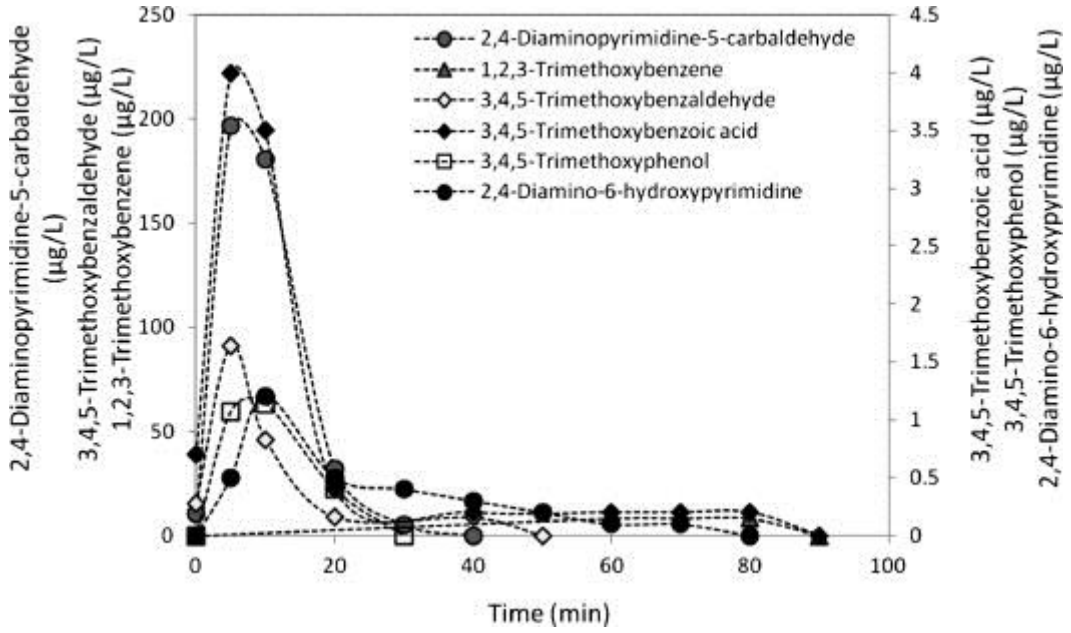


Figure 3. Time-course of aromatic by-products formed during the electro-Fenton treatment. Experimental conditions: $[TMP]_0$ = 0.2 mM, $[Fe^{2+}]_0$ = 0.69 mM, $[Na_2SO_4]$ = 50 mM, pH = 3, T = 18°C, I = 466 mA, V= 1 L.

