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# Optimizing Fenton process for the removal of amoxicillin from the aqueous phase using Taguchi method

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ABSTRACT

In recent years, antibiotics have been considered as serious contaminants due to their high consumption and persistence in the aquatic environment. Currently, amoxicillin is one of the most widely used antibiotics and its emission into the environment encounters numerous health and environmental hazards. The main objectives of this research were focused on assessing the feasibility of using Fenton reagent in removing amoxicillin and determining the optimal conditions using Taguchi method. In addition, its effect on the rate of mineralization, biodegradation, and the removal efficiency of COD were studied. The Taguchi method was used to optimize variables and their levels using Qualitek-4 (w32b) software. The optimum values of the response variables were predicted using signal-to-noise ratio ($S/N$). The influence of different parameters including the initial concentration of amoxicillin, $H_2O_2$ concentration, Fe(II) concentration, pH, and reaction time at four different levels on the removal of amoxicillin in the aqueous phase were investigated. The removal efficiencies at initial concentrations of amoxicillin 10, 100, 200, and 500 mg/L were 68.64, 95.385, 98, and 99.3%, respectively. Process optimization by Taguchi method suggests that the optimal conditions for the removal of amoxicillin in the aqueous phase are as follows: the initial amoxicillin concentration of 500 mg/L, Fe(II) concentration of 5.0 mg/L, $H_2O_2$ concentration of 500 mg/L, pH 3, and the reaction time of 15 min; and level of significance for the study parameters were 60.228, 26.369, 5.638, 4.373, and 3.392, respectively. The maximum removal efficiency of COD and mineralization rate were 71.3 and 36.3%, respectively. The biodegradation rate was also increased from 0 to 0.738. In conclusion, our study demonstrated that Fenton process may enhance the rate of amoxicillin degradation in polluted water and could be used as a pretreatment step for the biological removal. The results also indicate that the Taguchi experimental design can simply predict the optimal conditions for the removal of amoxicillin in the aqueous phase using Fenton process.

*Keywords:* Antibiotic; Amoxicillin; Taguchi method; Fenton process

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## 1. Introduction

Antibiotics are a large group of pharmaceuticals with various medical and veterinary applications [1]. The worldwide consumption of antibiotics is estimated between 100,000–200,000 ton annually [2]. These medicines are widely used for the prevention and treatment of human and animal infections. It is also consumed as nontherapeutic to promote the growth of farm animals and crops [3]. Antibiotic resistance is considered as a public health threat. The extensive use of these medicines has increased the potential of environmental contamination [2]. Antibiotics are persistent pollutants in the environment with a tendency of bioaccumulation. They might enter water resources through different pathways and endanger the life of aquatic organisms [4]. The toxic effects of antibiotics on aquatic organisms may disrupt the ecological balance [5].

Amoxicillin belongs to *β*-lactam class of antibiotics and is a broad-spectrum penicillin, which has various medical and veterinary applications with the highest consumption rate among antibiotics [6]. The absorption rate of amoxicillin in the body is 10–20% [7] and the rest is excreted and eventually enters the environment [8]. Research has shown that amoxicillin has toxic effects on algae and aquatic micro-organisms. These compounds eliminate the effective micro-organisms required in biological wastewater treatment [6] and decrease the efficiency of the treatment plants [9]. Antibiotics are resistant to biodegradation process; therefore, the conventional wastewater treatment methods, are not capable of removing these compounds [6]. Advanced oxidation processes (AOPs) is an efficient environmentally friendly method in which hydroxyl radicals ($OH^{\circ}$) are used to oxidize recalcitrant organic pollutants and convert them to harmless end products such as $H_2O$ and $CO_2$ [10]. AOPs lead to the oxidation and degradation of antibiotics. Fenton method is one of the highly applied treatment method due to high efficiency, simplicity of technology, very low costs, and insignificant toxicity of reactants [11]. In Fenton process, ferrous ions and hydrogen peroxide molecules are considered as reductants and oxidants, respectively [12]. Eq. (1) mainly occurred in this process [13]:

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

The efficiency of this process depends on many parameters including temperature, pH, hydrogen peroxide concentration, ferrous ion concentration, and reaction time [14]. The removal rate of amoxicillin in aqueous phase using sulfate radicals at 60 min ultrasonic irradiation was more than 98% [15]. Amoxicillin can be degraded in 10 min using nonthermal plasma [16]. Complete removal of metronidazole can be achieved using nano zero-valent iron after 5 min [17]. Elmolla and Chaudhuri [18] used the combination of photo-Fenton and sequencing batch reactor processes for the removal of amoxicillin and cloxacillin from wastewater. Under optimal conditions, 89% of soluble chemical oxygen demand was removed [18].

