Removal of Reactive Red 141 Dye from Synthetic Wastewater by Electrocoagulation Process: Investigation of Operational Parameters

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ABSTRACT
Release of textile industries waste especially their dying effluent impose a serious pollution on the environment. Reactive dyes are one of the most used dyes which are recalcitrant to conventional treatment processes. In the performed project, the effectiveness of electrocoagulation process was studied on decolorization.

RR141 was selected as model dye and treatment process was performed in a simple batch of electrocoagulation (EC) cell using iron electrodes. Central Composite Design (CCD) was used to plan study runs. Experiments were done under 5 levels of various operational parameters at bench scale. Initial concentration of dye was varied among 50 and 500ppm, pH ranging from 4-12; retention time was ranged between 3-30 minutes, 1-3cm was selected as the distance between electrodes, and current intensity studied under the range of 5-30 mA/cm^2.

EC treatment process of dyestuff wastewater was satisfactory at high levels of current density, pH, and retention time. While increasing the initial dye concentration and electrodes gap had a negative effect on decolorization performance. Determined optimal conditions to treat 200ml of sample were including pH: 9.68, electrode gap: 1.58cm, dye concentration: 180ppm, retention time: 10.82 minutes, and current intensity: 22.76mA/cm^2. Successful removal of the model dye about 99.88% was recorded in the mentioned values of variables.

Simple design and operation of the experiments can be an interesting option for implementation and applying of inexpensive electrocoagulation treatment process which was successful to reach nearly a complete decolorization.
Keywords: Central Composite Design, Dye Removal, Electrocoagulation, Iron Electrode, Reactive Red 141

INTRODUCTION
Textile industry is one of the water-intensive consumers in the world which imposes large amounts of wastewater to the environment. The effluent contains considerable quantities of various chemicals and dyes [1-2]. Annually production of dyes has been estimated at about 700 thousands to 1 million tons in the world which are used in multiple industries like cosmetics, leather, paper and textile [3-4]. Azo dyes with more than 50% production of all dyes are known as the largest group among the synthetic organic dyes [3,5]. Reactive azo dyes are known as the most problematic substances in textile industry wastewaters due to their high water solubility and low degradability [6]. It is estimated that about 50% of them are released to the effluents [3]. The hydrophilic characteristic of reactive dyes makes low expectance of their adsorption on biomass in biological treatment methods. High molecular weight and aromatic rings are from the other specification of these dyes which can imply on their toxicity to microorganisms. Complex structure of colors makes them stable and resistant to biodegradation. So they are considered threatening from the health point of view because of their carcinogenic and mutagenic properties, allergies effects and skin problem-making [3, 7]. Discharge wastewaters containing dyestuff prevents of light penetration to aqueous media like lakes and rivers, reduces the amount of dissolved oxygen and increases chemical oxygen demand in them, and thereby disrupts the aquatic life. In addition, colors can remain in the environment for long periods of time due to their high thermal and optical stability [2]. The presence of colored contaminants, even at levels less than 1mg/l, is visible and unfavorable in terms of physical properties [8]. Regarding extreme rate of colored effluents generation in Iran as one of the leading countries in the field of textile industry, development of practical scientific solutions for appropriate treatment of such wastewaters seems essential. Nowadays numerous methods have been tested for treating colored effluents. UV/H_2O_2 [9], UV/O_3 [10-11], Fenton, and Electro-Fenton [12] are some of

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mentioned ways that have been less applied as they require high cost, skilled operator, adding salt, excessive energy consumption, and absorbent regeneration [3]. However, if it would be possible to ignore the high operating costs of some new techniques, they exhibit a great efficiency for the removal of resistant organic compounds and synthetic organic materials. Hence they can be considered as proper options for separating dyestuff from wastewater with heavily load of contaminants. [13]. In recent years the electrocoagulation (EC) process has been investigated as an effective method for the wastewater treatment. In EC process reagents or chemicals aren't used and the electron is the main reagent. So the sludge generation is minimized compared to the other processes. Since it doesn't consume high values of current, its energy demand can be supplied by green processes, such as, solar, windmills and fuel cells. Destabilizing of small colloidal particles and eliminating of some coagulants which are hazardous chemicals are mentioned as the other advantages of this process [14]. In EC process metal ions can be generated in solution through applying a direct electric current. Electrolytic oxidation of anode leads to in situ generation of coagulants followed by hydroxyl ions and hydrogen production in cathode. The opposite charge of ions which neutral pollutants particles in solutions end to the final deposition of solids. Moreover, the formation of hydrogen bubbles allows suspended solids to float [10,15]. Based on literature the principal ways of pollutant removal in the EC process are coagulation, adsorption, precipitation and flotation [16].