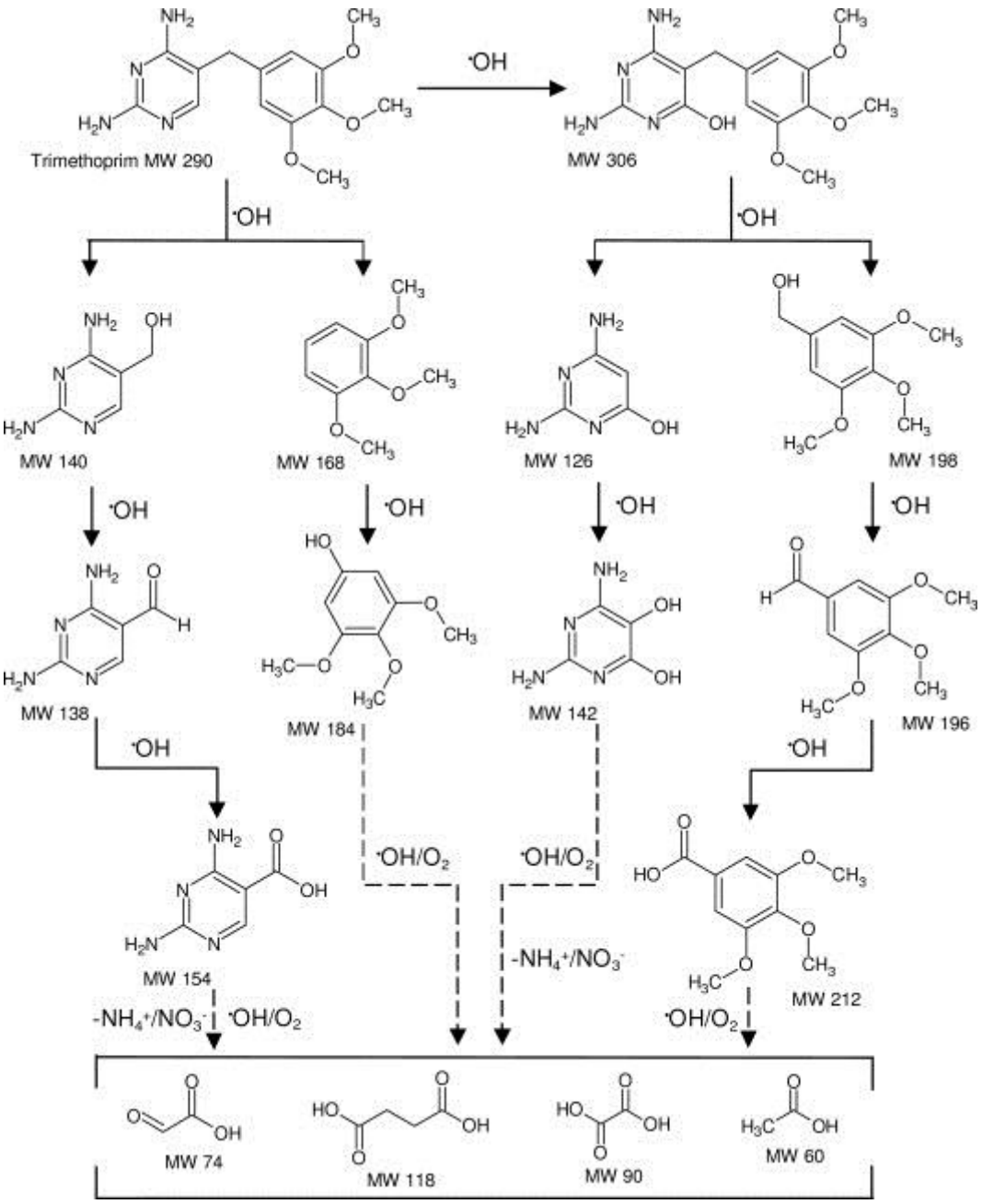


Figure 4. Pathway proposed for the degradation of TMP by the electro-Fenton process.

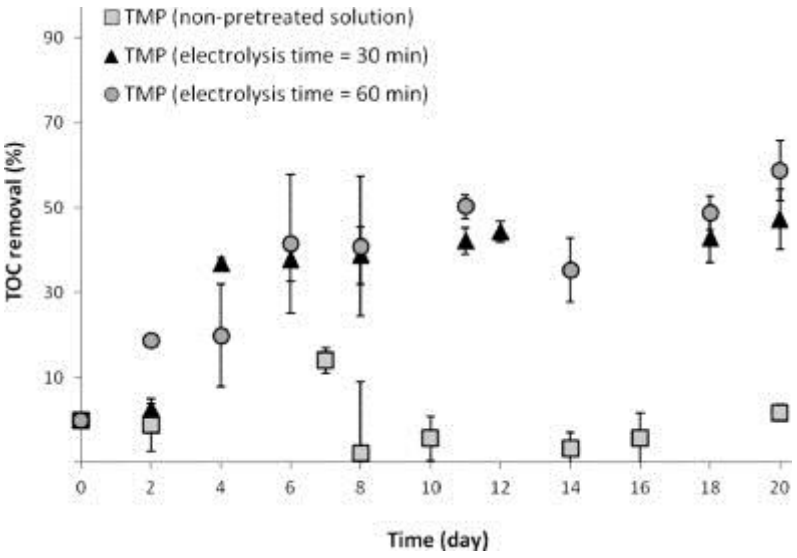


Figure 5. Mineralization during activated sludge cultures of non-pretreated TMP solution and solutions electrolyzed during 30 min and 60 min. Electro-Fenton pretreatment conditions: $[TMP]_0$ = 0.2 mM, $[Fe^{2+}]_0$ = 0.69 mM, $[Na_2SO_4]$ = 50 mM, pH = 3, T = 18°C, I = 466 mA, V= 1 L.

