Reactive oxygen and iron species monitoring to investigate the electro-Fenton performances. Impact of the electrochemical process on the biodegradability of metronidazole and its by-products

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Abstract

In this study, the monitoring of reactive oxygen species and the regeneration of the ferrous ions catalyst were performed during electro-Fenton (EF) process to highlight the influence of operating parameters. The removal of metronidazole (MTZ) was implemented in an electrochemical mono-compartment batch reactor under various ranges of current densities, initial MTZ and ferrous ions concentrations, and pH values. It was found that under 0.07 mA cm^{-2}, 0.1 mM of ferrous ions and pH=3, the efficiency of 100 mg L^{-1} MTZ degradation and mineralization were 100 % within 20 min and 40% within 135 min of electrolysis, respectively. The highest hydrogen peroxide and hydroxyl radical concentrations, 1.4 mM and 2.28 mM respectively, were obtained at 60 min electrolysis at 0.07 mA cm^{-2}. Improvement of the biodegradability was reached from 60 min of electrolysis with a BOD_5/COD ratio above 0.4, which was reinforced by a respirometric study, that supports the feasibility of coupling electro-Fenton and biological treatment for the metronidazole removal.

Keywords: Electro-Fenton process; Metronidazole; reactive oxygen species; ferrous ions regeneration; respirometry; biodegradability

1. Introduction

Metronidazole (MTZ, 2-methyl-5-nitroimidazole-1-ethanol) is a nitroimidazole antibiotic, that presents antibacterial and anti-inflammatory properties. MTZ is extensively used in Europe for the treatment of infectious diseases caused by a wide range of anaerobic bacteria and various protozoans, such as *Trichomonas vaginalis* and *Giardia lamblia* (Tally and Sullivan, 1981; Lau et al., 1992).

Because of its refractory character and a high solubility in water, MTZ is difficult to remove during conventional sewage treatment (Kümmerer, 2001). Biorecalcitrance of this compound was also demonstrated during culture of *Trametes Versicolor* fungus or using enzyme extract from this fungus (Becker et al., 2016). As a result, MTZ accumulates in animals' body, fish-farm waters and, more importantly, effluents from meat industries and hospitals (Kümmerer, 2001). Residual concentrations have consequently been detected in hospital effluents, wastewater, surface water and groundwater (Dantas et al., 2010; Vulliet and Cren-Olivé, 2011). The results spotted that untreated release of MTZ exposes humans' health at risk because of its potentially carcinogenic and mutagenic properties (Bendesky et al., 2002), its toxicity against aquatic organisms (Lanzky and Halting-Sørensen, 1997) and genotoxicity to humans since it causes a DNA damage in human lymphocytes (Ré et al., 1997).

Physico-chemical processes such as adsorption or ozonation can be performed to remove refractory pharmaceutical pollutants in aqueous effluent including MTZ. Indeed, in their study on MTZ adsorption, Rivera-Utrilla et al tested three different activated carbons. Results showed the high adsorption capacity of the different activated carbons. Adsorption phenomenon was more influenced by the activated carbons chemical properties rather than their structural properties. Moreover, pH and the electrolyte concentration had a little

53 influence in the adsorption process, indicating a minor role of the electrostatic interactions
54 between MTZ and the activated carbons (Rivera-Utrilla et al., 2009). Adsorption is an
55 efficient process for MTZ removal but not destructive and then needs further post-treatment to
56 eliminate completely the pollutant.

57 Sánchez-Polo et al studied the removal of four different nitroimidazoles by ozonation process.
58 Low reactivity of nitroimidazoles with ozone was obtained with specific rate constants below
59 350 M^{-1} s^{-1} and only 10-20% of organic carbon reduction (Sánchez-Polo et al., 2008).

60 Among physico-chemical processes, Advanced Oxidation Processes (AOPs) are considered as
61 promising technologies for water remediation, based on the *in-situ* generation of strong
62 oxidants such as hydroxyl radical that react non-selectively with most persistent organic
63 pollutants in aqueous solutions until they reach total mineralization (conversion into CO_2,
64 water and inorganic ions) (Latimer, 1952; Ku et al., 1999; Stasinakis, 2008; Brillas et al.,
65 2009).

66 Several AOPs were tested by Shemer and his co-workers (Shemer et al., 2006). They found
67 that the degradation of metronidazole by photolysis was low; the concentration decrease was
68 around 10% over a period of 5 min. The addition of H_2O_2 to the UV irradiation improved the
69 efficiency of the degradation and the best degradation yield reached 67% for the same
70 duration. On other way, during Fenton's process, the metronidazole degradation yield i.e the
71 concentration decrease was enhanced to 76% (Shemer et al., 2006). This process is well
72 known as one of the most efficient AOPs (Lopez et al., 2004). Its implementation and
73 maintenance are simple since that needs only hydrogen peroxide and ferrous ions (Eq. 1)
74 (Lopez et al., 2004; Lu et al., 2005; Pignatello et al., 2006). It had been widely applied for the
75 treatment and pre-treatment of wastewater (Trujillo et al., 2006).

76 $$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + {}^{\cdot}OH \quad (1)$$

When UV irradiation was applied in addition to the Fenton's reaction, quasi-total metronidazole degradation was obtained (over 90%)(Shemer et al., 2006). Solar photo-Fenton (Ammar et al., 2016) was also studied for the removal of metronidazole and based on COD degradation, oxidation yield reached 96%.

Some studies based on the electro-Fenton process were carried out for the degradation of metronidazole (Cheng et al., 2013; Pérez et al., 2015). This process is a combination of the classical Fenton treatment with electrochemical reactions. It is based on the *in-situ* generation of H_2O_2, brought by the continuous electrochemical reduction of dissolved oxygen as shown in Eq. 2. Hydrogen peroxide reacts then with ferrous ions in catalytic amount to produce hydroxyl radicals (Eq. 1) and ferrous ions were regenerated by their reduction at the cathode surface. (Eq. 3) (Brillas et al., 2009).

$$O_2 + 2H^+ + 2e^- \leftrightarrow H_2O_2 \quad (2)$$

$$Fe^{3+} + e^- \leftrightarrow Fe^{2+} \quad (3)$$

Mineralization yield obtained with electro-Fenton did not exceed 40% in each study, and increased to 53% with additional solar irradiation (Pérez et al., 2015).