 Mechanisms that occur during the electrocoagulation process" by iron electrodes "[15, 17] are presented in equations of 1 to 4:

$$Fe \rightarrow Fe^{+2} + 2e^- \qquad (1)$$
$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \qquad (2)$$
$$2H_2O + 2e^- \rightarrow 2OH^- + H_2 \quad (3)$$
$$Fe^{2+} + 3OH^- \leftrightarrow Fe(OH)_3 \quad (4)$$
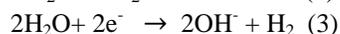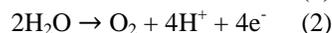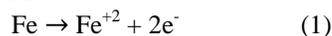
So far, various contaminants such as phenol [18-19], heavy metals [20-21], nitrate [22], phosphate [23], dyes [24], and many other organic compounds have been removed of aquatic environment by EC process. Pajootan *et al.* study showed a substantial elimination of colors acidic black 52 and acidic yellow 220 from synthetic wastewater using aluminum electrodes in a batch electrochemical reactor. Based on their findings by increasing the current density had a positive effect on dye removal while various concentrations of electrolyte from 0-8g/l did not affect the removal rate [24]. Pirkarami and Olya [25] investigated the impact of different parameters on the removal of Reactive

Red 120 in synthesized wastewater through electrocoagulation. They used anodes of iron, aluminum, and titanium while titanium was used as cathode. Experiments were conducted in 1L of effluent by applying a current density of 0-18A. Current density of 45 Am-2, iron anode and pH: 7 proved to be optimum conditions for dye removal.

In the study of El-Ashtoukhy and Amin [26] by electrocoagulation and anodic oxidation methods were studied for their ability on removing acid green dye 50. Based on results electrocoagulation was more economic than anodic oxidation, since its energy consumption ranged from 2.8to12.8 kWh/kg removed dye while in the case of anodic oxidation it ranged from 3.31to16.97 kWh/kg removed dye. Also, among the operational parameters pH had an important effect on colour removal in electrocoagulation process and maximum efficiency observed at pH ranged from 6.9-11.

In the present study, the efficiency of EC process in decolorization of synthetic wastewater was studied using iron electrodes. During this study the role of different influential operational parameters on dye removal was investigated at several levels.

MATERIALS AND METHODS
Experimental set up, Chemicals and Procedures
This experimental study was performed on a laboratory scale in a batch reactor. Chemicals were purchased from Merck products. Reactive Red 141 was selected as a representative of reactive azo dyes which are known for problem making in conventional methods of wastewater treatment. The molecular structure of RR 141 is presented in Fig.1. Electrochemical cell includes a 250ml beaker in which one pair of iron electrodes at dimensions of 0.2cm (thickness) * 3cm (width) * 11cm (height, immersion height = 5.5cm) was connected to direct current. Magnetic stirrer was used at 150 rpm to mix the samples. To study the role of various operational parameters includes initial dye concentration, pH, reaction time, electrodes distance and the current density on dye removal trend, reactor was fed of 200 ml wastewater in each experiment. KCl salt was used to reach electrical conductivity equal to 3mS / cm which previously was considered as the optimum amount of salt in providing needed current intensity. After performing each test electrodes, were polished using sandpaper and placed for 10 minutes in a solution of 15% wt HCl [27], then washed and dried before the next experiment. Residual concentration of color was measured by a spectrophotometer model 3220 optizen at 544nm wavelengths. Experiments were done thrice and results reported on average values. The obtained data finally were analysed by Design Expert software.
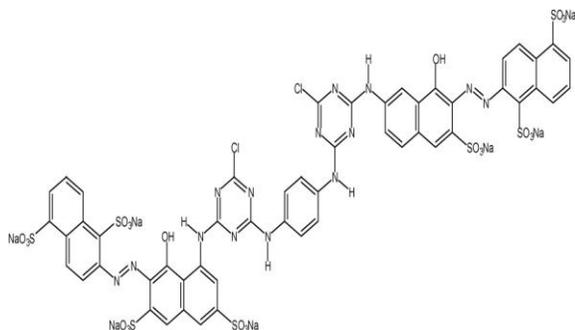
Fig.1: Molecular structure of dye Reactive Red 141

