Degradation of Oxytetracycline by Electrochemical, Fenton and Electro-fenton Processes Using SS316 and SS316/β-PbO2 Anodes: Process Optimization Using Rsm-ccd, Bioassay Test and Degradation Pathway

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Degradation of oxytetracycline by electrochemical, Fenton and electro-Fenton processes using SS316 and SS316/β-PbO_2 anodes: Process optimization using RSM-CCD, bioassay test and degradation Pathway

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Highlights:

- ✓ The bioassay was conducted to assess the biological toxicity the inlet and outlet solution.
- ✓ SS316/β-PbO_2 anode was used to improve the biodegradability of diazinon.
- ✓ Oxytetracycline antibiotics degradation by electrochemical oxidation, Fenton and electro-Fenton processes.
- ✓ Process optimization by RSM-CCD
- ✓ degradation mechanism of Oxytetracycline antibiotics.
- ✓ The electrofenton process was more efficient than the oxytetracycline antibiotic compared to the electrochemical processes and Fenton.
- ✓ The removal efficiency of oxytetracycline using SS316/ β-PbO_2 anode electrode was 100%.
- ✓ The bioassay method with microorganisms effectively showed the reduction of the toxicity of the effluent treated by the electrofenton process.

Abstract

The aim of the present study was to evaluate the efficiency of advanced oxidation processes (electrochemical, Fenton and electro-Fenton) in the removal of oxytetracycline using SS316 and SS316/β-PbO_2 anodes. This study was performed experimentally on a laboratory scale in a 250 mL reactor. First, experiments were designed for the electrochemical process using a central composite design, and the optimal conditions for the variables pH(3.53), electric current density(3.85mA/cm^2), initial concentration of oxytetracycline (20mg/L) and electrolysis time (42.35min) was obtained; then, under these conditions, the efficiency of Fenton process with $FeSO_4$ variable without the presence of electrodes was evaluated, and its optimal value was 0.3 g/L, and then in the presence of optimal values of the above 5 variables, the efficiency of electro-Fenton process with H_2O_2 changes were investigated and the optimal value of 0.12 was obtained for H_2O_2. The removal efficiencies of oxytetracycline in electrochemical, Fenton, and electro-Fenton processes were 84.7%, 73.4%, and 98.2%, respectively. Under optimal conditions, the SS316/β-PbO_2 anode electrode enhanced the oxytetracycline efficiency by electron-Fenton process to 100%. The results of bioassay with microorganisms showed that the reduction of toxicity of the effluent treated by electro-Fenton process for Pseudomonas aeruginosa and Staphylococcus aureus was 84.5% and 69%, respectively.

Keywords: AOP_s, Electrochemical, Electro-Fenton, Oxytetracycline, Stainless steel/β-PbO_2, Toxicity.

1. Introduction

Due to the increasing use of medicinal compounds, the study of compounds derived from treated wastewater is very important [1, 2]. Today, there are many concerns about medicinal compounds because the presence of these compounds in the ecosystem can make bacteria resistant to these compounds [3, 4], which occurs by causing genetic mutations in bacteria; thus, paying attention to

the presence of this contaminant in the environment and especially in aquatic environments are important [5, 6]. On the other hand, pharmaceutical compounds are abundant in the wastewater of hospitals, livestock and residential centers and have even been observed in surface water samples and these compounds have very strong chemical and physical properties that make these substances dangerous for non-target groups. However, it is noteworthy that some pharmaceutical compounds can be transferred to the natural environment through the waste of the pharmaceutical industry [7-9]. On the other hand, we know that the growth of the pharmaceutical industry in some countries has increased in recent years and the non-compliance of this industry with environmental standards has caused the effluents from this industry to be discharged into the domestic sewage network, which is an important issue because these compounds are only partially removed by treatment processes and their lack of optimal removal causes contamination of surface water resources [1, 10]. The global estimate for the use of antibiotics is between 100,000 and 200,000 tons per year, which is a significant number [11]. Among the types of antibiotics, oxytetracycline (from the tetracycline category) is of great importance for its widespread use in medicine and veterinary medicine [12]. Because it has the ability to eliminate various infections and is active against a wide range of bacteria and is mainly excreted in the urine, so the removal of this contaminant from sewage seems very necessary [13, 14]. In order to treat water and sanitation, various processes have been used in this field. Advanced oxidation processes have received much attention in recent years [15]. Of course, processes such as removal by UV/H_2O_2 [16], removal by ozone [17], activated carbon [18] and bio-nanocomposite method [19] can be mentioned, but each has advantages and disadvantages; for example, although activated carbon is very economically suitable and has good potential for treatment, however the limitations of the adsorption process related to the separation and regeneration of the adsorbent after treatment are considered [20]. Of course, in the meantime,

attention has been paid to electrochemical processes because compared to conventional processes, they have different applications in water and wastewater treatment processes in small systems, remotely or from the entry point [21, 22]. Moreover, the electrochemical method, electrical energy is used as the driving force [23]. It is noteworthy that in recent years, advanced oxidation processes in combination with other processes have received much attention. For example, electro-Fenton [24] is one of the most effective and environmentally friendly processes of EAOPs [25], which is actually a new and developed process of Fenton [26] in combination with electrochemical, and in this process, the contaminant is eliminated with the reagents of Fenton process and anodic oxidation processes [27, 28].

Nowadays, the use of statistical methods to optimize different processes due to their economics and simplicity is very important that the response surface methodology (RSM) is one of these widely used statistical models [29]. In this study, the central composite scheme (CCD) has been used to optimize the operational variables [30]. The present study considers the efficiency of advanced oxidation processes (electrochemical, Fenton and electro-Fenton) for the removal of oxytetracycline antibiotics from aqueous solutions using stainless steel and stainless steel/β-PbO_2 electrodes due to the widespread use of the antibiotic oxytetracycline and its entry into aqueous solutions. The experiments were designed using the response-surface methodology.

2. Materials and methods

2. 1. Required materials and sample preparation:

The oxytetracycline antibiotic powder was prepared from Sigma, and hydrogen peroxide (H_2O_2), ferrous sulfate ($FeSO_4$), lead nitrate, and nitric acid from Merck CO. Also, to adjust the pH of the solution containing the oxytetracycline antibiotics, H_2SO_4, and NaOH, with a purity of 98%, were

prepared from the Merck Company, Germany. To test for toxicity, standard strains of Pseudomonas aeruginosa and Staphylococcus aureus were obtained from the microbiology laboratory of Ardabil University of Medical Sciences. Stoke solution (1000 mg/L) of oxycetracycline antibiotic was prepared by dissolving 1 g of oxytetracycline antibiotic precursor in 1 liter of double distilled water. For determination of surface morphology of the PbO_2 deposited onto the stainless steel 316, scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDX) (model HITACHI S-4160, Japan) was employed. Analysis of the phase structure of the PbO_2 layer was done using X-ray diffraction (XRD pattern) using an X'Pert Pro diffractometer (Rigaku RINT2200, Japan) with a 2θ step width of 0.1° and a scan rate of 1520 s/0.1° at 40 kV and the electron probe current of 40 mA.

2-2) Design of experiments for electrochemical process:

In this study, the response surface methodology (RSM) with a central composite design was used to determine the optimal conditions and the effect of independent variables on response performance (removal of oxytetracycline antibiotic). The experiments were designed with four factors, i.e., pH (A), electric current intensity (B), initial antibiotic concentration (C), and electrolysis time (D). The number of experiments in this study was obtained using Equation No.1. Therefore, in this study, a total of 30 experiments were performed using 6 replications at the central point. These central points are for evaluating test error and measuring lack of fit. The efficiency of the quadratic polynomial model was expressed by correlation coefficient (R^2) and adjusted correlation coefficient (adjusted R^2). The significance of each sentence was expressed by regression equation, and significant expressions were determined by analysis of variance (ANOVA) for each answer.

$$N = 2K (K – 1) + C \quad (1)$$

In this equation, N is the number of experiments, K is the number of independent variables, and C is the number of central points.

2.3. Experimental procedure:

This experimental study was performed on a laboratory scale. In this research, in order to perform electrochemical, Fenton and Electro-Fenton processes, a cylindrical reactor with a volume of 250 ml was used; it was equipped with two stainless steel electrodes (one as a cathode and the other as an anode) with dimensions (60×20×1 mm) and a distance of 20 mm from each other and connected to the DC power supply. The actual schematic of the reactor used in this study were shown in Figure 1. The volume of each sample was 250 ml. For experiments, a certain amount of NaCl salt (0.2 g/L) was added to the reactor for improvement of ionic properties. In the electrochemical process, independent variables including pH (3-11), electric current intensity (1-4 mA/cm^2), initial concentration of oxytetracycline (20-100 mg/L), and electrolysis time (10-50). Minutes). After completion of the determined reaction, the sample was centrifuged at 30,000 rpm for 4 minutes, and after passing through a 0.45 micron filter, it was prepared to measure the residual concentration of antibiotics. Then, the residual concentration of oxytetracycline was determined by spectrophotometric device (model DR-5000, HACH Company, Germany) at 348 nm, and finally, the removal efficiency of oxytetracycline was obtained by equation No.2 [31]. Changes in ferrous sulfate (0.1, 0.2, 0.3, and 0.4 gr/L) without the presence of electrodes in the Fenton process were investigated using the optimal conditions determined in the electrochemical process (previous step) and the optimal amount of ferrous sulfate was obtained to remove oxytetracycline. Finally, in the presence of the optimal amount of ferrous sulfate (iron ion source) and the optimal amount of independent variables, changes in hydrogen peroxide (H2O2) in the electro-Fenton process were

obtained in a similar reactor and in the presence of stainless steel electrodes to achieve optimal removal efficiency.

$$\text{removal efficiency} \quad (\%) = \frac{C0-Ct}{C0} \times 100 \qquad (2)$$

In the above equation, C_0 and Ct are the initial and final concentrations of antibiotics in solution (mg/L), respectively.

2. 4. Preparation of stainless steel electrode/lead dioxide (SS316 / β-PbO_2)

Figure 2 shows the stainless steel (a) and stainless steel/lead dioxide (b) electrodes. To prepare stainless steel/lead dioxide electrodes, stainless steel electrodes with suitable dimensions were prepared. It was then mechanically polished with fine-grade sandpaper. Also, for degreasing the surface of stainless steel electrodes, it was placed in a mixture of diluted nitric acid and caustic soda for 2 hours in the presence of sound waves at a frequency of 40 kHz. It was thoroughly washed with distilled water. For the of lead oxide on the stainless steel electrode, a 500 ml electrolyte solution containing lead nitrate and nitric acid was used. To prepare the stainless steel/lead dioxide electrode, an electrolysis cell was specially designed in which the stainless steel electrodes were placed and the lead dioxide nanoparticles were deposited simultaneously and uniformly on each electrode. For preparing the electrodeposition solution, a 24 g of lead nitrate and 0.3 M nitric acid were used for on liter of distilled water. Then, for electrochemical coating of lead dioxide per square centimeter, 25 mA current was applied for 6-9 hours [32]. After the electrochemical coating process, the prepared electrodes were thoroughly washed with distilled water and their performance was evaluated under optimal conditions.

5-2) Bioassay using microorganisms

In order to test the toxicity of the bioassay method, two microorganisms, Gram-negative pseudomonas aeruginosa and Gram-positive Staphylococcus aureus, were used. MacConkey agar, nutrient agar and lactose broth were sterilized after preparation according to standard method instructions in an autoclave (temperature of 121°C, pressure of 15 ppm at 15 minutes). Next, Pseudomonas aeruginosa was cultured on McConkey agar medium and Staphylococcus aureus on nutrient agar medium and incubated (37 ° C for 24 hours). After growth of colonies, toxicity test was performed using broth lactose culture medium under optimal conditions of electro-Fenton process for oxytetracycline. For each bacterium (gram-negative and gram-positive) 15 test tubes (5 tubes for each of the inlet, outlet and control solutions) were considered. Each of the inlet and outlet solutions of the process consisted of 10 ml of broth lactose culture medium, 1 ml of the desired solution, and 1 loop of bacteria. The control sample contained lactose broth culture medium and 1 loop of bacteria. Microbial culture was performed in presence of the flame and one colony was taken with a loop for each tube, and after inoculation and dissolution in the wall of the test tube, they were placed in an incubator. Then, the spectrophotometer was set to zero with broth lactose culture medium at a wavelength of 600 nm and after every 2 hours, the absorption rate of three samples (inlet solution, outlet solution, and control) was read by spectrophotometer for 5 consecutive periods of 2 hours. The experiments were repeated in two steps to ensure the accuracy of the work. Finally, the growth inhibition percentage of the inlet and outlet solution for each bacterium in 5 different time intervals was obtained by Equation (3) and the 10-hour mean growth inhibition rate was calculated.

$$GI\ (\%) = [\ (\ 1- \ OD_{600S}/OD_{600B}\ ) \times 100] \qquad (3)$$

In the above equation, GI represents the growth inhibition percentage, and OD600S and OD600B are the optical density of the sample and the control at a wavelength of 600 nm, respectively.

187 2. 6. Investigation of by-products under optimal conditions:

188 LC-MS (Liquid chromatography-mass spectrometry) with Waters Alliance 2695 HPLC-
189 Micromass Quattro micro API Mass Spectrometer was used to determine the intermediates
190 obtained from the degradation of the oxytetracycline antibiotic using the electro-Fenton process
191 under optimal conditions.

192 **3. Results and Discussion**

193 **3.1. Determination of structural characteristics of SS316/β-PbO2 anode**

194 As mentioned above, evaluation of the surface morphology of PbO_2 electrodeposited on stainless
195 steel 316 (SS316) substrate was carried out by SEM technique (Fig. 3 (a, b)). An agglomeration
196 with limited individual particle boundary was observed for all particles. According to provided
197 images, the crystalline structures of β-PbO_2 in the electrodeposited PbO_2 microparticles on SS316
198 was identified and the tetragonal structure for the β-PbO_2 was detected. Moreover, according to
199 EDX results, the weight percentage of main elements existed in β-PbO_2, i.e., oxygen (O) and lead
200 (Pb) were 20.1% and 79.9%, respectively.

201 For estimation of the phases and crystallinity of PbO_2 and the deposited film, X-ray diffraction
202 patterns (XRD) measurements were applied. In Fig. 3c, the XRD of the PbO_2 layer deposited on
203 the SS316 interlayers was brought, in which the diffraction peaks of β form of PbO_2 is observed.
204 the tetragonal structure of β-PbO_2 is approved by all XRD results. we could observe the main
205 peaks at 2θ of 25.4°, 32.0º, 36.2°, 49.1º, and 62.5º for these SS316/ β-PbO_2 electrode; these are
206 related to the (110), (101), (200), (211), (220) plane of β-PbO_2, respectively.

207 **3. 2. Statistical analysis of central composite design**

208 CCD is a statistical technique used to collect mathematical and statistical data and to analyze model
209 in which response performance (antibiotic elimination efficiency) is influenced by independent

factors. Table 1 shows the results of the design matrix of 30 experiments (runs) with the effect of independent variables PH (A), electric current intensity (B), initial concentration of antibiotic (C), and electrolysis time (D) for removal of the oxytetracycline antibiotic. Table 2 shows the analysis of variance (ANOVA) for the removal of oxytetracycline by electrochemical process and the significance of the quadratic model. According to this table, the value of P-Value ($P < 0.0001$) indicates the significance of the model and the interaction of variables on response performance (removal of oxytetracycline). In addition, the value of F-Value equal to 81.58 indicates the significance of the variance of each variable against the error variance. The correlation coefficient of R-squared was 0.9870. In fact, this model explains 98.70% of the changes in response, and for a suitable model, the R^2 must be at least 0.80, and the significance and usefulness of the model id greater for 1 [33, 34]. Lack of Fit (0.28) describes the difference between the predicted values with the observed values, and the repetition of the errors of the real values, and the model is significant when it does not fit well. The coefficient of variation (CV) is a criterion for describing the range of changes in the data and the maximum acceptable value for the satisfaction of the results of the proposed model is 10%, which in this study was 4.10%. Adequate precision expresses the signal to noise ratio and is desirable when its value is more than 4 and its value in our results was 31/10, which indicates a high reliability [35]. Figure 4 shows the separate and interaction effects of each of the study variables. Among the variables, electric current intensity (B) and electrolysis time (D) had the greatest effect on the removal of oxytetracycline, respectively. These findings show that the regression model provides a good explanation for the relationship between the four factors and the response. Finally, after removing the non-significant expressions by the proposed model, Equation 4 was obtained by the quadratic polynomial model for the removal of oxytetracycline.

$$R = 20.72 + 7.25A + 35.25B - 1.36C + 0.47D - 1.07AB + 0.03AC + 0.005AD + 0.036BC + 0.023BD + 0.009CD - 0.56A^2 - 4.30B^2 + 0.005C^2 - 0.009D^2 \quad (4)$$

In Equation (4), R represents the removal efficiency of oxytetracycline, and A represents the pH. B represents the density of electric current, C represents the initial concentration of the antibiotic, and D represents the time of electrolysis.

