



## RESEARCH PAPER

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## A parametric study and mathematical modeling of electro-flocculation as harvesting process of *Dunaliella salina* microalgae for biodiesel production

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Article published on August 02, 2014

**Key words:** Microalgae, Electrolytic flocculation, Modeling, Harvesting, Recovery efficiency.

### Abstract

Electro-flocculation is one of the technologies which have been considered for microalgae harvesting by many researchers in recent years. In this paper, electro-flocculation has been used for the harvesting and recovery *Dunalila Salina* microalgae from the culture medium. The effect of current intensity, time for electro-flocculation, electrode gap, stirring speed and electrode material on harvesting and recovery microalgae was investigated in batch test, and the modeling of microalgae recovery process was conducted by response surface methodology with combining categorical and numeric factors based on the D-optimal design. The modified quadratic model was used to fit the microalgae recovery efficiency data obtained from each batch test. The coefficients of determination ( $R^2$ ), adjusted and predicted were more than 0.98, 0.96 and 0.90 respectively, which indicated that the modified quadratic model could describe the microalgae recovery efficiency in the batch tests of this study successfully. The results indicated that the linear effect of independent variable on the recovery efficiency is very statistically significant. Moreover with increasing the electric current intensity and time for electro-flocculation, or reduce the distance between the electrodes, the recovery efficiency has increased significantly. Also by increasing stirrer speed from 0 to 200 rpm the amount of recovery efficiency is increased, and by increasing stirrer speed from 200 to 400 rpm the amount of recovery efficiency has decreased. The results showed that aluminum electrodes on the recovery of microalgae from the culture medium are more efficient than iron electrodes.

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**Introduction**

Today’s energy system is unsustainable because of equity issues as well as environmental, economic and geopolitical concerns that have implications far into the future. Bioenergy is one of the most important components to mitigate greenhouse gas emissions and substitute for fossil fuels (Goldemberg and Johansson, 2004; Dincer, 2008). Renewable energy is one of the most efficient ways to achieve sustainable development.

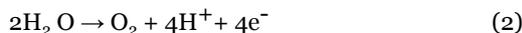
Biomass energy, as a green and renewable resource, has been considered to be one of the best ways to solve the global energy crisis (Chisti, 2007; Lei *et al.*, 2012). Microalgae is an economical and potential raw material of biomass energy (Pereira *et al.*, 2011), because it does not require a large area of land for cultivation, exhibits short growth period, possesses a high growth rate and contains more high-lipid materials than food crops (Weyer *et al.*, 2009). In general, an algae biomass production system includes growing microalgae in an environment that favors accumulation of target metabolites and recovery of the microalgae biomass for downstream processing (Cheng *et al.*, 2011). However, due to the small size (5~50 μm), negative surface charge (about -7.5~-40 mV) and low biomass concentration (0.5~5 g/L) of microalgae cells, harvesting microalgae biomass from growth medium is a challenge (Garzon-Sanabria *et al.*, 2012), which accounts for more than 30% of the total production cost from algae to biodiesel (Horiuchi, 2003). Therefore, it is necessary to develop effective and economic technologies for harvesting process. There are currently several harvesting methods, including mechanical, electrical, biological and chemical based. In mechanical based methods, microalgae cells are harvested by mechanical external forces, such as centrifugation, filtration, sedimentation, dissolved air flotation and usage of attached algae biofilms and ultrafiltration membranes (Valdivia-Lefort, 2011). Microalgae can easily be flocculated using metal coagulants such as Fe<sup>3+</sup> or Al<sup>3+</sup> salts (Ahmad *et al.*, 2006; Bernhardt and Clasen, 1991; Papazi *et al.*, 2009). In wastewater treatment,

electrocoagulation (EC) has been proposed as an alternative for chemical coagulants (Mollah *et al.*, 2001). In EC, iron or aluminum ions are released from a sacrificial anode through electrolytic oxidation. Compared to coagulation with Fe<sup>3+</sup> or Al<sup>3+</sup> salts, EC has the advantage that no anions such as chlorine and sulphate are introduced in the process water. The electrolytic oxidation of the sacrificial anode, however, requires electricity. Some studies have investigated the use of EC for removal of microalgae from drinking or wastewater (Alfajara *et al.*, 2002; Azarian *et al.*, 2007; Gao *et al.*, 2010).

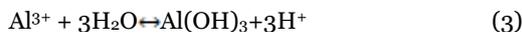
The main reactions occurring in EC process with different electrode materials are as follows (Vasudevan *et al.*, 2008 and Gao *et al.*, 2010):

When aluminum is used as electrode material,

Anode:



In solution:



Cathode:

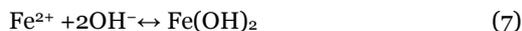


When iron is used as electrode material,

Anode:

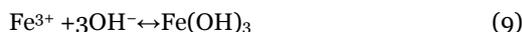
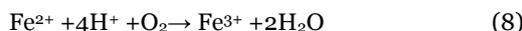


In solution:



If dissolved oxygen is presented in the solution, the reaction would be:

In solution:



Cathode:



The main objective of this work was to evaluate and mathematical modeling of the recovery efficiency of the *Dunaliella salina* microalgae by electro-flocculation (EF) for further biodiesel production. The effect of different electro-flocculation operation parameters were evaluated, such as: current density, current applied time, electrode gap, stirring speed and material of the electrodes.

