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# **Electro-Fenton pretreatment for the improvement of Tylosin biodegradability**

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## Abstract

The feasibility of an electro-Fenton process to treat tylosin (TYL), a non-biodegradable antibiotic, was examined in a discontinuous electrochemical cell with divided cathodic and anodic compartments. Only 15 min electrolysis were needed for total tylosin degradation using a carbon felt cathode and a platinum anode; while 6 h electrolysis were needed to achieve high oxidation and mineralization yields, 96% and 88 % respectively. Biodegradability improvement was shown since BOD<sub>5</sub>/ COD increased from 0 initially to 0.6 after 6 h electrolysis (for 100 mg L<sup>-1</sup> initial TYL). With the aim of combining electro-Fenton with a biological treatment, an oxidation time in the range 2 to 4 h has been however considered. Results of AOS (Average Oxidation State) and COD/TOC suggested that the pre-treatment could be stopped after 2 h rather than 4 h; while in the same time, the increase of biodegradability between 2 and 4 h suggested that this latter duration seemed more appropriate. In order to conclude, biological cultures have been therefore carried out for various electrolysis times. TYL solutions electrolyzed during 2 and 4 h were then treated with activated sludge during 25 days, showing 57 and 67 % TOC removal respectively, namely 77 and 88 % overall TOC removal if both processes were considered. Activated sludge cultures appeared therefore in agreement with the assessment made from the analysis of physico-chemical parameters (AOS and COD/TOC), since the gain in terms of mineralization expected from increasing electrolysis duration appeared too low to balance the additional energy consumption.

**Key words:** Tylosin, Electro-Fenton process, Degradation, Mineralization, Biological treatment.

## I. Introduction

The occurrence of a great number of pharmaceutical residues in the environment has been frequently reported in recent literature, receiving increasing attention as emerging contaminants [1-4]. Antibiotics are widely used in human and veterinary medicine to prevent or treat microbial infections, as well as growth factors in livestock production. After administration, fifty to ninety percent of these pharmaceuticals or their primary metabolites are excreted rapidly by humans as well as animals. After excretion, antibiotics are transferred to sewage treatment plants (STPs). Antibiotics may adsorb to the sewage sludge or leave the treatment plant unchanged with the STP discharge water [5].

Among veterinary pharmaceuticals, tylosin is a macrolide antibiotic produced by a strain of *Streptomyces fradiae*. It displays good anti-bacterial activity against most pathogenic gram-positive bacteria, and some gram-

negative bacteria, *Vibrio*, spirochete, coccidian, etc. It is one of the first-choice drugs against infections caused by mycoplasma [6]. The chemical structure of tylosin is given in Fig.1.

Tylosin is widely used for therapeutics and growth promotion in swine, beef cattle, and poultry production [7], while only a part of the administered antibiotic is metabolized; the part left is found back in its active form in animal's excreta, which is therefore found in wastewaters produced from livestock production. The absence of biodegradability of tylosin was previously demonstrated using a modified Sturm-test (OECD 301 B) [8]. The inhibitory effect of tylosin on anaerobic treatment in sequencing batch reactor have been studied by Shimada et al. [9]. The addition of tylosin (167 ppm) has induced a gradual decrease of methane production and the accumulation of metabolites such as propionate and acetate in the culture medium along with a pH decrease. As a consequence, tylosin addition has negatively impacted the overall system. In batch tests, the specific biogas production was also completely inhibited in the presence of tylosin. According to these authors [10], this failure was the consequence of the direct inhibition of propionate-oxidizing syntrophic bacteria, closely related to *Syntrophobacter* and the indirect inhibition of *Methanosaeta* by high propionate concentration and low pH. This inhibition was also noticed by Stasinakis [11].

The biologic deactivation of tylosin has been carried out by ozonation at a concentration of 1 mM [12]; this physico-chemical process has been studied for an application on municipal wastewater treatment.

Advanced oxidation processes (AOP) are considered good alternatives due to their high efficiency in oxidizing a great variety of organic compounds by the generation of highly oxidizing hydroxyl radicals [13-17]. Considering tylosin degradation, interesting results have been obtained with a photocatalytic treatment under UV irradiation using TiO<sub>2</sub> in suspension [18] or fixed on a non-woven paper [19]; 97% of tylosin was degraded in less than 60 min using 0.05 g L<sup>-1</sup> of TiO<sub>2</sub> suspension. When TiO<sub>2</sub> was fixed (25 g m<sup>-2</sup>), 2 h were needed to degrade 90.4% of the compound.