3. 3. The effect of independent variables

Effect of PH:

In advanced oxidation processes, including electrochemical methods, pH plays an important role in the removal of contaminants. According to Figure 5-a, with increasing pH, the removal efficiency of oxytetracycline decreased, so that at the acidic pH, a higher removal percentage was observed. According to Table 1, in run No.4 and at pH = 3, the removal efficiency is equal to 82.84%, while in the same conditions in run No.25 and at pH = 11, the removal efficiency has decreased to 61.55%. The effect of pH on the efficiency of the electrochemical process is manifested by its effect on the electrical production of metal hydroxyl. According to previous studies, acidic pH values provide better conditions for increasing the efficiency of the electrochemical process. Lowering the pH of the solution provides the conditions for increasing the production of hydroxyl radicals. Since the potential of these radicals to degrade pollutants in acidic environment is higher, so it leads to increased oxidation efficiency [36, 37].

Effect of current density:

One of the important and effective parameters in electrochemical processes is the electric current density because it plays an important role in the production of hydroxyl radicals for the degradation and removal of pollutants from aqueous solutions. In this study, the electric current density in the range of 1-4 mA/cm^2 was used to determine its efficiency on removal efficiency. As can be seen

in Figure 5-a, the removal efficiency has gradually increased with increasing electric current density. In a period of 30 minutes and under constant conditions of other variables, with increasing electric current density from 1 mA/cm2 in run No.12 to 4 mA / cm2 in run No.5, the removal efficiency was augmented from 32.41% to 73.17% (Table 1). The results of Jafarzadeh et al. showed that increasing the electric current density leads to increasing the efficiency of pollutant removal, which were consistent with the present study [38]. In another study by Martinez and Bahena, the electric current density exhibited a direct relationship with increasing the removal efficiency in oxidation processes [39].

Effect of initial concentration of the oxytetracycline antibiotic:

The initial concentration of the antibiotic provides an important driving force for overcoming all solute mass transfer resistances between the aqueous and solid phases. Figure 5-b shows that the rate of degradation of oxytetracycline in the electrochemical process is affected by its initial concentration, and with increasing the initial concentration of the oxytetracycline antibiotic from 20 mg/L to 100 mg/L, its removal efficiency has decreased. By increasing the initial concentration, the amount of contact and exposure of the contaminant to hydroxyl radicals decreases, and consequently, leads to more consumption of hydroxyl radicals and a decrease in removal efficiency [40]. Also, increasing the initial concentration leads to the production of more by-products of oxidation, which results in more consumption of hydroxyl radicals and ultimately reduction of the removal efficiency [41].

Effect of electrolysis time:

The findings of Figure 5-c indicate that with increasing electrolysis time, the efficiency of the electrochemical process for removal of oxytetracycline has increased significantly. By increasing the electrolysis time from 10 min to 50 min (thighs 3 and 6 in Table 1), the removal efficiency has

increased from 45.11% to 69.52%. With increasing electrolysis time, the electrodes have more opportunity to produce ferrous ions and increasing the ferrous ion solubility leads to the production of more hydroxyl radicals and finally, enhancement of the removal efficiency [42]. The study of Dargahi et al. showed that increasing the electrolysis time is directly related to the contaminant removal efficiency in the electrochemical process [43]. Findings from studies of other researchers have also shown that the removal efficiency of contaminants from aqueous solutions increases with increasing electrolysis time in oxidation processes [34, 44].

3.4. Optimization of process and investigation of the quality of the proposed model

To achieve the highest removal efficiency of oxytetracycline antibiotic from various experiments designed with a central composite design, the optimal conditions of each of the variables, e.g., pH, electric current density (mA/cm^2), initial concentration of oxytetracycline (mg/L), and time Electrolysis (min) was obtained for the quadratic polynomial model according to Table 3, and finally, it was estimated that under these optimal conditions, the removal efficiency of oxytetracycline by electrochemical process was equal to 84.7%. Various studies have also pointed out the importance and usefulness of the central composite design for optimizing independent variables and achieving the highest efficiency of pollutant removal [45, 46].

The quality of the proposed model was evaluated using graphs of values obtained from experimental values and predicted by the model and the normal distribution of data. As can be seen in Figure 6-a, the points are uniformly and homogenously aligned and there is no deviation in the data distribution, indicating a high correlation and normal distribution among the residual values. Figure 6 - b and c helps to understand the relationship between actual and predicted values and evaluates the fit of the model well. Since the residual values are randomly scattered in the standard deviation range and none of the points are out of range; thus, it provides a good agreement

between the actual results and the predicted values. As shown in Figure 6-d, the lowest point in the Box-Cox design with lambda equal to 1 (best lambda) indicates that there is no need to transfer the response of the proposed model [47, 48].

5-3) Evaluation of efficiency of Fenton and electro-Fenton processes

After determining the optimal conditions for the electrochemical process using a central composite design, the effect of different concentrations of $FeSO_4$ (0.1, 0.2, 0.3, and 0.4 gr/L) as a source of divalent iron ions on removal efficiency was investigated at different times in the Fenton process under optimal conditions, and the results of which are shown in Figure 7-a. By increasing the amount of FeSo4 from 0.1 g/L to 0.4 g/L, the removal efficiency increased and the highest removal efficiency was obtained in the FeSo4 amount equal to 0.3 g/L, and this amount was selected as the optimal value in the Fenton process, and removal efficiency in this case was 73.4%. Increasing the concentration of FeSo4 leads to the production of more divalent iron ions in solution, which in turn increases the contaminant removal efficiency by producing more hydroxyl radicals [49]. The results of Yang et al. showed that increasing the dose of FeSo4 to some extent increases the production of hydroxyl radicals and increases the removal efficiency, which were consistent with the present study [50].

According to Figure 7-b, changes in different concentrations of hydrogen peroxide (H_2O_2) (0.03, 0.06, 0.09, 0.12, and 0.15 mg/L) in the electro-Fenton process were investigated using the optimal process conditions of the electrochemical and optimal amount of FeSO4 obtained in the Fenton process. The results showed that with increasing the amount of hydrogen peroxide, the removal efficiency in the electro-Fenton process increases consequently. In this study, the optimal amount of H2O2 equal to 0.12 mg/L was obtained, and in this case, the oxytetracycline removal efficiency by electro-Fenton process reached 98.2%. A normal increase in concentration of hydrogen

peroxide leads to produce hydroxyl radicals and increase the power of oxidizing due to reaction with the ferrous ion, and radicals generated during access times more pollutants were causing further degradation of oxytetracycline and increased removal efficiency [51]. Ahmadzadeh and Dolatabadi, in their study, showed that increasing the concentration of hydrogen peroxide increases the efficiency of the electro-Fenton process to remove antibiotics [52].

6-3) Comparison of process efficiency, the effect of SS316/β-PbO_2 anode on removal efficiency

As can be seen in Figure 8-a, the efficiency of each of the electrochemical processes, Fenton and electro-Fenton, has been investigated under optimal conditions at different times. According to the results in the optimal time of 42.35 minutes, the removal efficiencies of Fenton and electrochemical processes were 73.4% and 84.7%, respectively, while the efficiency in the electro-Fenton process reached 98.2%, and since this process could offer greatest effect on oxytetracycline antibiotic removal, it was selected as the optimal process.

Coating of the anode electrode using lead dioxide (β-PbO_2) is one of the effective and practical methods to remove contaminants using oxidation processes that can affect the removal efficiency [43]. In this study, the stainless steel anode was coated with lead dioxide and then under the optimal conditions of the electro-Fenton process, the effect of the SS316/β-PbO_2 anode electrode in comparison with the SS316 anode was investigated, and the relevant results in Figure 8-b showed that the SS316/β-PbO_2 anode was only more effective in removing oxytetracycline than SS316, so that at an optimal time of 42.35 min, the removal efficiency for SS316/β-PbO_2 was 100%.

7-3) Calculation of electrical energy consumption

The amount of energy consumed under optimal conditions for the outlet solution treated by the electro-Fenton process was calculated using Equation (5) [53, 54]. The results showed that the energy

consumption for the three voltages (6.5, 11.5 and 15.5 volts) were equal to 0.91, 3.86, and 8.24 kwh/m3, respectively. Based on the results, increasing the amount of voltage was led to an increase in the removal efficiency of oxytetracycline, and the amount of electrical energy consumed was enhanced (Eq. 5).

$$E_{EC} = \frac{UItEC}{Vs} \times 10^{-3} \quad (5)$$

In the above equation, E_{EC} represents the electrical energy consumed (kwh/m^3), U is the reactor voltage (volts), I is the electric current intensity (amperes), t_{EC} is the electrolysis time (hour), and Vs is the reactor volume (m^3).

8-3) Results of toxicity test

Figure 9 shows the results of the toxicity test of the inlet solution and the effluent treated by the electro-Fenton process. The results showed that after 10 hours, the growth rate of both bacteria in the control sample and the effluent increased and consequently the toxicity decreased, while the growth of bacteria in the inlet solution was less and its toxicity was high. Results 10-hour mean growth inhibition under optimal electro-Fenton process conditions (pH = 3.53, current density = 3.85 mA/cm^2, concentration of oxytetracycline = 20 mg/L, electrolysis time = 42.35 min, $FeSO_4$ = 0.3 gr/L, and H_2O_2 = 0.12 mg/L) showed that the toxicity for Pseudomonas aeruginosa decreased from 75.6% in the reactor inlet solution to 11.7% in the outlet solution (84.5% reduction in toxicity) and for Staphylococcus aureus, was declined from 41.4% in the inlet solution to 12.8% in the outlet solution (69% reduction in toxicity). Nadafi et al. used standard strains of Escherichia coli and Staphylococcus aureus to evaluate for toxicity using a bioassay; their results showed that the reduction of Escherichia coli and Staphylococcus toxicity in the outlet solution was 86.7% and 72.3%, respectively [55]. Dirany et al., by conduction a study on the sulfomethoxazole antibiotic

toxicity by bioassay with microorganisms, noted that the toxicity of the effluent treated by the electro-Fenton process was reduced by about 75% compared to the inlet solution [56]. In the present study, the electro-Fenton process effectively reduced the toxicity of aqueous solution containing oxytetracycline after treatment, and the bioassay method using microorganisms could be used as an efficient and cost-effective technique to evaluate the toxicity of aqueous solutions.

3) Intermediates from LC-MS analysis

Intermediates obtained from the degradation of the oxytetracycline antibiotic were identified by LC-MS (Figure 10). According to the spectral data obtained from the method used, the mechanism of the oxytetracycline compound removal in the presence of hydroxide ions was presented in Table 4 and as schematic 1. Initially, the oxytetracycline compound undergoes a demethylation reaction in the presence of active hydroxide ions, and the (4S, 4aR, 5S, 5aR, 6S, 12aR) -4-amino-1,5,6,10,11,12a-hexahydroxy-6 -methyl-3,12-dioxo-3,4,4a, 5,5a, 6,12,12a-octahydrotetracene-2-carboxamide compound with a molecular mass of 432 is produced. The second demethylation process is performed and the compound with IUPAC name of (4S, 4aR, 5S, 5aR, 6S, 12aR) -4-amino-1,5,6,10,11,12a-hexahydroxy-3,12- produces dioxo-3,4,4a, 5,5a, 6,11,11a, 12,12a-decahydrotetracene-2-carboxamide and mass of 420. This compound forms compounds with masses of 404 and 361, in two steps and by losing the functional group of hydroxide and $CONH_2$, respectively. The compound with mass of 361 can produce a compound with mass of 268 by losing all the functional groups of hydroxide and amine. From this compound, it can produce two compounds with masses of 240 and 200, which in turn, compounds called 1- (1,2,3,4-tetrahydronaphthalen-2-yl) ethan-1-one with mass 174 and 1,2,3,4-tetrahydronaphthalene-2-carbaldehyde with mass 160 and finally 1,2,3,4-tetrahydronaphthalene with mass 132 are produced. At the end of the process, compounds with lower molecular masses such as toluene,

benzene, carbonic acid and acetic acid are produced, the degradation of which leads to water and carbon dioxide.

10-3) Study of process kinetics

Kinetic models were used to evaluate the speed of chemical reactions, in which the determination of the reaction rate of liquids and gases is expressed in terms of the concentration of the substance (pollutant) per unit time. The basis of the reaction rate is the decrease of the reactant concentration per unit time or the increase of the product concentration per unit time [57]. In this study, a pseudo-first-order kinetic model under optimal electro-Fenton process conditions was used to estimate the process kinetics. Equation (6) was used to calculate the pseudo-first-order kinetic model for the removal of the oxytetracycline antibiotic.

$$\text{Ln}\left(\frac{[OxyTET]0}{[OxyTET]e}\right) = \text{kt} \qquad (6)$$

In the above equation, $[\text{OxyTET}]_0$ and $[\text{OxyTET}]_e$ represent the initial and final concentrations of the antibiotics oxytetracycline and k, respectively. The constant k was calculated by plotting the Ln $([\text{OxyTET}]_0/[\text{OxyTET}]_t)$ versus the electrolysis time. Equation (7) was also used to determine the half-life of the pollutant.

$$t_{1/2} = \frac{0/693}{\text{k}} \qquad (7)$$

The results of the evaluation of the oxytetracycline degradation under optimal conditions with a pseudo-first-order kinetic model were shown in Table 5 and Figure 11. As can be seen, the value of regression coefficient (R^2) for the pseudo-first order kinetic model was determined to be 0.9965, which indicates the suitability of the kinetic model to express the data for the degradation of oxytetracycline [58, 59]. According to Table 5, the kinetic parameters represent a good linear

relationship, and also the degradation of oxytetracycline by the electro-Fenton process follows pseudo-first-order kinetics; accordingly, the studied system has a very good potential for the degradation of oxytetracycline. In the present study, the velocity constant (k) of the pseudo-first-order model for degradation of oxytetracycline by electro-Fenton process was 0.073 min^{-1}, and the half-life ($t_{1/2}$) of oxytetracycline removal was 9.5 min.

4) Conclusion

The findings of this study showed that the efficiency of electrochemical processes, Fenton and electro-Fenton for the removal of the antibiotic oxytetracycline under optimal test conditions were 84.7%, 73.4%, and 98.2%, respectively; thus, the electro-Fenton process as an optimal process was selected for oxytetracycline removal. Optimal conditions for pH, electric current density, initial oxytetracycline concentration, electrolysis time, $FeSO_4$ and H_2O_2 concentrations are 3.53, 3.85 mA/cm^2, 20 mg/L, 42.35 min, 3 g/L, and 0.12 mg/L, respectively. Under the optimal conditions of the electro-Fenton process, the oxytetracycline antibiotic removal efficiency by the SS316/β-PbO_2 anode electrode reached 100%, and this anode had a significant effect on the removal efficiency compared to SS316 alone. The bioassay method using microorganisms effectively reduced the toxicity of effluent containing oxytetracycline treated by the electro-Fenton process; thus this method can be used as an effective tool to investigate the toxicity of pollutants from aqueous solutions. Intermediates obtained from the degradation of the antibiotic oxytetracycline were identified and determined by LC-MS. Results from the kinetic studies of process showed that the removal of oxytetracycline using the electro-Fenton process with R^2 = 0.9965 follows quasi-first-order kinetics.