Experimental design and Statistical analyses

The Response Surface Methodology (RSM) was applied to calculate the number of samples. In RSM as a complex of statistical techniques for process optimization, a desired response is affected by a number of variables. This method sets up a polynomial of data and explains the relationships between them. Among different ways provided by this program to design study, Central Composite Design (CCD) was selected, because the maximum information with the fewest number of runs can be available through the distribution of test points in the optimum range taken from pretest results [28]. A 25 factorial design with ten axial points and eight central points made 50 experiments. It can be seen in the formula 5:

Number of experiment $= 2^k + 2k + c$ (5)

In which K shows the number of independent variables, and C is the number of the central point. It must be mentioned that experiments are conducted triplicate and response is reported on an average value. In CCD, the actual values of factors in high and low levels were defined through $+\alpha$ and $-\alpha$ coded levels. Three other levels were determined by software and all of them are presented in Table 1. The response of dye removal performance is shown in equation 6 as a function of the independent variables in the form of a polynomial regression model:

$$Y = b_0 + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} \sum_{j=1}^{4} b_{ij} x_i x_j + \sum_{i=1}^{4} b_{ii} x_i^2 \quad (6)$$

Where Y is the response variable, b_0 implies on constant coefficient, b_i refers to regression coefficient calculated from Y data, x_i assigned for coded levels of independent variables, $x_i x_j$ and x_i^2 introduce the interaction coefficients, and second-order term respectively [29-30]. All the experiments were conducted using 0.05 mol.l-1 KCl salt as supporting electrolyte. This value was selected based on performing experiments using different concentrations of salt (0.01, 0.03, 0.05, 0.08, and 0.1 mol.l^{-1}) for determination its optimum quantity. The supporting electrolyte enhances the electrical conductivity in the solution which reduces the energy consumption of the process [31].

Table1: Variables in coded and actual levels

Variables	Coded sign	Actual sign	levels				
			$-\alpha$	-1	0	+1	$+\alpha$
pH	X_1	A	4	6.32	8	9.68	12
Retention time (min)	X_2	B	3	10.82	16.50	22.17	30
Initial dye concentration (ppm)	X_3	C	50	180.39	275	369.6	500
Electrodes Distance (cm)	X_4	D	1	1.58	2	2.42	3
Current Intensity (mA/cm^2)	X_5	E	5	12.24	17.5	22.75	30

RESULTS

Model fitting and ANOVA results

The observed and predicted results obtained in 50 experiments according to RSM design are given in table 2. The maximum and minimum amounts of dye removal have recorded 99.88% and 75.22% respectively. Large variations in obtained response suggest that experimental parameters were chosen at proper ranges. To explain the effect of variables in system the model was evaluated at 95% confidence level. In table 3 model P-value less than 0.0001 imply on a very significant model. As table 3 shows P-value < 0.05 for the main effects of A, B, C, D, E, intervention effects of BD and BE, and second order effect of E2 reflects the significance of these parameters on dye removal rate. Lack of fit wasn't

considerable with a P-value of 0.5220. Model goodness can also be concluded from the F ratio 20.63 for the regression in table 3. The high value of F- test indicates on a very significant model. Another test is applied to validate the model was linear regression. Obtained results of this analysis are presented in table 4. A very low difference is observable between R-squared value and adjusted R-squared value in table 2. R^2 adjusted value is another sign of model significance. To reach a well fitting among data and model, the difference between the predicted and adjusted R-squared must not exceed from 0.2. Adequate precision compares the predicted values of design points with the mean predicted error. It must be 4 at the lowest level [32]), so based on table 4 the modified model has a good precision.

