

EDTA and electricity synergetic catalyzed $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ process for amoxicillin oxidation

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ABSTRACT

Three oxidation processes for amoxicillin wastewater pretreatment such as Electro- $\text{Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$ (EDTA: ethylenediaminetetraacetic acid), $\text{Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$ and Electro- $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ were simultaneously discussed at pH of 7.0 (± 0.1). It was found that the above processes could achieve 78%, 64%, 33% chemical oxygen demand (COD_{Cr}) removal, and 86%, 70%, 47% amoxicillin degradation respectively. Moreover, the results of biodegradability (biological oxygen demand (BOD_5)/ COD_{Cr} ratio) showed that the Electro- $\text{Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$ process was a promising way to pretreat antibiotic wastewater due to the biodegradability of the effluent improved to 0.48 compared with the cases of $\text{Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$ (0.40) and Electro- $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ process (0.12). Therefore, it was reasonable to note that EDTA and electricity showed synergetic effect on the oxidation process. Additionally, infrared spectra (IR) were applied to concisely propose a potential degradation way of amoxicillin. The characteristic changes of H_2O_2 and EDTA in the oxidation process were also investigated in detail.

Key words | amoxicillin, catalyzed oxidation, degradation, electro- $\text{Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$

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INTRODUCTION

Amoxicillin belongs to the β -lactam antibiotic group, which at present stands for a great majority of the antibiotics used in medical treatments. According to Huang *et al.* (2001), the predicted amoxicillin concentration in municipal wastewater of excluding metabolism was up to $27,000 \text{ ng L}^{-1}$, while that of $16,000 \text{ ng L}^{-1}$ including metabolism. This could do potential adverse effects on the ecosystem and possibly on human health because of their toxicity to various microorganisms and recalcitrant degradability (Andreozzi *et al.* 2004). As a consequence, pre-treatment of amoxicillin or other antibiotic formulation effluents has attracted tremendous attention (Arslan-Alaton & Dogruel 2004).

The Fenton process ($\text{Fe}^{2+}/\text{H}_2\text{O}_2$), one of the advanced oxidation processes (AOPs), is widely used as a suitable treatment method for highly concentrated wastewater, due to its effectiveness in producing HO^\cdot (Neyens & Baeyens

2003). However, it has a strict acidic limitation of pH 3.0 and causes a lot of iron sludge to be generated at the end of the reaction, which needs further treatment because the pH value of real wastewater generally ranges from 6.0 to 9.0. Undoubtedly, troublesome acidification and subsequent neutralization will result in two additional steps and are expensive for any scheme (Ndjou'ou *et al.* 2006). Thus, how to improve the Fenton reagent's oxidation activity at ambient circumstance becomes a universally concerned topic in the advanced oxidation processes (AOPs).

In the previous study, an enhanced version of the classical Fenton reaction, Electro-Fenton system is known to provide a highly efficient means of generating $\text{Fe}(\text{II})$ (Gozmen *et al.* 2003). As for amoxicillin, even though a variety of AOPs (O_3/OH^- , $\text{H}_2\text{O}_2/\text{UV}$, $\text{Fe}^{2+}/\text{H}_2\text{O}_2$, $\text{Fe}^{3+}/\text{H}_2\text{O}_2$, $\text{Fe}^{2+}/\text{H}_2\text{O}_2/\text{UV}$ and $\text{Fe}^{3+}/\text{H}_2\text{O}_2/\text{UV}$) (Arslan-Alaton & Dogruel 2004) have been applied for

the oxidative pre-treatment of real amoxicillin formulation effluents, yet, there hitherto has been little investigation involving amoxicillin treatment with Electro-Fe³⁺(EDTA)/H₂O₂. It is of great importance that EDTA used as catalyst with Fenton or Fenton like system has been proved to provide high efficiency for organics degradation (Zhou *et al.* 2008).

Therefore, in the present work, we attempt to elucidate the special characteristics of amoxicillin degradation using Electro-Fe³⁺(EDTA)/H₂O₂ process under ambient circumstance with another two modified processes, namely Fe³⁺(EDTA)/H₂O₂ and Electro-Fe³⁺/H₂O₂ as control experiments. Multivariate experimental design has been used to accomplish such a goal including amoxicillin degradation rate, chemical oxygen demand (COD_{cr}), biological oxygen demand (BOD₅), biodegradability (BOD₅/COD_{cr}) as well as the characteristic changes of H₂O₂ and EDTA in the oxidation process. Additionally, Infrared Spectra (IR) was applied to concisely propose a potential degradation way of amoxicillin due to its powerful sensitivity and high resolution.

METHODS

Chemicals

Reagents used in the work were purchased from Sinopharm Chemical Reagent Co., Ltd., China, including H₂O₂ (30% w/v), Na₂SO₄, K₂Ti(C₂O₄)₃, FeCl₃·7H₂O, Na₂EDTA (99.9%), H₂SO₄, NaOH, sodium acetate (99%), acetate (89.5%), ammonium acetate (analytical grade), methanol, acetonitrile (HPLC grade). Amoxicillin reference standard and amoxicillin trihydrate C₁₆H₁₉N₃O₅S·3H₂O, were kindly provided to us by Xiangtan Centric Hospital, China. All of the reagents used without further purified. The water used in this work was deionized and purified by Millipore (USA) pure water system.