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Authorship contribution statement

Abdollah Dargahi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Supervision, Funding acquisition. Mehdi Vosoughi: Methodology, Validation, Resources, Writing - original draft, Writing - review & editing. Somayeh Biparva Haghighi: Analyses, writing and text revision. Kamal Hasani: Methodology, Validation, Formal analysis,

Investigation, Resources, Writing - original draft. Sama Hosseini and Heliya Gholizadeh: Validation, Formal analysis, Writing - original draft, Writing - review & editing, Project.

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Table 1. Matrix results of experimental design for removal of Oxytetracycline antibiotic via electrochemical process

Run	A	B	C	D	Removal (%)	
	pH	current density (mA/cm^2)	Concentration (mg/L)	electrolysis time (min)	Actual Value	Predicted Value
1	7	2.5	20	10	74.7	73.98
2	7	2.5	60	30	62.7	66.38
3	3	2.5	60	50	69.52	69.73
4	3	2.5	20	30	82.84	82.69
5	3	4	60	30	73.17	72.77
6	3	2.5	60	10	45.11	47.72
7	7	4	60	10	55.78	54.74
8	11	2.5	60	10	35.78	37.17
9	3	2.5	100	30	60.23	57.89
10	11	2.5	60	50	61.76	60.75
11	7	2.5	60	30	63.24	66.38
12	3	1	60	30	32.41	32.48
13	11	1	60	30	35.7	35.61
14	11	4	60	30	50.68	50.11
15	7	2.5	60	30	67.9	66.38
16	7	2.5	60	30	70.63	66.38
17	7	2.5	100	50	81.9	82.12
18	7	1	60	10	31.2	28.72
19	7	2.5	60	30	69.66	66.38
20	7	1	60	50	50.2	50.14
21	7	2.5	60	30	64.15	66.38
22	7	1	20	30	59.86	60.53
23	7	4	100	30	72.34	73.27
24	7	4	60	50	77.54	78.92
25	11	2.5	20	30	61.55	62.78
26	7	2.5	20	50	83	82.26
27	11	2.5	100	30	59.22	58.26
28	7	2.5	100	10	44.55	44.80
29	7	4	20	30	83.8	83.51
30	7	1	100	30	39.55	41.45

Table 2. ANOVA results pertaining to Oxytetracycline elimination

Source	Sum of Squares	df	Mean Square	F-value	p-value	Status
Model	7086.49	14	506.18	81.58	< 0.0001	significant
A-pH	286.07	1	286.07	46.11	< 0.0001	significant
B-current density	2252.01	1	2252.01	362.97	< 0.0001	significant
C-Concentration	644.75	1	644.75	103.92	< 0.0001	significant
D-electrolysis time	1559.52	1	1559.52	251.36	< 0.0001	significant
AB	166.15	1	166.15	26.78	0.0001	significant
AC	102.82	1	102.82	16.57	0.0010	significant
AD	0.6162	1	0.6162	0.0993	0.7570	not significant
BC	19.58	1	19.58	3.16	0.0959	not significant
BD	1.90	1	1.90	0.3069	0.5877	not significant
CD	210.98	1	210.98	34.00	< 0.0001	significant
A^2	550.50	1	550.50	88.73	< 0.0001	significant
B^2	642.20	1	642.20	103.51	< 0.0001	significant
C^2	437.35	1	437.35	70.49	< 0.0001	significant
D^2	87.70	1	87.70	14.14	0.0019	significant
Residual	93.07	15	6.20	-	-	-
Lack of Fit	33.56	10	3.36	0.2820	0.9581	not significant
Pure Error	59.51	5	11.90	-	-	-
Cor Total	7179.56	29	-	-	-	-

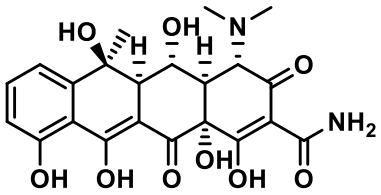
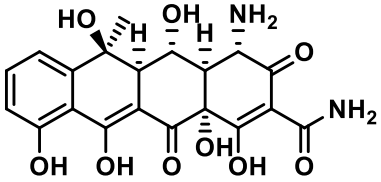
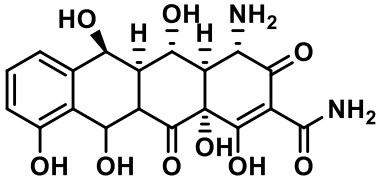
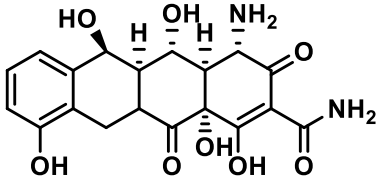
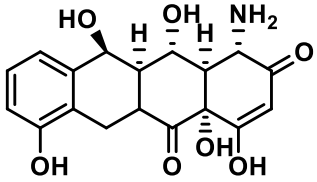
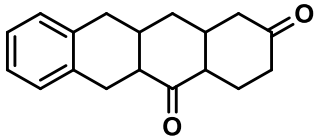
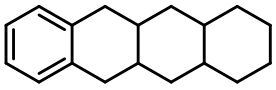
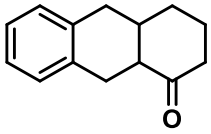
R^2 = 0.9870 **Std. Dev = 2.49**
Adjusted R^2 = 0.9749 **Mean = 60.69**
Predicted R^2 = 0.9611 **C.V (%) = 4.10**
Adeq Precision = 31.10

Table 3. Optimal condition of independent variables for Oxytetracycline removal via electrochemical process.

Factor	Low	High	Optimum
pH	3.0	11.0	3.53
current density (mA/cm^2)	1.0	4.0	3.85
Concentration (mg/L)	20.0	100.0	20
electrolysis time (min)	10.0	50.0	42.35
Optimum Value %		84.7 %	

673

674 Table 4. Identified products by LC-MS in the degradation of Oxytetracycline antibiotic by the
675 Electro-Fenton process.

Structure	IUPC name	Molecular Weight
	(4S,4aR,5S,5aR,6S,12aR)-4-(dimethylamino)-1,5,6,10,11,12a-hexahydroxy-6-methyl-3,12-dioxo-3,4,4a,5,5a,6,12,12a-octahydrotetracene-2-carboxamide	460
	(4S,4aR,5S,5aR,6S,12aR)-4-amino-1,5,6,10,11,12a-hexahydroxy-6-methyl-3,12-dioxo-3,4,4a,5,5a,6,12,12a-octahydrotetracene-2-carboxamide	432
	(4S,4aR,5S,5aR,6S,12aR)-4-amino-1,5,6,10,11,12a-hexahydroxy-3,12-dioxo-3,4,4a,5,5a,6,11,11a,12,12a-decahydrotetracene-2-carboxamide	420
	(4S,4aR,5S,5aR,6S,12aR)-4-amino-1,5,6,10,12a-pentahydroxy-3,12-dioxo-3,4,4a,5,5a,6,11,11a,12,12a-decahydrotetracene-2-carboxamide	404
	(1S,4aR,11S,11aR,12S,12aR)-1-amino-4,4a,7,11,12-pentahydroxy-5a,6,11,11a,12,12a-hexahydrotetracene-2,5(1H,4aH)-dione	361
	4,4a,5a,6,11,11a,12,12a-octahydrotetracene-2,5(1H,3H)-dione	268
	1,2,3,4,4a,5,5a,6,11,11a,12,12a-dodecahydrotetracene	240
	3,4,4a,9,9a,10-hexahydroanthracen-1(2H)-one	200

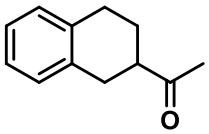
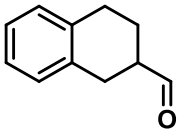
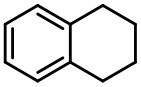
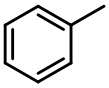
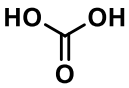
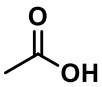
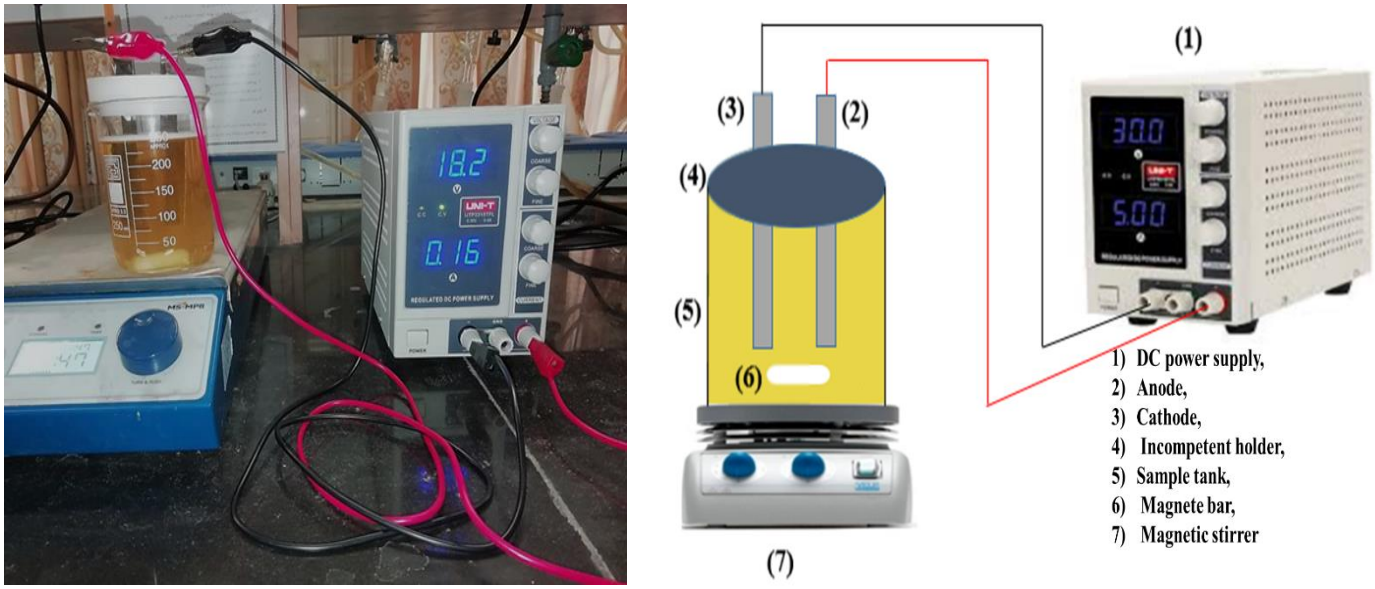
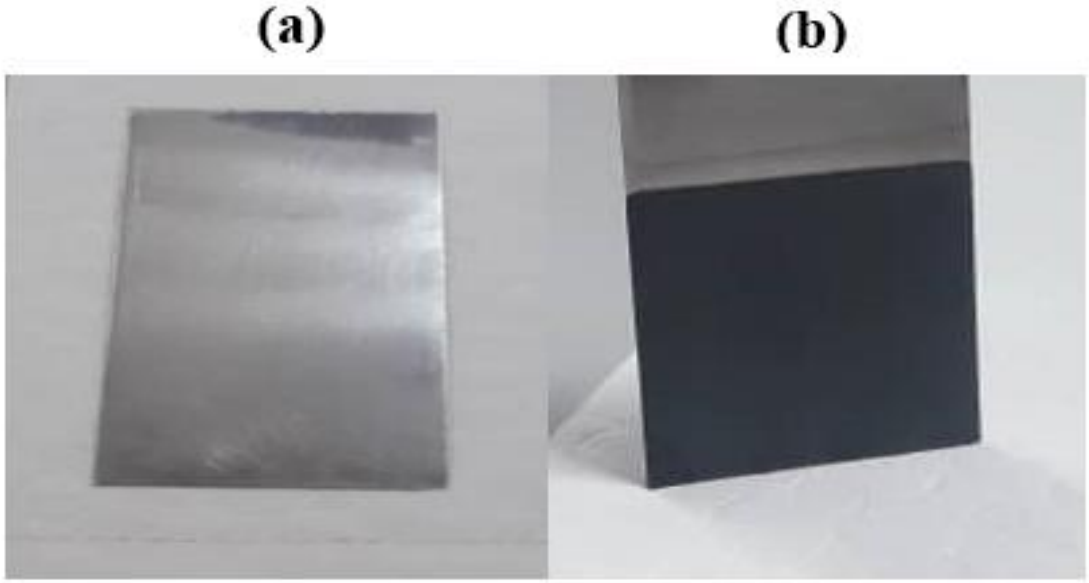
	1-(1,2,3,4-tetrahydronaphthalen-2-yl)ethan-1-one	174
	1,2,3,4-tetrahydronaphthalene-2-carbaldehyde	160
	1,2,3,4-tetrahydronaphthalene	132
	toluene	92
	benzene	78
	carbonic acid	62
	acetic acid	60

Table 5. The kinetics for the removal of Oxytetracycline by Electro-Fenton process in optimum condition (pH = 3.53, current density = 3.85 mA/cm^2, concentration of Oxytetracycline = 20 mg/L, electrolysis time = 42.35 min, $FeSO_4$ = 0.3 gr/L, H_2O_2 = 0.12 mg/L)

kinetics	**k (min^{-1})**	**R^2**	**$t_{1/2}$ (min)**
pseudo-first-order model	0.073	0.9965	9.5



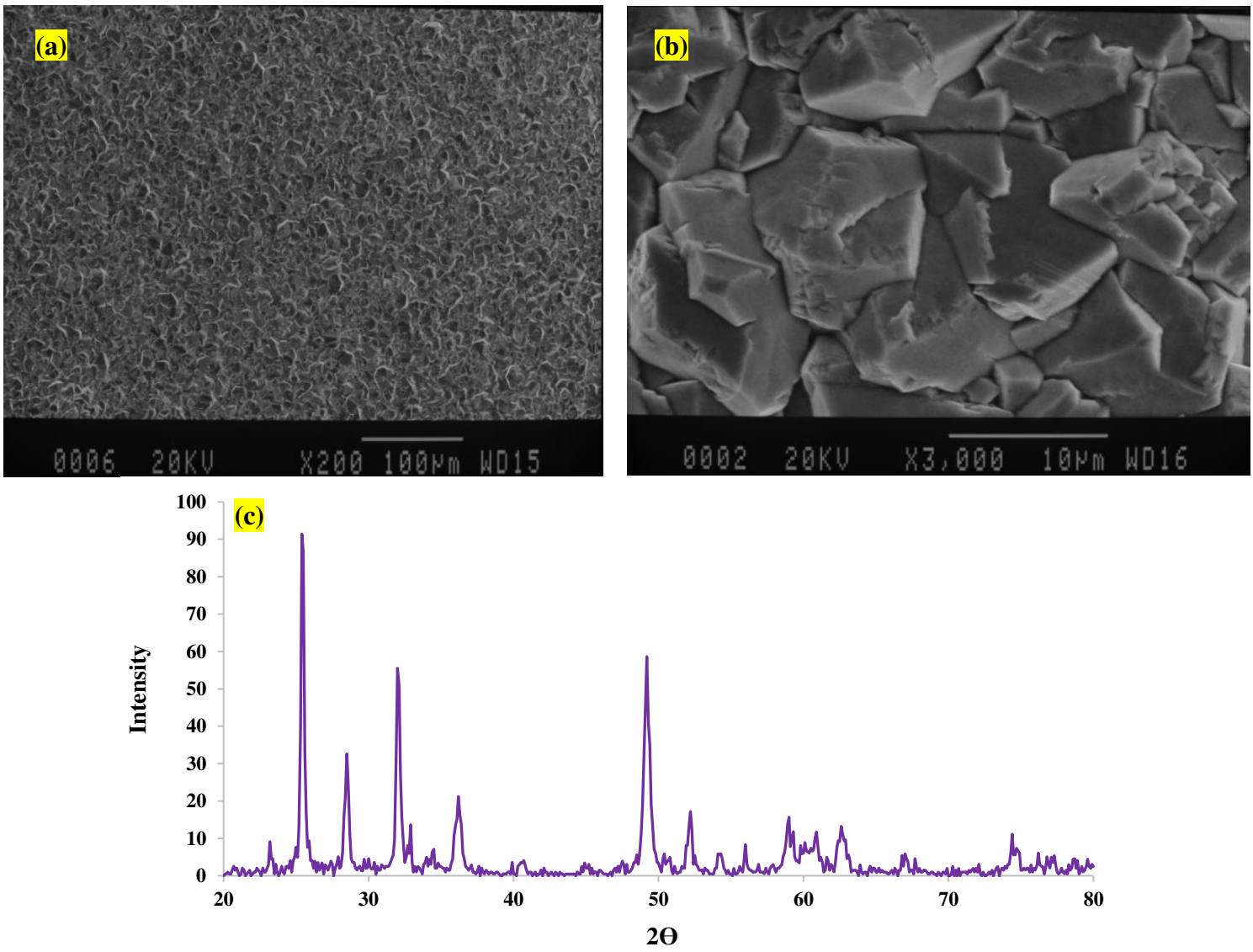
690 **Fig 1:** Schematic of the reactor used for removal of oxytetracycline by oxidation processes