Even though AOPs have been shown to be highly efficient, their operation is still quite expensive. An attractive option is a short AOP pretreatment which let expect the formation of biodegradable intermediates of the recalcitrant pollutants, which can be subsequently degraded in a biological process, with the aim of reducing energy costs. For example, in a previous study, after tylosin degradation by photocatalysis, 56% COD decrease was reached by means of a biological culture [19]. Therefore, before examination of the combination of an AOP with a conventional biological treatment for pollutant removal, the relevance of an AOP pre-treatment has to be checked by the monitoring of some specific parameters such as the target compound concentration or that of global parameters like total organic carbon (TOC), chemical oxygen demand (COD), biodegradability of the

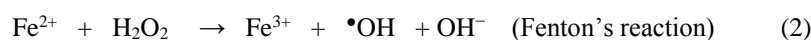
pretreated pollutant solution through biological oxygen demand (BOD<sub>5</sub>) measurements. The evolution of global parameters like TOC, COD, COD/TOC and AOS also provide useful information on mineralization and oxidation; while the BOD<sub>5</sub> on COD ratio approximates effluent biodegradability, since a value of 0.4 is considered by several authors as the boundary of biodegradability [20-22].

Heterogeneous photocatalysis is interesting in water purification because TiO<sub>2</sub>, the most used catalyst, is not expensive and no more chemical reagents are needed to degrade organic pollutants at neutral pH [19]. Another AOP, electro-Fenton, an indirect electrochemical advanced oxidation process derived from Fenton reaction, is also a promising technology for the treatment of wastewaters [23,24]. Indeed, it does not involve the use of harmful chemical reagents due to the fact that the reactants are in situ electro-generated; moreover the method is easy to handle and the reactors involved are simple.

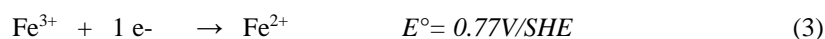
H<sub>2</sub>O<sub>2</sub> is continuously generated by reduction of the dissolved molecular O<sub>2</sub> in mildly acidic aqueous medium (Eq. (1)) using various cathodes materials [23].



Hydroxyl radical (OH•) and Fe<sup>3+</sup> ions are then generated from the classical Fenton's reaction between Fe<sup>2+</sup> ions and H<sub>2</sub>O<sub>2</sub>



Fe<sup>2+</sup> ions in catalytic amount were consumed by Fenton's reaction in the homogeneous medium (Eq. (2)) and are regenerated at the cathode by reduction of Fe<sup>3+</sup> ions (Eq. (3))



Electro-Fenton was developed and widely applied for oxidation of various organic pollutants [24-29]. Recently, integrated processes such as electro-Fenton / electro-oxidation allowed a higher production of hydroxyl radicals and then a higher mineralization of the effluent [30,31]. Among the studied materials, boron-doped diamond [30], Ce /SnO<sub>2</sub>-Sb coated titanium [31] can be used as efficient anode materials. Air-diffusion cathodes were implemented for a better oxygen supply at the electrode surface and then a higher production of hydrogen peroxide [30]. Another way to increase the hydroxyl radicals production is to integrate UVA radiations in the photoelectro-Fenton process as in the photoelectron-Fenton process [32].

The aim of this study was therefore to examine the relevance of the electro-Fenton process for the removal of tylosin and its possible combination with a biological treatment. Previous studies showed biodegradability enhancement of solutions after electro-Fenton oxidation of antibiotics [27,33]. The combination between electro-Fenton and a biological treatment has been already tested on landfill leachate [34], olive oil mill wastewater [35],

formaldehyde [36] but not on such toxic effluent as those containing antibiotics except a previous study on tetracycline from our lab [29]. In order to select the most appropriate pretreatment duration, the first step was to study the performance of an electro-Fenton pre-treatment through the monitoring of antibiotic degradation, its oxidation and mineralization, and especially the monitoring of global parameters, like COD/TOC, AOS and biological oxygen demand.