Experimental setup

All experiments were conducted in a non-membrance electrobath reactor (10 cm × 10 cm × 10 cm) with an effective volume of 1,000 mL equipped with two

self-prepared electrodes. An SPR (Ru -Ir -TiO₂, 20 × 30 mm) was employed as the anode and graphite as the cathode (20 × 30 mm). In addition, a galvanostat (HYD) from Shanghai Huyi Science & Technology Co., Ltd., and a digital magnetic stirrer (85-2) from Jiangsu Jintan Environment Equipment Technology Factory, China. Amoxicillin solution was prepared by using deionized and super-purified water and a certain molar concentration Na₂SO₄ electrolyte solution. The pH of the reaction solution was adjusted to be a certain value by using 1.0 M H₂SO₄ or 1.0 M NaOH before reaction, respectively, probed by a digitally portable pH meter (pH330i, Germany WTW). During the reaction, water samples were collected at certain intervals for analyses. Potential reactions by OH radical and hydrogen peroxide in the collected effluents were quenched by using a solution of 6.0 M NaOH (Potter & Roth 1993).

Analytical methods

Estimation of amoxicillin concentration

All analyses were done in duplicate. Amoxicillin concentration was determined by HPLC (Agilent 1100) with ODS C₁₈ reversed phase chromatography column (150 mm × 2.1 mm, i.d. 3.5 μm) with a UV-detector (255 nm) (Holstege *et al.* 2002; Sara *et al.* 2004). The mobile phase consisted of methanol and buffer solution ammonium acetate (1.0 mol L⁻¹) that were carried as 40%: 60%. Both, methanol and buffer solution were degassed by filtering through a 0.45 μm cellulose acetate membrane filter. The flow rate was 0.2 mL min⁻¹. The injection volume was at 20 μL. The HPLC analysis was conducted at 30°C. Each determination required 30 min. The samples were taken out at certain intervals from solution and raised pH to 10.0 filtering the solution to removal Iron. And then extracted with chloroform (10 mL of effluent solution and 1 mL of CHCl₃), filtered with 0.45 μm to removal inorganic impurities and blow-dried with nitrogen flows. After this procedure, the sample was detected with FTIR Spectrometer (Spectrum One system, Perkin Elmer, USA) or for HPLC analysis.

Estimation of H₂O₂ concentration

H₂O₂ was only measured in the aqueous solution, which was verified by its complete miscibility in water and low

volatility. H_2O_2 solution was prepared with 30% H_2O_2 calibrated by KMnO_4 titration, which had been calibrated by $\text{Na}_2\text{C}_2\text{O}_4$ titration. Whereas, the H_2O_2 concentration during the reaction was quantified by UV-vis spectra at 400 nm after color was developed with potassium titanium oxalate ($\text{K}_2\text{Ti}(\text{C}_2\text{O}_4)_3$) in 1.0 M H_2SO_4 solution to prevent any interference caused by the organic disturbance (Sellers 1980). The limit of detection of H_2O_2 was 0.04 mmol L^{-1} .

Estimation of COD_{cr} and BOD_5

Chemical oxygen demand (COD) was determined by using microwave assisted potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) oxidation method (Jardim & Rohwedder 1989; Dharmadhikari *et al.* 2005). At pre-selected time intervals, samples were taken into the tubes containing sodium hydroxide solution to quench the reaction by increasing pH *ca.* 10.0. Then it was diluted and further stood for 30 min. The supernatant was withdrawn to measure COD_{cr} . A WTW OxyTop system (WTW, Germany) was used for biological oxygen demand (BOD_5) determinations. In all biological analyses, hydrogen peroxide and iron in solution were previously removed to avoid errors in the measurement.

Estimation of Fe^{3+} EDTA concentration

The chelatable Fe^{3+} (EDTA) in sample filtrates was determined by reverse phase high performance liquid chromatography (HPLC, Agilent 1100) with UV light detection ($\lambda = 258 \text{ nm}$, Hewlett-Packard 1050 series), which referred to the method developed by Nowack (2002) and Noradoun & Cheng (2005). The mobile phase consisted of 92% 0.02 M formate buffer (formic acid sodium salt 99%, formic acid 89.5%) and 8.0% acetonitrile containing 0.001 M tetrabutylammoniumbromide (TBA-Br). The pH was adjusted to 3.5. The standard Fe^{3+} (EDTA) complex was created by adding equivalent $\text{FeCl}_3 \cdot 7\text{H}_2\text{O}$ to the EDTA sample prior to HPLC analysis. Controls showed that neither EDTA nor Fe^{3+} salts alone gave UV absorbance at 258 nm. All samples were filtered using $0.22 \mu\text{m}$ nylon syringe filters prior to analysis.

RESULTS AND DISCUSSION

Amoxicillin removal at various initial pH

Based on large investigations, an optimum operation conditions were chosen as followings: Amoxicillin of 250 mg L^{-1} ; H_2O_2 of 64.7 mmol L^{-1} ; Fe^{3+} : EDTA molar ratio of 1:1; EDTA of 0.80 mmol L^{-1} ; electrical current of 0.20 A and temperature of 25°C .

