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Electro-Fenton pretreatment for the improvement of tylosin biodegradability

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

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31   **Abstract**

32   The feasibility of an electro-Fenton process to treat tylosin (TYL), a non-biodegradable antibiotic, was examined

33   in a discontinuous electrochemical cell with divided cathodic and anodic compartments. Only 15 min electrolysis

34   were needed for total tylosin degradation using a carbon felt cathode and a platinium anode; while 6 h

35   electrolysis were needed to achieve high oxidation and mineralization yields, 96% and 88 % respectively.

36   Biodegradability improvement was shown since BOD_5/ COD increased from 0 initially to 0.6 after 6 h

37   electrolysis (for 100 mg L^{-1} initial TYL). With the aim of combining electro-Fenton with a biological treatment,

38   an oxidation time in the range 2 to 4 h has been however considered. Results of AOS (Average Oxidation State)

39   and COD/TOC suggested that the pre-treatment could be stopped after 2 h rather than 4 h; while in the same

40   time, the increase of biodegradability between 2 and 4 h suggested that this latter duration seemed more

41   appropriate. In order to conclude, biological cultures have been therefore carried out for various electrolysis

42   times. TYL solutions electrolyzed during 2 and 4 h were then treated with activated sludge during 25 days,

43   showing 57 and 67 % TOC removal respectively, namely 77 and 88 % overall TOC removal if both processes

44   were considered. Activated sludge cultures appeared therefore in agreement with the assessment made from the

45   analysis of physico-chemical parameters (AOS and COD/TOC), since the gain in terms of mineralization

46   expected from increasing electrolysis duration appeared too low to balance the additional energy consumption.

47

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

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51   **I. Introduction**

52   The occurrence of a great number of pharmaceutical residues in the environment has been frequently reported in

53   recent literature, receiving increasing attention as emerging contaminants [1-4]. Antibiotics are widely used in

54   human and veterinary medicine to prevent or treat microbial infections, as well as growth factors in livestock

55   production. After administration, fifty to ninety percent of these pharmaceuticals or their primary metabolites are

56   excreted rapidly by humans as well as animals. After excretion, antibiotics are transferred to sewage treatment

57   plants (STPs). Antibiotics may adsorb to the sewage sludge or leave the treatment plant unchanged with the STP

58   discharge water [5].

59   Among veterinary pharmaceuticals, tylosin is a macrolide antibiotic produced by a strain of *Streptomyces*

60   *fradiae*. It displays good anti-bacterial activity against most pathogenic gram-positive bacteria, and some gram-

2

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_2 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^{-1} of TiO_2 suspension. When TiO_2 was fixed (25 g m^{-2}), 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

91 pretreated pollutant solution through biological oxygen demand (BOD_5) measurements. The evolution of global

92 parameters like TOC, COD, COD/TOC and AOS also provide useful information on mineralization and

93 oxidation; while the BOD_5 on COD ratio approximates effluent biodegradability, since a value of 0.4 is

94 considered by several authors as the boundary of biodegradability [20-22].

95 Heterogeneous photocatalysis is interesting in water purification because TiO_2, the most used catalyst, is not

96 expensive and no more chemical reagents are needed to degrade organic pollutants at neutral pH [19]. Another

97 AOP, electro-Fenton, an indirect electrochemical advanced oxidation process derived from Fenton reaction, is

98 also a promising technology for the treatment of wastewaters [23,24]. Indeed, it does not involve the use of

99 harmful chemical reagents due to the fact that the reactants are in situ electro-generated; moreover the method is

100 easy to handle and the reactors involved are simple.

101 H_2O_2 is continuously generated by reduction of the dissolved molecular O_2 in mildly acidic aqueous medium

102 (Eq. (1)) using various cathodes materials [23].