For process intensification purpose in terms of mineralization, an alternative for the MTZ removal was proposed. It concerned the improvement of biodegradability by the electro-Fenton process prior to a subsequent biological mineralization (Mansour et al., 2012; Ferrag-Siagh et al., 2014; Annabi et al., 2016; Olvera-Vargas et al., 2016; Ganzenko et al., 2017).

The interest of coupling physico-chemical process and biodegradation was already highlighted (Scott and Ollis, 1995). In their study, the authors explained that the processes order depends on the nature of the effluent to be treated. In presence of wastewater concentrated in biorecalcitrant compounds, a chemical oxidation is required in order to produce biodegradable by-products that will be then metabolized during the biological process. Two sequences for the combination between electro-Fenton and biodegradation were

discussed for the removal of both 5-fluorouracil and caffeine at 0.1 mM (Ganzenko et al., 2017). Both sequences were efficient for the removal of organic compounds. Biological pre-treatment performances in a SBR reactor depended on microbial acclimation time and biomass concentration. Residual organic load was then removed by electro-Fenton but a part of the produced hydroxyl radicals was loss due to their attack on microorganism. During the pre-pretreatment, pollutant biosorption could occur (Fontmorin et al., 2013) but was not considered in this study. Moreover, the concentration of pollutants has an influence of the biological treatment since for concentration higher than their inhibitory threshold for microorganisms; biodegradation could be less efficient (Yahiat et al., 2011). When electro-Fenton was performed as pre-treatment, biodegradability was improved by increasing the electrolysis time and the current intensity. However, regarding the economic feasibility of the electrochemical pre-treatment, long electrolysis duration with a low current intensity was a better option.

An increase of the BOD_5/COD ratio was obtained after the MTZ electro-Fenton treatment (Cheng et al., 2013), but below the limit of biodegradability (Alonso Salles et al., 2010).

In this study, in order to reach the limit of biodegradability, the influence of some operating parameters was considered in regard to the production and the monitoring of reactive oxygen species (hydrogen peroxide, hydroxyl radicals) and in regard to the behavior of iron species in solution during electrolysis. The electrochemical behavior of metronidazole was also examined in order to understand its rule in the compound degradation and mineralization. Moreover, the estimation of biodegradability by means of the BOD_5/COD ration was supported by a respirometric study implemented during 21 d.

2. Materials and methods

2.1. Chemicals and materials

MTZ (2-methyl-5-nitroimidazole-1-ethanol) with 99% of purity was purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). $FeSO_4 \cdot 7H_2O$ (purity 99%) and Na_2SO_4 (purity 99%) were used as a catalyst source and inert supporting electrolyte respectively, and were obtained from Acros Organics (Thermo Fisher Scientific, Geel, Belgium). Acetonitrile (purity 99.9%) (HPLC grade) was also obtained from Sigma–Aldrich. All solutions were prepared with ultrapure water and all other chemicals used for analysis were purchased from Acros and Sigma.

2.2. Electrochemical Apparatus and Procedures

Experiments were performed at ambient temperature (20°C) in a discontinuous reactor containing 250 mL of solution and were previously detailed (Abou Dalle et al., 2017). An ammeter power supply (Microsonic systems, Microlab MX 20V-2A, France) delivered cathodic current intensity throughout the electrolysis between two electrodes. A tri-dimensional piece of graphite felt of 42 cm^3 geometrical volume was used as cathode (Le Carbone Lorraine RVG 4000 Mersen, Paris la Défense, France) and the anode was a platinum cylinder (32 cm^2). Compressed air was bubbled into the solution for 10 min before the electrolysis to saturate the solution with O_2 and then throughout the experiments. $FeSO_4 \cdot 7H_2O$ was added into the cell just before the beginning of the electrolysis. 50 mM Na_2SO_4 was added as supporting electrolyte.

2.3Analytical procedure

2.3.1. Electrochemical analysis

Current-potential curves were performed in a conventional three electrodes electrochemical cell as previously detailed (Fourcade et al., 2013). The electrochemical apparatus was a SP150 BioLogic potentiostat/galvanostat (BioLogic SAS, Clais, France) with EC-Lab® software.

2.3.2. High Performance Liquid Chromatography (HPLC).

The degradation of Metronidazole was measured by HPLC using Waters 996 system equipped with Waters 996 PDA (Photodiode Array Detector) and Waters 600LCD Pump. The separation was achieved on Waters C 18 (5 μm; 4.6 × 250 mm) reversed-phase. The eluent consisted of an acetonitrile/water mixture (20/80, v/v) with 0.1% formic acid delivered at a flow rate of 1 mL min^{-1}. The detection of Metronidazole was carried out at 318 nm and the retention time was 4.3 min.

2.3.3. Total organic carbon (TOC) measurement.

TOC was measured by TOC-$V_{CPH/CPG}$ Total Organic Analyzer Schimadzu. CO_2 produced from the combustion and the conversion of Organic Carbon compounds was identified by non-dispersive Infra-Red Detector (NDIR).

2.3.4. Hydrogen peroxide quantification.

Hydrogen peroxide concentration was iodometrically measured with sodium thiosulfate as titrant, by amperometric titration with a double Pt indicator electrode and an applied potential difference of 100 mV (Potentio-amperometric TPA4 titrator, Tacussel, Lyon, France) (Abou Dalle et al., 2017).

2.3.5. Hydroxyl radical's quantification

Dimethyl sulfoxide (DMSO) was used to trap hydroxyl radicals (Abou Dalle et al., 2017). It reacts quickly with a hydroxyl radical forming methanesulfonic acid and sulfate (Jahnke, 1999). DIONEX DX120 ion chromatography was used to detect the formed methanesulfonic acid. A DIONEX DX120 chromatograph was provided with a conductivity detector; the stationary phase is constituted by an anion exchange column AS19 (4 x 250 mm) and the mobile phase was constituted of a KOH solution in water. A gradient elution mode was adopted for the analyses. 10 mM of KOH was considered during the first 10 min; then the amount increased linearly to reach 45 mM from 10 to 25 min; and finally an amount of 45

177 mM of KOH was maintained from 25 to 35 min of the analysis. The flow rate remained
178 constant at 1 mL min^{-1}.