In order to validate the biodegradability results assessed from the BOD<sub>5</sub>/COD ratio, subsequent biological cultures were then considered.

## **2. Material and methods**

### **2.1 Chemicals and reagents**

Tylosin tartrate (Fluka 5 g packaging, purity > 95 %) was obtained from Sigma-Aldrich (St Quentin Fallavier, France). Solution pH was adjusted by adding sulphuric acid (H<sub>2</sub>SO<sub>4</sub>; 96 % purity) or sodium hydroxide (NaOH) solutions obtained from Sigma Aldrich and Fisher, respectively. Methanol (MeOH) was UPLC grade from Fisher Scientific (Illkirch, France); Formic acid was Fisher Scientific. Catalyst heptahydrated ferrous sulfate (FeSO<sub>4</sub>·7H<sub>2</sub>O) and anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) were supplied by Acros Organics (Geel, Belgique). Ultra pure water (Purelab Options-Q7/15, Elga, 18.2MΩ cm) was used in all experiments. All the reagents and materials used in this study were of analytical grade.

### **2.2 Electro-Fenton oxidation experiments**

Tylosin oxidation was performed at room temperature in cylindrical glass cell of 1 L (10 cm inner diameter), equipped with a carbon-felt cathode whose surface was 194 cm<sup>2</sup> piece (Carbone Lorrain, RVG 4000-Mersen, Paris La Defense, France) (13 × 6 cm) of 1 cm thickness. It was placed on the inner wall of the cell. Its specific area, measured by the BET method, was 0.7 m<sup>2</sup> g<sup>-1</sup> and its density was 0.088 g cm<sup>-3</sup>. A cylindrical glass of 150 mL (5 cm diameter) was placed in the center of the cell, equipped with a cylindrical platinum anode (5 cm × 1 cm) and the electrolytic solution of 0.05 M of Na<sub>2</sub>SO<sub>4</sub> at pH=3. The two electrodes were connected to a DC Amperemeter power supply (Microsonic systems, Microlab MX 300V- 1A, France). Continuous saturation of the solution by O<sub>2</sub> at atmospheric pressure was ensured by bubbling compressed air, which was beforehand acidified by passage through a solution of 0.5 M H<sub>2</sub>SO<sub>4</sub>, at a flow rate of 4.5 L min<sup>-1</sup>, and starting 30 min before electrolysis to reach a stationary O<sub>2</sub> concentration. The TYL concentration was 100 mg L<sup>-1</sup>, 0.1 mM Fe<sup>2+</sup> catalyst

concentration was added and the current intensity was maintained constant at 300 mA. Experiments were performed at pH 3.0 (Hanna Instruments pH-meter 211, Lingolsheim, France, Alsace), namely close to the optimal pH of 2.8 reported for the Fenton's reaction Eq. (2) [28,37]; it was maintained constant throughout experiments by 2 M H<sub>2</sub>SO<sub>4</sub> addition. The working volume was 600 mL and the agitation rate was 1500 rpm, to ensure an efficient mass transfer towards/from the electrodes. These experimental conditions are taken from a previous study on the removal of tetracycline and gave the optimal results regarding molecule degradation and its mineralization [29].

## **2.3 Analysis**

Samples were filtered through 0.45 µm membrane filter and tested for chemical oxygen demand (COD), biological oxygen demand (BOD<sub>5</sub>), total organic carbon (TOC) and filtered through 0.2 µm membrane filter for measurement of antibiotics residual concentration by UPLC.

### **2.3.1 Ultra Performance Liquid Chromatography (UPLC)**

The residual tylosin concentration during treatment was monitored by Waters Acquity UPLC<sup>®</sup> H-Class (Ultra Performance Liquid Chromatography). A C<sub>18</sub> BEH column (Bridged Ethylene Hybrid), 1.7 µm (2.1 × 50mm) operated at 45°C. The separation was performed according to the following gradient elution with methanol (eluant A) and formic acid 0.1 % in ultra-pure water, milli Q-water (eluant B): 10/90% from 0 to 1 min, from 1 to 4.5 min elution was linearly modified from 10/90 % to 98/2 %, maintained at 98/2 % from 4.5 to 5 min, from 5 to 5.5 min elution was linearly modified from 98/2 % to 10/90 % where it was maintained from 5.5 to 10 min for solvents A and B, respectively.