103 $$O_2 \; + \; 2\,H^+ \; + \; 2\,e\text{-} \; \rightarrow \; H_2O_2 \qquad E°=0.69 \; V/SHE \qquad\qquad (1)$$
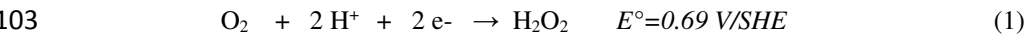
104 Hydroxyl radical ($OH^•$) and Fe^{3+} ions are then generated from the classical Fenton's reaction between Fe^{2+} ions

105 and H_2O_2

106 $$Fe^{2+} \; + \; H_2O_2 \; \rightarrow \; Fe^{3+} \; + \; {}^{•}OH \; + OH^{-} \quad \text{(Fenton's reaction)} \qquad (2)$$
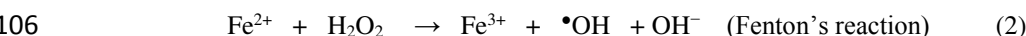
107 Fe^{2+} ions in catalytic amount were consumed by Fenton's reaction in the homogeneous medium (Eq. (2)) and are

108 regenerated at the cathode by reduction of Fe^{3+} ions (Eq. (3))

109 $$Fe^{3+} \; + \; 1\,e\text{-} \; \rightarrow \; Fe^{2+} \qquad\qquad E°= 0.77V/SHE \qquad\qquad (3)$$
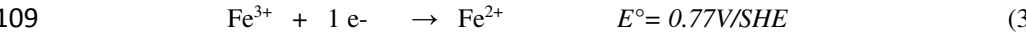
110 Electro-Fenton was developed and widely applied for oxidation of various organic pollutants [24-29]. Recently,

111 integrated processes such as electro-Fenton / electro-oxidation allowed a higher production of hydroxyl radicals

112 and then a higher mineralization of the effluent [30,31]. Among the studied materials, boron-doped diamond

113 [30], Ce /SnO_2-Sb coated titanium [31] can be used as efficient anode materials. Air-diffusion cathodes were

114 implemented for a better oxygen supply at the electrode surface and then a higher production of hydrogen

115 peroxide [30]. Another way to increase the hydroxyl radicals production is to integrate UVA radiations in the

116 photoelectro-Fenton process as in the photoelectron-Fenton process [32].

117 The aim of this study was therefore to examine the relevance of the electro-Fenton process for the removal of

118 tylosin and its possible combination with a biological treatment. Previous studies showed biodegradability

119 enhancement of solutions after electro-Fenton oxidation of antibiotics [27,33]. The combination between electro-

120 Fenton and a biological treatment has been already tested on landfill leachate [34], olive oil mill wastewater [35],

4

121 formaldehyde [36] but not on such toxic effluent as those containing antibiotics except a previous study on

122 tetracycline from our lab [29]. In order to select the most appropriate pretreatment duration, the first step was to

123 study the performance of an electro-Fenton pre-treatment through the monitoring of antibiotic degradation, its

124 oxidation and mineralization, and especially the monitoring of global parameters, like COD/TOC, AOS and

125 biological oxygen demand.

126 In order to validate the biodegradability results assessed from the BOD_5/COD ratio, subsequent biological

127 cultures were then considered.

128

129 **2. Material and methods**

130

131 **2.1 Chemicals and reagents**

132 Tylosin tartrate (Fluka 5 g packaging, purity > 95 %) was obtained from Sigma-Aldrich (St Quentin Fallavier,

133 France). Solution pH was adjusted by adding sulphuric acid (H_2SO_4; 96 % purity) or sodium hydroxide (NaOH)

134 solutions obtained from Sigma Aldrich and Fisher, respectively. Methanol (MeOH) was UPLC grade from

135 Fisher Scientific (Illkirch, France); Formic acid was Fisher Scientific. Catalyst heptahydrated ferrous sulfate

136 ($FeSO_4$, $7H_2O$) and anhydrous sodium sulfate (Na_2SO_4) were supplied by Acros Organics (Geel, Belgique). Ultra

137 pure water (Purelab Options-Q7/15, Elga, 18.2MΩ cm) was used in all experiments. All the reagents and

138 materials used in this study were of analytical grade.