The oxidation of amoxicillin as a function of pH was shown in Figure 1, COD_{cr} abatement rates were obtained for the amoxicillin effluent at varying initial pH. As expected, the highest COD_{cr} removal rates still occurred at pH 3.0 except for the relative longer of reaction time comparison of the classical Fenton, which was in good agreement with earlier reported values (Neyens & Baeyens 2003; Arslan-Alaton & Dogruel 2004). It was evident that a change in the pH of the solution to either side of the value of 3.0 leads to a decrease in the extent of oxidation. The following mechanisms also likely explain this observation: at the strong acidic medium, the active species $[\text{Fe}(\text{H}_2\text{O})_5\text{OH}]^{2+}$ is easily changed into $[\text{Fe}(\text{H}_2\text{O})_6]^{2+}$, which has lower catalysis than that of $[\text{Fe}(\text{H}_2\text{O})_5\text{OH}]^{2+}$ thus the concentration of hydroxyl radicals $\cdot\text{OH}$ are relative lower so the oxidation of amoxicillin become relative slower at $\text{pH} < 3.0$ (Burbano *et al.* 2005). When the value of pH raises beyond 3.0, the decrease in oxidation rate could be explained based on the formation of $\text{Fe}(\text{OH})_3$, leading to a decrease in Fe^{3+} concentration and, which has lower

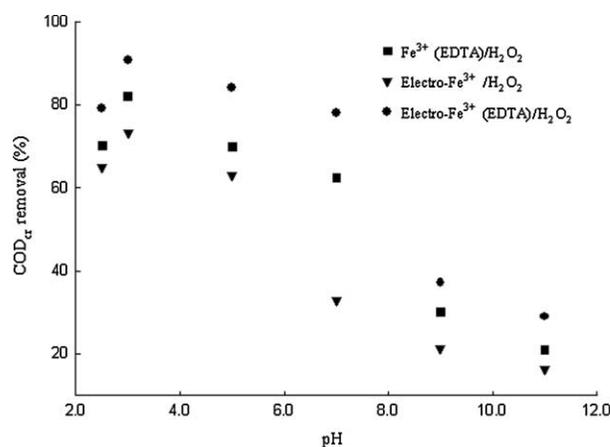


Figure 1 | Initial pH effect on COD_{cr} removal. Experimental conditions: Amoxicillin of 250 mg L^{-1} ; H_2O_2 of 64.7 mmol L^{-1} ; Fe^{3+} to EDTA molar ratio of 1:1; EDTA of 0.80 mmol L^{-1} ; electrical current of 0.20 A; temperature of 25°C .

catalytic activity in the decomposition of H_2O_2 , subsequently, the oxidation rate (Malik 2004).

As shown in Figure 1, at the neutral and alkaline medium, with the elapse of time, COD_{cr} removal rates decreased with increasing pH value, yet, once EDTA and electrical current added into $\text{Fe}^{3+}/\text{H}_2\text{O}_2$, different situation occurred at pH of 7.0. This demonstrated that EDTA and electrical current played different crucial roles in the oxidation process due to 78%, 64% and 33% COD_{cr} removal achieved in Electro- $\text{Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$, $\text{Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$ and Electro- $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ processes respectively. And as a pre-treatment, 78% COD_{cr} removal carried the point as expected. Moreover, the prolonged reaction time would helpfully provide a full description of the characteristic change of H_2O_2 and EDTA in the total reaction process. Thus, pH of 7.0 (± 0.1) and the reaction time of 300 min were selected as the optimum operation parameters.

Effect of EDTA

From Figure 2(a), the results obtained by treating amoxicillin with Electro- $\text{Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$ and Electro- $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ system were reported so as to investigate the characteristics of COD_{cr} and amoxicillin degradation. As shown in Figure 2(a), only 47% amoxicillin degradation and 33% COD_{cr} removal were found in the Electro- $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ system after 300 min's operation, while 86% and 78%

of those were achieved in the Electro- $\text{Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$ system respectively. Moreover, in the Electro- $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ system, the formulation effluent was in dark color with more sediment, which must contribute from ferric ion (III) precipitated at the neutral pH. Whereas, in Electro- $\text{Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$ system, ethylenediaminetetraacetic acid (EDTA) had been used to minimize iron dosage and enhance iron cycling which facilitated $\text{Fe}^{2+}(\text{EDTA})$ to catalyze H_2O_2 and produce HO^\cdot radicals to oxidize amoxicillin. And from Figure 2(b), it was clearly indicated that excessive or inadequate EDTA on the treatment efficiency, i.e., a molar ratio $\text{Fe}^{3+}:\text{EDTA}$ higher than 1:1, may inhibit the formation of $[\text{FeII}(\text{EDTA})(\text{O}_2)]^{2-}$, which, as indicated before, is indispensable for the production of H_2O_2 . Furthermore, more excessive EDTA addition will also lead to an environmental concern. On the other hand, inadequate EDTA would cause ferric ion (III) precipitated at the neutral pH, so the appropriate molar ratio $\text{Fe}^{3+}:\text{EDTA}$ was the key operation condition in this experiment, then $\text{Fe}^{3+}:\text{EDTA}$ (1:1) was selected as the best molar ratio.