179 **2.3.6. Iron species quantification.**

180 Total dissolved iron species were quantified by atomic absorption spectrometry (AA140,
181 VARIAN spectrometer, Palo Alto, USA) with air/acetylene flame at 372 nm. Ferrous ions
182 were quantified by the orthophenanthroline complexometric method, detailed in a previous
183 work (Ben Hammouda et al., 2016).

184 **2.3.7. Biochemical oxygen demand (BOD_5) measurements**

185 The inoculated activated sludge was obtained from a municipal wastewater treatment plant
186 (Beaurade, Rennes, France).The activated sludge was cultivated under oxygen flow on a
187 previously detailed mineral medium (Fontmorin et al., 2012). Before inoculation, the
188 activated sludge was washed three times with tap water and twice with ultrapure water to
189 remove all carbon residues. A verification of the elimination of these latter was carried out by
190 analyzing the TOC values.

191 BOD_5 measurements were carried out in Oxitop IS6 (WTW, Alès, France) under a previously
192 detailed protocol (Fontmorin et al., 2012).

193 **2.3.8. Respirometric experiments**

194 Experiments were carried out in 3L bioreactor (Bioflow 3000, New Brunswick scientific)
195 coupled to a respirometric device measuring Oxygen Uptake Rates (OUR) of activated sludge
196 (Spérandio and Paul, 2000; Adouani et al., 2015). This latter was collected from the biological
197 basin of Pontivy (Brittany, France) Waste Water Treatment Plant.

198 2.5 L of settled sludge, after sifting at 400 µm, were aerated in the bioreactor; pH and
199 temperature were controlled to 7.7 and 25°C respectively. Dissolved oxygen uptake rate was
200 measured continuously in the bioreactor. Biological activity of heterotrophic biomass can be
201 then characterized as well as its behavior (a possible microorganism's inhibition for example)

in the presence of 500 mg L^{-1} MTZ or its electrolyzed solutions. First, endogenous respiration was measured (OUR_{endo}). To characterize heterotrophic biomass activity, a biodegradable carbon source, here sodium acetate (0.1 g), was firstly added in the culture medium when OUR_{endo} was stable and exogenous respiration $(OUR)_{exo}$ was measured. MTZ or electrolyzed solution was then added to reach TOC values in the range 5-50 mg C L^{-1} in the bioreactor. When the OUR_{endo} remained stable, sodium acetate was added again, the response of OUR_{exo} was compared with the previous one. To characterize autotrophic biomass activity, sodium acetate was replaced by a mineral substrate (NH_4Cl 0.1 g). These tests were performed during 21 d. The analysis of the different curve patterns (OUR function of time) provided information on the metabolization of each substrate.

3. Results and discussion

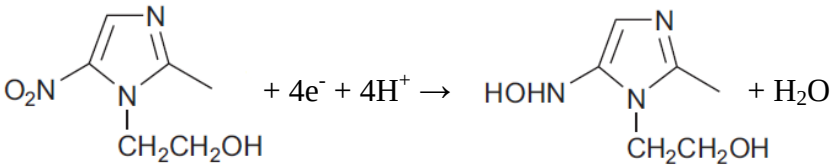
3.1. Evolution of MTZ degradation and mineralization

Metronidazole degradation and mineralization were first studied during electrolysis at 0.04 mA cm^{-2} in a 50 mM sodium sulfate solution at pH 3 with 0.1 mM of ferrous ions and for an initial concentration of 100 mg L^{-1}. The degradation kinetic was rapid compared to the mineralization kinetic since an almost total degradation of the target compound was obtained at 25 min. After 135 min of electrolysis, mineralization yield was only 26% (Fig 1a). The recalcitrance of metronidazole by-products during electro-Fenton treatment was already noticed by Pérez et al. (Pérez et al., 2015) and could explain the moderate mineralization yield obtained. In their study, the identified heterocyclic by-products included an imidazole ring that is considered as stable because of the presence of two nitrogen atoms in the aromatic cycle. In the imidazole ring, a free electron doublet associated to the pyrrole nitrogen is delocalized in the cycle and electrons from pyridine nitrogen are localized in its sp^2 orbital. This particular configuration confers stability to the imidazole ring (Clayden et al., 2002) and

could then confer its recalcitrance toward hydroxyl radical attack. In the same way, cyanuric acide is well known as recalcitrant to hydroxyl radical oxidation due to the electron deficiency of the triazine ring linked to the higher electronegativity of nitrogen compared to that of carbon (Liu, 2014).

Different evolutions for MTZ concentration during electrolysis conducted at 0.07 mA cm^{-2} with 0.1 mM of ferrous ions were plotted according to the initial target compound concentration in the range of 100-500 mg L^{-1} (Fig. SM-1). As almost total degradation was obtained after 20 min for 100 mg L^{-1} MTZ initial concentration, the time needed for a total degradation increased to 30 min doubling the initial concentration. If we considered a pseudo first order kinetic for the global degradation as mentioned in most of the studies on the electro-Fenton process (Brillas et al., 2009), apparent initial kinetic constant (k_{app}) deduced from the 10 first min of electrolysis (Table 1) highlighted a higher reaction kinetic for an initial concentration of 100 mg L^{-1}. Then k_{app} decreased with the initial MTZ concentration. These results were in accordance with previous studies (Ferrag-Siagh et al., 2014; Annabi et al., 2016) and can be explained by a gradual acceleration of competitive reactions between the degradation of the target compound and that of its by-products.

The electrochemical behavior of metronidazole was also studied in order to study the contribution of a direct oxidation and/or reduction of the target molecule at the electrode surface during the electro-Fenton process. For this purpose, current potential curves were plotted with a vitreous carbon rotating disk electrode in Na_2SO_4 50 mM supporting electrolyte at pH 3 with 100 mg L^{-1} of the target compound and under nitrogen atmosphere. No MTZ oxidation was highlighted (data not show) while a signal for MTZ reduction was observed around - 0.4 V/SCE (Fig. 1b). The MTZ electrochemical reaction was already highlighted by Saïdi et al. (Saïdi et al., 2014) in acidic aqueous medium H_2SO_4 (0.5 M) and corresponded to the four-electrons reduction of the nitro group to the corresponding hydroxylamine (Eq. 4).



