Mineralization of synthetic and industrial pharmaceutical effluent containing trimethoprim by combining electro-Fenton and activated sludge treatment

Dorsaf Mansour, Florence Fourcade, Isabelle Soutrel, Didier Hauchard, Nizar Bellakhal, Abdeltif Amrane

To cite this version:

Dorsaf Mansour, Florence Fourcade, Isabelle Soutrel, Didier Hauchard, Nizar Bellakhal, et al.. Mineralization of synthetic and industrial pharmaceutical effluent containing trimethoprim by combining electro-Fenton and activated sludge treatment. Journal of the Taiwan Institute of Chemical Engineers, 2015, In press. &lt;10.1016/j.jtice.2015.02.022&gt;. &lt;hal-01132840&gt;

HAL Id: hal-01132840

https://hal-univ-rennes1.archives-ouvertes.fr/hal-01132840

Submitted on 19 May 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Mineralization of synthetic and industrial pharmaceutical effluent containing trimethoprim by combining electro-Fenton and activated sludge treatment

Dorsaf Mansour\textsuperscript{a,b,c,d}, Florence Fourcade\textsuperscript{a,d}, Isabelle Soutrel\textsuperscript{b,d}, Didier Hauchard\textsuperscript{b,d}, Nizar Bellakhal\textsuperscript{c}, Abdeltif Amrane\textsuperscript{a,d*}

\textsuperscript{a}Université de Rennes 1, Ecole Nationale Supérieure de Chimie de Rennes, CNRS, UMR 6226, 11 allée de Beaulieu, CS 50837, 35708 Rennes Cedex 7, France
\textsuperscript{b}Ecole Nationale Supérieure de Chimie de Rennes, CNRS, UMR 6226, 11 allée de Beaulieu, CS 50837, 35708 Rennes Cedex 7, France
\textsuperscript{c}Unité de recherche de Catalyse d’Electrochimie de Nanomatériaux et leurs applications et de didactique CENAD, Institut National des Sciences Appliquées et de Technologie (INSAT), B.P.N°676, 1080 Tunis Cedex, Tunisie
\textsuperscript{d}Université Européenne de Bretagne, 5 boulevard Laënnec, 35000, France

*Corresponding author: Phone: (+33) 2 23 23 81 55; Fax: (+33) 2 23 23 81 20; abdeltif.amrane@univ-rennes1.fr
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 (’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 H₂O₂/ozonation, photo-Fenton, heterogeneous photocatalysis and electro-Fenton [11–15].

\[
\begin{align*}
\text{RH} + \cdot \text{OH} & \rightarrow R' + H_2O \\
\text{RX} + \cdot \text{OH} & \rightarrow RX'' + \text{OH}^- \\
\text{ArX} + \cdot \text{OH} & \rightarrow \text{OHArX}'
\end{align*}
\]

(1) (2) (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$_2$O$_2$ in an acidic medium through the electrochemical reduction of O$_2$ at the cathode (Eq. (4)). The generated H$_2$O$_2$ reacts with the added Fe$^{2+}$ ions to produce hydroxyl radicals (•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.

\[
\begin{align*}
\text{O}_2 + 2e^- + 2H^+ & \rightarrow \text{H}_2\text{O}_2 \quad (E^\circ = 0.69 \text{ V, Standard Hydrogen Electrode: SHE}) \quad (4) \\
\text{Fe}^{2+} + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{•OH} \quad (\text{Fenton’s reaction}) \quad (5) \\
\text{Fe}^{3+} + e^- & \rightarrow \text{Fe}^{2+} \quad (E^\circ = 0.77 \text{ V, SHE}) \quad (6) \\
2 \text{H}_2\text{O} & \rightarrow \text{O}_2 + 4e^- + 4\text{H}^+ \quad (7)
\end{align*}
\]

As mentioned above, the chosen target compound is trimethoprim (C$_{14}$H$_{18}$N$_4$O$_3$, 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). FeSO₄.7H₂O (purity 99%) and Na₂SO₄ (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\textsubscript{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\textsubscript{2}HPO\textsubscript{4}, delivered at a flow rate of 1 mL min\textsuperscript{-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\textsuperscript{®} 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\textsubscript{2}Cr\textsubscript{2}O\textsubscript{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\textsubscript{CPH/CPN} Total Organic Analyzer Shimadzu. Organic carbon compounds were combusted and converted to CO\textsubscript{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 (BOD₅) measurements