Additionally, the oxidation of amoxicillin in this system underwent a three-stage reaction; the first stage occurs relatively slow and then swiftly, the last stage appears less rapidly. The following mechanisms maybe account for this phenomenon:

First, as described by Equations of (1) and (2), when Fe^{3+} was initially used, a lag time for the Fe^{3+} reduction preceded the decomposition of H_2O_2 . Moreover, $\text{Fe}(\text{III})$ has

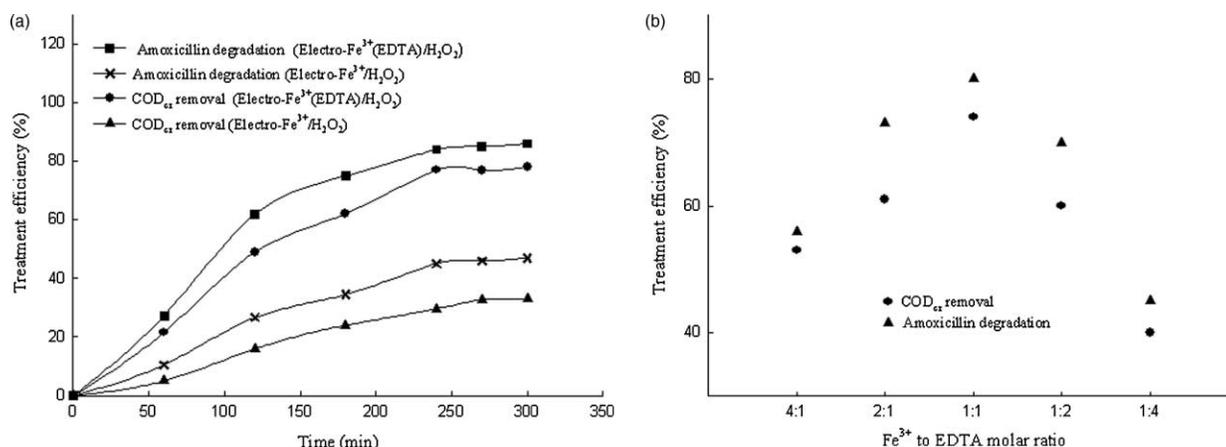
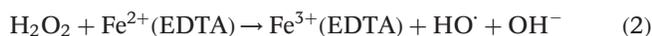
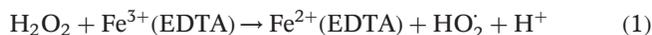


Figure 2 | (a) Effect of EDTA on treatment efficiency. Experimental conditions: Amoxicillin of 250 mg L^{-1} ; H_2O_2 of 64.7 mmol L^{-1} ; Fe^{3+} to EDTA molar ratio of 1:1; EDTA of 0.80 mmol L^{-1} ; electrical current of 0.20 A ; temperature of 25°C ; (b) Fe^{3+} to EDTA molar ratio effect on treatment efficiency. Experimental conditions: Amoxicillin of 250 mg L^{-1} ; H_2O_2 of 64.7 mmol L^{-1} ; Fe^{3+} to EDTA molar ratio of 4:1, 2:1, 1:1, 1:2, 1:4; initial EDTA and Fe^{3+} of 0.80 mmol L^{-1} ; electrical current of 0.20 A ; temperature of 25°C .

lower catalytic activity than Fe(II) and may form a complex with the degradation intermediates (Chen *et al.* 2002). The HO₂[·] hydro-peroxyl radicals developed by reaction (1) are less reactive toward organic compounds. And the slow generation of Fe(II) from reaction (1) limits the HO[·] radicals production by Equation (2) which is the specific characteristics of Fe³⁺/H₂O₂ Fenton like system, without exception of Electro-Fe³⁺(EDTA)/H₂O₂. Consequently, the oxidation rate at the first stage of reaction should be relatively slow due to the slow production of Fe(II) from Fe(III).



Then, with the reaction going on, the more and more Fe²⁺(EDTA) produced then caused largely quantity of HO[·] radicals thus accelerates the oxidation of amoxicillin. It can be clearly seen from the results obtained in Figure 3, when Fe³⁺(EDTA) was added the characteristic UV-vis absorbance of initial amoxicillin near at 230 nm and 272 nm disappear and results in blue shift of the total absorbance curve, so it possibly attributed to the complexation of Fe(III) and the oxidation intermediate of amoxicillin occurred. This Fe(III)-organic complex may be highly active in catalytic decomposition of hydrogen peroxide to produce an oxidant capable of oxidizing amoxicillin. This may be another reason of increase in the oxidation rate (Malik 2004).

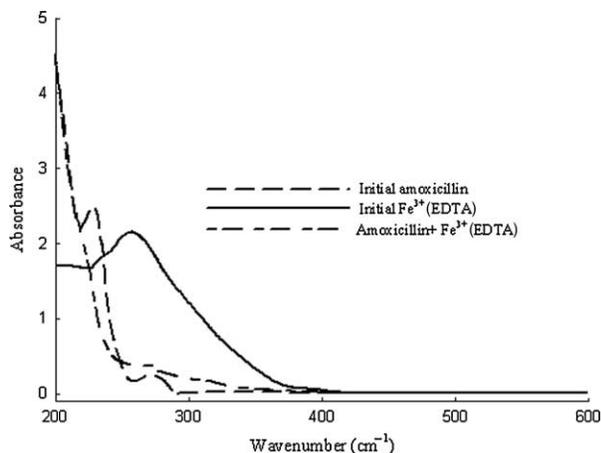


Figure 3 | The UV-vis spectra of initial amoxicillin, Fe³⁺(EDTA) and amoxicillin/Fe³⁺EDTA.

The last stage must ascribe to the largely consumption of H₂O₂, which does not constitute an effective source of HO[·] radical in aqueous solution, thus shows poor degradation rate of COD_{cr} removal and amoxicillin degradation until the reaction reaches equilibrium.