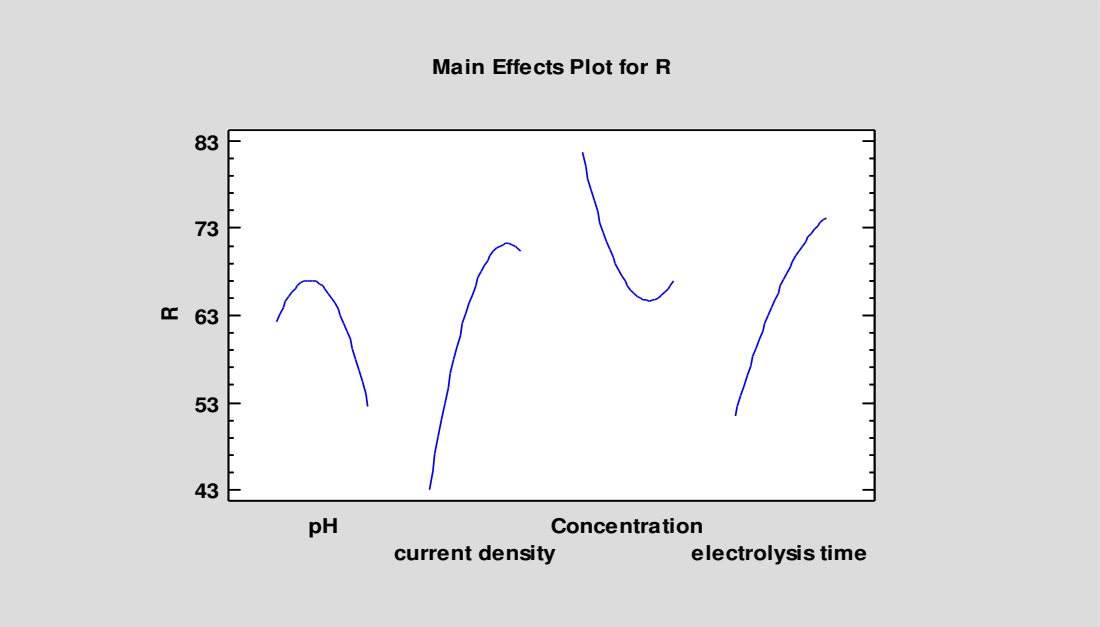
691

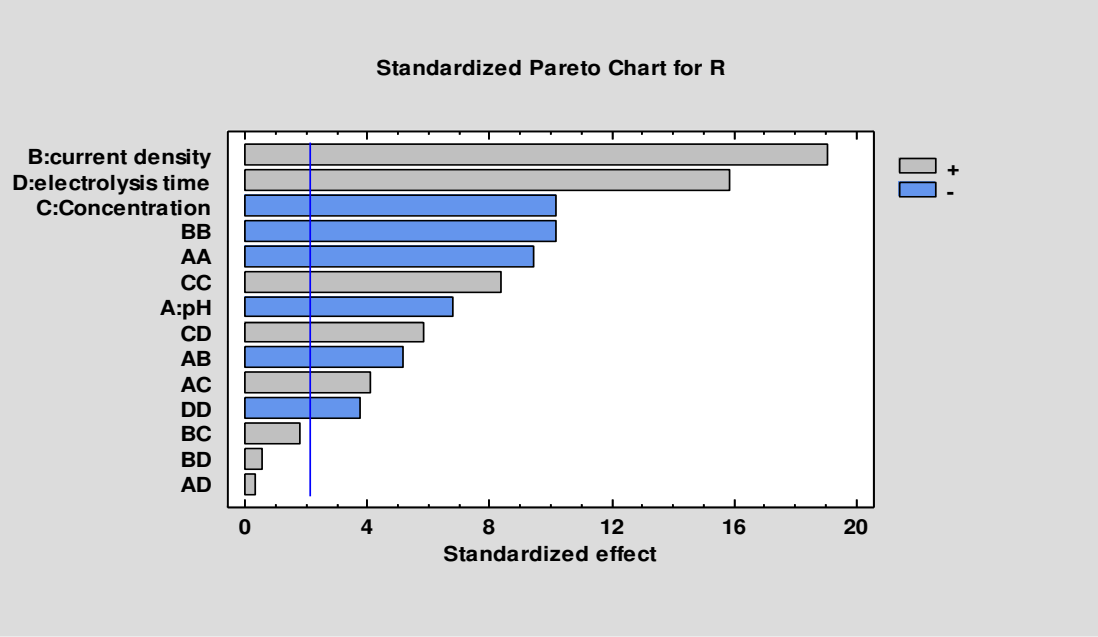
692 **Fig 2.** (a) Image of stainless steel electrode and (b) Stainless steel electrode coated with lead

693 dioxide (SS316/PbO_2) for removal of the oxytetracycline antibiotic under optimal test conditions



694 **Fig. 3.** (a, b) SEM micrographs of the surface of SS316/β-PbO_2 at different magnifications; (c)
695 XRD analysis of SS316/β-PbO_2 anode.





696

697 **Fig.4.** Separate effects of variables and the pareto plot for determining the effect of each term on
698 the response (removal of Oxytetracycline by electrochemical process).

699

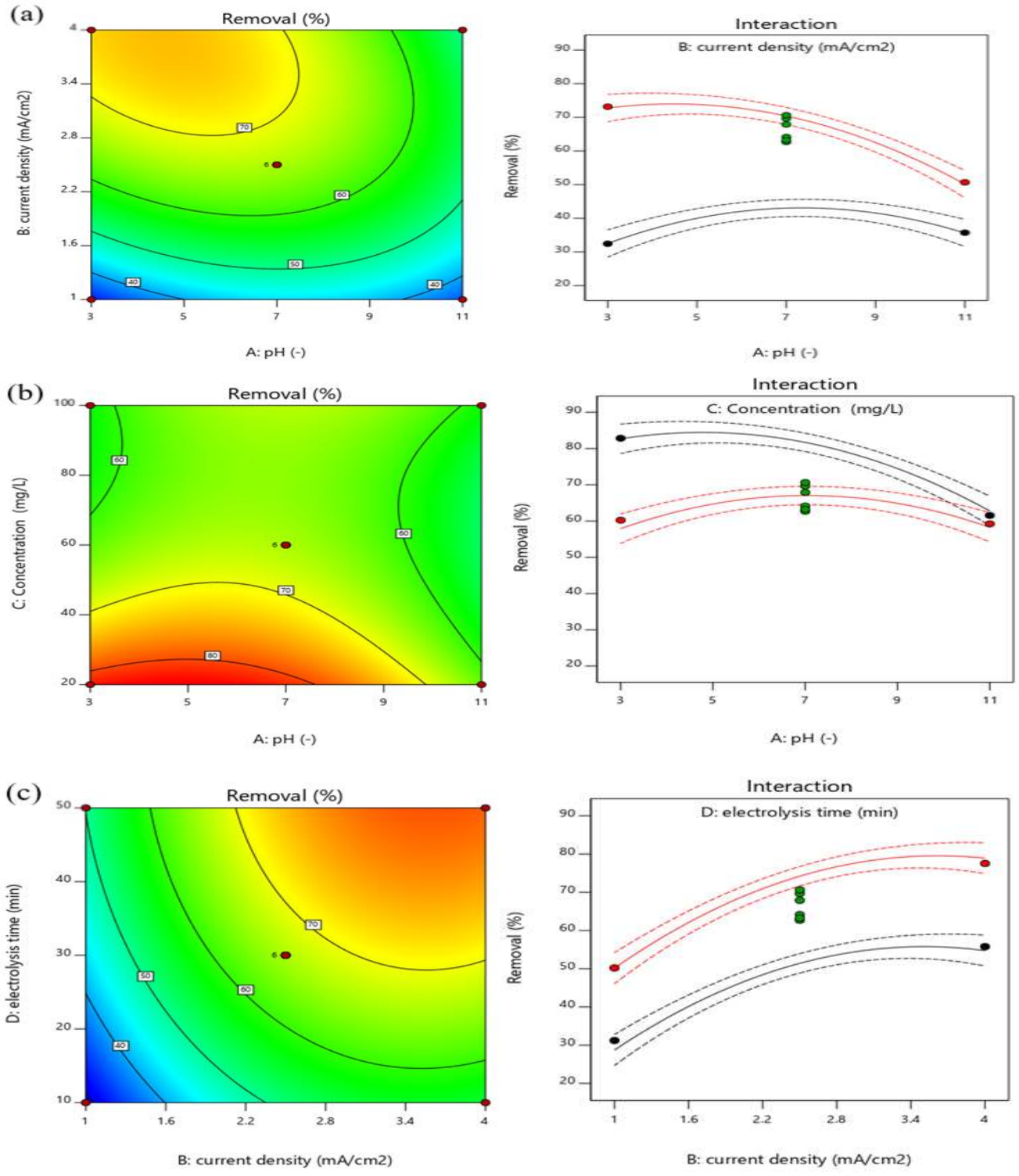


Fig. 5. (a) Contour plots and interaction effects of pH and current density (mA/cm^2), (b) Contour plots and interaction effects of pH and initial concentration of Oxytetracycline (mg/L), (c) Contour plots and interaction effects of current density (mA/cm^2) and electrolysis time (min).

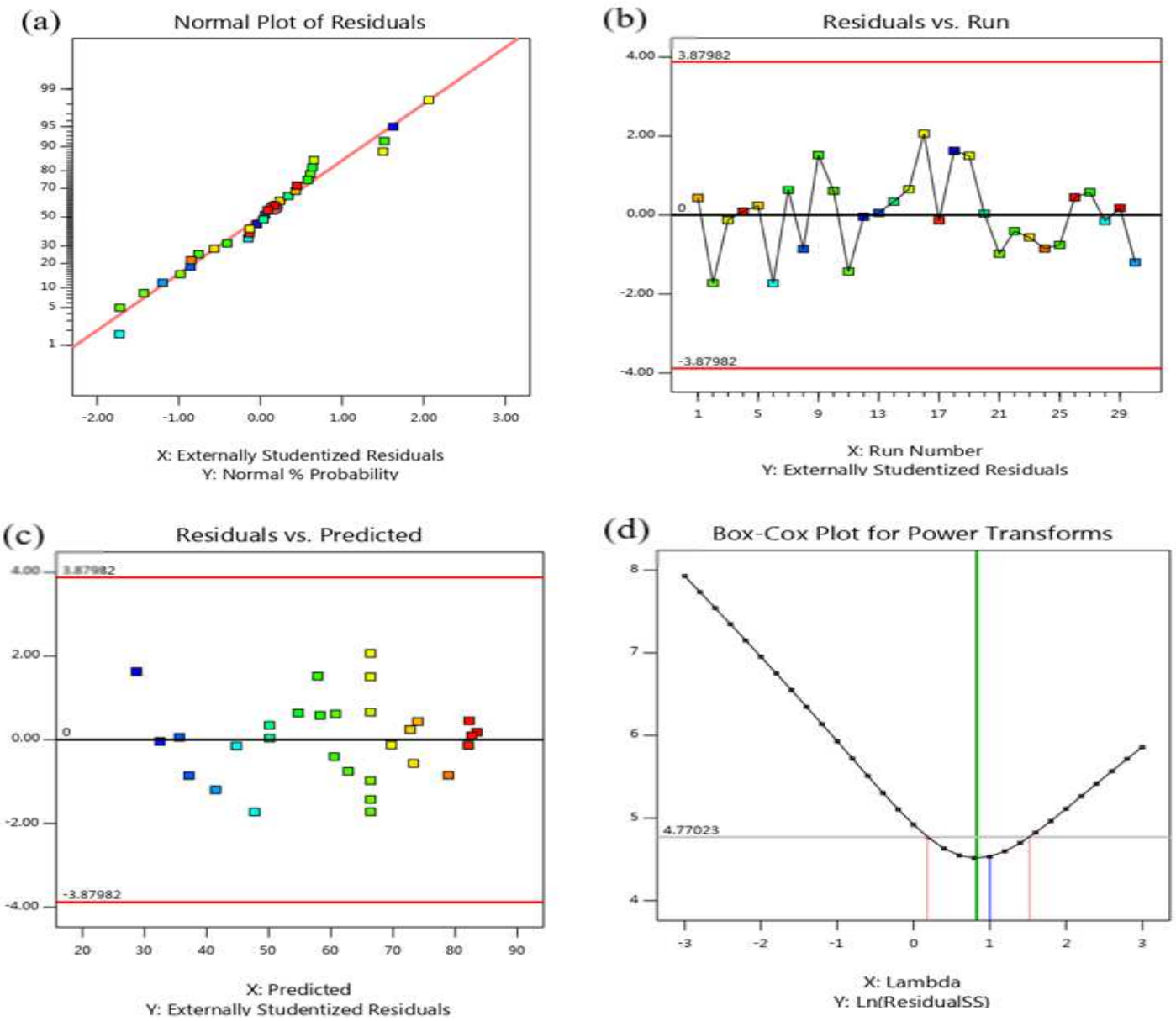
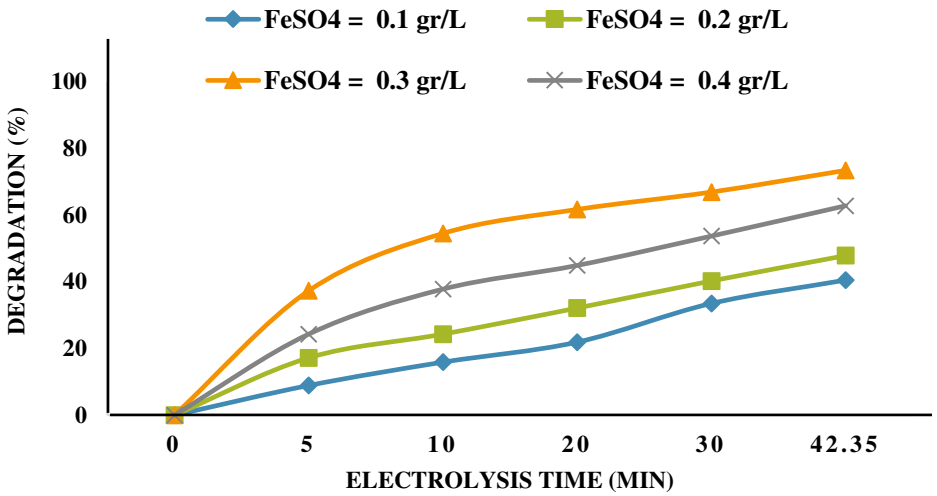


Fig. 6. The diagnostic plots for validation of obtained model: (a) Normal probability distribution of residuals, (b) Externally studentized residuals versus run order, (c) Residuals versus predicted and (d) Box-Cox Plot.