Many studies have used AOPs for the removal of antibiotics, but the application of Taguchi statistical method for designing the experiment by Fenton process has not yet been reported. Since the consumption of antibiotics particularly amoxicillin is very high in Iran, there is a concern regarding the effect of antibiotics in water resources on people's health and environment. Therefore, the objectives of the study were to (i) evaluate the feasibility of using Fenton reagent in the removal of amoxicillin in the aqueous phase; (ii) assess the removal efficiency of biochemical oxygen demand (BOD), COD, and dissolved organic carbon (DOC) as well as the biodegradation rate ($BOD_5$/COD) using AOPs; and (iii) determine the optimal conditions using Taguchi method so that the standard limit can be achieved by further complementary treatment.

## 2. Materials and methods

### 2.1. Chemicals and analytical method

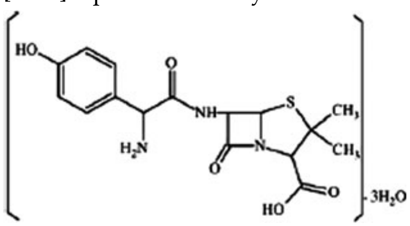
The Taguchi method was used to optimize variables and their levels by conducting experiments on a real time basis using Qualitek-4 (w32b) software. The optimum values of the response variables were predicted using signal-to-noise ratio ($S/N$). The $S/N$ ratio was used to measure the effect of the response variables and to determine the percent removal of amoxicillin (according to Eq. (2)). In this equation, the amounts of $Y_n$ and $n$ are the measured response and the number of repetition (2 in this case) for each test, respectively.

$$\frac{S}{N} = -10 \ \log \frac{(1/Y_1^2 + 1/Y_2^2 + \cdots + 1/Y_n)^2}{n} \quad (2)$$

The level of significance is 95% ($\alpha = 0.05$).

In this research, amoxicillin trihydrate of analytical standard was supplied by Iran Antibiotic Company. Other chemicals were purchased from Merck (Germany). The basic characteristics of amoxicillin trihydrate are shown in Table 1. All stock solutions were prepared weekly in highly purified double-distilled water and stored in dark containers at 4°C to prevent any degradation.

Table 1
Basic characteristics of amoxicillin trihydrate

| Formula | $C_{16}H_{19}N_3O_5S$-$3H_2O$ |
|---|---|
| Density | 0.320 g/mL |
| Molar mass | 350.3 mol |
| Boiling point | 743.2˚C |
| Melting point | 194˚C |
| Solubility | 3,430 mg/L |
| IUPAC name | (2S,5R,6R)-6-[[(2R)-2-amino-2-(4hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2 carboxylic acid |
| Structure |  |
| Molecular weight | 419.408 g/mol |

For amoxicillin detection in the aqueous phase, the Knauer Model high-performance liquid chromatography (Germany) system along with UV detector (Wellchrom K-2600, Knauer, Germany), degasser pump (Wellchrom HPLC pump k-1001, Knauer, Germany), container of solvents (Wellchrom solvent organizer K-1500, Knauer, Germany), and HPLC-specific oven were used (Water and Wastewater Organization, Shiraz, Iran). $C_{18}$ column (Ultrasep ES, PEST, B-690/06) with dimensions of 250 mm × 3 mm × 5 μm was also used. Wavelength of the UV detector was set at 240 nm to detect amoxicillin. Column temperature was maintained at 55˚C. The mobile phase consisted of acetonitrile and highly ultrapure distilled water containing $KH_2PO_4$ (0.025 M) buffer solution with the ratio of 20 and 80%, respectively. The flow rate of the mobile phase was set at 0.5 mL/min. Data obtained from HPLC were recorded and analyzed by Chromgate software. Retention time of amoxicillin at optimal condition was 8.30 min. The detection limit for the sample was 0.01 mg/L.

COD and $BOD_5$ of all samples were measured according to APHA Standard Method No. 5220D and No. 5210D, respectively [19]. To measure COD, pH was increased up to 10 in order to decrease the interference of hydrogen peroxide [20]. pH was measured by pH Meter (Metrohm, Swiss). DOC was measured by TOC Analyzer (N/C 3000, Analytic Jena, Germany).