Effect of electric current

As shown in Figure 4(a), COD_{cr} removal and amoxicillin degradation efficiency were raised from 64% to 78%, and 70% to 86% respectively when applied current to Fe³⁺(EDTA)/H₂O₂ system, which These indicated that electricity could conduct an enhancement of the degradation power. This was due to the higher electro-regeneration of ferrous ion from ferric ion with increasing current, which increased the efficiency of Fenton chain reactions because in this case the rate-determining step is the reduction of Fe(III) to Fe(II). Compared with no electricity system there were two pathways to regeneration of Fe²⁺(EDTA): one way is that by electron transform described with reaction (3)



Within the vicinity of the electrode causes amplification of its cyclic voltametric reduction wave (Mark *et al.* 2003). Another way is that Fe³⁺(EDTA) was reduced by H₂O₂ to produce Fe²⁺(EDTA) Naturally, the catalyst of Fe²⁺ was increased and then facilitated enhancement of the oxidation process.

However, higher electrical current would not cause an appreciable enhancement in COD_{cr} removal or amoxicillin degradation (as shown in Figure 4(b)), which will ascribe to the adverse effects of electrode reactions at higher current such as the discharge of oxygen at the anode *via* reaction (4) and the evolution of hydrogen at the cathode *via* reaction (5) would become pronounced.



These competitive reaction would inhibit Fe²⁺(EDTA) and hydroxyl radicals production so the optimum electricity was selected as 0.2 A.

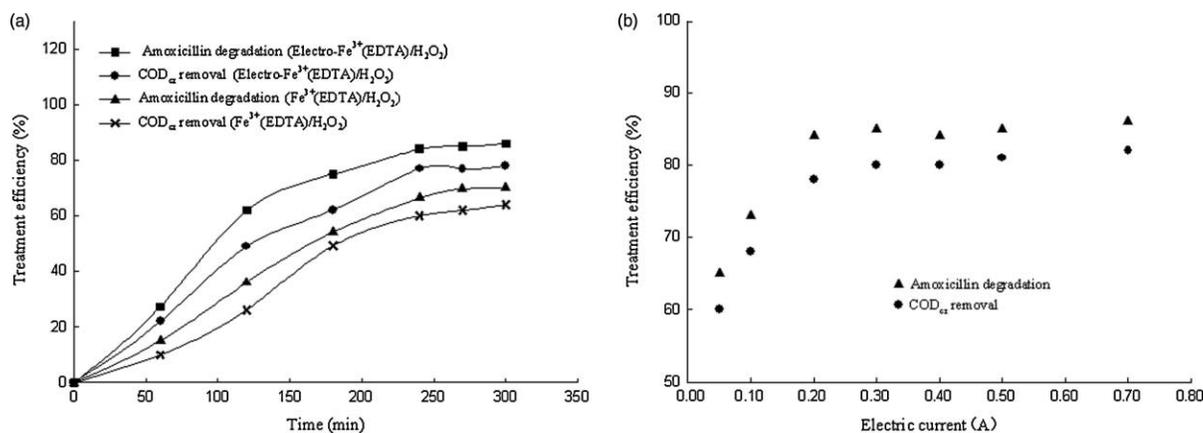


Figure 4 | (a) Effect of electricity on treatment efficiency. Experimental conditions: Amoxicillin of 250 mg L⁻¹; H₂O₂ of 64.7 mmol L⁻¹; Fe³⁺ to EDTA molar ratio of 1:1; EDTA of 0.80 mmol L⁻¹; electrical current of 0, 0.20 A; temperature of 25°C; (b) Electric current effect on treatment efficiency. Experimental conditions: Amoxicillin of 250 mg L⁻¹; H₂O₂ of 64.7 mmol L⁻¹; Fe³⁺ to EDTA molar ratio of 1:1; EDTA of 0.80 mmol L⁻¹; electrical current of 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 0.70 A; temperature of 25°C.

Additionally, the electricity contributes to promote Fe(III)-organic complex oxidation activity. This is accounted for the fact that in E-Fenton process COD was removed by both oxidation and coagulation. The latter was due to the formation of ferric hydroxide during neutralization step of E-Fenton process.

Biodegradability (BOD₅/COD_{cr})

The results of the biodegradability (BOD₅/COD_{cr}) in Electro-Fe³⁺(EDTA)/H₂O₂, Fe³⁺(EDTA)/H₂O₂ and Electro-Fe³⁺/H₂O₂ system were demonstrated in Figure 5. BOD₅ were of 55.5, 38.5, and 24.5 mg L⁻¹ respectively. Obviously, the (BOD₅/COD_{cr}) in Electro-Fe³⁺(EDTA)/H₂O₂ was superior to the other system and arrived to 0.48, while 0.40 in Fe³⁺(EDTA)/H₂O₂ and 0.12 in Electro-Fe³⁺/H₂O₂ system. The possible reason could attribute to the different oxidative pathway of amoxicillin which caused distinct products. And this result also could demonstrate that EDTA and electricity do synergetic effect on the amoxicillin degradation and COD_{cr} removal due to the intermediate products were less toxicity and easily to be degraded by biological treatment. Obviously, EDTA did greater efforts than that of electricity due to the higher treatment efficiency achieved in Electro-Fe³⁺(EDTA)/H₂O₂ such as enhancement of 45% COD_{cr} removal and 39% amoxicillin degradation compared with the case of Electro-Fe³⁺/H₂O₂. However, comparison of system Electro-Fe³⁺(EDTA)/H₂O₂ and Fe³⁺(EDTA)/H₂O₂, only

to 14% COD_{cr} removal and 16% amoxicillin degradation enhancement respectively. It was reasonable to point out that the Electro-Fe³⁺(EDTA)/H₂O₂ system was potentially superior to other two systems in improving the biodegradability of amoxicillin effluents.