The flow-rate was 0.5 ml min<sup>-1</sup> and the injection volume was 5 µL. The retention time was 2.85 min and tylosin detection was made at a wavelength of 285 nm with a PDA detector (photodiodes array) allowing an analysis between 210 and 400 nm.

### **2.3.2 Chemical Oxygen Demand (COD)**

Chemical oxygen demand (COD) was measured by means of Nanocolor-CSB 40 and 160 tests from Macherey-Nagel (Düren, Germany). COD concentration was measured by NANOCOLOR<sup>®</sup> photometer.

### **2.3.3 Total Organic Carbon (TOC)**

Total organic carbon (TOC) was measured via a TOC-meter SHIMADZU TOC-VCPH analyzer (Kyoto, Japan). Total organic carbon (TOC) present in the samples was calculated by the subtraction of the inorganic carbon (IC) value from the total carbon (TC) value. Analyses were duplicated.

#### **2.3.4 Biological Oxygen Demand (BOD<sub>5</sub>)**

BOD<sub>5</sub> measurements were carried out in OxiTop IS12 WTW (Alès, France). Activated sludge was obtained from a municipal wastewater treatment plant (Beaurade, Rennes, France) and was used to inoculate the flasks. Before used for inoculation, sludge was washed three times with drinking water and two times with distilled water to avoid any nutrients other than those contained in the culture media; after each washing, sludge was centrifuged at 3000 rpm for 10 min (Jouan, Thermo Fisher Scientifics, Saint Herblain, France). A given amount of sludge was resuspended in distilled water, in order to achieve an initial microbial concentration of 0.05 g L<sup>-1</sup>. The following mineral basis was used for all experiments (g L<sup>-1</sup>): MgSO<sub>4</sub>·7H<sub>2</sub>O, 22.5; CaCl<sub>2</sub>, 27.5; FeCl<sub>3</sub>, 0.15; NH<sub>4</sub>Cl, 2.0; Na<sub>2</sub>HPO<sub>4</sub>, 6.80; KH<sub>2</sub>PO<sub>4</sub>, 2.80. The BOD<sub>5</sub> value was initially estimated based on the COD value experimentally measured according to the following ratio, BOD<sub>5</sub> = COD/1.46. The volumes of sample, of activated sludge solution and nitrification inhibitor (10 mg L<sup>-1</sup> solution of N-allylthiourea) which have to be added in the shake flask were then deduced from the expected range of BOD<sub>5</sub> values. 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<sup>-1</sup>) and glucose (150 mg L<sup>-1</sup>). Before use, NaOH 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 deduce the biological oxygen demand corresponding to the endogenous respiration (negligible BOD<sub>5</sub> value) [29]. All sample flasks were duplicated.

#### **2.4 Biological treatment**

Biological treatments were carried out in aerobic conditions using activated sludge purchased from a local wastewater treatment plant (Station de Beaurade, Rennes, France). Before use, activated sludge was washed three times with tap water and twice with distilled water. After each washing, activated sludge was centrifuged at 3000 rpm for five minutes (Jouan, Thermo Fisher Scientifics, Saint Herblain, France). The supernatant was then separated from the sludge to remove any residual carbon or mineral source. Cultures were carried out in duplicate experiments for 25 days at 25°C in 500 ml Erlenmeyer flasks, which were magnetically stirred (300 rpm), closed with a cellulose cap to ensure oxygenation and loaded with 400 mL of



non-treated tylosin (100 mg L<sup>-1</sup>) or treated tylosin solutions (100 mg L<sup>-1</sup> initial concentration), namely electrolyzed during 2 h and 4 h. The following mineral supplementation was added in the flasks (mg L<sup>-1</sup>): KH<sub>2</sub>PO<sub>4</sub>, 43.8; Na<sub>2</sub>HPO<sub>4</sub>, 33.4; NH<sub>4</sub>NO<sub>3</sub>, 3; CaCl<sub>2</sub>, 27.5; MgSO<sub>4</sub>·7H<sub>2</sub>O, 22.5; and trace mineral solution (mg L<sup>-1</sup>): FeSO<sub>4</sub>·7H<sub>2</sub>O, 1.36; CuSO<sub>4</sub>·2H<sub>2</sub>O, 0.24; ZnSO<sub>4</sub>·5H<sub>2</sub>O, 0.25; NiSO<sub>4</sub>·6H<sub>2</sub>O, 0.11; MnSO<sub>4</sub>·H<sub>2</sub>O, 1.01. Before use, NaOH was added to achieve a neutral pH (7.0 ± 0.2) and the flasks were inoculated with 0.5 g L<sup>-1</sup> of activated sludge.