139

140 **2.2 Electro-Fenton oxidation experiments**

141 Tylosin oxidation was performed at room temperature in cylindrical glass cell of 1 L (10 cm inner diameter),

142 equipped with a carbon-felt cathode whose surface was 194 cm^2 piece (Carbone Lorrain, RVG 4000-Mersen,

143 Paris La Defense, France) (13 × 6 cm) of 1 cm thickness. It was placed on the inner wall of the cell. Its specific

144 area, measured by the BET method, was 0.7 m^2 g^{-1} and its density was 0.088 g cm^{-3}. A cylindrical glass of 150

145 mL (5 cm diameter) was placed in the center of the cell, equipped with a cylindrical platinum anode (5 cm × 1

146 cm) and the electrolytic solution of 0.05 M of Na_2SO_4 at pH=3. The two electrodes were connected to a DC

147 Amperemeter power supply (Microsonic systems, Microlab MX 300V- 1A, France). Continuous saturation of

148 the solution by O_2 at atmospheric pressure was ensured by bubbling compressed air, which was beforehand

149 acidified by passage through a solution of 0.5 M H_2SO_4, at a flow rate of 4.5 L min^{-1}, and starting 30 min before

150 electrolysis to reach a stationary O_2 concentration. The TYL concentration was 100 mg L^{-1}, 0.1 mM Fe^{2+} catalyst

concentration was added and the current intensity was maintained constant at 300 mA. Experiments were

152 performed at pH 3.0 (Hanna Instruments pH-meter 211, Lingolshieim, France, Alsace), namely close to the

153 optimal pH of 2.8 reported for the Fenton's reaction Eq. (2) [28,37]; it was maintained constant throughout

154 experiments by 2 M H_2SO_4 addition. The working volume was 600 mL and the agitation rate was 1500 rpm, to

155 ensure an efficient mass transfer towards/from the electrodes. These experimental conditions are taken from a

156 previous study on the removal of tetracycline and gave the optimal results regarding molecule degradation and

157 its mineralization [29].

158

159 **2.3 Analysis**

160 Samples were filtered through 0.45 µm membrane filter and tested for chemical oxygen demand (COD),

161 biological oxygen demand (BOD_5), total organic carbon (TOC) and filtered through 0.2 µm membrane filter for

162 measurement of antibiotics residual concentration by UPLC.

163

164 **2.3.1 Ultra Performance Liquid Chromatography (UPLC)**

165 The residual tylosin concentration during treatment was monitored by Waters Acquity UPLC® H-Class (Ultra

166 Performance Liquid Chromatography). A C_{18} BEH column (Bridged Ethylene Hybrid), 1.7 µm (2.1 × 50mm)

167 operated at 45°C. The separation was performed according to the following gradient elution with methanol

168 (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

169 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

170 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

171 for solvents A and B, respectively.

172 The flow-rate was 0.5 ml min^{-1} and the injection volume was 5 µL. The retention time was 2.85 min and tylosin

173 detection was made at a wavelength of 285 nm with a PDA detector (photodiodes array) allowing an analysis

174 between 210 and 400 nm.

175

176 **2.3.2 Chemical Oxygen Demand (COD)**

177 Chemical oxygen demand (COD) was measured by means of Nanocolor-CSB 40 and 160 tests from Macherey-

178 Nagel (Düren, Germany). COD concentration was measured by NANOCOLOR® photometer.

179

180 **2.3.3 Total Organic Carbon (TOC)**

181 Total organic carbon (TOC) was measured via a TOC-meter SHIMADZU TOC-VCPH analyzer (Kyoto, Japan).

182 Total organic carbon (TOC) present in the samples was calculated by the subtraction of the inorganic carbon (IC)

183 value from the total carbon (TC) value. Analyses were duplicated.