Potential degradation mechanisms of amoxicillin

H₂O₂ and EDTA evaluation in the oxidation process

As shown in Figure 6, H₂O₂ concentration took on multi-stage variation versus time in the oxidation process. This observed situation could be explained by the following

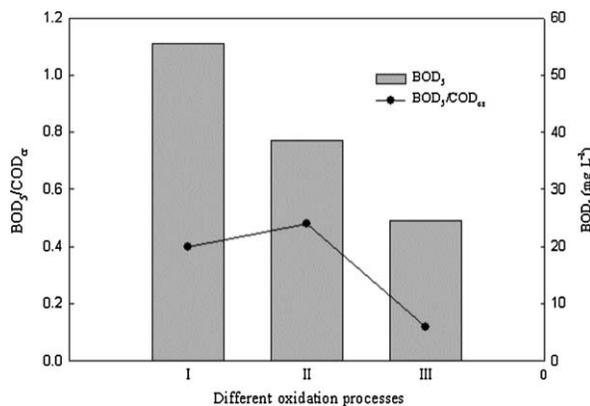


Figure 5 | The biodegradability (BOD₅/COD_{cr}) and COD_{cr} removal achieved with different oxidation processes. I: Treated with Fe³⁺(EDTA)/H₂O₂ process; II: Treated with Electro-Fe³⁺(EDTA)/H₂O₂ process; III: Treated with Electro-Fe³⁺/H₂O₂ process. Experimental conditions: Amoxicillin of 250 mg L⁻¹; H₂O₂ of 64.7 mmol L⁻¹; Fe³⁺ to EDTA molar ratio of 1:1; EDTA: 0.80 mmol L⁻¹; electrical current 0.20 of A; temperature of 25°C; initial pH of 7.0.

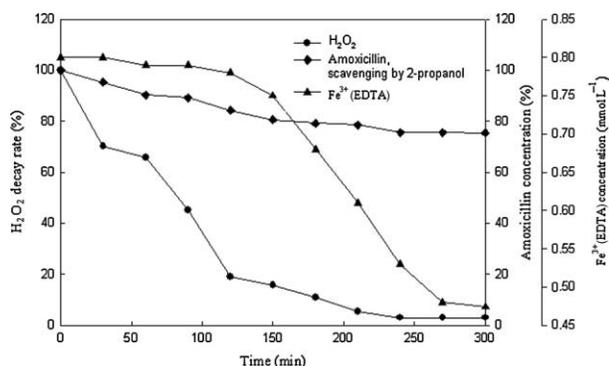
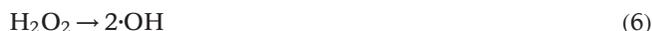
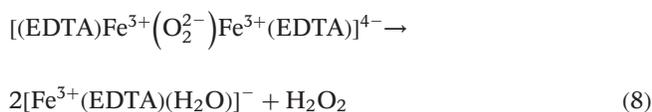


Figure 6 | H_2O_2 decay rate, changes of Fe^{3+} (EDTA) concentration and amoxicillin concentration scavenging by 2-propanol. Experimental conditions: Amoxicillin of 250 mg L^{-1} ; initial H_2O_2 of 64.7 mmol L^{-1} ; Fe^{3+} to EDTA molar ratio of 1:1; EDTA: 0.80 mmol L^{-1} , temperature of 25°C ; initial pH of 7.0.

multi-step mechanism: the dramatically decay trend of H_2O_2 could be accounted for the large amount of H_2O_2 contributed to produce $\text{HO}_2^-/\text{O}_2^-$ reacting with Fe^{3+} (EDTA) described as reaction (1) and (2) (Noradoun & Cheng 2005) or electro-oxidation reaction (6) (Anotai *et al.* 2006)

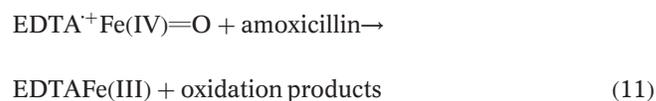


With the oxidation process going on, the decreasing pH facilitates reaction (7) and (8) to produce H_2O_2 , so the newly generated H_2O_2 increased the concentration of H_2O_2 in the aqueous solution, then the gentle decay trend of the degradation curve occurred in the oxidation process.



Furthermore, the last decreasing step was attributed to amoxicillin and EDTA further degraded, which caused H_2O_2 consumption to a low level. However, combining with Figures 2, 4 and 6, it was clearly shown that from reaction time 200 min to 300 min, despite the concentration of H_2O_2 was almost depletion, yet about 10% amoxicillin degradation and 18% COD_{cr} removal efficiency were still achieved. Considering the above observations, the following mechanism ideas could give a potential explanation and borrowing some mechanistic ideas from the literature

(Malik 2004), the reaction mechanism of Fe^{3+} EDTA catalyzed peroxide oxidation of amoxicillin could perhaps be hypothesized as



So the high valence oxide $\text{EDTA}^+\text{Fe(IV)=O}$ continue to carry out the potential oxidation of amoxicillin until the reaction equilibrium.