### 3. Results and discussion

#### 3.1 Degradation and mineralization kinetics of tylosin

The concentration of TYL decreased exponentially (Fig.2) and the concentration-time curve followed a pseudo-first order kinetic (inset in Fig.2), which allowed deducing the apparent rate constant for TYL degradation (slope of the straight line) according to Eq. (4):

$$-\frac{d[TYL]}{dt} = k_{abs}[\bullet OH][TYL] = k_{app}[TYL] \quad (4)$$

Where  $k_{abs}$  is the absolute rate constant of the oxidation of TYL by  $\bullet OH$  and  $k_{app}$  is the apparent rate constant of this reaction.

Complete degradation of TYL was achieved in 15 min at 300 mA, with a ferrous concentration of 0.1 mM, sodium sulfate concentration of 0.05 M and pH 3. This high and rapid degradation could be attributed to the break of ether functions in the molecule [19]. Mineralization of aqueous TYL solutions during treatment was monitored through the TOC evolution (Fig.3). TOC values decreased exponentially with time, until reaching significant mineralization yields, 45, 62 and 88% after 2, 4 and 6 h electrolysis respectively (table 1). The same trend has been observed during the electro-Fenton treatment of sulfachloropyridazine with a carbon felt cathode and a platinum anode [25]. Indeed, 59.2 ppm (0.2 mM) of sulfachloropyridazine has been degraded after about 10 min of oxidation with a current intensity of 300 mA and a mineralization yield of 79% has been obtained after 4h.

The aim of the electro-Fenton step was to increase its biodisponibility leading to more readily biodegradable by-products of the target compound. This can be expected, owing to the total tylosin degradation, while partial

mineralization was observed indicating a significant remaining amount of residual carbon available for a subsequent biological treatment.

### 3.2 COD analysis and estimation of the pre-treatment duration

A decrease of the chemical oxygen demand generally involves a chemical oxidation of the target compound and hence a modification of its chemical structure which could lead to an increase of its biodegradability. On the other hand, a limited mineralization is required to ensure sufficient residual organic carbon for subsequent biologic treatment. As a consequent a favorable trend is a decrease of the ratio COD/TOC [20,21].

For increasing electrolysis time, the COD values decreased from 136 mg O<sub>2</sub> L<sup>-1</sup> for the non-treated target compound to 46, 30 and 5 mg O<sub>2</sub> L<sup>-1</sup> after 2, 4 and 6 h oxidation times, respectively (Table 1). The evolution of the COD on TOC ratio was favorable, since the COD/COT decreased. This ratio decreased up to 2 h of electrolysis; it remained then constant from 2 to 4 h and finally decreased up to 6 h of electrolysis. It can be supposed that between 2 and 4 h, the chemical structure of compounds in solution did not vary significantly [38].

In the same way, the Average Oxidation State (AOS) [21], has been calculated according to equation 5:

$$AOS = \frac{4(TOC - COD)}{TOC} \quad (5)$$

Where TOC and COD are expressed in moles of C per liter and moles of O<sub>2</sub> per liter, respectively. The maximum value +4 corresponds to the most oxidized state of carbon, CO<sub>2</sub>, and the minimum value -4 corresponds to the most reduced state of carbon, CH<sub>4</sub>.

When the AOS remains constant, Parra et al. [39] suggest that the chemical nature of the intermediates does not vary anymore. In these conditions, the AOS of the treated solution could be an indirect indication of the ability of the pre-treatment to improve the biodegradability of the solution.

From these results, it seems more judicious to stop the pre-treatment after 2 h rather than after 4 h of electrolysis. Concerning a more extended electrolysis, electro-Fenton could be considered alone, since 96% of oxidation and 88% of mineralization were obtained after 6 h of treatment.