184

185 **2.3.4 Biological Oxygen Demand (BOD$_5$)**

186 BOD$_5$ measurements were carried out in OxiTop IS12 WTW (Alès, France). Activated sludge was obtained from

187 a municipal wastewater treatment plant (Beaurade, Rennes,France) and was used to inoculate the flasks. Before

188 used for inoculation, sludge was washed three times with drinking water and two times with distilled water to

189 avoid any nutrients other than those contained in the culture media; after each washing, sludge was centrifuged

190 at 3000 rpm for 10 min (Jouan, Thermo Fisher Scientifics, Saint Herblain, France). A given amount of sludge

191 was resuspended in distilled water, in order to achieve an initial microbial concentration of 0.05 g L^{-1}. The

192 following mineral basis was used for all experiments (g L^{-1}): MgSO$_4$·7H$_2$O, 22.5; CaCl$_2$, 27.5; FeCl$_3$, 0.15;

193 NH$_4$Cl, 2.0; Na$_2$HPO$_4$, 6.80; KH$_2$PO$_4$, 2.80. The BOD$_5$ value was initially estimated based on the COD value

194 experimentally measured according to the following ratio, BOD$_5$ = COD/1.46. The volumes of sample, of

195 activated sludge solution and nitrification inhibitor (10 mg L^{-1} solution of N-allylthiourea) which have to be

196 added in the shake flask were then deduced from the expected range of BOD$_5$ values. Similar protocol was

197 applied for the control sample except that it was replaced by a solution of easily biodegradable compounds,

198 namely glutamic acid (150 mg L^{-1}) and glucose (150 mg L^{-1}). Before use, NaOH was added to achieve neutral

199 pH (7.0 ± 0.2). Similar protocol was also considered for the blank solution, for which the sample was replaced

200 by water to deduce the biological oxygen demand corresponding to the endogenous respiration (negligible BOD$_5$

201 value) [29]. All sample flaks were duplicated.

202

203 **2.4 Biological treatment**

204 Biological treatments were carried out in aerobic conditions using activated sludge purchased from a local

205 wastewater treatment plant (Station de Beaurade, Rennes, France). Before use, activated sludge was washed

206 three times with tap water and twice with distilled water. After each washing, activated sludge was centrifuged at

207 3000 rpm for five minutes (Jouan, Thermo Fisher Scientifics, Saint Herblain, France). The supernatant was then

208 separated from the sludge to remove any residual carbon or mineral source.

209 Cultures were carried out in duplicate experiments for 25 days at 25°C in 500 ml Erlenmeyer flasks, which were

210 magnetically stirred (300 rpm), closed with a cellulose cap to ensure oxygenation and loaded with 400 mL of

211 non-treated tylosin (100 mg L^{-1}) or treated tylosin solutions (100 mg L^{-1} initial concentration), namely

212 electrolyzed during 2 h and 4 h. The following mineral supplementation was added in the flasks (mg L^{-1}):

213 KH_2PO_4, 43.8; Na_2HPO_4, 33.4; $NHNO_3$, 3; $CaCl_2$, 27.5; $MgSO_4,7H_2O$, 22.5; and trace mineral solution (mg L^-

214 ^{1}): $FeSO_4,7H_2O$,1.36; $CuSO_4$, $2H_2O$, 0.24; $ZnSO_4,5H_2O$, 0.25; $NiSO_4$, $6H_2O$, 0.11; $MnSO_4$, H_2O, 1.01.

215 Before use, NaOH was added to achieve a neutral pH (7.0 ± 0.2) and the flasks were inoculated with 0.5 g L^{-1} of

216 activated sludge.

217

218

219 **3. Results and discussion**

220

221 **3.1 Degradation and mineralization kinetics of tylosin**

222 The concentration of TYL decreased exponentially (Fig.2) and the concentration-time curve followed a pseudo-

223 first order kinetic (inset in Fig.2), which allowed deducing the apparent rate constant for TYL degradation (slope

224 of the straight line) according to Eq. (4):

225

226 $$-\frac{d[TYL)]}{dt} = k_{abs}[^{\bullet}OH][TYL] = k_{app}\,[TYL] \tag{4}$$

227

228 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

229 this reaction.