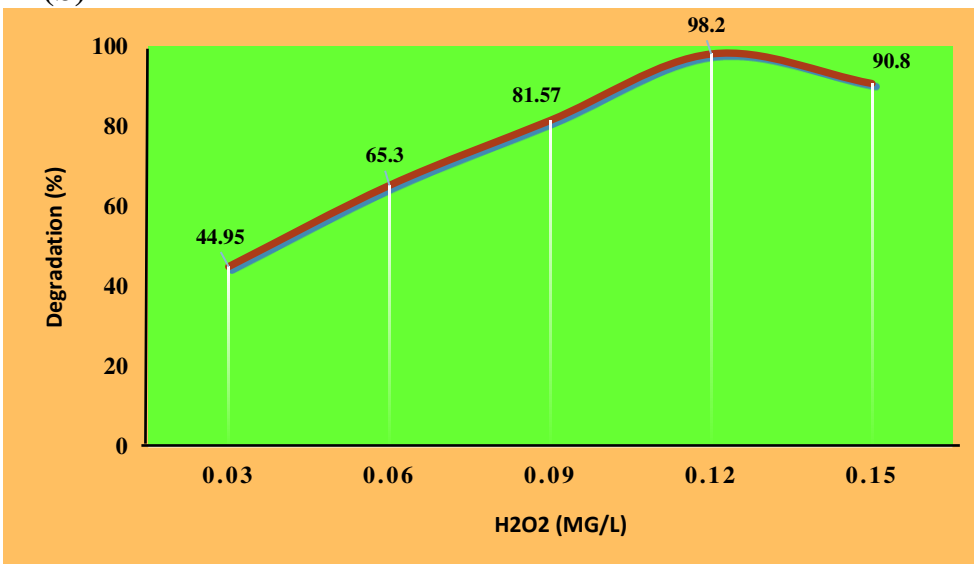
(a)



The changes of FeSo4 amounts in optimal condition via Fenton process

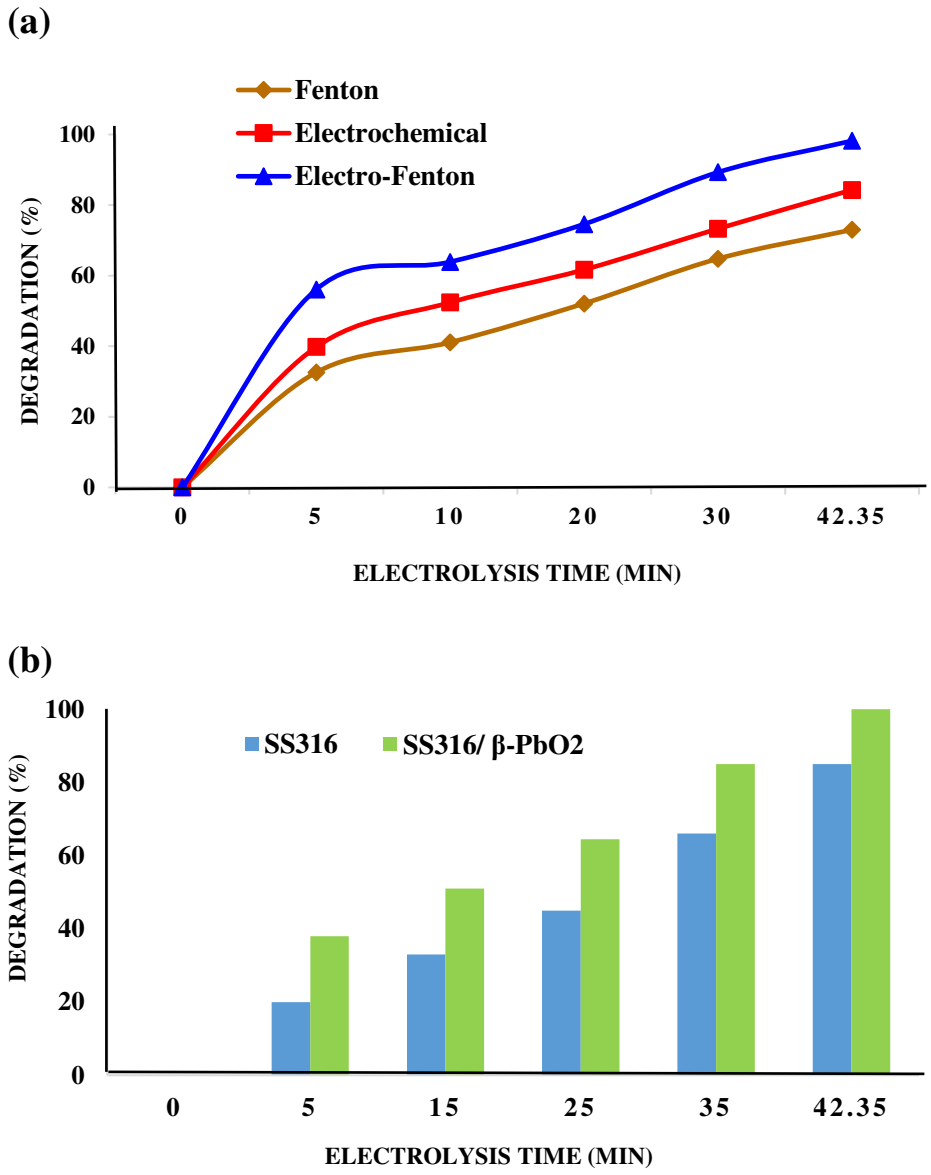
(pH = 3.53, concentration = 20 mg/L)

(b)

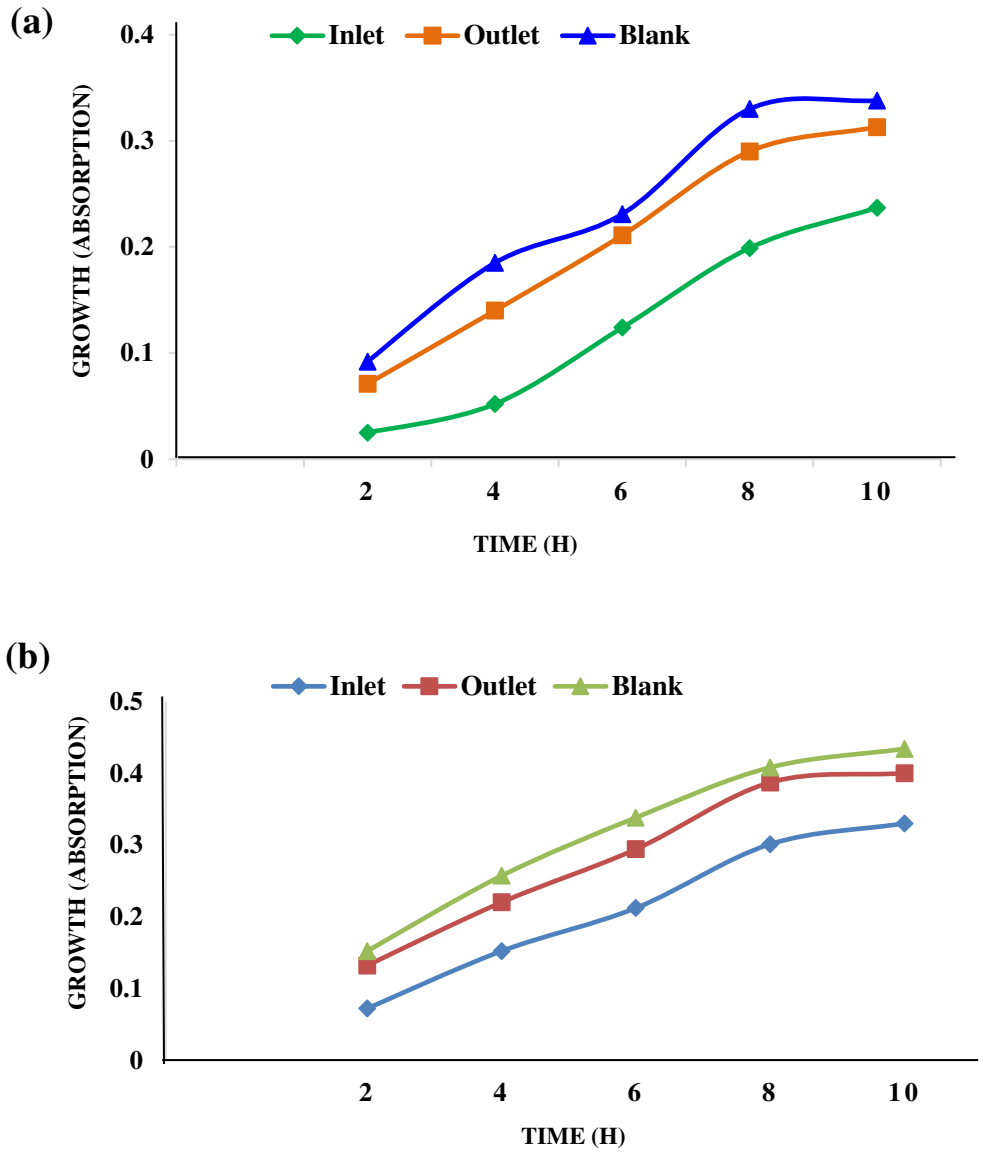


709

710 Fig. 7. (a) Influence of FeSo4 amounts via Fenton process and (b) H_2O_2 values in elimination
711 of Oxytetracycline in electro-Fenton process under optimum condition (pH= 3.53, current
712 density = 3.85 mA/cm^2, concentration = 20 mg/L, electrolysis time = 42.35 min and FeSO4 = 0.3
713 gr/L). (a: Optimize amount of FeSO4 = 0.3 gr/L, b: Optimize value of H2O2 = 0.12 mg/L)



714 Fig. 8. (a) The effect of AOPs processes on the degradation of Oxytetracycline antibiotic, (b)
715 The elimination of Oxytetracycline antibiotic by the SS316 and SS316/β-PbO_2 anodes (pH =
716 3.53, current density = 3.85 mA/cm^2, concentration of Oxytet = 20 mg/L, electrolysis time = 42.35 min,
717 $FeSO_4$ = 0.3 gr/L, H_2O_2 = 0.12 mg/L).



718 Fig. 9. (a) Growth trend of Pseudomonas aeruginosa (gram-negative) and (b) Staphylococcus
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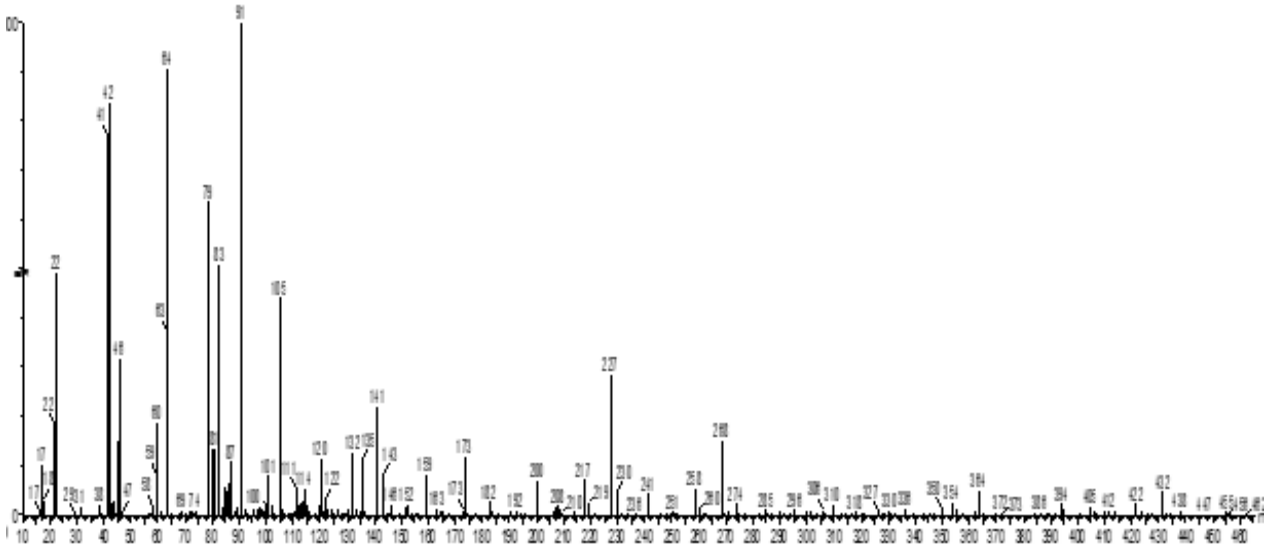
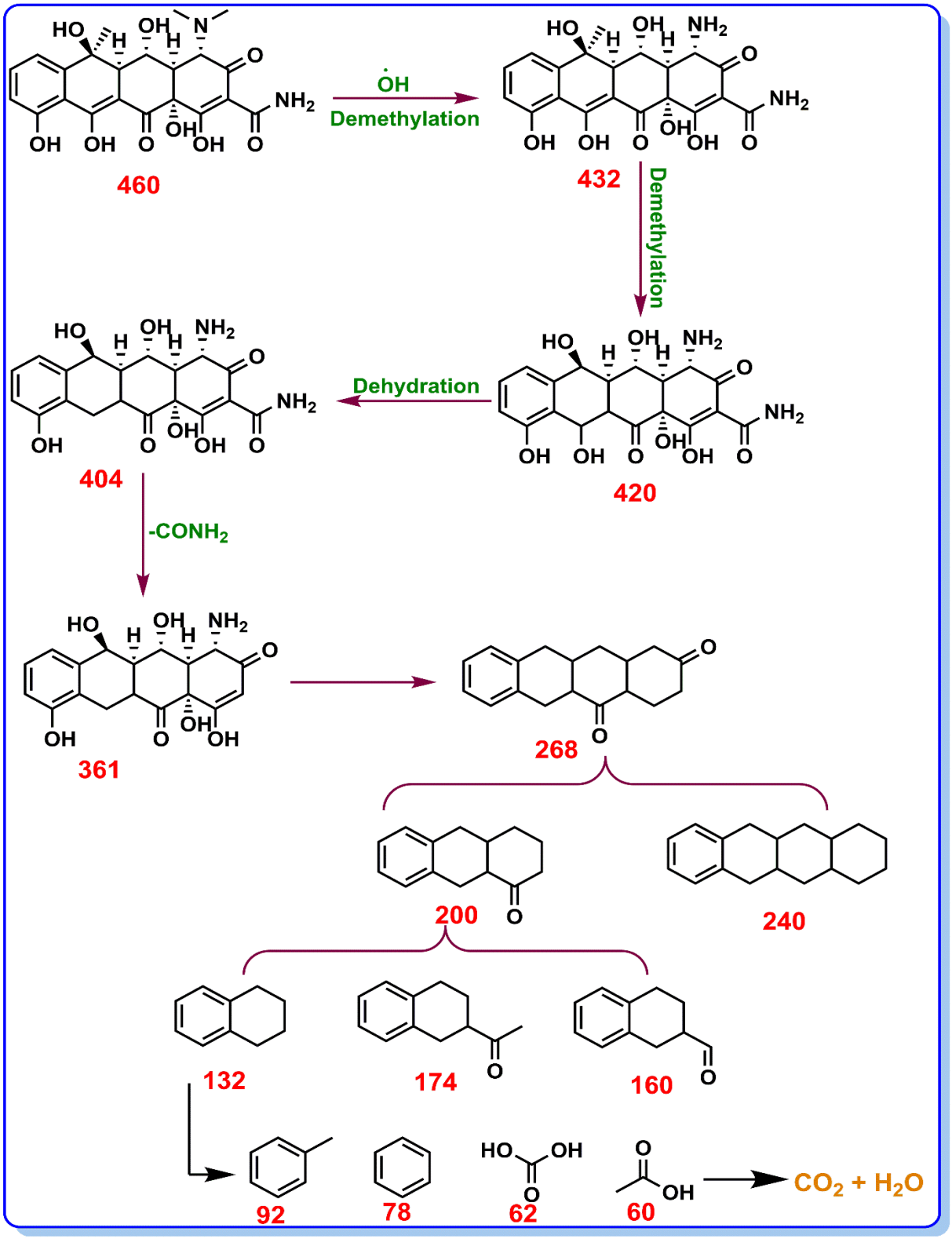
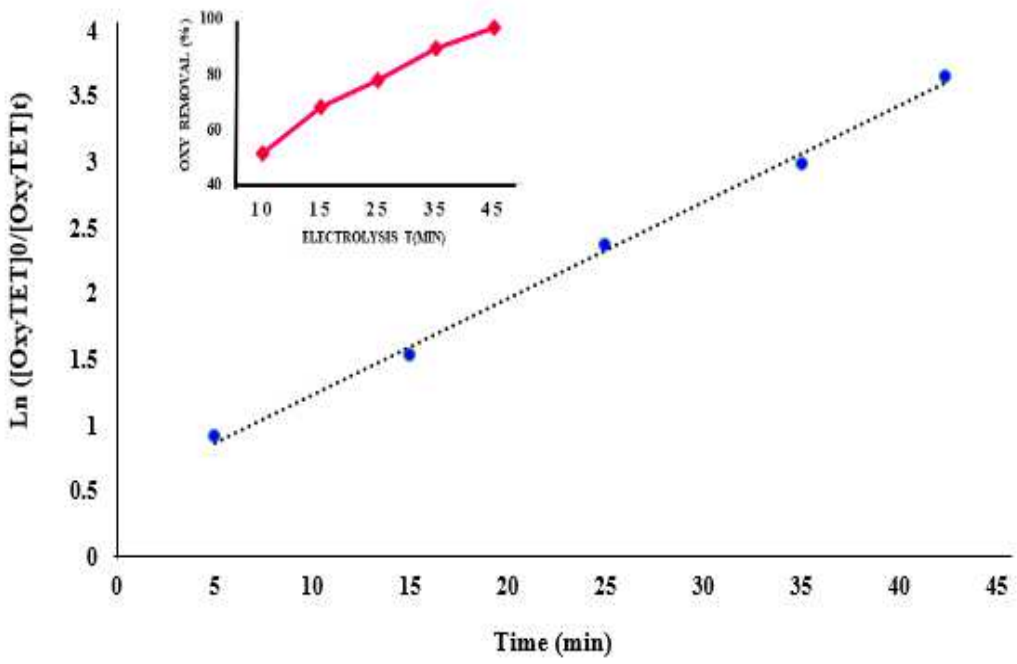