From Figure 6, addition of 2-propanol (an $\cdot\text{OH}$ scavenger) resulted in negligible amoxicillin oxidation (only 25% amoxicillin degradation) over 300 min in relation to control data (Figure 4(a)). These results demonstrate that $\cdot\text{OH}$ is the primary reactant in amoxicillin oxidation and also further confirm that other oxidants may possible exist in the oxidation process.

Also from Figure 6, the concentration of Fe^{3+} (EDTA) was also elucidated in the oxidation process with the elapse of time. The concentration of Fe^{3+} EDTA changed little in 120 minutes reaction, this process almost occurred during the dramatically decreasing and increasing stage of H_2O_2 concentration, which further proved that EDTA firstly played a role of catalysis and further decreased to a low level as amoxicillin was degraded into stable medium products during the oxidation process. Another likely explanation for the observed results was that the gradually decreasing pH (as shown in Figure 7) was helpful for EDTA degradation because EDTA was subject to Fenton oxidation at acidic conditions (Walling *et al.* 1970). So, it was possible that the initial higher pH inhibit the oxidation of EDTA during the initial reaction step even there was a large amount of $\text{HO}\cdot$ production, which is in agreement with results obtained by Seibig & van Eldik (1997). The following mechanisms also likely explain this observation:

- (1) EDTA and Fe^{3+} formed stable complex thereof the solubility of Fe^{3+} was improved in the aqueous solution, and then made activated medium Fe^{2+} effectively catalyzed H_2O_2 to produce hydroxyl radical;

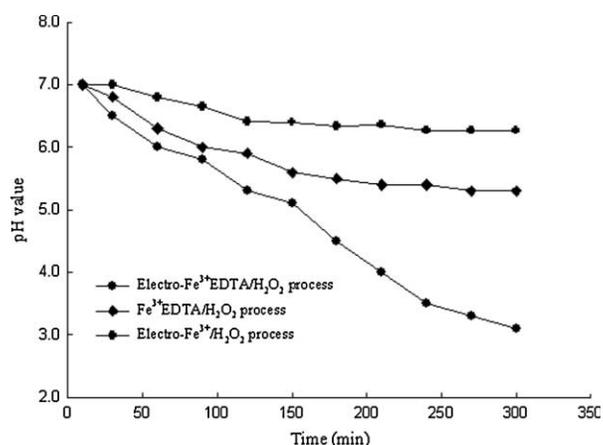


Figure 7 | pH changes in the oxidation process. Experimental conditions: Amoxicillin of 250 mg L^{-1} ; initial H_2O_2 of 64.7 mmol L^{-1} ; Fe^{3+} to EDTA molar ratio of 1:1; EDTA: 0.80 mmol L^{-1} , temperature of 25°C ; initial pH of 7.0.

- (2) The chelatable $\text{Fe}^{3+}(\text{EDTA})$ could provide ligand orbit to coordinate with H_2O_2 or decrease the oxidation-reduction potential ($E(\text{Fe}^{3+}/\text{Fe}^{2+}) = 0.77\text{V}$, $E(\text{Fe}^{3+}/\text{Fe}^{2+}-\text{EDTA}) = 0.12\text{V}$ vs NHE) from 0.77 to 0.12 V (Sun & Pignatello 1993; Gozmen *et al.* 2003) and thus the reactivity of Fe^{3+} was improved heavily during the oxidation process.
- (3) Regeneration of $\text{Fe}^{3+}(\text{EDTA})$ or $\text{Fe}^{2+}(\text{EDTA})$ within the vicinity of electrode, which would be benefit to the generation of HO^\cdot so that an appreciable enhancement in COD_{cr} removal could be achieved.

It was necessary to note that in the control experiment EDTA was selected as the model contamination, the COD_{cr} removal was less than 5.0 mg L^{-1} , so it is reasonable to deduce that EDTA in this oxidation process was just degraded into low weight molecules and not mineralization at all. So it could further prove EDTA acted as catalyst and reactant as well. This also demonstrates that EDTA or its degraded products are less toxicity and facilitating microorganism metabolism thus the biodegradability and oxidation efficiency of amoxicillin are potentially improved.

A potential degradation way of amoxicillin

Figure 7 shows that a gradual decrease of pH from 7.0 to 5.3 in $\text{Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$ system with reaction time evolved, 5.3 in $\text{Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$, but that only down to 6.3 in the $\text{Electro-Fe}^{3+}/\text{H}_2\text{O}_2$ system. The pH profile implied that low molecule weight organic acids or acidic

medium products were possibly formed during the oxidation process because the $\text{Electro-Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$ system could not be greatly responsible for the pH decrease. Additionally, these results also indicated that $\text{Electro-Fe}^{3+}(\text{EDTA})/\text{H}_2\text{O}_2$ oxidation process was an attractive treatment method for the degradation of β -lactam antibiotics in aqueous solution.