### 3.3 Biodegradability of the electrolyzed solution

The biodegradability test was performed with a solution electrolyzed under the following conditions: 100 mg L<sup>-1</sup> TYL, 0.1 mM [Fe<sup>2+</sup>], 5 · 10<sup>-2</sup> M [Na<sub>2</sub>SO<sub>4</sub>], 300 mA and pH 3. The BOD<sub>5</sub> values increased from 0 mg O<sub>2</sub> L<sup>-1</sup> initially (non-treated tylosin) to 14 – 15 mg O<sub>2</sub> L<sup>-1</sup> after 2 and 4 h oxidation time and then decreased to 3 mg O<sub>2</sub>

L<sup>-1</sup> after 6 h oxidation time, leading to BOD<sub>5</sub>/COD ratios increasing from 0 initially to 0.3, 0.5 and 0.6 for 2, 4 and 6 h electrolysis, respectively (Table 1). These results showed the biodegradability of the electrolyzed effluent, since a value of 0.4 was reached, confirming the relevance of the electro-Fenton pre-treatment. It should however be noted that in the case of a long pre-treatment time (6 h), the high BOD<sub>5</sub> on COD ratio (Table 1) should be balanced by the high mineralization yield also obtained (88% – Fig.3), indicating a low residual amount of organic content (6.8 mg L<sup>-1</sup> – Table 1) available for the subsequent biological treatment. The optimal oxidation time should be therefore most likely between 2 and 4 h, since after 2 and 4 h electrolysis the treated solution was almost biodegradable (BOD<sub>5</sub>/COD = 0.3) and biodegradable (BOD<sub>5</sub>/COD = 0.5) for moderate mineralization yields, 45 and 62% (Fig.3), respectively. Previous results of AOS and COD/TOC (Table 1) seemed to suggest that the pre-treatment could be stopped after 2 h rather than 4 h; however, this latter electrolysis time seemed more appropriate since biodegradability increased between 2 and 4 h. In order to conclude, biological treatments have been therefore carried out for various electrolysis times.

### 3.4 Biological treatment

The negligible TYL biosorption on the sludge can be noted in Fig. 4 and the recalcitrance of tylosin (100 mg L<sup>-1</sup>) was confirmed at the examination of Fig.5, since the ratios of the total organic carbon on its initial value (TOC<sub>t</sub>/TOC<sub>0</sub>) remained constant even after 25 days of culture. It was also in agreement with previous findings [8], showing a maximum tylosin adsorption capacity of 7.7 mg per g of activated sludge, namely less than 4 mg L<sup>-1</sup> at best for 0.5 g L<sup>-1</sup> activated sludge as considered in this work, leading to less than 4% tylosin biosorption (100 mg L<sup>-1</sup> initial concentration).

Contrarily, the electrolyzed TYL solutions appeared partially biodegradable by activated sludge (Fig.5). Indeed, after only two days of culture, TOC decrease were about 46 and 56% for solutions pretreated during 2 and 4 h (Fig.5), leading to 31 and 40% biodegradation if the adsorbed amounts were taken into account (Fig.4), respectively. A significant part of the by-products resulting from electrolysis were therefore readily biodegradable. It can be noticed that at equilibrium, reached in less than 3 h, the part of adsorbed by-products were close to 15 and 16% for 2 and 4 h electrolysis pre-treatment (Fig.4). Biodegradation continued at lower rates throughout cultures, and after 25 days of activated sludge culture, final TOC decrease were close to 57 and 67 % for 2 and 4 h pretreatment, respectively (Fig.5), namely 42 and 51 % biodegradation after subtraction of the adsorption part.

It can be noticed that the mineralization of by products was similar to that obtained after photocatalysis [19] for similar pre-treatment durations.

If the pretreatment was also taken into account, the overall TOC decrease were therefore 77 and 88% after 25 days activated sludge culture of TYL solutions electrolyzed 2 and 4 h respectively. They corresponded to the sum of 45 and 62% TOC removal during electrolysis and 32 and 26% TOC removal during biological treatment related to the initial TOC amounts, respectively.