### 2.2. Experimental setup

Although full factorial is an accurate method with high precision, it is not recommended due to high energy, time, and cost [21]. Therefore, fraction of full factorial method was selected for this study, in which the optimal condition is determined through statistical evaluation of responses. Recently, Taguchi method has been widely used in biological studies for the purpose of experimental optimization and process controls [22]. Taguchi's statistical method and Qualitek-4 (QT4) software were used for the experimental design. Using this software, 16 tests were designed randomly to reduce the errors. Amoxicillin's reduction rate was analyzed using QT4 software. Afterward, the most effective parameters in removing amoxicillin in the aqueous phase, the rate of efficiency, and the level of precision and optimal conditions were determined.

In this study, five parameters including the initial concentration of amoxicillin, $H_2O_2$ concentration, Fe(II) concentration, pH, and reaction time were selected at four different levels to determine the removal efficiency of amoxicillin at optimal conditions in the aqueous phase by Fenton process (Table 2). The levels were selected according to data reported in an earlier study [23]. Two replications were used for each sample. A blank sample without Fenton reagent was also used without adequately removing amoxicillin, with a rate of degradation less than 1%.

## 3. Method

One liter samples were used in 2 L volume reactor using Jar test apparatus (Hach, USA). Test was performed in a batch reactor at 185 rpm to ensure complete mixing and to achieve a homogeneous mixture during the reaction. The experiments were done in

Table 2
Parameters and the selected levels of Fenton process (Fe(II) /$H_2O_2$) for the removal of amoxicillin in the aqueous phase

| Parameter | Level 1 | Level 2 | Level 3 | Level 4 |
|---|---|---|---|---|
| $Fe^{2+}$ (mg/L) | 0 | 5 | 25 | 50 |
| $H_2O_2$ (mg/L) | 10 | 50 | 250 | 500 |
| pH | 3 | 3.5 | 4 | 4.5 |
| Reaction time (min) | 2 | 5 | 10 | 15 |
| Amoxicillin (mg/L) | 10 | 100 | 200 | 500 |

ambient temperature (23–25˚C). Temperature variation during the experiments was not significant.

The samples were analyzed to determine the removal rate of amoxicillin, $BOD_5$, COD, and DOC at each interval time. To determine the residual amoxicillin, 20 mL of the sample was filtered using 0.20 μm acetate cellulose membrane (Glasco, Germany), and 100 μL was injected to HPLC. To measure $BOD_5$, COD, and DOC, samples were passed through Whatman filter paper (0.45 μm in diameter).

## 4. Results and discussion

According to Table 3, the average, minimum, and maximum reduction rates of amoxicillin by Fenton process were 82.91, 59.85, and 99.3%, respectively. The analysis of the results revealed the effect of each parameter on amoxicillin's reduction rate and determined the optimal conditions for the process as well. Fig. 1 showed amoxicillin chromatograms before and after the Fenton process (Run No. 5)

### 4.1. Effect of amoxicillin's initial concentration

The initial concentrations of amoxicillin at four levels (10, 100, 200, and 500 mg/L) play a significant role in the performance of Fenton process and consequently have a considerable effect on the reduction rate of the antibiotic in the aqueous phase. The effect of initial amoxicillin concentration on its reduction rate in the aqueous phase has been shown in Fig. 2. As initial amoxicillin concentration increased from 10 to 500 mg/L, the removal efficiency increased from 65.684 to 92.889% (Fig. 2), respectively. The optimal initial concentration of amoxicillin is at level four (500 mg/L) and level of significance for this parameter was 60.228. The obtained results demonstrate that the increase in amoxicillin's concentration leads to an improvement in its removal efficiency by the Fenton process. The removal efficiency of amoxicillin at the initial concentrations 10, 100, 200, and 500 mg/L was determined to be 65.684, 92.242, 85.043, and 92.889%, respectively.

Furthermore, amoxicillin reduction rate by Fenton process is limited at its lower initial concentration (10 mg/L). Therefore, level four with the initial concentration of 500 mg/L is considered as the optimal condition for removing amoxicillin by Fenton process.

### 4.2. Effect of $H_2O_2$ concentration

The effect of $H_2O_2$ concentration on amoxicillin reduction rate has been shown in Fig. 2. The removal efficiency for the four levels (10, 50, 250, and 500 mg/L of hydrogen peroxide) was 72.907, 83.212, 89.773, and 89.967%, respectively. Therefore, an increase in the concentration of $H_2O_2$ can lead to an increase in amoxicillin removal efficiency.