(4)

During MTZ electrolysis in the same operating conditions as for electro-Fenton but in the presence of tert-butanol, well-known as an hydroxyl radical's scavenger (Ma and Graham, 2000), kinetic of MTZ degradation was slower since an almost total degradation was obtained for around 40 min (not shown) compared to 25 min for electrolysis without tert-butanol. The electric charge (Q) was calculated using the Faraday's law (Eq. 5) with F the Faraday constant, N the mol number of degraded MTZ and n the 4 electrons reduction of metronidazole. According to the consumed electric charge during 40 min of electrolysis in the presence of tert-butanol (Eq. 6), the current efficiency was about 47%.

$$Q = n F N \quad (5)$$

$$Q = i t \quad (6)$$

It can be therefore supposed that metronidazole degradation was due to both the electrochemical reduction at the cathode surface and the oxidation by hydroxyl radicals. Under nitrogen atmosphere to avoid the presence of oxygen in the solution and then the production of hydrogen peroxide, mineralization yield was negligible (not shown). This result confirms that metronidazole mineralization was mainly due to the action of hydroxyl radicals produced by the Fenton reaction.

3.2. Effect of the applied cathodic current density

3.2.1. Effect of the applied cathodic current density on MTZ degradation and mineralization

Current density is one of the main parameters governing the efficiency of the electro-Fenton process, governing the formation of H_2O_2 and then that of hydroxyl radicals (Oturan et al., 2008; Özcan et al., 2009; Feng et al., 2014). Cathodic current density affects the organic

matter treatment performance (Brillas et al., 2000). Its impact on the degradation and the mineralization of MTZ was therefore examined. The figure 2a shows the evolution of metronidazole concentration as a function of time for different cathodic current densities ranging from 0.04 to 0.45 mA cm^{-2}. The obtained results showed an almost total degradation except for 0.04 mA cm^{-2} after 15 min of electrolysis (Fig. 2a). In the range 0.07 to 0.22 mA cm^{-2}, the k_{app} were nearly similar, in a short range of values, 0.22 to 0.24 min^{-1} (Table 1), while for 0.04 mA cm^{-2}, the apparent constant kinetics was lows, about 0.08 min^{-1}. The weak maximum value observed for 0.07 mA cm^{-2} should however be noticed.

This faster oxidation at 0.07 mA cm^{-2} can be ascribed to an acceleration of the H_2O_2 formation rate according to reaction (Eq. 2) and was confirmed by the hydrogen peroxide quantification (Fig. 3b). A good regeneration of ferrous ions from reaction (Eq. 3), as mentioned in paragraph 3.2.2., can also explain the better efficiency of the electro-Fenton process at 0.07 mA cm^{-2} if compared to higher current densities (Brillas et al., 2009). At higher current densities, the production of hydrogen peroxide was lower (Fig. 3b).

As observed in Fig. 3a, plotting the concentration decrease according to the electric charge (Q) highlighted the presence of competitive reactions since the necessary electric charge for a total degradation increased with the current density. At 0.07 mA cm^{-2}, total degradation was obtained for an electric charge of 120 C. When current density increased to 0.45 mA cm^{-2} total MTZ degradation required more than 600 C. Concurrent reactions such as the direct 4 electrons oxygen reduction (Eq. 7), hydrogen peroxide reduction (Eq. 8) or formation of hydrogen (Eq. 9) at the cathode and the oxidation of H_2O_2 on the anode (Eq.10) in the undivided cell can impede the accumulation of hydrogen peroxide (Brillas et al., 2009; Dirany et al., 2012; Ferrag-Siagh et al., 2013). Because of these competing reactions, the constant rate k_{app} (Table 1) cannot be proportional to the current density (Annabi et al., 2016).

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

$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$ (8)

$2H_3O^+ + 2e^- \rightarrow H_2 + 2H_2O$ (9)

$H_2O_2 \rightarrow O_2 + 2e^- + 2H^+$ (10)

Mineralization yields remained limited irrespective of the applied current density (Fig. 2b) due to the recalcitrance of metronidazole by-products as mentioned before. For instance, for a current density of 0.04 mA cm^{-2}, the TOC removal was only 26 % after 135 min of treatment. TOC removal increased to 40 % with a current density of 0.07 mA cm^{-2}. This highest mineralization yield was also in accordance with the highest concentration of hydrogen peroxide obtained, 1.4 mM (Fig. 3b); while a lower production of hydrogen peroxide was observed for a further increase of the current density.

3.2.2. Effect of the current density on the concentration of iron species.

The evolution of the ferrous and ferric ions concentrations in the solution during the electro-Fenton process at different current densities is presented in Fig. 4a. The total concentration of iron ions remained constant throughout the electrolysis for 0.04 mA cm^{-2}; while a decrease of this concentration of about 15% was observed when the current density increased to 0.07 mA cm^{-2}. For 0.22 and 0.45 mA cm^{-2}, an almost total disappearance of the total iron ions in solution was observed from 60 min of electrolysis. Indeed, for high current densities, water reduction can lead to a local alkalinization of the solution caused by hydroxyl anions formation during H_2 release and then to the precipitation of iron as $Fe(OH)_3$ (Eq. 11) (Brillas et al., 2009; Petrucci et al., 2016).

$2H_2O + 2e^- \leftrightarrow H_{2\,(g)} + 2OH^-$ (11)

The adsorption of iron hydroxide onto the electrode surface (Petrucci et al., 2016) or the reduction of iron ions to metallic iron considering standard potentials for iron redox couples, could be checked even if no deposit on the electrode surface was observed after electrolysis. All these phenomena could then partly explain a lower efficiency for these current densities.

In order to study the regeneration of ferrous ions, electrolysis at 0.04 and 0.07 mA cm^{-2} were carried out under N_2 inert atmosphere, in order to avoid dissolved O_2 and then the electro-generation of H_2O_2 (Fig. 4b). An initial amount of ferric ions of 0.1 mM was added into the reactor at pH 3. At the initial time, without electrodes polarization, the slight amount of ferrous ions detected can be explained by redox equilibrium between ferrous and ferric ions in solution. Since the electrodes polarization, ferrous ions were detected very quickly in solution at a value of 0.06 mM for both current densities, and remained then stable throughout electrolysis. 60% of ferric ions were reduced at the cathode surface and as the concentration of total iron ions in solution remained quasi constant in solution, 40% of ferric ions remained in solution; this could be explained by an equilibrium between ferrous and ferric ions implemented in solution throughout the electrolysis. In addition, the evolution of ferrous ions generation was similar for both current intensities, as for the total concentration of iron species. The difference observed between 0.04 and 0.07 mA cm^{-2} concerning mineralization can be then explained by the higher production of hydrogen peroxide measured for the latter current density.