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Table2: Designed experiments, actual and predicted results of RR141 dye removal in CCD

Run order	X_1	X_2	X_3	X_4	X_5	Actual dye removal %	Predicted dye removal %
1	6.31	10.82	180.39	1.58	12.24	83.4	85.5
2	9.68	10.82	180.39	1.58	12.24	92.67	88.27
3	6.31	22.17	180.39	1.58	12.24	96.48	94
4	9.68	22.17	180.39	1.58	12.24	96.03	96.77
5	6.31	10.82	369.6	1.58	12.24	76	81.28
6	9.68	10.82	369.6	1.58	12.24	85.40	84
7	6.31	22.17	369.6	1.58	12.24	90	89.78
8	9.68	22.17	369.6	1.58	12.24	86.16	92.53
9	6.31	10.82	180.39	2.42	12.24	80.71	80.25
10	9.68	10.82	180.39	2.42	12.24	83.44	83
11	6.31	22.17	180.39	2.42	12.24	93.40	93.36
12	9.68	22.17	180.39	2.42	12.24	94.77	96.12
13	6.31	10.82	369.6	2.42	12.24	75.22	76
14	9.68	10.82	369.6	2.42	12.24	76.78	78.77
15	6.31	22.17	369.6	2.42	12.24	88	89.13
16	9.68	22.17	369.6	2.42	12.24	96.61	91.88
17	6.31	10.82	180.39	1.58	22.75	96.4	98.17
18	9.68	10.82	180.39	1.58	22.75	99.88	100
19	6.31	22.17	180.39	1.58	22.75	96.44	95.94
20	9.68	22.17	180.39	1.58	22.75	97.56	98.69
21	6.31	10.82	369.6	1.58	22.75	90.58	93.93
22	9.68	10.82	369.6	1.58	22.75	97	96.69
23	6.31	22.17	369.6	1.58	22.75	94.86	91.70
24	9.68	22.17	369.6	1.58	22.75	90.40	94.46
25	6.31	10.82	180.39	2.42	22.75	93.5	92.90
26	9.68	10.82	180.39	2.42	22.75	94.47	95.66
27	6.31	22.17	180.39	2.42	22.75	95.22	95.29
28	9.68	22.17	180.39	2.42	22.75	97	98
29	6.31	10.82	369.6	2.42	22.75	87.65	88.67
30	9.68	10.82	369.6	2.42	22.75	90.4	91.42
31	6.31	22.17	369.6	2.42	22.75	87.7	91
32	9.68	22.17	369.6	2.42	22.75	92.85	93.8
33	4	16.5	275	2	17.5	93	89.18
34	12	16.5	275	2	17.5	98.89	95.73
35	8	3	275	2	17.5	85.67	85.99
36	8	30	275	2	17.5	97.37	98.93
37	8	16.5	50	2	17.5	99.6	97.49
38	8	16.5	500	2	17.5	97	87.42
39	8	16.5	275	1	17.5	95.89	95.97
40	8	16.5	275	3	17.5	86.48	88.94
41	8	16.5	275	2	5	76.446	76.63
42	8	16.5	275	2	30	97.84	93.97
43	8	16.5	275	2	17.5	91.17	92.46
44	8	16.5	275	2	17.5	90.78	92.46
45	8	16.5	275	2	17.5	92	92.46
46	8	16.5	275	2	17.5	94.32	92.46
47	8	16.5	275	2	17.5	92.8	92.46
48	8	16.5	275	2	17.5	96.4	92.46
49	8	16.5	275	2	17.5	97	92.46
50	8	16.5	275	2	17.5	87.75	92.46

Table3: ANOVA results for dye removal efficiency

source	Sum of squares	Degrees of freedom	Mean square	F value	$P>F$
Model	1632.9	8	204.12	20.63	<0.0001
X_1	82.21	1	82.21	8.31	0.0062
X_2	320.4	1	320.4	32.40	<0.0001
X_3	194.2	1	194.2	19.63	<0.0001
X_4	94.6	1	94.6	9.567	0.0036
X_5	575.2	1	575.2	58.16	<0.0001
X_2X_4	42.63	1	42.63	4.31	0.0442
X_2X_5	229.9	1	229.9	23.25	<0.0001
X_5^2	93.6	1	93.6	9.464	0.0037
Residual	405.5	41	9.89	-	-
Lack of fit	338.8	34	9.96	1.05	0.5220
Pure Error	66.68	7	9.53	-	-