The results showed that an increase in hydrogen peroxide concentration leads to an increase in the reduction rate of amoxicillin. Based on the data obtained in the present study, $H_2O_2$ concentration of 500 mg/L was optimal for amoxicillin degradation. The removal efficiency and level of significance were 89.967% and 5.638, respectively. Many investigations showed a positive effect of $H_2O_2$ on amoxicillin reduction by UV/$H_2O_2$ [24]. Adding extra $H_2O_2$ concentration (more than 500 mg/L) will act as the scavenger for hydroxyl radical and form $HO_2^{\circ}$, which has a lower oxidative potential compared to $OH^{\circ}$ [21]. The decomposition of hydrogen peroxide into oxygen and water occurred at a concentration of more than optimal. Therefore, it can be concluded that high concentrations of $H_2O_2$ act as an inhibitor for the formation of $OH^{\circ}$ and consequently reduce the efficiency of the process [13].

### 4.3. Effect of ferrous ion ($Fe^{2+}$)

The removal efficiency of amoxicillin increased from 87.4 to 89.153% as the Fe(II) concentration increased from 0 to 5 mg/L. However, as the concentration increased from 5 to 50 mg/L, there was a decrease in the reduction rate of the amoxicillin degradation (9.922%). In the current study (Fe(II)/$H_2O_2$), increase in Fe(II) increased the production rate of hydroxyl radical. According to Fig. 3, optimal ferrous ion concentration, amoxicillin removal efficiency, and level of significance were 5 mg/L, 89.153%, and 26.369, respectively. The removal efficiency decreased from 89.153 to 79.231% as ferrous ion concentration increased from 5 to 50 mg/L. Therefore, amoxicillin reduction rate increased with the increase in Fe(II) concentration up to a specific level (0–5 mg/L) and

Table 3
The experiments designed by Taguchi method and the percent reduction rate of amoxicillin

| Trial No. | Investigated parameter in Fenton process: Level of amoxicillin | Level of $H_2O_2$ | Level of $Fe^{2+}$ | Level of pH | Level of reaction Time | Removal efficiency of amoxicillin (%): First run | Second run | Average of two runs |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 1 | 58.00 | 70.00 | 64.00 |
| 2 | 1 | 2 | 2 | 2 | 2 | 57.70 | 62.00 | 59.85 |
| 3 | 1 | 3 | 3 | 3 | 3 | 62.10 | 62.60 | 62.35 |
| 4 | 1 | 4 | 4 | 4 | 4 | 68.28 | 69.00 | 68.64 |
| 5 | 2 | 1 | 2 | 3 | 4 | 74.55 | 82.39 | 78.47 |
| 6 | 2 | 2 | 1 | 4 | 3 | 94.22 | 94.31 | 94.26 |
| 7 | 2 | 3 | 4 | 1 | 2 | 95.37 | 95.40 | 95.38 |
| 8 | 2 | 4 | 3 | 2 | 1 | 95.30 | 95.30 | 95.30 |
| 9 | 3 | 1 | 3 | 4 | 2 | 61.00 | 63.00 | 62.00 |
| 10 | 3 | 2 | 4 | 3 | 1 | 78.80 | 82.00 | 80.40 |
| 11 | 3 | 3 | 1 | 2 | 4 | 98.50 | 98.60 | 98.55 |
| 12 | 3 | 4 | 2 | 1 | 3 | 97.60 | 98.90 | 98.25 |
| 13 | 4 | 1 | 4 | 2 | 3 | 75.40 | 75.60 | 75.50 |
| 14 | 4 | 2 | 3 | 1 | 4 | 96.30 | 97.90 | 97.10 |
| 15 | 4 | 3 | 2 | 4 | 1 | 97.12 | 97.18 | 97.15 |
| 16 | 4 | 4 | 1 | 3 | 2 | 99.30 | 99.30 | 99.30 |

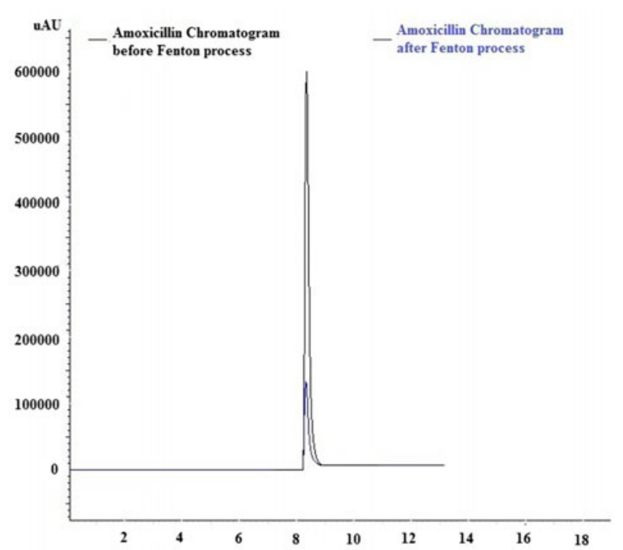



Fig. 1. Amoxicillin chromatograms before and after Fenton process (Run No. 5).