230 Complete degradation of TYL was achieved in 15 min at 300 mA, with a ferrous concentration of 0.1 mM,

231 sodium sulfate concentration of 0.05 M and pH 3. This high and rapid degradation could be attributed to the

232 break of ether functions in the molecule [19]. Mineralization of aqueous TYL solutions during treatment was

233 monitored through the TOC evolution (Fig.3). TOC values decreased exponentially with time, until reaching

234 significant mineralization yields, 45, 62 and 88% after 2, 4 and 6 h electrolysis respectively (table 1). The same

235 trend has been observed during the electro-Fenton treatment of sulfachloropyridazine with a carbon felt cathode

236 and a platinum anode [25]. Indeed, 59.2 ppm (0.2 mM) of sulfachloropyridazine has been degraded after about

237 10 min of oxidation with a current intensity of 300 mA and a mineralization yield of 79% has been obtained after

238 4h.

239 The aim of the electro-Fenton step was to increase its biodisponibility leading to more readily biodegradable by-

240 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_2 L^{-1} for the non-treated target compound to 46, 30 and 5 mg O_2 L^{-1} 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:

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

(5)

Where TOC and COD are expressed in moles of C per liter and moles of O_2 per liter, respectively. The maximum value +4 corresponds to the most oxidized state of carbon, CO_2, and the minimum value -4 corresponds to the most reduced state of carbon, CH_4.

When the AOS remains constant, Parra et al. [39] suggest that the chemical nature of the intermediates does no 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^{-1} TYL, 0.1 mM [Fe $^{2+}$], 5 10^{-2} M [Na_2SO_4], 300 mA and pH 3. The BOD_5 values increased from 0 mg O_2 L^{-1} initially (non-treated tylosin) to 14 – 15 mg O_2 L^{-1} after 2 and 4 h oxidation time and then decreased to 3 mg O_2

270 L^{-1} after 6 h oxidation time, leading to BOD$_5$/COD ratios increasing from 0 initially to 0.3, 0.5 and 0.6 for 2, 4

271 and 6 h electrolysis, respectively (Table 1). These results showed the biodegradability of the electrolyzed

272 effluent, since a value of 0.4 was reached, confirming the relevance of the electro-Fenton pre-treatment. It should

273 however be noted that in the case of a long pre-treatment time (6 h), the high BOD$_5$ on COD ratio (Table 1)

274 should be balanced by the high mineralization yield also obtained (88% – Fig.3), indicating a low residual

275 amount of organic content (6.8 mg L^{-1} – Table 1) available for the subsequent biological treatment. The optimal

276 oxidation time should be therefore most likely between 2 and 4 h, since after 2 and 4 h electrolysis the treated

277 solution was almost biodegradable (BOD$_5$/COD = 0.3) and biodegradable (BOD$_5$/COD = 0.5) for moderate

278 mineralization yields, 45 and 62% (Fig.3), respectively. Previous results of AOS and COD/TOC (Table 1)

279 seemed to suggest that the pre-treatment could be stopped after 2 h rather than 4 h; however, this latter

280 electrolysis time seemed more appropriate since biodegradability increased between 2 and 4 h. In order to

281 conclude, biological treatments have been therefore carried out for various electrolysis times.

282

283 **3.4 Biological treatment**

284 The negligible TYL biosorption on the sludge can be noted in Fig. 4 and the recalcitrance of tylosin (100 mg L^{-1})

285 was confirmed at the examination of Fig.5, since the ratios of the total organic carbon on its initial value

286 (TOC$_t$/TOC$_0$) remained constant even after 25 days of culture. It was also in agreement with previous findings

287 [8], showing a maximum tylosin adsorption capacity of 7.7 mg per g of activated sludge, namely less than 4 mg

288 L^{-1} at best for 0.5 g L^{-1} activated sludge as considered in this work, leading to less than 4% tylosin biosorption

289 (100 mg L^{-1} initial concentration).