BOD₅ measurements were carried out in Oxitop IS6 (WTW, Alès, France). The following mineral basis was used for all experiments (g L⁻¹): MgSO₄·7H₂O, 22.50; CaCl₂, 27.50; FeCl₃, 0.15; NH₄Cl, 2.00; Na₂HPO₄, 6.80; KH₂PO₄, 2.80. Washed activated sludge was added in order to have 0.05 g L⁻¹ initial concentration of dry matter. The BOD₅ value was initially estimated based on the COD value experimentally measured, BOD₅ = COD/1.46. The range of expected BOD₅ 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 BOD₅ 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 C₁₈ 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 (N₂) 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 m² 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 cm³ min⁻¹ to saturate the aqueous solution with
oxygen.
The pH of the solutions was adjusted to 3 by sulfuric acid (H₂SO₄). A catalytic quantity of
FeSO₄.7H₂O 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\textsubscript{2}SO\textsubscript{4}. The electrolytic
solution was in circulation with the help of a peristaltic pump (flow rate of 2 L min\textsuperscript{-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\textsuperscript{-1}): Na\textsubscript{2}HPO\textsubscript{4}, 334; K\textsubscript{2}HPO\textsubscript{4}, 208; KH\textsubscript{2}PO\textsubscript{4}, 85; CaCl\textsubscript{2}, 27.4; MgSO\textsubscript{4}.7H\textsubscript{2}O,
22.6; NH\textsubscript{4}Cl, 2; FeCl\textsubscript{3}.6H\textsubscript{2}O, 0.26; and the initial pH was adjusted to 7. Activated sludge was
added in order to have 1 g L\textsuperscript{-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\textsuperscript{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]:

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

(8)

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

\[
U_{i(0)} = \frac{\text{upper limit of } U_i + \text{lower limit of } U_i}{2}
\]  

(9)

\[
\Delta U_i = \frac{\text{upper limit of } U_i - \text{lower limit of } U_i}{2}
\]  

(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:

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

(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

\[
B = (X^T X)^{-1} X^T Y
\]  

(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:

\[ Y = 98.9 + 2.7X_1 + 1.5X_2 + 13.2X_3 - 2.2X_1^2 - 3.4X_2^2 - 13.2X_3^2 + 0.8X_1X_2 + 0.7X_1X_3 - 2.6X_2X_3 \]  

(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 (R² = 0.98 and R²_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 Fe²⁺ 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 Fe²⁺ 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 'OH by the excessive ferrous ions according to equation (14), decreasing the quantity of this radical and hence inhibiting the degradation reaction [8,27].

$$\text{Fe}^{2+} + \cdot \text{OH} \rightarrow \text{Fe}^{3+} + \text{OH}^-$$  \hspace{1cm} (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$_2$O (Eq. (15)), which inhibits H$_2$O$_2$ 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$_2$O$_2$ [30]. This situation reduces the production of hydroxyl radicals and consequently, decreases the removal efficiency of trimethoprim.

$$\text{O}_2 + 4\text{e}^- + 4\text{H}^+ \rightarrow 2\text{H}_2\text{O}$$  \hspace{1cm} (15)

$$2\text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{H}_2 + 2\text{OH}^-$$  \hspace{1cm} (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 BOD₅ 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 BOD₅/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 Na₂SO₄ 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
cromatography 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 \textsuperscript{1}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 \textsuperscript{1}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 \textsuperscript{1}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 \textsuperscript{1}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 BOD5/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 Fe²⁺ 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 O₂ 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 BOD₅/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 BOD₅/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 (15th 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 BODs/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 BODs/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


Table 1 – Doehlert matrix experiments and experimental results.