Fig.10. LC/MS chromatographs after degradation of Oxytetracycline antibiotic via Electro-Fenton process under optimum conditions.



Scheme 1. Proposed pathway for degradation of Oxytetracycline antibiotic by the Electro-Fenton process.



726

727 **Fig. 11.** Kinetics of Oxytetracycline removal at the optimum conditions (pH = 3.53, current
728 density = 3.85 mA/cm^2, concentration of Oxytetracycline = 20 mg/L, electrolysis time = 42.35 min,
729 $FeSO_4$ = 0.3 gr/L, H_2O_2 = 0.12 mg/L) with pseudo- order model.

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731

732

Figures

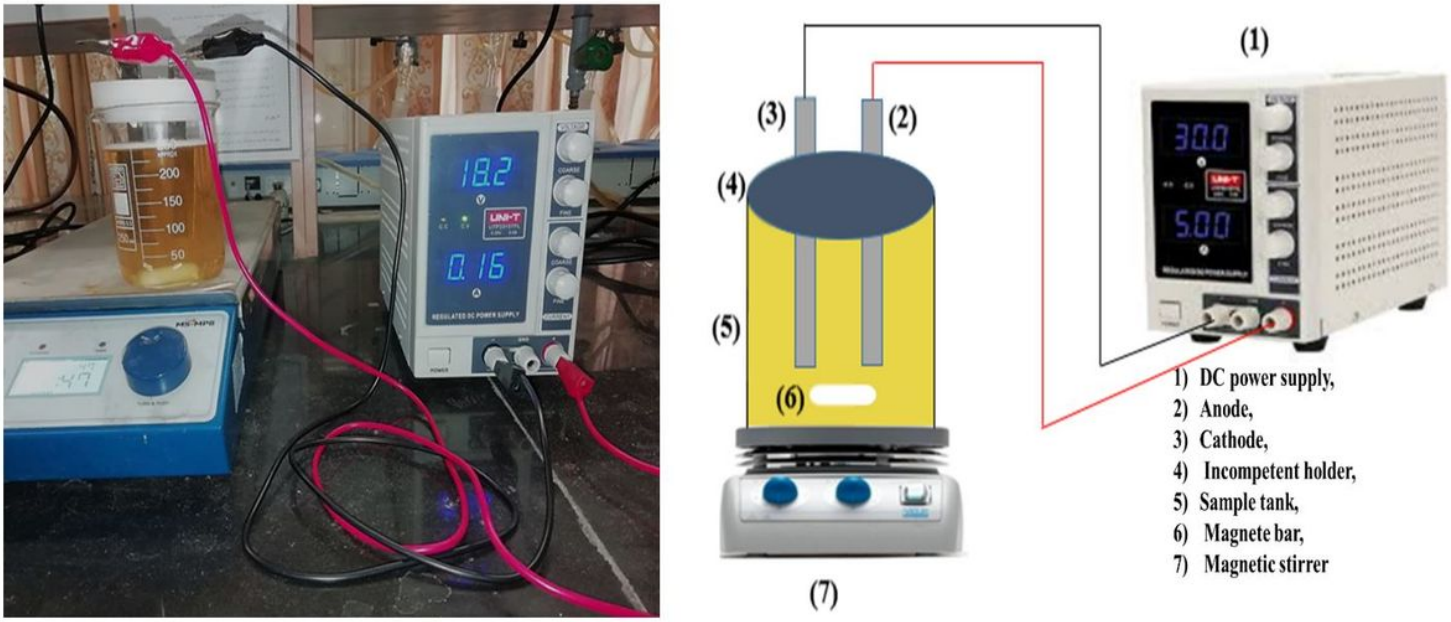


Figure 1

Schematic of the reactor used for removal of oxytetracycline by oxidation processes

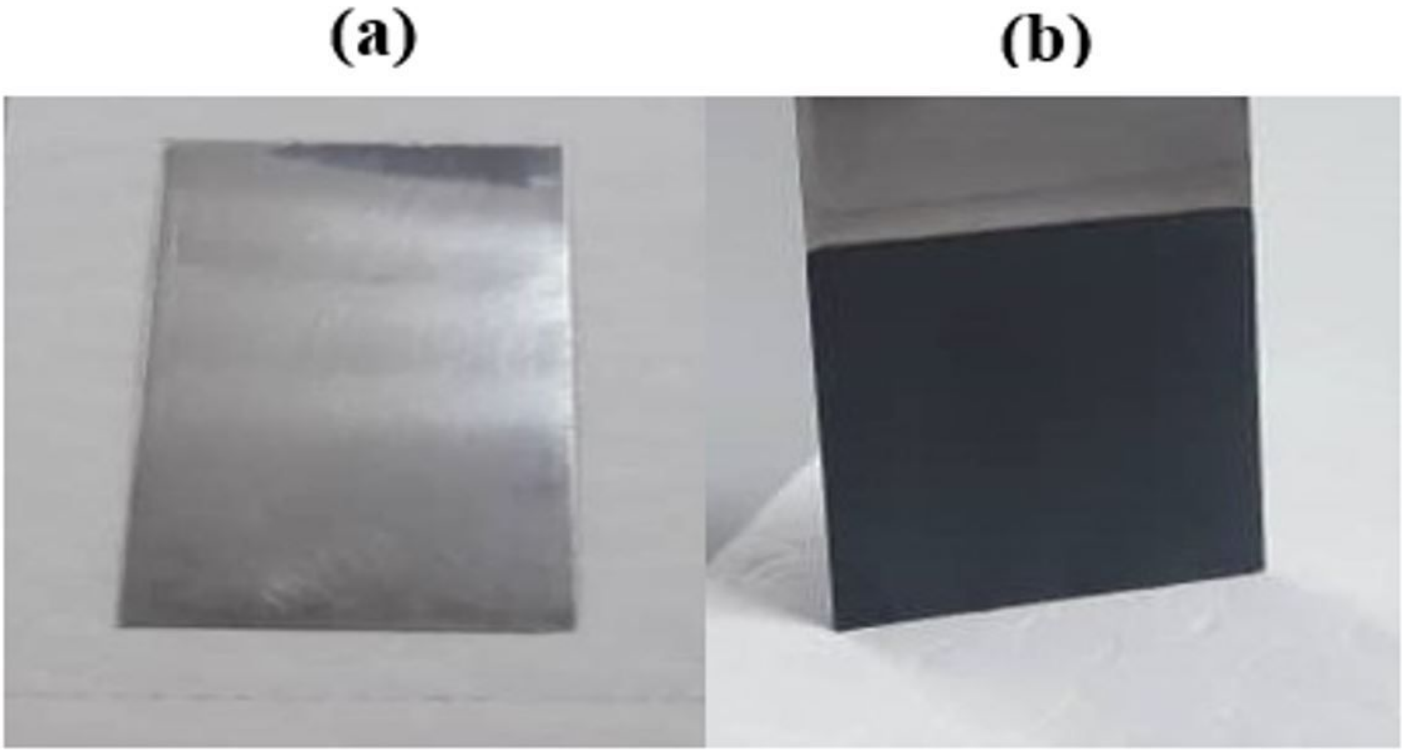


Figure 2

(a) Image of stainless steel electrode and (b) Stainless steel electrode coated with lead dioxide (SS316/PbO2) for removal of the oxytetracycline antibiotic under optimal test conditions

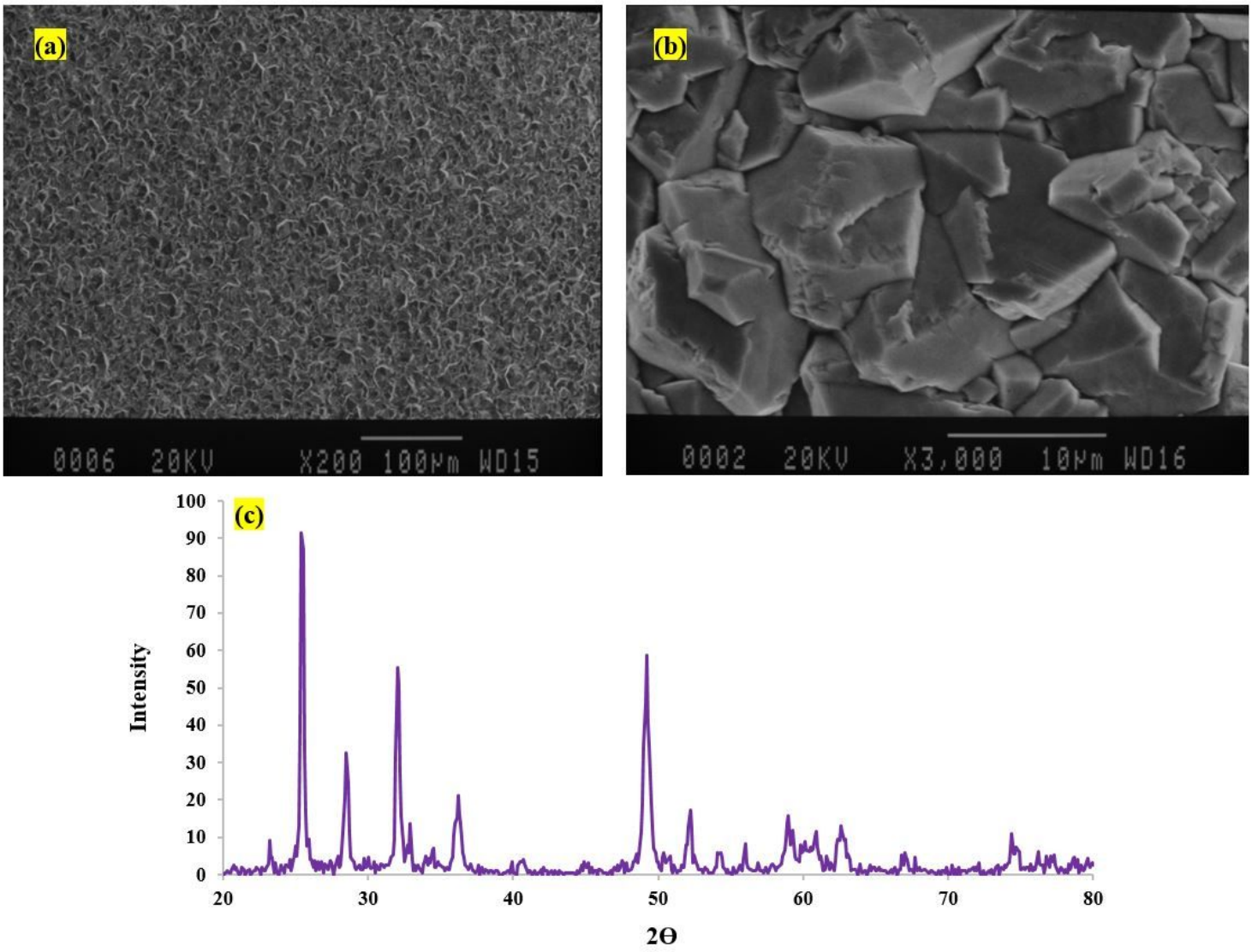
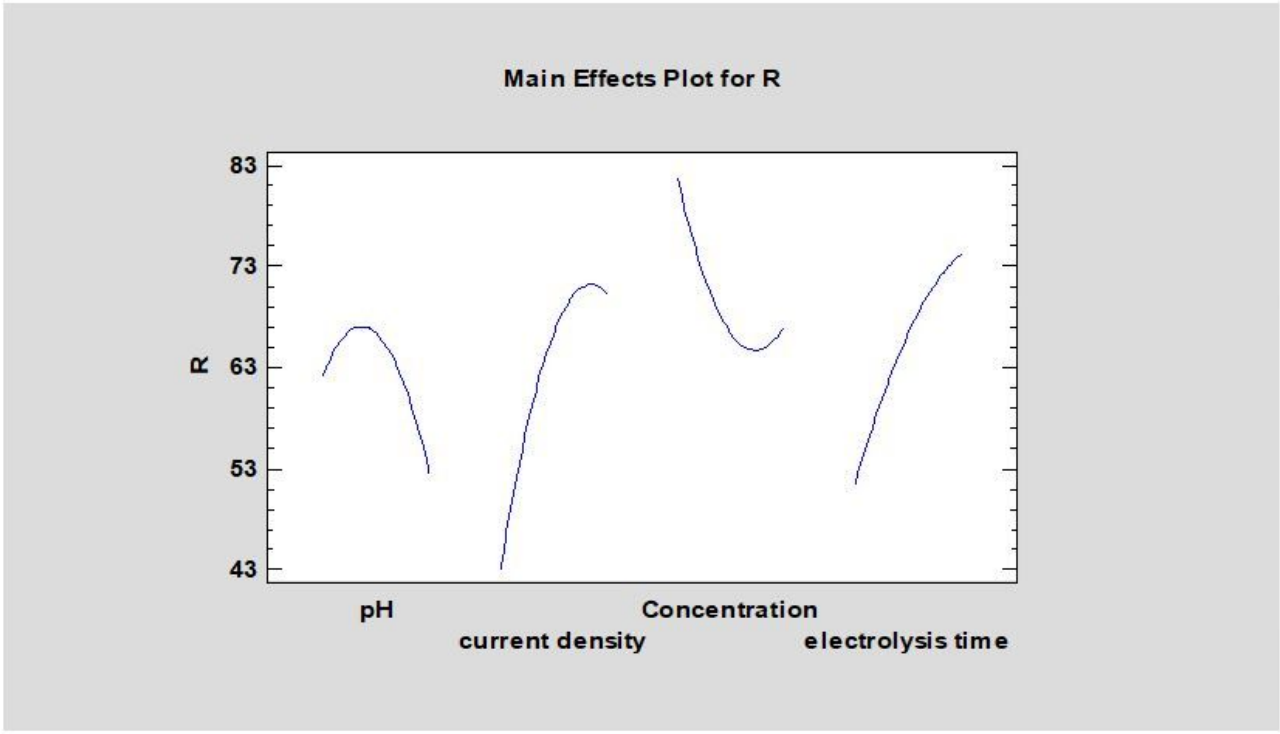


Figure 3

(a, b) SEM micrographs of the surface of SS316/β-PbO2 at different magnifications; (c) XRD analysis of SS316/β-PbO2 anode.