3.3. Influence of the initial catalyst concentration

In order to evaluate the effect of the catalyst concentration on the degradation and the mineralization of Metronidazole, several experiments were performed in the presence of 100 mg L^{-1} of MTZ at pH 3 and an applied current density of 0.07 mA cm^{-2}. The degradation of the target compound was slighthly influenced by the concentration of ferrous ions in the

studied range from 0.05 to 1 mM (Fig. SM-2). Indeed, for 15 min of electrolysis, degradation yields were in a short range of values, 90 to 99% with a slight peak (99%) for 0.1 mM of catalyst. Because of the electrochemical reduction of metronidazole at the cathode surface and a good regeneration of ferrous ions at these current densities, the influence of the ferrous ions amount on the metronidazole degradation can be considered as low in this range of concentrations. MTZ mineralization also showed a slight peak in the range of catalyst concentrations tested; it increased from 20% to 32% at 120 min of electrolysis for increasing Fe^{2+}concentrations from 0.05 to 0.1 mM respectively (Fig. SM-2). This increase could be attributed to the increase of the production of hydroxyl radicals from 1.52 to 2.28 mM respectively (Table 2). The MTZ mineralization and the production of hydroxyl radicals remained then constant for 0.5 mM and then decreased for 1 mM of ferrous ions. For this latter ferrous concentration, mineralization yield was 23% after 120 min and the concentration of produced hydroxyl radical decreased to 1.56 mM for 60 min of electrolysis respectively (Fig. SM-2 and Table 2). This decrease can be explained by competition effects between hydroxyl radicals resulting from the Fenton's reaction and the excess of ferrous ions (Panizza and Cerisola, 2009), according to Eq. 12.

$$Fe^{2+} + {}^{\bullet}OH \leftrightarrow Fe^{3+} + OH^{-} \quad (12)$$

It can be therefore deduced that the optimal value for ferrous ions was 0.1 mM. This value was in accordance with other studies, such as the mineralization by the electro-Fenton process of tetracycline (Ferrag-Siagh et al., 2013) , direct orange 61 (Hammami et al., 2007) and pentachlorophenol (Oturan et al., 2001). However, the influence of this parameter is not essential in this range of concentrations since the difference between the mineralization yields for all tested ferrous ions concentrations did not exceed 12%.

3.4. Effect of the pH

To consider the effect of pH on the electro-Fenton process, degradation and mineralization of MTZ were studied at different pH values from 2 to 9. The degradation of metronidazole was achieved by the Fenton's reaction and such reaction is highly related to the pH (Brillas et al., 2009). Moreover, it was also shown that the electrochemical reduction of metronidazole depends also on the pH (Saïdi et al., 2014), in agreement with the impact of the pH on the degradation rate of metronidazole experimentally observed (Table 1). Indeed, for acidic media, values of k_{app} (0.18 and 0.17 min^{-1} for pH 2 and 3 respectively) were higher compared to those obtained in neutral and basic media (0.08, 0.08, 0.05 min^{-1} for pH 5, 7 and 9 respectively). The mineralization yields decreased from 32% to 24% after 120 min when pH decreased from 3 to 2 respectively (Fig. SM-3). At higher pH values, the degradation of metronidazole was very slow (Fig. SM-3) and reached 83.0%, 80.7%, 65.4% after 30 min, while mineralization yields were 17.0%, 8.3%, and 5.9% after 2 hours for pH 5, pH 7 and pH 9 respectively. Optimal mineralization yield (32%) was obtained at pH 3. Higher amounts of hydroxyl radicals were obtained at acidic pH (Table 2) and the optimal production of hydroxyl radicals was reached at pH 3, that is consistent with the related literature (Qiang et al., 2003; Ramirez et al., 2005; Zhou et al., 2007; Ramirez et al., 2009). At pH values lower than 3, ferric ions can form complexes with hydrogen peroxide leading to lower production of hydroxyl radicals (1.95 ± 0.09 mM – Table 2). Moreover, The H_2 evolution can be favored at acidic pH (Petrucci et al., 2016) and hydrogen peroxide would form an oxonium ion (Eq. 13) which inhibits the formation of hydroxyl radicals (Sun et al., 2009; Zhou et al., 2013). For higher values of pH, the precipitation of ferric ions (Eq. 14) and then a lower production of hydroxyl radicals (Table 2) leads to a loss of efficiency (Brillas et al., 2009; Masomboon et al., 2009; Nidheesh and Gandhimathi, 2012).

$$H_2O_2 + H^+ \leftrightarrow H_3O_2^+ \quad (13)$$

400 $Fe^{3+} + 3OH^- \leftrightarrow Fe(OH)_3$ (14)

401

402 **4. Biodegradability of the electrolyzed solution of MTZ**

403 Once the solution was treated by electro-Fenton, since organic carbon was still present in
404 solution due to low mineralization yield, a combined process involving a subsequent
405 biological treatment could be considered in order to complete the mineralization of the
406 electrolyzed solution. To assess the relevance of such combined process, the biodegradability
407 should be first determined, it corresponded to the BOD_5 on COD ratio; a solution is
408 considered as easily biodegradable from a ratio of 0.4 (Alonso Salles et al., 2010).
409 Table 3 reported the time-course of the ratio values of 100 mg L^{-1} MTZ electrolysis at 0.07
410 mA cm^{-2} and with 0.1 mM of ferrous ions. BOD_5 /COD ratio values suggested that the initial
411 MTZ solution was not biodegradable. After 30 min of electrolysis, even if a total degradation
412 of MTZ was observed (Fig. 1), the solution remained not biodegradable with a ratio of 0.24,
413 showing that the MTZ by-products, at this time of electrolysis, were relatively resistant to
414 micro-organisms since these components were structurally close to the target compound
415 (Mansour et al., 2012). However, the slight improvement of biodegradability can be due to
416 short linear aliphatic carboxylic acids resulting from the loss of lateral ethanol and acetic acid
417 group during the oxidation of MTZ and its heterocyclic intermediates (Pérez et al., 2015) that
418 can be metabolized by microorganisms. The solution became biodegradable since the ratio
419 value reached 0.7 for 120 min of electrolysis (Table 3). It can be therefore supposed that a
420 large part of recalcitrant by-products was oxidized beyond 60 min since the ratio reached 0.46
421 and can be therefore considered as an optimal oxidation time to minimize the energy
422 consumption. Respirometric experiments showed that no inhibition of heterotrophic and
423 autotrophic biomasses was observed whether after the injection of a non-electrolyzed solution
424 of MTZ or an electrolyzed one. Whereas no response in OUR curve was observed with a non-