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Coded variables</th>
<th>Real variables</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$</td>
<td>$X_2$</td>
<td>$X_3$</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>-1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>$\frac{1}{2}$</td>
<td>$\frac{\sqrt{3}}{2}$</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>$\frac{1}{2}$</td>
<td>$-\frac{\sqrt{3}}{2}$</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>$\frac{1}{2}$</td>
<td>$-\frac{\sqrt{3}}{2}$</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>$-\frac{1}{2}$</td>
<td>$\frac{\sqrt{3}}{2}$</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>$\frac{1}{2}$</td>
<td>$\frac{\sqrt{3}}{6}$</td>
<td>$\frac{\sqrt{6}}{3}$</td>
</tr>
<tr>
<td>8</td>
<td>$-\frac{1}{2}$</td>
<td>$-\frac{\sqrt{3}}{6}$</td>
<td>$-\frac{\sqrt{6}}{3}$</td>
</tr>
<tr>
<td>9</td>
<td>$\frac{1}{2}$</td>
<td>$-\frac{\sqrt{3}}{6}$</td>
<td>$-\frac{\sqrt{6}}{3}$</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>$\frac{\sqrt{3}}{3}$</td>
<td>$-\frac{\sqrt{6}}{3}$</td>
</tr>
<tr>
<td>11</td>
<td>$-\frac{1}{2}$</td>
<td>$\frac{\sqrt{3}}{6}$</td>
<td>$\frac{\sqrt{6}}{3}$</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>$-\frac{\sqrt{3}}{3}$</td>
<td>$\frac{\sqrt{6}}{3}$</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>991.773</td>
<td>9</td>
<td>110.197</td>
<td>28.721</td>
<td>0.00088</td>
</tr>
<tr>
<td>Residual</td>
<td>19.184</td>
<td>5</td>
<td>3.8368</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1010.957</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R^2 = 0.98; R^2_{Adjusted} = 0.95.$
Table 3 – Chromatographic identification of the products generated during the electro-Fenton degradation of trimethoprim.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chromatography</th>
<th>Chemical structure</th>
<th>Retention time (min)</th>
<th>ESI mode</th>
<th>Precursor ion (m/z)</th>
<th>Product ions (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,3-trimethoxybenzene (MW 168)</td>
<td>UPLC-MS/MS</td>
<td><img src="image" alt="1,2,3-trimethoxybenzene structure" /></td>
<td>1.98</td>
<td>Positive</td>
<td>169</td>
<td>138</td>
</tr>
<tr>
<td>2,4-diaminopyrimidine-5-carbaldehyde (MW 138)</td>
<td>UPLC-MS/MS</td>
<td><img src="image" alt="2,4-diaminopyrimidine-5-carbaldehyde structure" /></td>
<td>0.69</td>
<td>Positive</td>
<td>139</td>
<td>69</td>
</tr>
<tr>
<td>3,4,5-trimethoxyphenol (MW 184)</td>
<td>UPLC-MS/MS</td>
<td><img src="image" alt="3,4,5-trimethoxyphenol structure" /></td>
<td>0.91</td>
<td>Negative</td>
<td>183</td>
<td>153</td>
</tr>
<tr>
<td>2,4-diamino-6-hydroxypyrimidine (MW 126)</td>
<td>UPLC-MS/MS</td>
<td><img src="image" alt="2,4-diamino-6-hydroxypyrimidine structure" /></td>
<td>0.58</td>
<td>Positive</td>
<td>127</td>
<td>68</td>
</tr>
<tr>
<td>3,4,5-trimethoxybenzaldehyde (MW 196)</td>
<td>UPLC-MS/MS</td>
<td><img src="image" alt="3,4,5-trimethoxybenzaldehyde structure" /></td>
<td>1.63</td>
<td>Positive</td>
<td>197</td>
<td>138</td>
</tr>
<tr>
<td>3,4,5-trimethoxybenzoic acid (MW 212)</td>
<td>UPLC-MS/MS</td>
<td><img src="image" alt="3,4,5-trimethoxybenzoic acid structure" /></td>
<td>0.72</td>
<td>Negative</td>
<td>211</td>
<td>152</td>
</tr>
<tr>
<td>Acetic acid (MW 60)</td>
<td>IC</td>
<td><img src="image" alt="Acetic acid structure" /></td>
<td>4.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyoxylic acid (MW 74)</td>
<td>IC</td>
<td><img src="image" alt="Glyoxylic acid structure" /></td>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxalic acid (MW 90)</td>
<td>IC</td>
<td><img src="image" alt="Oxalic acid structure" /></td>
<td>5.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinic acid (MW 118)</td>
<td>IC</td>
<td><img src="image" alt="Succinic acid structure" /></td>
<td>14.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure captions
**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$_2$SO$_4$] = 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: $[\text{TMP}]_0 = 0.2$ mM, $[\text{Fe}^{2+}]_0 = 0.69$ mM, $[\text{Na}_2\text{SO}_4] = 50$ mM, pH = 3, $T = 18^\circ$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: $[\text{TMP}]_0 = 0.2 \text{ mM}$, $[\text{Fe}^{2+}]_0 = 0.69 \text{ mM}$, $[\text{Na}_2\text{SO}_4] = 50 \text{ mM}$, 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: $[\text{TMP}]_0 = 0.2$ mM, $[\text{Fe}^{2+}]_0 = 0.69$ mM, $[\text{Na}_2\text{SO}_4] = 50$ mM, pH = 3, $T = 18^\circ$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, [Fe²⁺]₀ = 0.69 mM, [Na₂SO₄] = 50 mM, pH = 3, T = 18°C, I = 466 mA, V = 1 L.