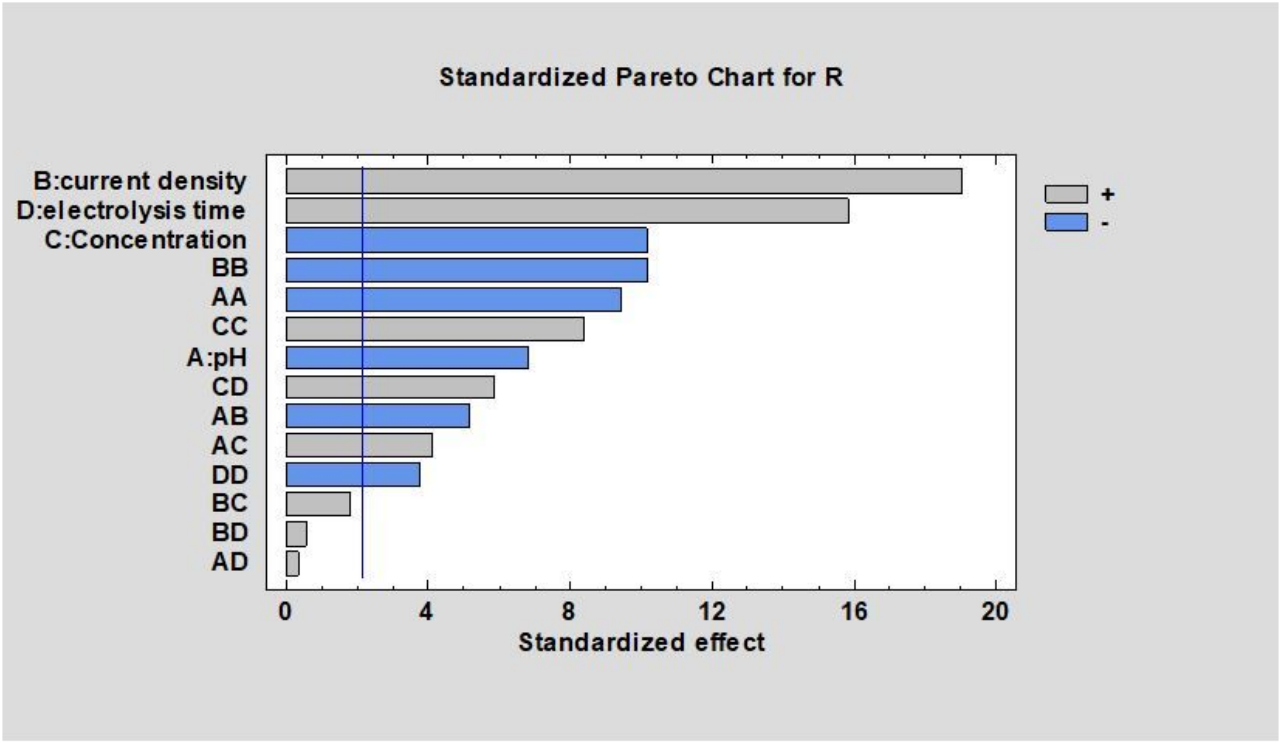
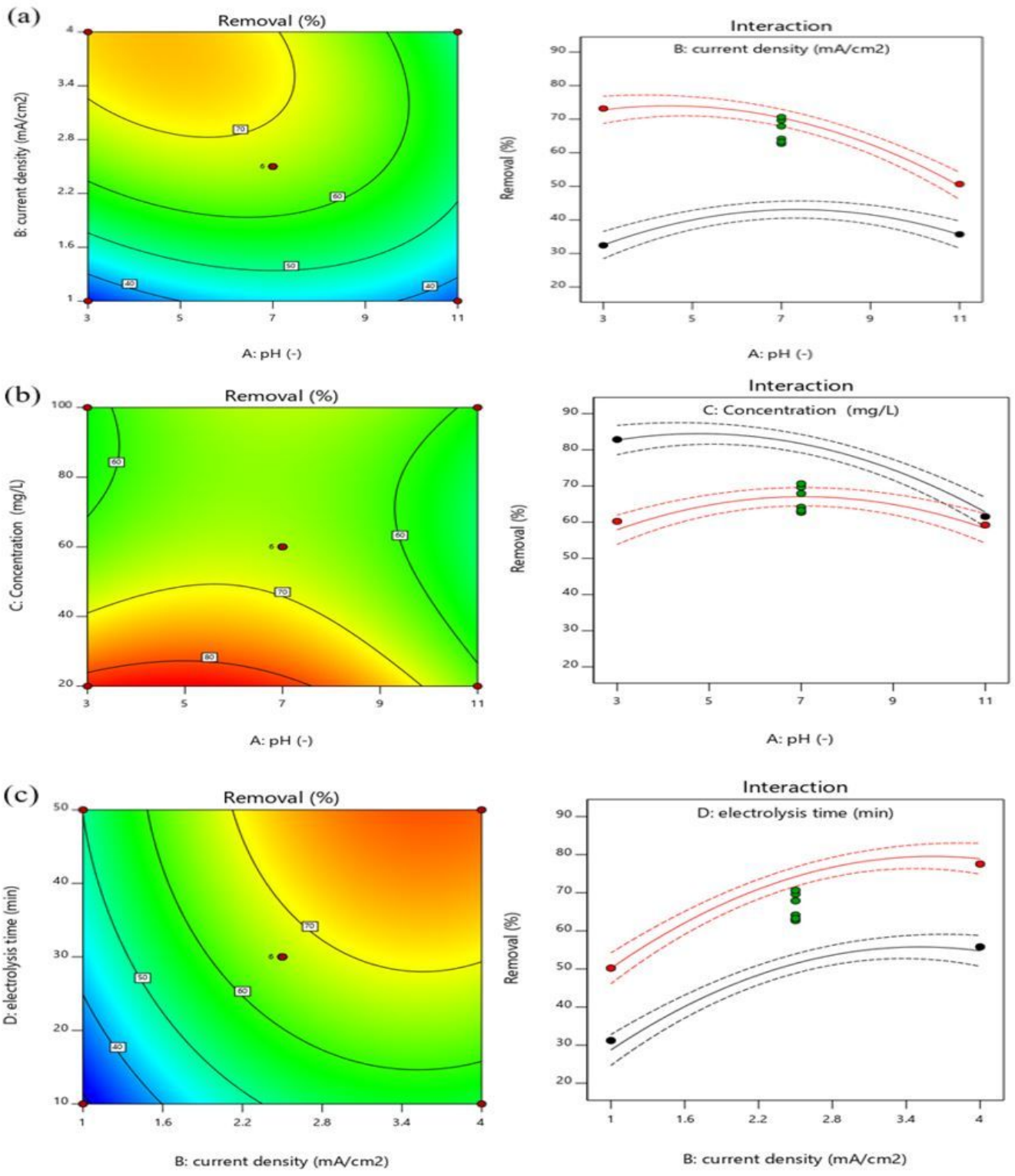


Figure 4

Separate effects of variables and the pareto plot for determining the effect of each term on the response (removal of Oxytetracycline by electrochemical process).



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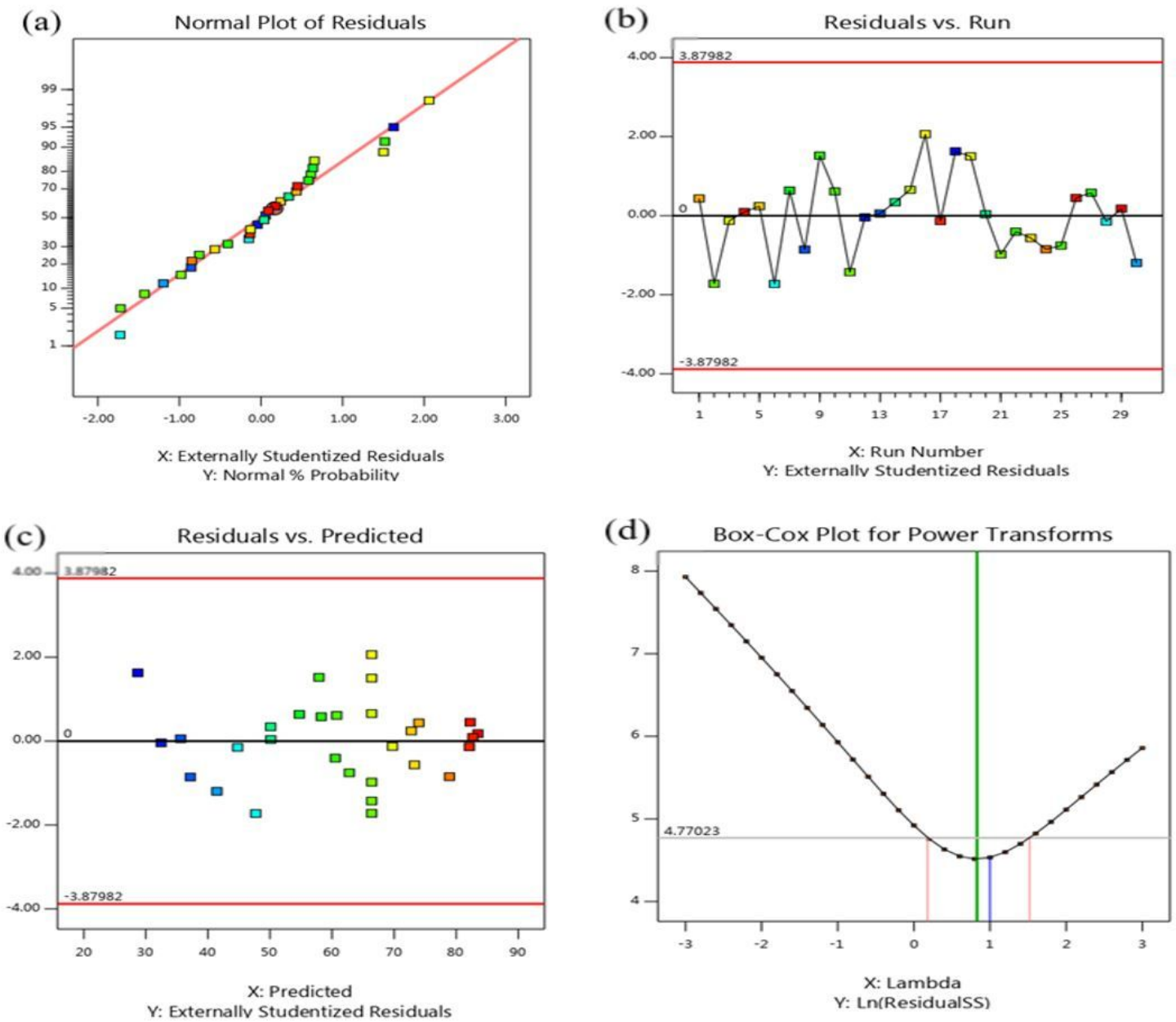

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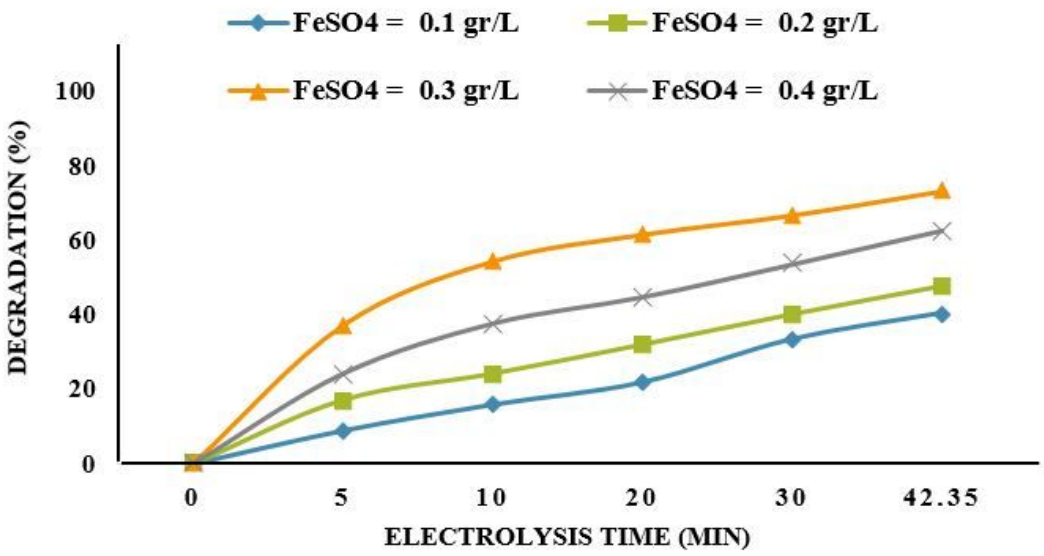
(a)



The changes of FeSo4 amounts in optimal condition via Fenton process

(pH = 3.53, concentration = 20 mg/L)

(b)

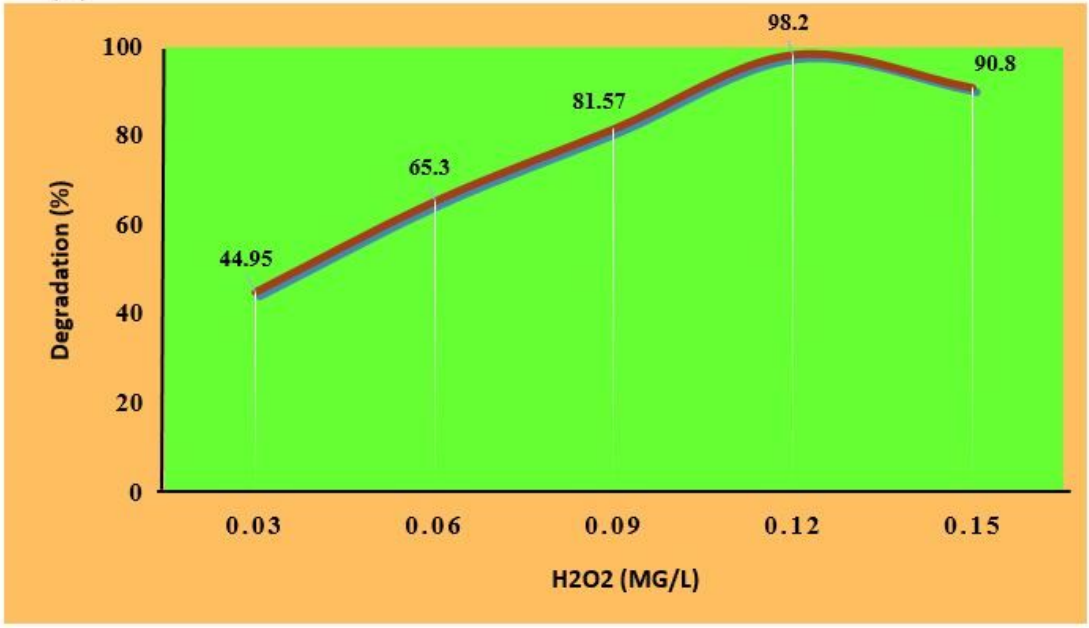


Figure 7

(a) Influence of FeSo4 amounts via Fenton process and (b) H2O2 values in elimination of Oxytetracycline in electro-Fenton process under optimum condition (pH= 3.53, current density = 3.85 mA/cm2, concentration = 20 mg/L, electrolysis time = 42.35 min and FeSO4 = 0.3 gr/L). (a: Optimize amount of FeSO4 = 0.3 gr/L, b: Optimize value of H2O2 = 0.12 mg/L)