electrolyzed solution of MTZ at the beginning of the test, direct response was observed with the first injection of the electrolyzed solution of MTZ which supported the BOD_5 results (data not show). Moreover, after 18 d, a response in OUR curve was observed with a non-electrolyzed solution of MTZ (data not show), which would prove that biomass could be acclimatized to MTZ and perform its direct degradation. The biodegradability improvement after electro-Fenton treatment of recalcitrant compounds was also noticed in several reports (Alonso Salles et al., 2010; Mansour et al., 2012; Ferrag-Siagh et al., 2014; Annabi et al., 2016). Even if the respirometric study assumed a possible biodegradation of pure MTZ after 18 d, the electro-Fenton pretreatment should allow to significantly decrease the biodegradation duration.

4. Conclusion

The degradation and mineralization of metronidazole was studied by the electro-Fenton process in a mono-compartment discontinuous reactor including a carbon felt cathode and a platinum anode. Both the MTZ electrochemical reduction at the cathode surface and the Fenton reaction were responsible for the degradation of the target compound. However, mineralization was mainly due to the production *in-situ* of hydroxyl radicals. The influence of some operating parameters such as initial Metronidazole's concentration, current intensity, initial Fe^{2+} concentration, and pH value was examined. The highest generation of hydrogen peroxide was obtained at 0.07 mA cm^{-2} with a good regeneration of ferrous ions and at this current density, production of hydroxyl radicals was the highest at pH 3 with 0.1 mM ferrous ions. These conditions were considered as optimal and allowed a total degradation of MTZ and 40% mineralization within 20 min and 135 min of electrolysis, respectively. Additionally, an improvement of the biodegradability to 0.46 was reached from 60 min of electrolysis and was supported by respirometry, indicating the relevance of the electro-Fenton process as a pretreatment prior to a subsequent biological treatment to complete the mineralization.

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686

687 **Figures Legends**

688 **Figure 1: a)** Time-courses of MTZ degradation (with or without tert-butanol) and its
689 mineralization during electrolysis. Experimental conditions: C_0 100 mg L^{-1}, I =0.04 mA cm^{-2},
690 [Fe^{2+}] = 0.1 mM, pH = 3, [Na_2SO_4] =0.05 M. **b)** Current-potential curve obtained with a
691 vitreous carbon rotating electrode (S = 3.2 10^{-2} cm^2 and 1500 rpm), r = 5 mV s^{-1}, under
692 nitrogen atmosphere and T = 298 K, for 100 mg L^{-1} MTZ (continuous line) at pH 3 in Na_2SO_4
693 0.1 M (dashed line).

694 **Figure 2**: Effect of the applied current density on the MTZ degradation **(a)** and its
695 mineralization **(b)**. Experimental conditions: C_0 =100 mg L^{-1}, [Fe^{2+}] =0.1 mM, pH=3,
696 Na_2SO_4=50 mM, V=250 mL.

697 **Figure 3:** Evolution of MTZ degradation with the electric charge for different current
698 densities **(a)** and effect of the current density on the accumulated H_2O_2 production after 1 hour
699 of electrolysis **(b)**. Experimental conditions: C_0 =100 mg L^{-1}, [Fe^{2+}] =0.1 mM, pH=3,
700 Na_2SO_4=50 mM, V=250 mL.

701 **Figure 4**: **(a)** Effect of the applied current density on the total iron ions concentration.
702 Experimental conditions: C_0 =100 mg L^{-1}, [Fe^{2+}] =0.1 mM, pH=3, Na_2SO_4=50 mM.
703 **(b)** Regeneration of Fe^{2+} at 0.04 and 0.07 mA cm^{-2}. Experimental conditions C_0 =100 mg L^{-1},
704 [Fe^{3+}] =0.1 mM, pH=3, Na_2SO_4=50 mM.

705

706

Table 1 : Apparent kinetic rate constants K_{app} obtained during the EF treatment at various initial MTZ concentrations,Fe (II) concentration, pH and applied current intensity.