Table 4: Regression coefficients for modified model

Regression index	R^2	R^2 adjusted	R^2 predicted	Adequate precision
Dye removal efficiency	0.8011	0.7623	0.6910	18.66

Effect of parameters on response using RSM

RSM was used to determine the relation between response and operating parameters include pH, retention time, initial dye concentration, electrode distance, and current intensity. Below equation gives the modified model for the most important variable dye removal efficiency:

$$Removal(\%) = 92.46 + 1.38X_1 + 2.72X_2 - 2.12X_3 - 1.48X_4 + 3.64X_5 + 1.15X_{2,4} - 2.68X_{2,5} - 1.27X_5^2$$

The constant coefficient of 92.46 shows the average value of dye removal rate of the 50 assays. The most powerful effect belongs to the current intensity as it can be observed in the related coefficient of +3.64.The second most important effect on dye removal is the retention time with a positive coefficient of + 2.72. All the main effects are also represented in Fig. 2. As perturbation plot shows dye removal efficiency has a direct relation with pH of the solution, retention time and current intensity. While increasing the initial concentration of dye and electrodes distance has a negative effect on dye removal percent. In the perturbation plot increasing the amount of level (-1 to+1) of pH (A), retention time (B), and current intensity (E), causes better removal efficiency around 2.8, 5.44, and 7.3% respectively. Contrarily a reduction in removal percent about 4.23, and 2.96 % can be seen when dye concentration (C) and electrodes gap (D) rise to level +1.

Note: for describing the behavior of any parameter others must be considered in the central points (level 0). For example, when factor pH in level -1 (6.32) moves to level +1(9.68), B, C, D, and E are constant at 16.5 min, 275 ppm, 2cm, and 17.5 mAcm^{-2} respectively.
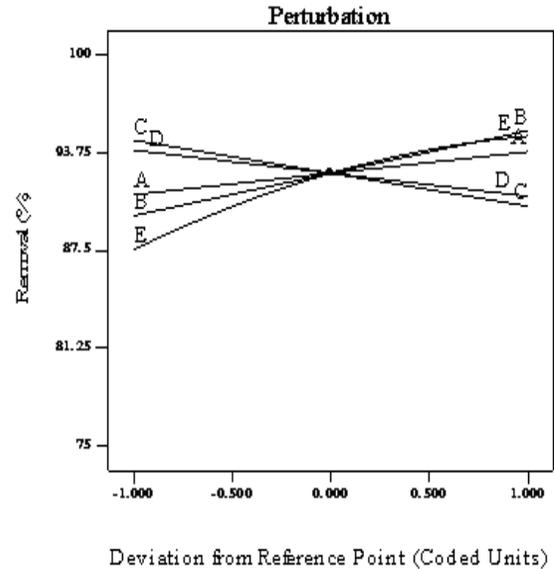
Fig.2: Perturbation plot for the main effects

Significant Interaction effects

Among the interaction terms, X2X4 and X2X5 have the most significant effects. Fig.3 explains the concurrent effect of retention time, and distance between electrodes on dye removal percent while the other parameters are constant at their central points. In this plot, increasing the time of reaction from 10.82 to 22.18 minutes in the constant gap of 1.58 cm, lead to a better dye removal almost 3.12%. The least efficient of dye removal can be observed using 2.42cm of electrodes distance at 10.82 minutes of retention time. In different electrodes distances, a same behavior of removal efficiency can be seen in higher levels of reaction time. There are 95.5% and 94.8% of dye removal using electrodes at distances 1.58 and 2.42cm, both in retention time of 22.18 minutes. Fig. 4 shows in the effect of retention time, and current intensity on dye removal efficiency. As it makes clear there is a direct relationship between these operational parameters and dye removal rate. The highest removal efficiency of 94.84% has been achieved in the 22.18 minutes of retention time, 22.76 mA/cm^2 of current density, pH equal to 8, 275 ppm of dye dose, and electrode distance of 2cm. When retention time moved from level -1 to level +1 at the applied current of 22.76 mA/cm2, the removal efficiency of 82.14% rose to 93.47%. Also during 10.82 minutes of reaction time, when the level of