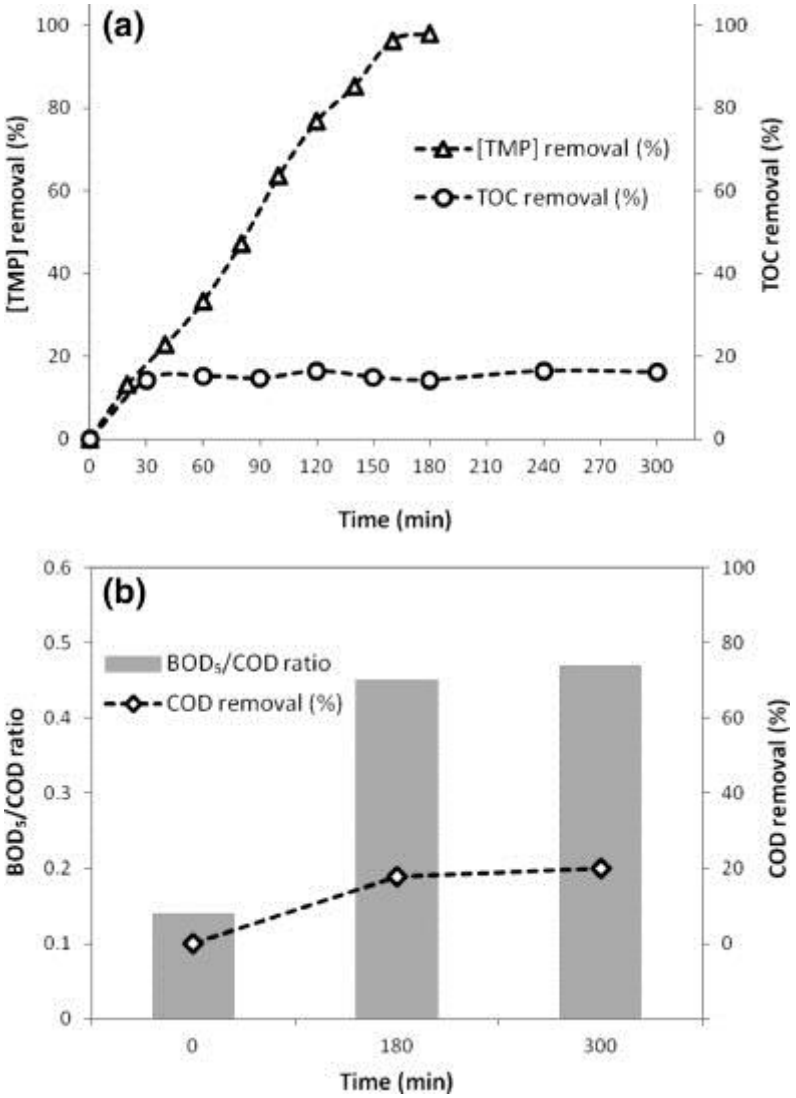


Figure 6. Pretreatment of the industrial pharmaceutical effluent: (a) Time evolution of TMP and TOC removals; (b) BOD_5/COD ratio and COD removal. Experimental conditions: $[TMP]_0$ = 0.2 mM, $[Fe^{2+}]_0$ = 0.69 mM, $[Na_2SO_4]$ = 50 mM, pH = 3, T = 18°C, I = 466 mA, V= 1 L.

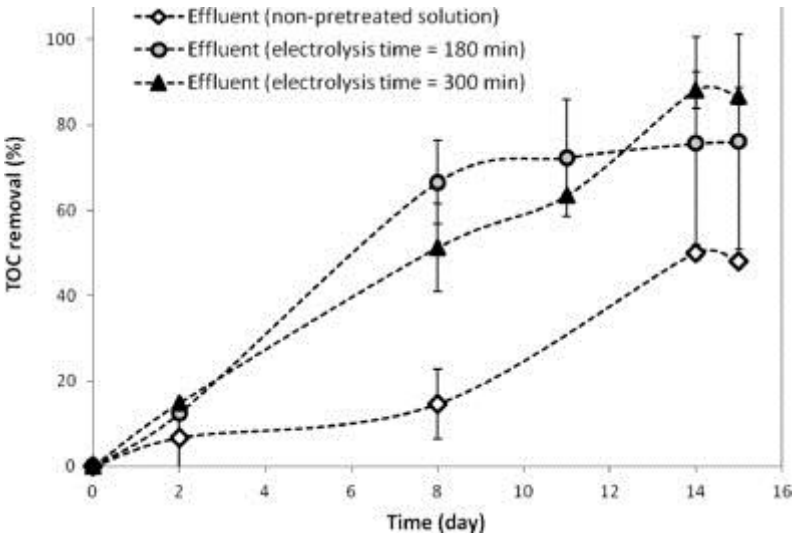


Figure 7. Mineralization during activated sludge cultures of non-pretreated industrial effluent and effluent electrolyzed during 180 and 300 min. Electro-Fenton pretreatment conditions: $[TMP]_0$ = 0.2 mM, $[Fe^{2+}]_0$ = 0.69 mM, $[Na_2SO_4]$ = 50 mM, pH = 3, T = 18°C, I = 466 mA, V= 1 L.