As shown in Figure 8, the IR spectra of initial amoxicillin and the degraded effluent were showed at the range of $400\text{--}4,000 \text{ cm}^{-1}$, with the elapse of time developed, the characteristic vibration at $1,770\text{--}1,815 \text{ cm}^{-1}$ assigned to the $\text{C}=\text{O}$ of β -lactam ring and $1,686 \text{ cm}^{-1}$ attributed to the $\text{C}=\text{O}$ stretching vibration of secondary amido greatly decreased, which indicates that the β -lactam ring was opened by hydroxyl radical. Additionally, the special absorptions of $1,583$, $1,519$ and $1,464 \text{ cm}^{-1}$ belong to benzene stretching (Zhu 2005) obviously weakened, it was possible that the benzene ring was also attacked by hydroxyl radical and aromatic ring was cracked and changed into aliphatic hydrocarbon. Moreover, the bands at $2,922$, $2,850$, $1,450$, $1,377$, 756 , 698 cm^{-1} assigned to the C-H bending vibration of $-\text{CH}_2$ and $-\text{CH}_3$ relatively strengthened, respectively, which further indicated amoxicillin was degraded into other low weight molecules.

Taking into account the above observations and borrowing some mechanistic ideas from the literature (Song *et al.* 2008), the reaction mechanism of $\text{Fe}(\text{III})$ chelate catalyzed peroxide oxidation of amoxicillin could perhaps be hypothesized as the following way (as shown in Figure 9). Amoxicillin, as the only salicylic type of β -lactam

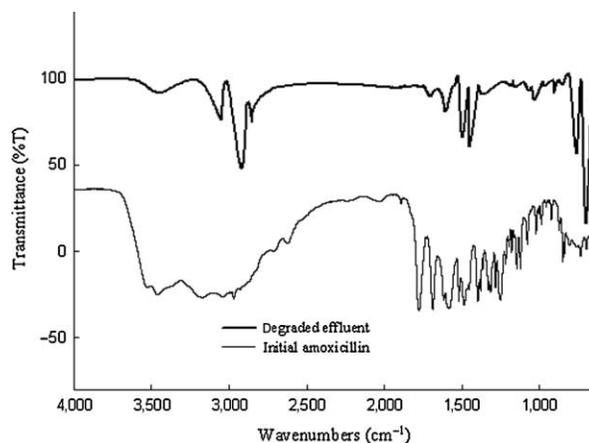


Figure 8 | IR spectra of amoxicillin and degraded effluent.

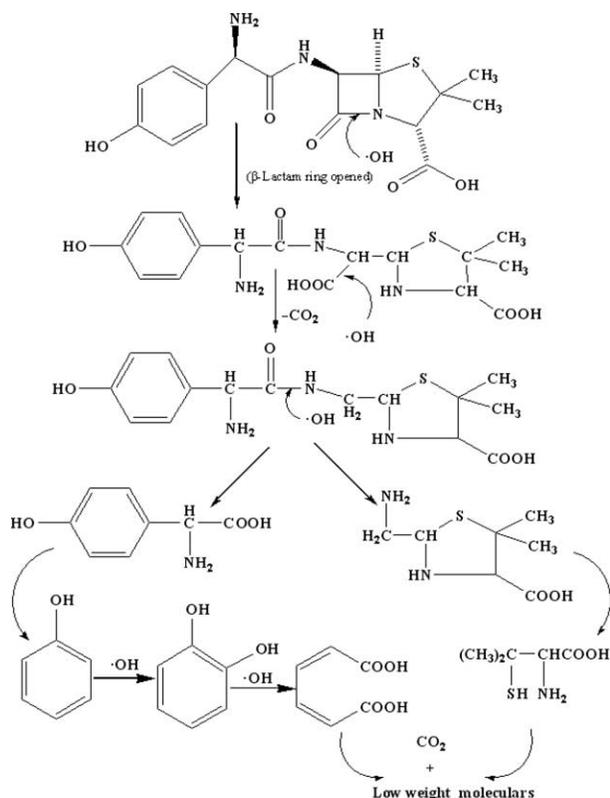


Figure 9 | A potential degradation way of amoxicillin.

antibiotics, β -lactam ring was sensitive to hydroxyl radical and was easily attacked and opened. On the other hand, reflecting to the influence of $-\text{OH}$, amoxicillin was difficult to be degraded as compared with other similar antibiotics, the major degradation pathway appears to involve hydroxyl radical addition to the benzene ring following the degradation way of phenolic compounds.

CONCLUSIONS

According to this investigation, the following conclusions could be drawn:

- (1) As expected, the Electro- Fe^{3+} (EDTA)/ H_2O_2 process has been proven to be a feasible method for amoxicillin pretreatment because of an appreciable enhancement in COD_{cr} removal, amoxicillin degradation and a profound improvement of biodegradability ($\text{BOD}_5/\text{COD}_{\text{cr}}$) compared with that cases with Electro- $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ process and Fe^{3+} (EDTA)/ H_2O_2

under initial pH of $7.0(\pm 0.1)$. EDTA contributed much more to COD_{cr} removal and amoxicillin degradation than those of electricity did.

- (2) Electro- Fe^{3+} (EDTA)/ H_2O_2 process was shown multi-step mechanism, except for hydroxyl radicals oxidation and coagulation processes, possibly involving Fe(III)-organic complex, high valence oxide $\text{EDTA}^+\text{Fe(IV)=O}$ promotion mechanism and electricity catalyzed mechanism.
- (3) IR was applied to concisely propose a potential degradation pathway of amoxicillin due to its high selective and sensitive resolution for determination of organic matter.

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