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current density enhanced from -1 to +1, can be observed an increase in removal efficiency from 82.14% to 95.07%.
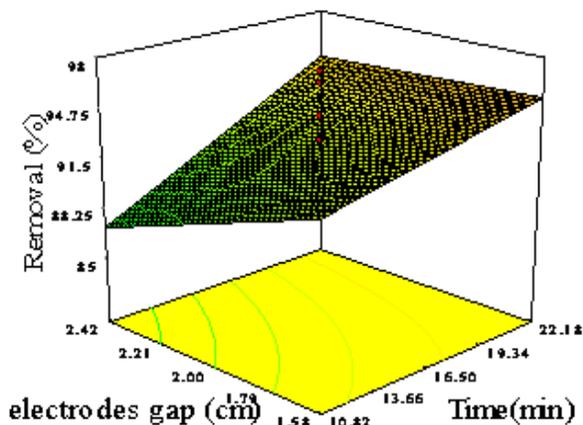
Fig.3: 3D surface plot of retention time and electrode gap effects on dye removal efficiency
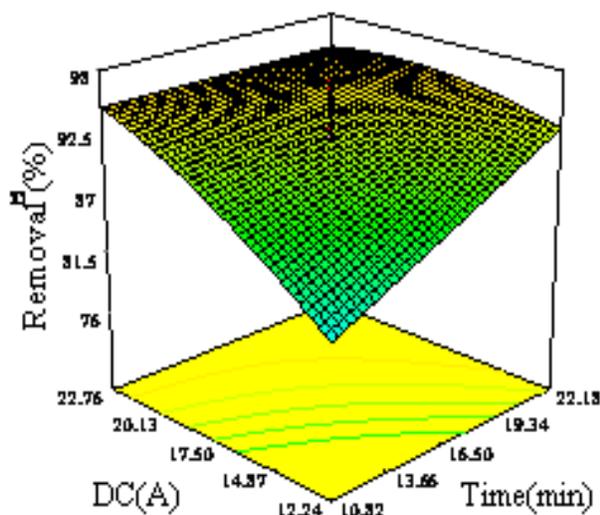
Fig.4: 3D surface plot of current density and retention time effects on dye removal efficiency

Electrical energy consumption

The amount of consumed energy in the experiments (E: KWhm-3) was calculated through equation 7:

$$E = \left(\frac{UIt}{V}\right) \qquad (7)$$

U is a sign of applied voltage on the electrolysis cell (V), I index refers to the current density (A), t imply on retention time (minute), and V is the volume of sample (L). With respect to optimal conditions considered at level +1 belonging to pH and current intensity, and level -1 for the retention time and

initial dye concentration, the amount of energy consumption was measured 5.85KWhm^{-3}.

DISCUSSION

In this project pH was investigated in the range of 4 to 12. The importance of pH regarding the electrocoagulation process has been well known as it experiences an increase during the process. Depending obtained results pH had a positive direct effect on the response of pollutant removal efficiency. The best results for dye removal were observed in alkaline pH values. It may be caused by the reaction between metal (iron electrode) and hydroxide ions. In alkaline pH the cited reaction is more severe and $Fe(OH)_3$ the most influential factor on pollutant removal is the dominant species in the solution. But it has been worth to notice that the form of Fe-species formed in the effluent can't justify the effect of pH. Based on the literature, most of the cationic ferric– hydroxy complexes which participate in dye coagulation are predominant in the acidic or neutral pH. It can be explained through the influence of pH on changing the structure of the reactive dyes. This is likely due to the formation of a molecule which can be chelating for the metal ion in the chemical composition of dye. In acidic pH in the presence of iron, this metal is chelated instead of the dye–causing metal in the color structure [33]. Another mechanism must not be neglected in which in parallel to pH raising the inactive iron oxide layer formed on electrodes is destroyed. In our study of the best removal percentage of reactive dye occurred in pH: 9.68. Phalakornkule *et al.* [33] also reported the most removal rate for reactive dye in the pH: 9.6. Song *et al.* [34] achieved over 96% of dye removal conducting experiments in pH: 10. Mohammadi *et al.* (35) achieved the best performance in alkaline pH for the removal of nitrates using iron electrodes. Also Ezechi *et al.* [36] observed the most reduction of boron in alkaline pH value.