290 Contrarily, the electrolyzed TYL solutions appeared partially biodegradable by activated sludge (Fig.5). Indeed,

291 after only two days of culture, TOC decrease were about 46 and 56% for solutions pretreated during 2 and 4 h

292 (Fig.5), leading to 31 and 40% biodegradation if the adsorbed amounts were taken into account (Fig.4),

293 respectively. A significant part of the by-products resulting from electrolysis were therefore readily

294 biodegradable. It can be noticed that at equilibrium, reached in less than 3 h, the part of adsorbed by-products

295 were close to 15 and 16% for 2 and 4 h electrolysis pre-treatment (Fig.4). Biodegradation continued at lower

296 rates throughout cultures, and after 25 days of activated sludge culture, final TOC decrease were close to 57 and

297 67 % for 2 and 4 h pretreatment, respectively (Fig.5), namely 42 and 51 % biodegradation after subtraction of

298 the adsorption part.

299   It can be noticed that the mineralization of by products was similar to that obtained after photocatalysis [19] for

300   similar pre-treatment durations.

301   If the pretreatment was also taken into account, the overall TOC decrease were therefore 77 and 88% after 25

302   days activated sludge culture of TYL solutions electrolyzed 2 and 4 h respectively. They corresponded to the

303   sum of 45 and 62% TOC removal during electrolysis and 32 and 26% TOC removal during biological treatment

304   related to the initial TOC amounts, respectively.

305   From this, only 10% increase was obtained for two supplementary hours of electrolysis. Therefore the gain in

306   terms of mineralization did not balance the additional energy costs. It can be also noticed that the increase of

307   biodegradability between 2 and 4 h seems to be related to the increase of mineralization during this time

308   (decrease of TOC values from 32.2 to 22.0 mg.L^{-1}), BOD_5 values remaining constant. From this, the oxidative

309   pretreatment induced a modification of the chemical nature of the organic compounds, leading to an

310   improvement of the biodegradability. However, the physico-chemical mineralization of part of these by products

311   did not favor significantly the activated sludge culture.

312

313   **4. Conclusion**

314   The efficiency of the electro-Fenton process for the removal of tylosin antibiotic was shown. Under the

315   considered operating conditions, namely 100 mg L^{-1} tylosine, an applied current of 300 mA, an Fe^{2+} catalyst

316   concentration of 0.1 mM and an electrolyte Na_2SO_4 concentration of 0.05 M, at pH 3, total tylosin degradation

317   was achieved after 15 minutes and followed a pseudo first-order reaction; while mineralization yields were 45,

318   62 and 88% after 2, 4 and 6 h electrolysis respectively.

319   A decrease of the COD/TOC ratio and an increase of the AOS were experimentally observed during the

320   pretreatment step showing favorable trend for biodegradability improvement, as confirmed by the increase of the

321   BOD_5/COD ratio, from 0 initially to 0.6 after 6 h electrolysis (for 100 mg L^{-1} initial TYL concentration), namely

322   above the limit of biodegradability (0.4).

323   In view of these results, two possibilies can be envisaged. Electro-Fenton process can be carried out upstream of

324   an existing biological step. The preconized electrolysis duration was then estimated at 2 h with regards to the

325   evolution of the ratios COT/TOC and AOS, even if after 2 h the ratio BDO_5/COD continued to increase. Indeed,

326   considering mineralization improvement during the biological treatment, the gain obtained was too low to

327   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.

336

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Table 1. TOC, COD, AOS, COD/TOC, BOD_5 and biodegradability determination of tylosin and treated tylosin.