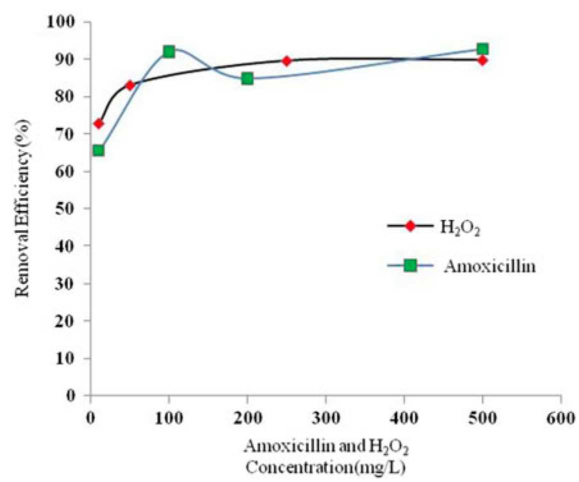



Fig. 2. The effects of concentration of amoxicillin and $H_2O_2$ on the removal efficiency of amoxicillin in the aqueous phase using Fenton process.

then began to decrease (5–50 mg/L). The reduction of the pollutant is basically proportional to the formation of $OH^{\circ}$. The extra Fe ions react with the hydroxyl radical and therefore, reduce the efficiency of the process [13,25] (Eq. (3)).

$$Fe^{2+} + OH^{\circ} \rightarrow Fe^{3+} + HO^{-} \tag{3}$$

Therefore, the second level (Fe(II) = 5 mg/L) is reported as the optimal concentration in the removal efficiency of amoxicillin by Fenton process.

### 4.4. Effect of pH

Data regarding the effect of pH shows that as pH increased from 3.0 to 4.5, the rate of amoxicillin reduction decreased (Fig. 4). Based on our data, pH of 3

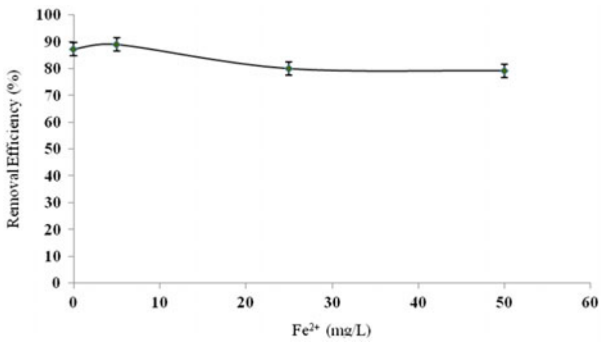



Fig. 3. The effect of $Fe^{2+}$ concentration on the removal efficiency of amoxicillin in the aqueous phase using Fenton process.

was optimal for amoxicillin degradation with a reduction rate of more than 88.9%. The level of significance for this parameter was 4.373. pH is one of the most important factors affecting chemical and biological processes, especially advanced oxidation efficiency. pH has a considerable effect on the solubility of amoxicillin, as well as the mechanism of hydroxyl radical production [20]. The Fenton and photo-Fenton reactions depend on the pH. The feasibility of hydroxyl radical production and oxidation efficiency also depend on pH [26]. The formation of $OH^{\circ}$ is prevented at pH less than 3 because of the reaction of the $OH^{\circ}$ with $H^{+}$ ions [27]. The rate of amoxicillin degradation reduced at higher pH, because of the formation of ferric hydroxide which in turn reduced the potential of hydroxyl radical production as well [28]. Additionally, high pH values intensify the formation of $HO_2^{2-}$ ions and destruction of $OH^{\circ}$ by carbonate and bicarbonate ions [29]. Reduction in amoxicillin degradation at pH over 3 might be attributed to the lower solubility of iron, reducing the potential of hydroxyl radical formation and degradation of