From this, only 10% increase was obtained for two supplementary hours of electrolysis. Therefore the gain in terms of mineralization did not balance the additional energy costs. It can be also noticed that the increase of biodegradability between 2 and 4 h seems to be related to the increase of mineralization during this time (decrease of TOC values from 32.2 to 22.0 mg.L<sup>-1</sup>), BOD<sub>5</sub> values remaining constant. From this, the oxidative pretreatment induced a modification of the chemical nature of the organic compounds, leading to an improvement of the biodegradability. However, the physico-chemical mineralization of part of these by products did not favor significantly the activated sludge culture.

#### 4. Conclusion

The efficiency of the electro-Fenton process for the removal of tylosin antibiotic was shown. Under the considered operating conditions, namely 100 mg L<sup>-1</sup> tylosine, an applied current of 300 mA, an Fe<sup>2+</sup> catalyst concentration of 0.1 mM and an electrolyte Na<sub>2</sub>SO<sub>4</sub> concentration of 0.05 M, at pH 3, total tylosin degradation was achieved after 15 minutes and followed a pseudo first-order reaction; while mineralization yields were 45, 62 and 88% after 2, 4 and 6 h electrolysis respectively.

A decrease of the COD/TOC ratio and an increase of the AOS were experimentally observed during the pretreatment step showing favorable trend for biodegradability improvement, as confirmed by the increase of the BOD<sub>5</sub>/COD ratio, from 0 initially to 0.6 after 6 h electrolysis (for 100 mg L<sup>-1</sup> initial TYL concentration), namely above the limit of biodegradability (0.4).

In view of these results, two possibilities can be envisaged. Electro-Fenton process can be carried out upstream of an existing biological step. The preconized electrolysis duration was then estimated at 2 h with regards to the evolution of the ratios COT/TOC and AOS, even if after 2 h the ratio BDO<sub>5</sub>/COD continued to increase. Indeed, considering mineralization improvement during the biological treatment, the gain obtained was too low to balance the additional energy consumed.

328 In view of comparison, the implementation of electro-Fenton as sole mineralization process should also be  
329 examined. The physico-chemical treatment of tylosin during 6 h led to 96% and 88% oxidation and  
330 mineralization, respectively. Indeed, from an energetic standpoint and at this step of the study, a sole electro-  
331 Fenton treatment seems more appropriate than the combination of 2 h electro-Fenton pre-treatment followed by a  
332 biological treatment. However, none of these processes has been optimized in order to reduce duration especially  
333 regarding the biological treatment. Therefore, the choice of the most relevant method for tylosin depollution  
334 appeared therefore premature and irrelevant at this stage. For this purpose and after its optimization, further  
335 works should be conducted dealing with the energy cost of such combined process.

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**Table 1.** TOC, COD, AOS, COD/TOC, BOD<sub>5</sub> and biodegradability determination of tylosin and treated tylosin.

Treatment time (h)	TOC (mg L <sup>-1</sup> )	COD (mgO <sub>2</sub> L <sup>-1</sup> )	AOS	COD/TOC	BOD <sub>5</sub> (mgO <sub>2</sub> L <sup>-1</sup> )	BOD <sub>5</sub> /COD
0	58.60	136	0.52	2.32	0	0
2	32.24	46	1.87	1.42	14	0.3
4	22.00	30	1.95	1.36	15	0.5
6	6.80	5	2.90	0.73	3	0.6

### Figure legends

**Figure 1.** Chemical structure of tylosin tartrate.

**Figure 2.** Degradation time-course of 100 mg L<sup>-1</sup> TYL during electro-Fenton treatment; experimental conditions: [Fe<sup>2+</sup>] = 0.1 mM; I = 300 mA; [Na<sub>2</sub>SO<sub>4</sub>] = 5 · 10<sup>-2</sup> M; pH = 3; V = 600 mL.

**Figure 3.** Time-courses of tylosin mineralization during electro-Fenton pre-treatment (initial concentration = 100 mg L<sup>-1</sup>) for 300 mA current intensity, at pH 3, V = 600 mL, [Na<sub>2</sub>SO<sub>4</sub>] = 5 · 10<sup>-2</sup> M and [Fe<sup>2+</sup>] = 0.1 mM.

**Figure 4.** Time-courses of biosorption on activated sludge at 25°C and initial pH 7 of tylosin solutions (100 mg L<sup>-1</sup> initial concentration) electrolyzed during 2 h (○) and 4 h (Δ) at pH 3, I = 300 mA, V = 600 mL, [Na<sub>2</sub>SO<sub>4</sub>] = 5 · 10<sup>-2</sup> M and [Fe<sup>2+</sup>] = 0.1 mM.