Treatment time (h)	TOC (mg L^{-1})	COD (mgO_2 L^{-1})	AOS	COD/TOC	BOD_5 (mgO_2 L^{-1})	BOD_5/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^{-1} TYL during electro-Fenton treatment; experimental conditions: [Fe $^{2+}$]= 0.1 mM; I= 300 mA; [Na_2SO_4] = 5 10^{-2} M; pH = 3; V= 600 mL.

Figure 3. Time-courses of tylosin mineralization during electro-Fenton pre-treatment (initial concentration = 100 mg L^{-1}) for 300 mA current intensity, at pH 3, V= 600 mL, [Na_2SO_4] = 5 10^{-2} M and [Fe $^{2+}$] = 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^{-1} initial concentration) electrolyzed during 2 h (○) and 4 h (Δ) at pH 3, I = 300 mA, V= 600 mL, [Na_2SO_4] = 5 10^{-2} M and [Fe $^{2+}$] = 0.1 mM.

Figure 5. Time-courses of (TOC_t/TOC_0) values during activated sludge culture at 25°C and an initial pH 7 of 100 mg L^{-1} tylosin (□) and tylosin solutions electrolyzed during 2 h (○) and 4h (Δ) at pH 3, I = 300 mA, V= 600 mL, [Na_2SO_4] = 5 10^{-2} M and [Fe $^{2+}$] = 0.1 mM.
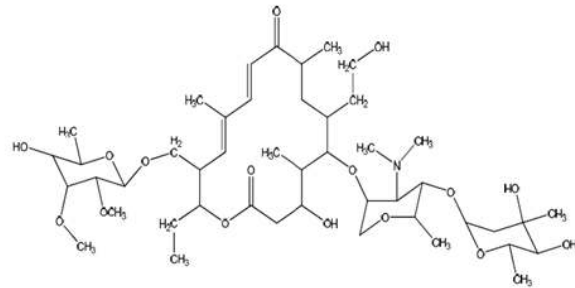
17

Figure 1

Figure 2

Inset: $y = 0{,}3083x + 0{,}1214$
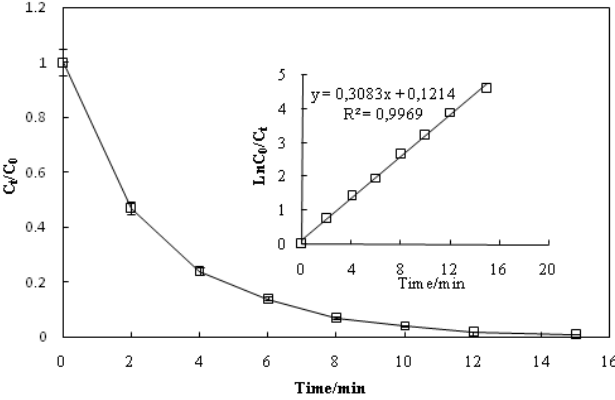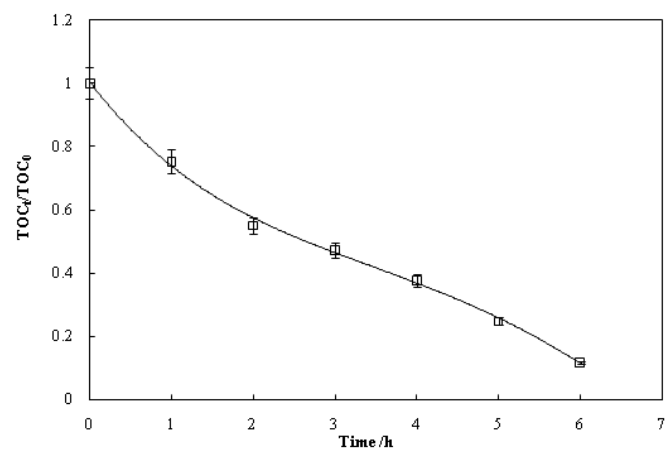$R^2 = 0{,}9969$

Axis labels: C_t/C_0, Time/min, LnC_0/C_t, Time/min

19

Figure 3

Figure 4

Figure 5