The results showed that dye removal efficiency was affected by the initial dye concentration and reaction time. According to table 2, 10.82 minutes of operating time was needed to achieve nearly complete dye removal percent (99.88%). An increase in time from 10.82 to 22.17 minutes enhanced the removal rate from 89.74% to 95.18%. Electrolysis time of the EC process influences the treatment efficiency as it determines the production rate of Fe ions from iron electrodes [37]. A linear falling of decolorization efficiency from 94.57 to 90.34% was observed when the initial dye concentration increased from 180 ppm to 369 ppm. A possible explanation can be the insufficient formation of ferric– hydroxide complexes from the iron electrode for the coagulation of the more number of dye molecules at higher

concentration of dye [38]. Based on observed results in the presence of higher concentrations of dye, higher voltage or a longer retention time is necessary to compensate for the reduction of efficiency. Findings of researchers confirm results of this study. Carvalho et al study in removing the methylene blue reveals that, optimal reaction time which is 10 minutes at 50mg/l of dye concentration, increases to 15 minutes with a senary dose of dye [39]. Also Pajootan and colleagues studied on Acid Yellow 220 removal from synthetic wastewater. They found that optimal reaction time in the presence of 200 mg/l of initial dye concentration was 3 minutes and when the concentration of dye was triple this time enhanced to 8 minutes [24]. These are similar to the results of present work.

Nearly doubling the value of current density increased the decolorization rate from 87.55% to 94.84%. Applying the current of 22.76mAcm^{-2} led to a removal rate of 99.88% after 10.82 minutes of the treatment process. Dye removal efficiency was highly affected by the current density. It can be explained through the reaction between hydroxyl anions formed as a result of water reduction in the cathode and the multivalent iron cations generated at anode. This leads to mechanisms of surface complex formation and electrostatic adsorption of contaminants. In other words, a very large surface of $Fe(OH)_3$ can enhance the adsorption of dissolved organic compounds or colloidal particles, resulting in trapping and final deposition of them. [40]. Additionally, superior removal of pollutants is expected from the increase of current density, as it increases the rate of bubble generation. More bubbles with lower size are proper for the separation of pollutants by flotation [16].

Dye removal efficiency was disproportional to the distance between electrodes. The increase in the electrode gap reduced the elimination efficiency. Falling in removal percent was also observed in Kim *et al.* study [41] in which they investigated the effect of 0.5-3 cm gap among anode and cathode on the dye removal rate. Expanding the distance from 1 to 3cm in our study reduced the removal efficiency about 2.96%, but this increase in their study led to a reduction of 20% in dye removal percent. However, it must be noted that Kim et al study was conducted under continuous flow reactor while our work is performed in batch condition. In our study as the distance between electrodes increased the resistance between them increased, and resulted in the reduction of conductivity and power consumption. This reduction prevents the sufficient generation of iron and hydroxide ions which is necessary for flocking and dye removal. In addition, it can be expected from the increasing of the gap between the electrodes which reduces the number of collisions between iron and hydroxyl ions. As a result fewer flocs form. Also in this condition the possibility of collision between dye molecule and hydroxide polymers reduces. Thus electrostatic attraction as one of the mechanisms involved in dye elimination confronts a diminution [36, 40].

CONCLUSION
A series of experiments were conducted to find how different operational parameters have effectiveness on dye removal from synthetic wastewater. The results demonstrated that the removal efficiency of dye was proportional to the current density and reaction time, while it showed a converse behavior regarding electrode gap and initial dye concentration. It can be concluded that EC treatment consisting iron electrodes in optimal conditions of pH: 9.68, electrode gap: 1.58cm, initial dye concentration: 180 ppm, retention time: 10.82 minutes, and current intensity: 22.76mA/cm2 have the capability of removing 99.88% of recalcitrant RR141 dye. Actually the optimal operating parameters were considered that points in which the maximum dye removal was obtained. However, regarding economical issues operating the treatment system in more retention times instead of applying high intensities of current can be a better choice. Further investigations may determine how this process can affect other types of dye compounds.

ETHICAL ISSUES
Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

COMPETING OF INTEREST
The authors have declared that no competing interest exists.

AUTHORS'CONTRIBUTIONS
All the authors made an equal participation.

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