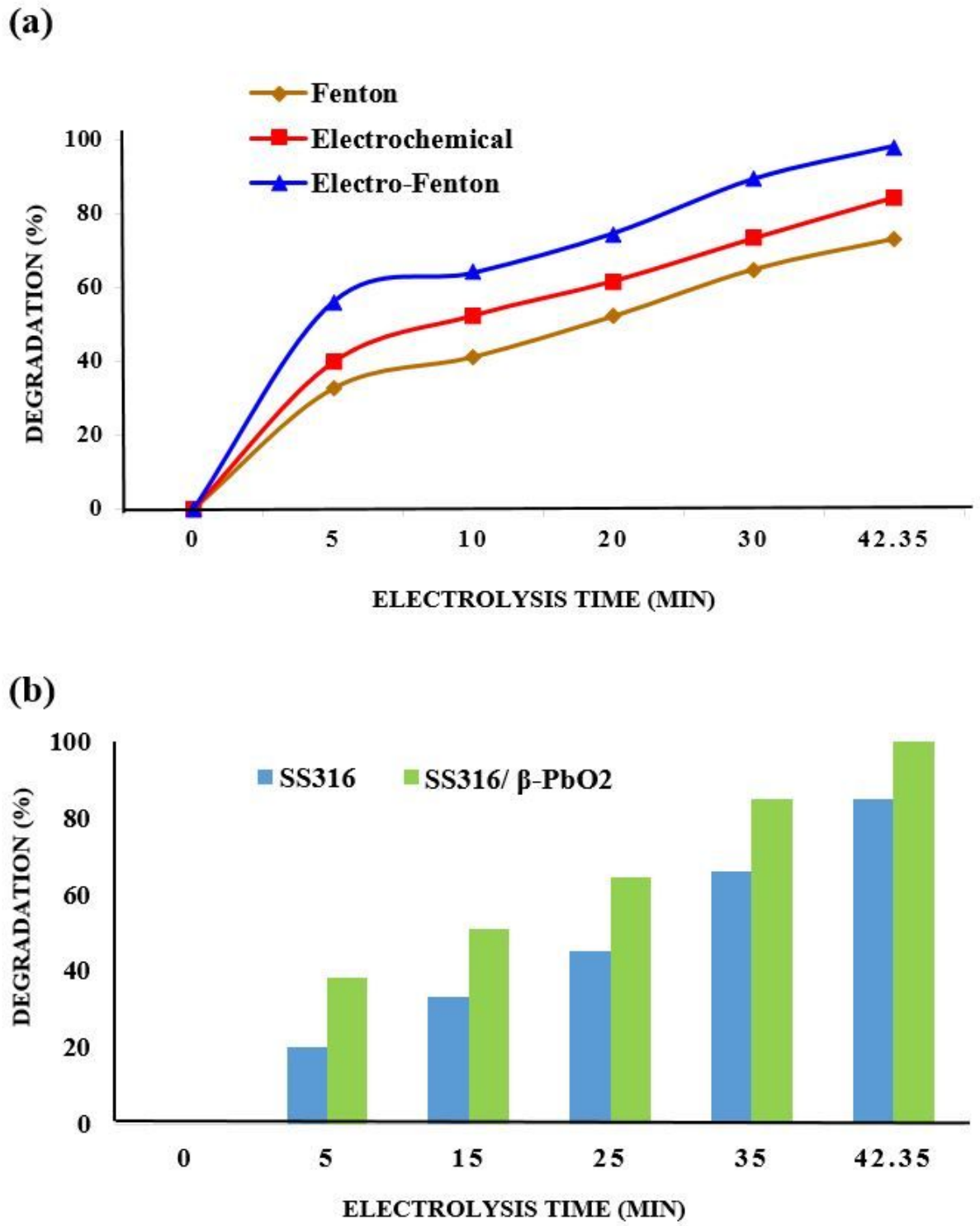
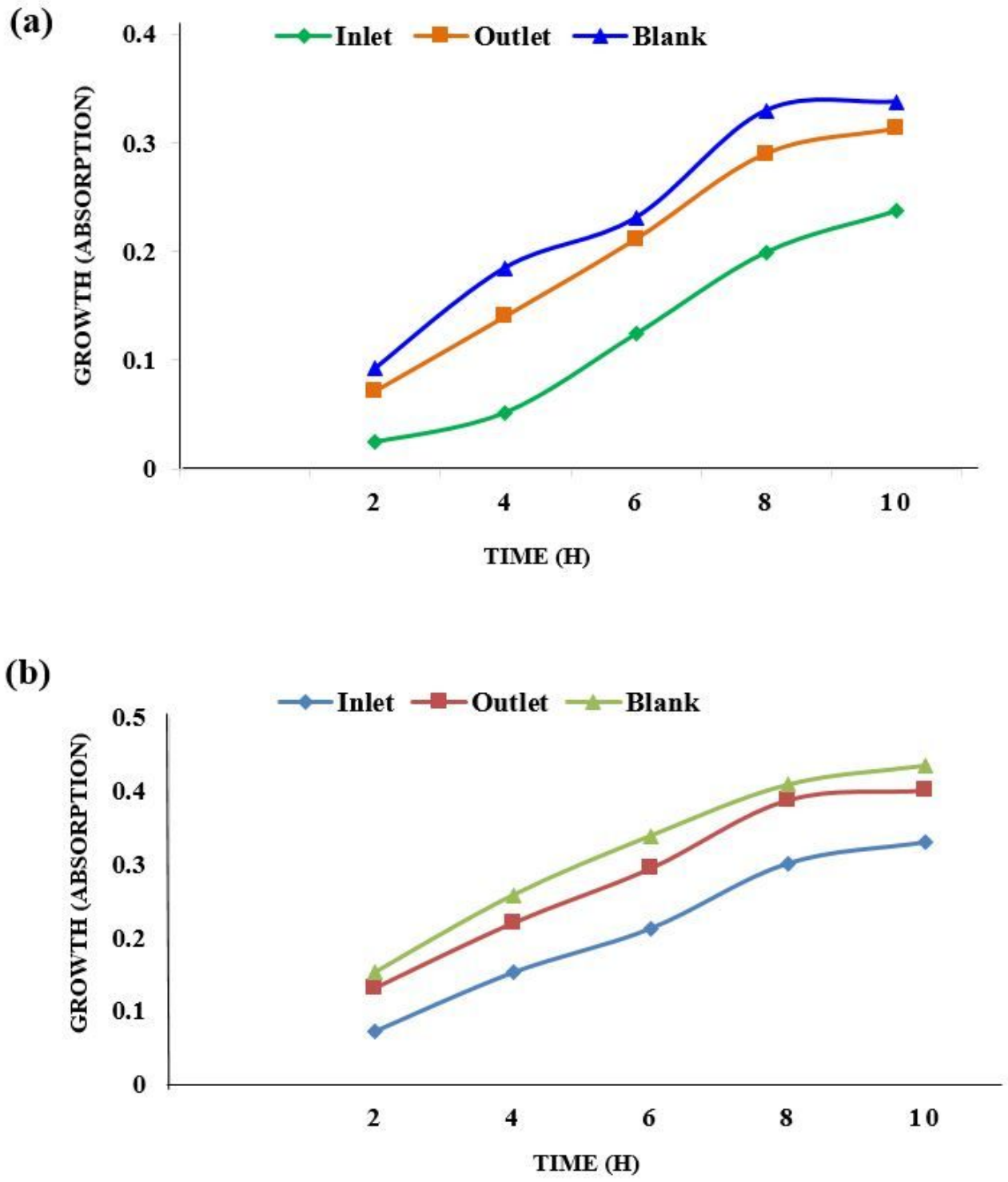


Figure 8

(a) The effect of AOPs processes on the degradation of Oxytetracycline antibiotic, (b) The elimination of Oxytetracycline antibiotic by the SS316 and SS316/β-PbO2 anodes (pH = 3.53, current density = 3.85 mA/cm2, concentration of Oxytet = 20 mg/L, electrolysis time = 42.35 min, FeSO4 = 0.3 gr/L, H2O2 = 0.12 mg/L).



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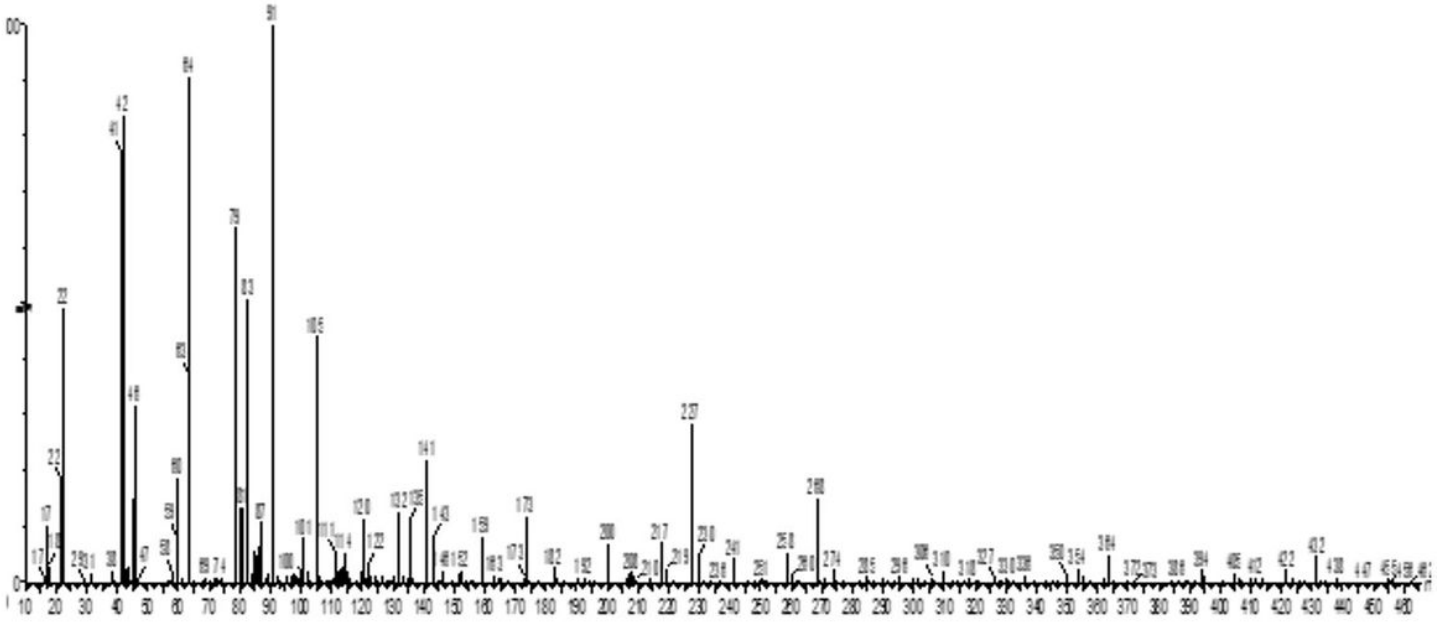

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LC/MS chromatographs after degradation of Oxytetracycline antibiotic via Electro-Fenton process under optimum conditions.

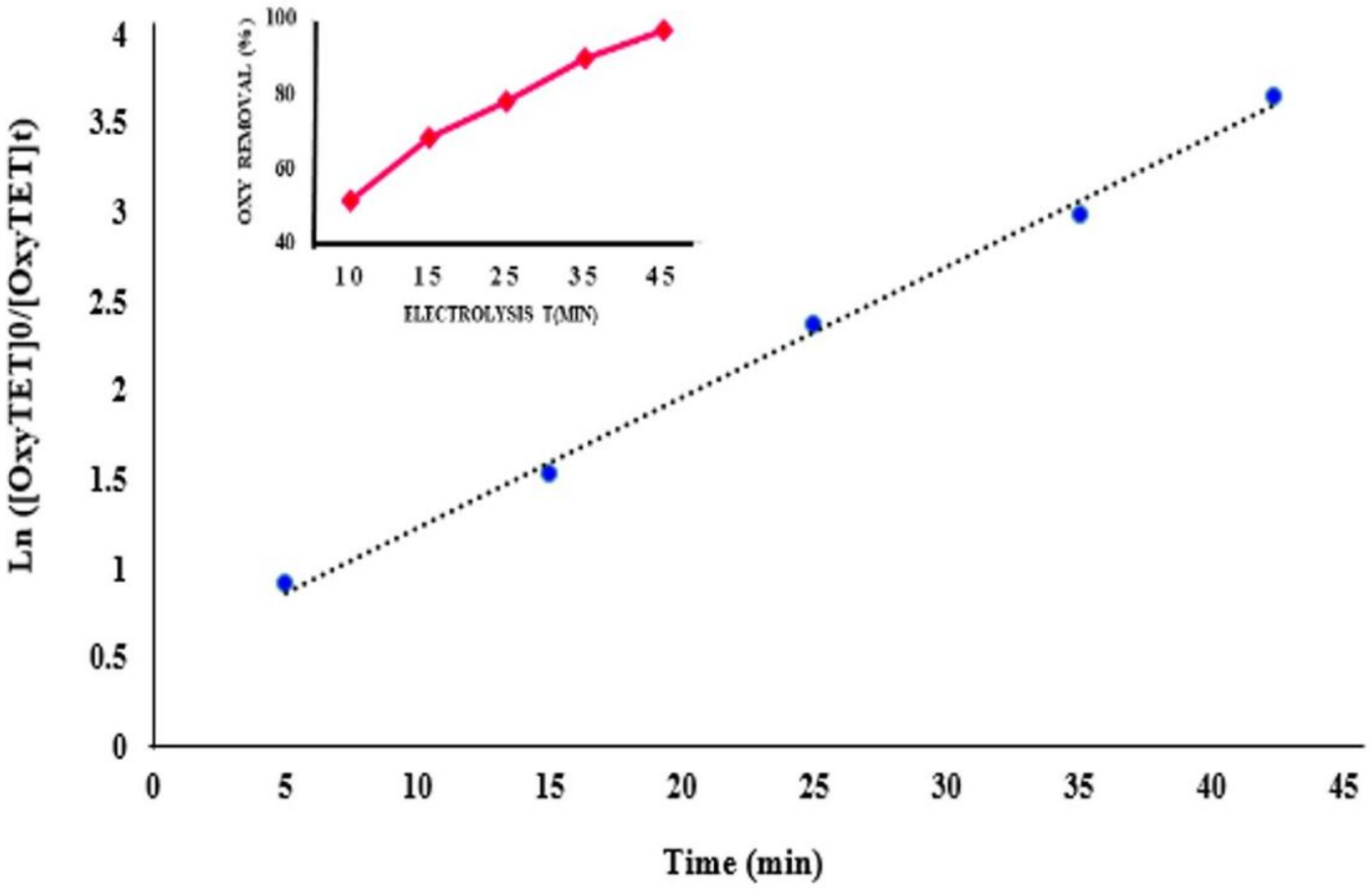

Figure 11

Kinetics of Oxytetracycline removal at the optimum conditions (pH = 3.53, current density = 3.85 mA/cm2, concentration of Oxytetracycline = 20 mg/L, electrolysis time = 42.35 min, FeSO4 = 0.3 gr/L, H2O2 = 0.12 mg/L) with pseudo- order model.

Supplementary Files

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- scheme1.jpg