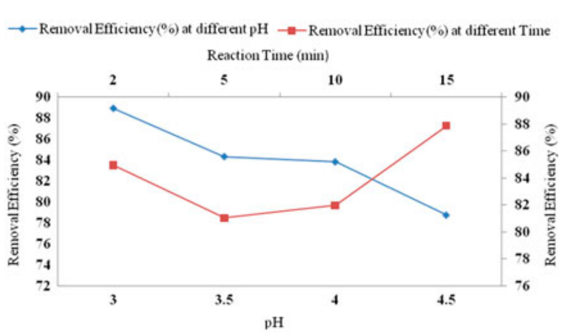



Fig. 4. The effects of pH and reaction time on the removal efficiency of amoxicillin in the aqueous phase using Fenton process.

hydrogen peroxide. On the other hand, hydrogen peroxide decreased the reaction with ferrous ions at higher pH leading to the formation of oxonium ions ($H_3O^{2+}$) [24].

### 4.5. Effect of reaction time

The effect of reaction time on the removal efficiency of amoxicillin in the aqueous phase was studied at four levels 2, 5, 10, and 15 min (Fig. 4). According to Fig. 4, amoxicillin removal efficiency at the reaction times of 2, 5, 10, and 15 min was 84.941, 81.071, 81.993 and 87.855%, respectively. Data regarding the effect of reaction time shows that as the time increased from 5 to 15 min, the rate of reduction increased by 6.784%. However, as the time increased from 2 to 5 min, a reduction rate in amoxicillin degradation was seen (3.87%). Taguchi statistical analysis showed that the maximum removal efficiencies occurred at 2 and 15 min; however, the removal efficiency was slightly higher at 15 min. Based on our study, 15 min reaction time was optimal for the degradation when the concentrations of amoxicillin and hydrogen peroxide were low. The level of significance for the study parameter was 3.392. In this case, increasing the reaction time may promote $OH^{\circ}$ production and subsequently enhance the degradation of amoxicillin. However, antibiotic reduction was higher at 2 and 5 min when the concentrations of amoxicillin and hydrogen peroxide were high due to destruction of hydroxyl radical overtime. The optimization of reaction time is one of the most important parameters in studying the removal processes. Basically, an optimal reaction time is a very important parameter for any chemical reaction. At equilibrium, amoxicillin degradation reached a plateau. If the reaction time exceeds equilibrium, the process will be no longer economical [21]. One study showed that the complete reduction of amoxicillin occurred at 30 min by Fenton oxidation process [6].

### 4.6. COD removal efficiency

COD removal efficiency has been presented in Fig. 5. The maximum removal efficiencies of COD at the initial concentrations of amoxicillin 10, 100, 200, and 500 mg/L were 48, 71.3, 69.45, and 56.2%, respectively. Our study revealed that a higher concentration of amoxicillin required higher doses of hydrogen peroxide and ferrous ions to achieve the effective removal efficiency. The results showed that despite complete degradation of amoxicillin, COD was not completely removed. This might be due to the dissociation of

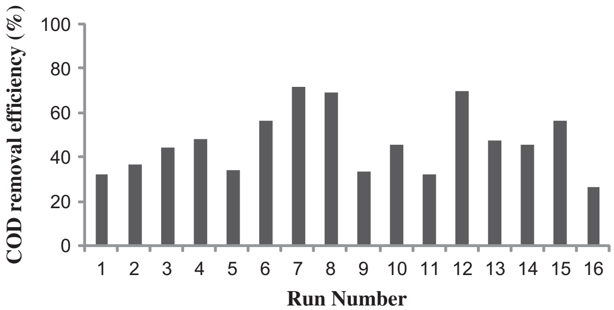



Fig. 5. COD removal efficiency (%) for each set of experiment (Run number) designed by Taguchi method.

OH° at higher concentrations of hydrogen peroxide and ferrous ion and/or the production of persistent byproducts. At low amoxicillin concentrations, an increase in hydrogen peroxide and ferrous ions doses did not remove COD completely. Basically, a low concentration of amoxicillin acts as the limiting parameter for the Fenton process. Analysis showed that maximum COD removal efficiency of 71.3% was achieved at pH 3 with the reaction time of 5 min and the concentration of amoxicillin, hydrogen peroxide, and ferrous ions of 250, 100, and 50 mg/L, respectively.