**Figure 5.** Time-courses of (TOC<sub>t</sub>/TOC<sub>0</sub>) values during activated sludge culture at 25°C and an initial pH 7 of 100 mg L<sup>-1</sup> tylosin (□) and tylosin solutions electrolyzed during 2 h (○) and 4h (Δ) at pH 3, I = 300 mA, V = 600 mL, [Na<sub>2</sub>SO<sub>4</sub>] = 5 · 10<sup>-2</sup> M and [Fe<sup>2+</sup>] = 0.1 mM.

Figure 1

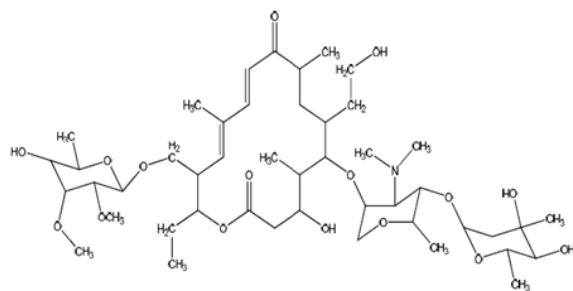


Figure 2

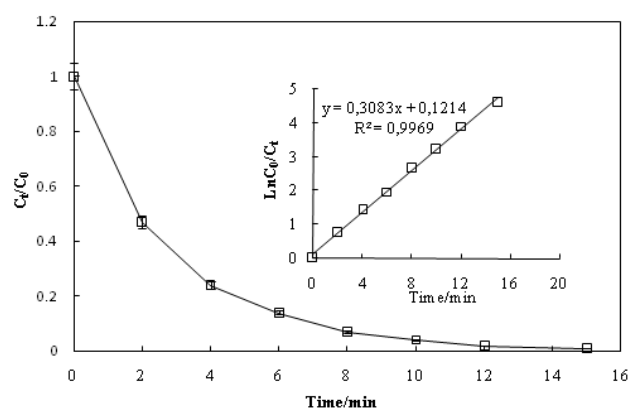


Figure 3

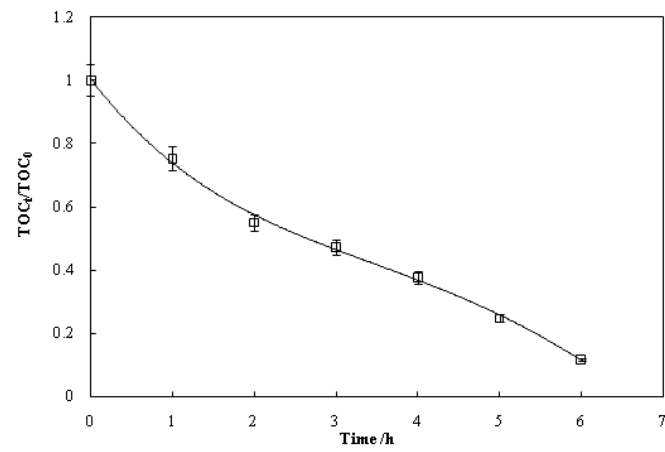


Figure 4

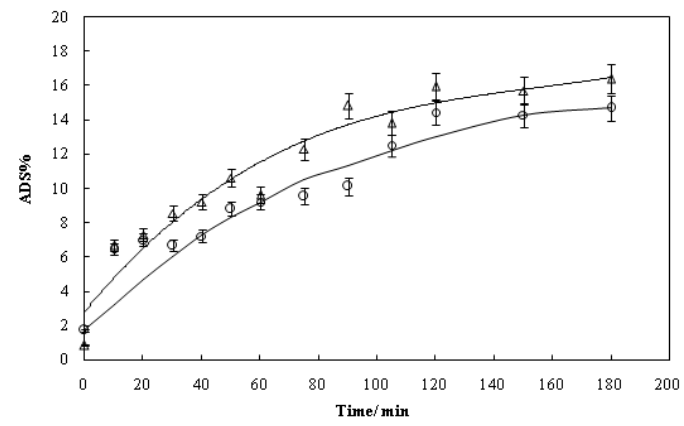


Figure 5