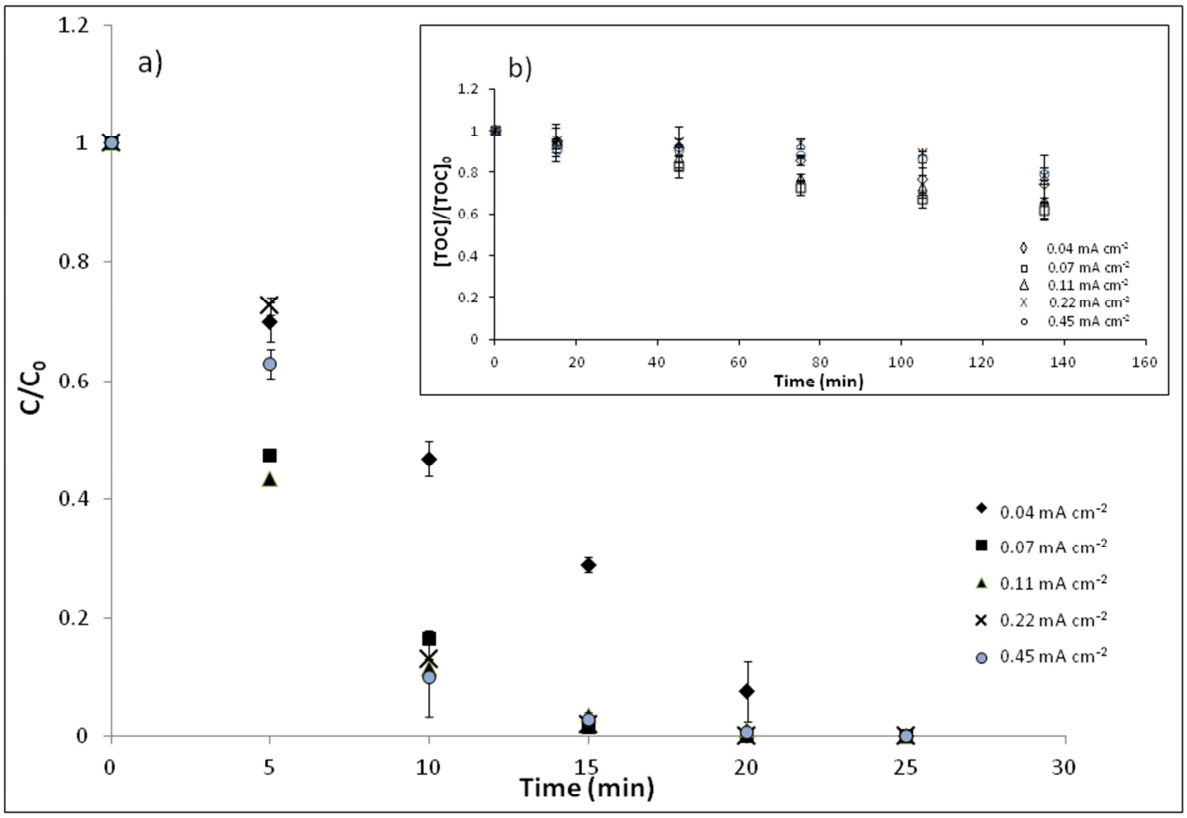
[MTZ] $_{initial}$ (mg L^{-1})	**[Fe^{2+}] (mM)**	**Current density (mA cm^{-2})**	**pH**	**kapp (min^{-1})**
100	0.10	0.07	3	0.24
200	0.10	0.07	3	0.06
300	0.10	0.07	3	0.04
500	0.10	0.07	3	0.04
100	0.10	0.04	3	0.08
100	0.10	0.11	3	0.22
100	0.10	0.22	3	0.23
100	0.10	0.45	3	0.23
100	0.05	0.07	3	0.15
100	0.5	0.07	3	0.19
100	1.00	0.07	3	0.14
100	0.10	0.07	2	0.18
100	0.10	0.07	5	0.08
100	0.10	0.07	7	0.08
100	0.10	0.07	9	0.08

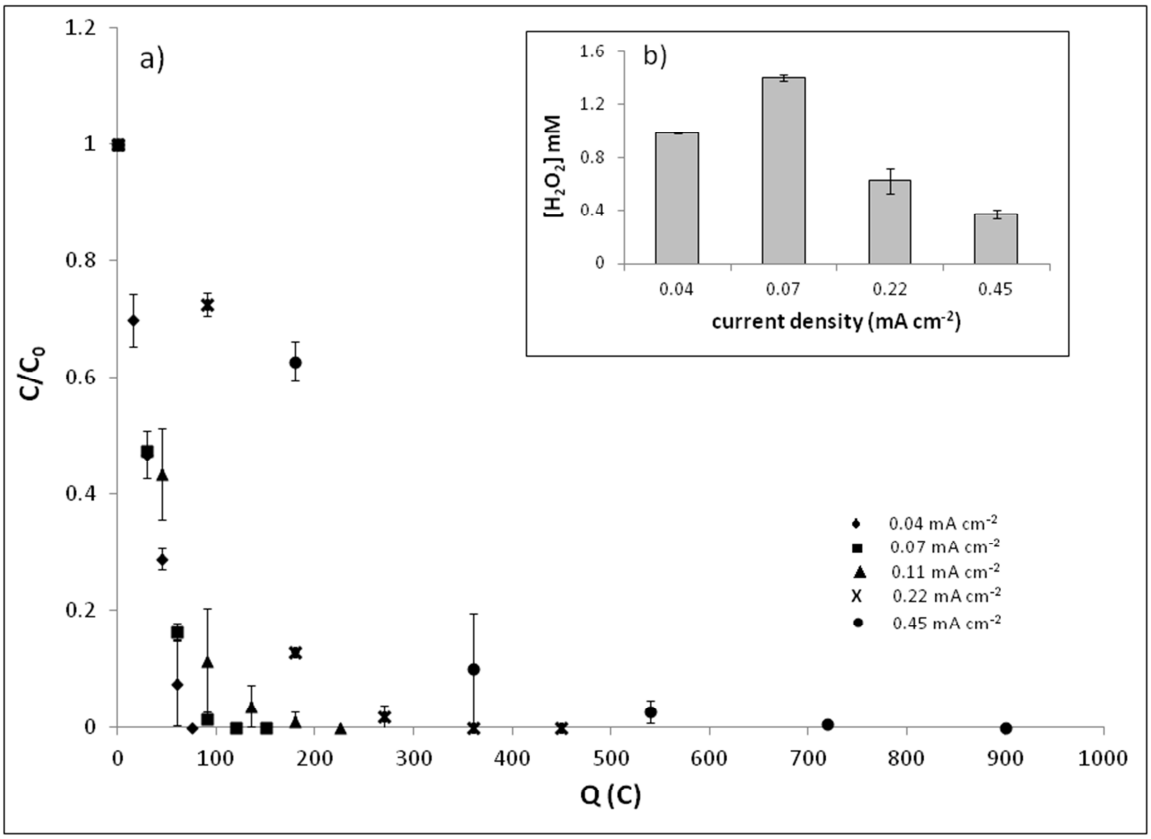
Table2 : Accumulated hydroxyl radicals production obtained during electro-Fenton treatment at various ferrous ion concentrations and various pH