### 4.7. DOC removal efficiency and the rate of mineralization

In this study, DOC was used to determine the rate of mineralization. At the initial amoxicillin concentrations of 10, 100, 200, and 500 mg/L, DOC was 6, 40, 87.5, and 193.125 mg/L, respectively. DOC removal efficiency for the Fenton process is shown in Fig. 6. According to Fig. 6, the removal efficiency for different sets of experiments were in the range of 2.3 (Run No. 10) to 36.3% (Run No. 3). In this study, the maximum rate of mineralization (36.3%) was achieved at pH 4 and the reaction time of 10 min and the concentration of amoxicillin, hydrogen peroxide, and ferrous ions of 10, 250, and 25 mg/L, respectively. By increasing amoxicillin concentration from 10 to 100 mg/L (at a constant concentration of hydrogen peroxide and ferrous ions 250 and 50 mg/L, respectively) the maximum DOC reduction was achieved (36.3%). However, DOC removal was decreased to 32.87% at pH 3 and reaction time of 5 min with a constant concentration of amoxicillin, hydrogen peroxide, and ferrous ions.

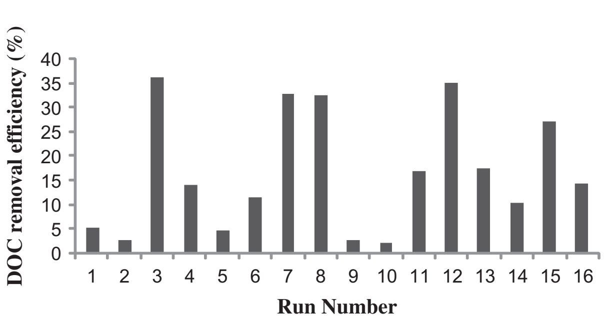



Fig. 6. DOC removal efficiency (%) for each set of experiment (Run number) designed by Taguchi method.

Despite the complete degradation of amoxicillin by Fenton process, DOC removal efficiency was less than 40% which might be due to the formation of persistent byproducts during the process. The higher concentrations of hydrogen peroxide (500 mg/L) and ferrous ions (50 mg/L) will be needed to achieve better mineralization compared to amoxicillin degradation by Fenton process. Inversely, the mineralization rate was decreased by increasing the concentration of the antibiotic. At high concentrations of hydrogen peroxide, DOC removal efficiency increased. Therefore, the concentration of hydrogen peroxide acted as a limiting parameter for the mineralization process. For instance, increasing the concentration of hydrogen peroxide from 10 to 250 mg/L subsequently leads to a significant removal of DOC (at constant concentration of amoxicillin and ferrous ions 10 and 25 mg/L, respectively). In addition, the removal efficiency of DOC at concentrations of hydrogen peroxide more than optimal is decreased due to reduction of OH°. In the current study, increasing Fe(II) increased the production rate of hydroxyl radical and DOC removal efficiency increased as well. However, the addition of hydrogen peroxide as an oxidant was only capable of removing a very low amount of DOC. Our results revealed that amoxicillin mineralization needs higher concentrations of $H_2O_2$ and Fe(II) than that required for its degradation. Despite complete degradation of amoxicillin by the Fenton process, the maximum rate of mineralization was only 36.3% indicating the formation of persistent byproducts. Complete mineralization of amoxicillin by Fenton process can be presented in the following reaction (Eq. (4)) [23]:

$$\begin{aligned} &C_{16}H_{19}N_3O_5S + 47H_2O_2 + Fe^{2+} \\ &\quad \rightarrow 16CO_2 + 54H_2O + 3HNO_3 + H_2SO_4 + Fe^{2+} \end{aligned} \tag{4}$$

Theoretically, 4.38 mg/L of hydrogen peroxide per mg/L of amoxicillin is required for complete mineralization. Based on this study, the mineralization rate was only 32.36% at pH 3.5, the reaction time of 2 min and the concentration of amoxicillin, hydrogen peroxide, and ferrous ions of 10, 500, and 25 mg/L, respectively. Therefore, the reduction in the mineralization rate might be attributed to the formation of persistent byproducts. Rozas et al. showed that the complete removal of ampicillin was accomplished by Fenton and photo-Fenton processes, but the rate of mineralization was higher in photo-Fenton when compared with Fenton process due to the formation of less persistent byproducts [30].