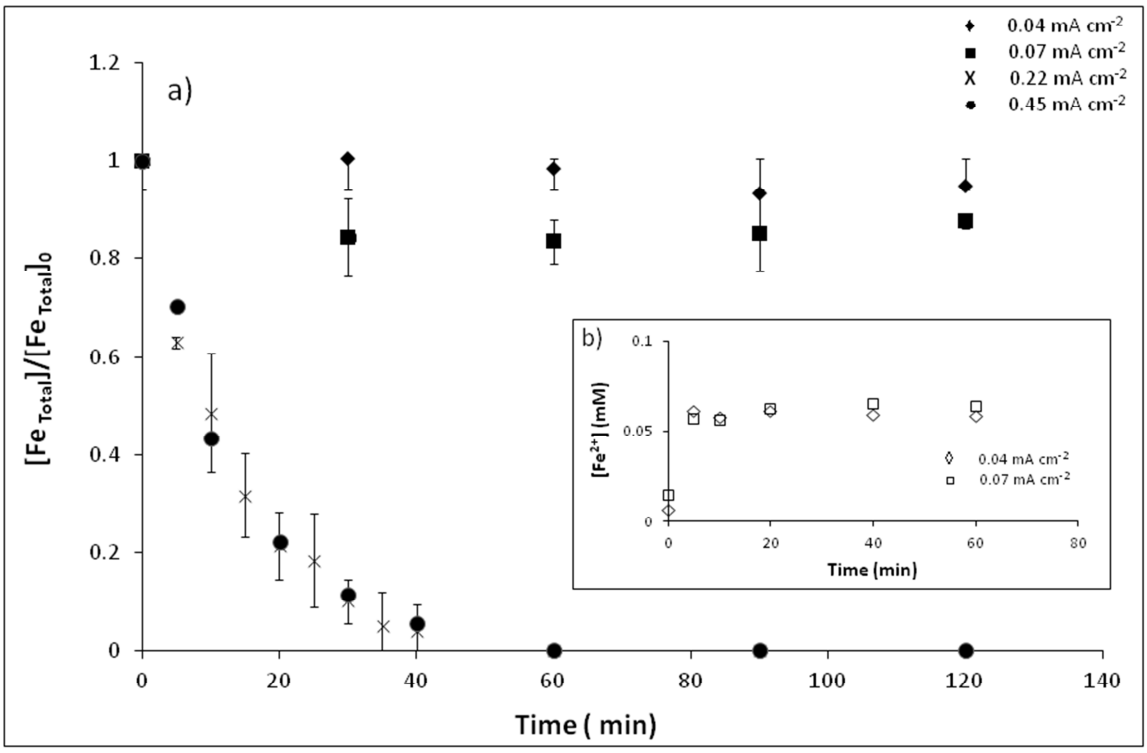
[MTZ] $_{initial}$ (mg L^{-1})	**[Fe^{2+}] (mM)**	**Current density (mA cm^{-2})**	**pH**	**[$^{\bullet}OH$] (mM)**
100	0.05	0.07	3	1.52 ± 0.03
100	0.1	0.07	3	2.28 ± 0.01
100	0.5	0.07	3	2.20 ± 0.03
100	1.00	0.07	3	1.56 ± 0.02
100	0.10	0.07	2	1.95 ± 0.09
100	0.10	0.07	5	0.33 ± 0.02
100	0.10	0.07	7	0.29 ± 0.04
100	0.10	0.07	9	0.21 ± 0.02

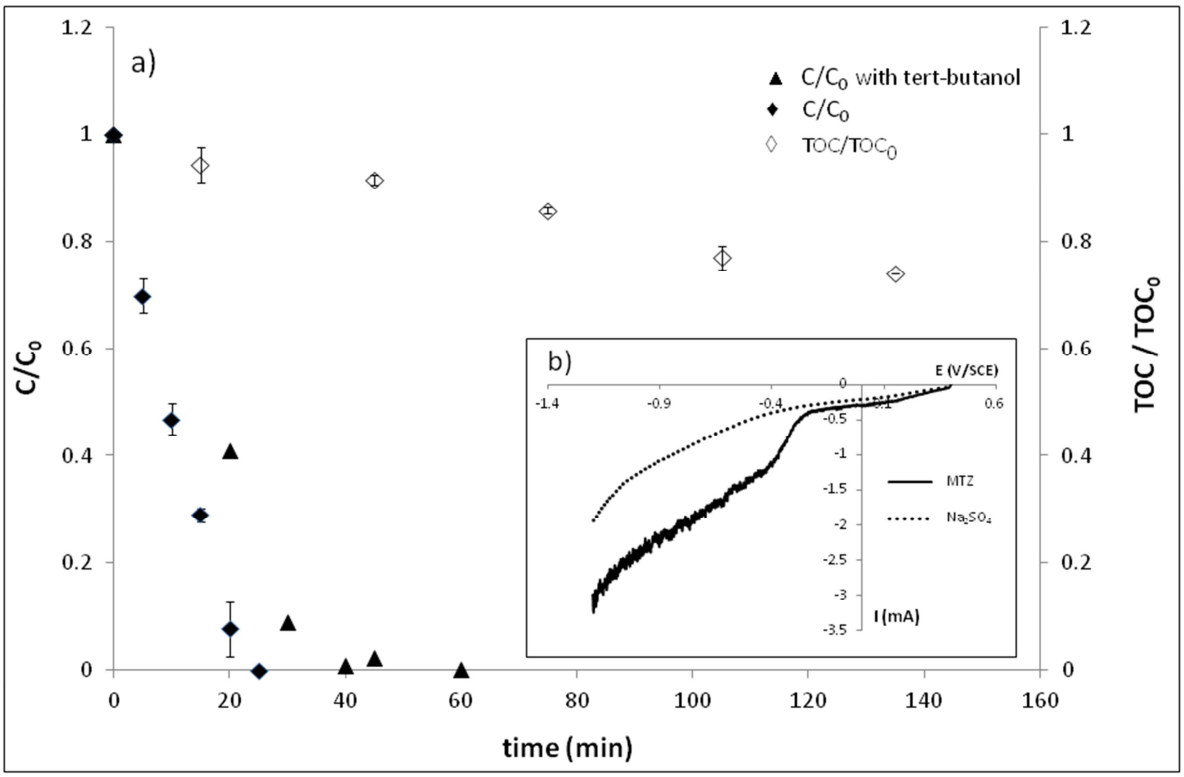
Table 3: Biodegradability of by-product of MTZ. Experimental Conditions: C0 = 100 mg L^{-1} [Fe^{2+}] =0.1 mM, Na_2SO_4=50 mM, V=250 mL , and 0.07 mA cm^{-2} of current density.

Time (min)	**BOD_5/COD**
0	0.01
30	0.24
60	0.46
90	0.46
120	0.70









Highlights

- MTZ electroreduction involved in its degradation not in its mineralization
- Good regeneration of ferrous ions up to 0.07 $mA.cm^{-2}$
- Highest $^{\bullet}OH$ production for 0.07 $mA.cm^{-2}$, pH 3 and 0.1 mM of ferrous ions
- Limit of biodegradability reached for 60 min of electrolysis according to BOD_5 test
- Results from BOD_5 reinforced by respirometric study implemented during 21 days