### 4.8. Biodegradability rate ($BOD_5$/COD ratio)

$BOD_5$/COD ratio is an important indicator for the study of biodegradability of industrial wastewaters. A ratio in the range of 0.4–0.8 indicates a good biodegradability of wastewater. The $BOD_5$/COD ratio of amoxicillin in the aqueous phase before the Fenton process was zero, indicating no biodegradability. Data regarding $BOD_5$/COD ratio has been presented in Fig. 7. The maximum, minimum, and average $BOD_5$/COD ratios were 0.738 (Run No. 4), 0.0385 (Run No. 13), and 0.304, respectively. We have shown that as the concentration of amoxicillin in the aqueous phase increased from 10 to 500 mg/L, the biodegradability ratio decreased from 0.738 to 0.088. The biodegradability reduction is probably due to the production of persistent byproducts. Hydrogen peroxide increased at amoxicillin concentrations of 10 to 200 mg/L, further increasing the biodegradability of amoxicillin. However, increasing hydrogen peroxide even at the concentration of 500 mg/L did not improve the biodegradability rate of amoxicillin (at the concentration of 500 mg/L). This might be due to the decomposition of $OH^{\circ}$ and formation of persistent byproducts. The optimal condition for the highest biodegradability of amoxicillin ($BOD_5$/COD ratio = 0.738) was observed at pH 4 and reaction time of 15 min and the concentration of amoxicillin, hydrogen peroxide, and ferrous ions of 10, 500, and 50 mg/L, respectively.

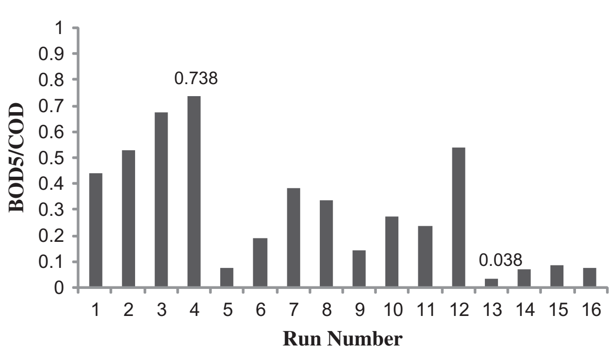



Fig. 7. Biodegradability rate ($BOD_5$/COD ratio) for each set of experiment (Run number) designed by Taguchi method.

Elmolla and Chaudhuri [20] showed that the complete degradation of mixed antibiotics occurred in 2 min by Fenton process. $BOD_5$/COD ratio increased to 0.37 and the rate of degradation of COD and DOC increased to 81.4 and 54.3%, respectively [20]. Although complete degradation of amoxicillin occurred at 2.5 min and the mineralization increased to only 37% at 15 min reaction time by AOPs [23], the removal efficiency of amoxicillin and DOC was 90 and 18%, respectively at 20 min reaction time by ozonation process [31].

Comparing data obtained in the current study with other researches showed that complete removal of amoxicillin in the aqueous phase occurred in just 5 min. Moreover, higher mineralization rate, COD removal efficiency, and $BOD_5$/COD ratio (36.3, 71.3, and 0.738%, respectively) were obtained in our research.

## 5. Conclusion

In conclusion, Fenton process may enhance the rate of amoxicillin degradation in polluted water and could be used as a pretreatment step for the biological removal of the antibiotic in the aqueous phase. The variance analysis suggests that the optimal conditions for amoxicillin reduction rate in the aqueous phase using Fenton method (Fe(II)/$H_2O_2$) are as follows: the initial amoxicillin concentration of 500 mg/L, Fe(II) concentration = 5 mg/L, $H_2O_2$ concentration = 500 mg/L, pH 3, and the reaction time of 15 min; and the level of significance for the study parameters were 60.228, 26.369, 5.638, 4.373, and 3.392, respectively. The results obtained from the study revealed that the biodegradability of amoxicillin ($BOD_5$/COD) increased from 0 to 0.738 following Fenton process and the rate of biodegradation increased as well. Despite complete degradation of amoxicillin, complete removal of DOC was not achieved due to the formation of persistent byproducts. The maximum removal of DOC was 36.3%. The optimum ratio of $H_2O_2$/$Fe^{2+}$/amoxicillin needs to be determined to have higher mineralization. Maximum removal efficiency of COD was 71.3%. Our results showed that Fenton process can be used for the removal of amoxicillin as a pretreatment step which increased the biodegradability rate for further biological treatment. Removal of DOC can be increased by the application of AOPs such as photo-Fenton, electro-Fenton, photoelectron-Fenton, and preozonation. To the best of our knowledge, no report has been submitted till date applying Taguchi experiment design method for the optimization of Fenton process for the removal of amoxicillin. Hence, the present study provides the analytical methodology and data for removing the antibiotic in the aqueous phase.

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