## Materials and methods

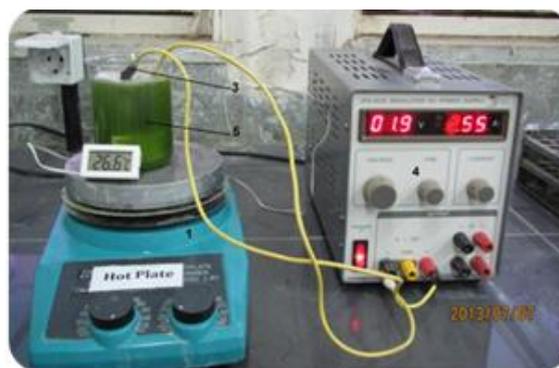
### *Microalgae strain, Medium and Culture*

*Dunaliella salina* was used for this electro-flocculation experiment. The starting culture was obtained from the collection of microalgae in Biotechnology Research Center, Tabriz. For the cultivation of *Dunaliella salina* was prepared Johnson medium in sterile conditions (Johnson *et al.*, 1968). The culture medium was included: NaCl, MgCl<sub>2</sub>.6H<sub>2</sub>O, CaCl<sub>2</sub>.2H<sub>2</sub>O, Na<sub>2</sub>SO<sub>4</sub>, SO<sub>4</sub> K<sub>2</sub>, Tris-Base, KNO<sub>3</sub> (1M/L), CoCl<sub>2</sub>.6H<sub>2</sub>O, MnCl<sub>2</sub>.4H<sub>2</sub>O, NaMoO<sub>4</sub>.2H<sub>2</sub>O, Na<sub>2</sub>EDTA, FeCl<sub>3</sub>.6H<sub>2</sub>O, CuSO<sub>4</sub>.7H<sub>2</sub>O, ZnSO<sub>4</sub>.7H<sub>2</sub>O, KH<sub>2</sub>PO<sub>4</sub> (100mM). Primary cultivation was performed in 250 ml Erlenmeyer flask with 10% of Stock microalgae in-semination. From one germinator device (Grouc Company Model, 400 GC, Iran) which is equipped with light, temperature, humidity and ventilation control systems was used as the culture room. The optimum temperature for cultivation was adjusted, 25°C; pH, 7.5; Light intensity, 3500 Lux (light intensity was measured by a light meter pro, model of TES 1339). For measuring electrical conductivity was used the EC meter (Metrohm, 712). During the cultivation, aeration was designed as continuously by an air pump and for drawing the growth chart, counting the frequency of cells by Hemacytometer (Lam Neubauer) and optical density by spectrophotometer (JENWAY, 6305) at a wavelength 550 and 680 nm on a daily basis was measured.

### *The Electro-flocculation system*

As shown Fig. 1, the experiments were carried out in a batch reactor with an effective volume of 250 mm which is made of Pyrex glass. Two electrodes with

dimensions of 5×5 cm and a surface area of 25 cm<sup>2</sup> with distance 2 cm from bottom of the reactor in vertically state and in different stages were placed inside the reactor with distance of 1, 2 and 3 cm. The electrode material were used from the aluminum electrodes with 99.5% purity (Al-1050) and iron electrodes (ST 37-2). The impurities level in the surface of the electrodes before using in the reactor is cleaned by sanding, and then the electrodes were put for 15 min in a solution of dilute hydrochloric acid (15 wt%), then were washed with distilled water after brushing, and were dried in the oven-device. The Voltage and required current in the reactor were provided with a digital DC power supply (Afzar Azma, Model JPS-403D). Order to establish a uniform mixture within the reactor, was used from a magnetic stirrer.



**Fig. 1.** Picture of experimental set-up. (1) magnetic stirrer; (2) magnetic; (3) anode and cathode electrodes; (4) DC Power supply; (5) microalgae culture.

### *Experimental design and procedure*

Response surface methodology with combining categorical and numeric factors based on the D-optimal design was used to design the experiment in this study.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques that are useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response. The purpose of applying this method is variance analysis model and the optimization of the

response (response variable). The first step is finding a suitable approximation function between a set of independent and response variables. This approximation function is usually a polynomial function of independent variables, such as which is used in the Eq. (11) (Montgomery, 2001).

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i < j} \beta_{ij} x_i x_j + \epsilon \quad (11)$$

where  $y$  represents the value of response;  $\beta_0, \beta_i, \beta_{ii}, \beta_{ij}$  regression coefficients;  $k$  the number of independent variables; and  $\epsilon$  the amount of errors. In the response surface method in order to estimate the parameters of the function, the independent variables must be have coded values these can be coded with Eq. (12) (Montgomery, 2001).

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (12)$$

Where  $x_i$  is the coded value of the  $i$ th test variable,  $X_i$  is the actual value of the  $i$ th independent variable,  $X_0$  is the actual center point value of the  $i$ th independent variable, and  $\Delta X_i$  is the step size of the  $i$ th independent variable.

In this study, Recovery efficiency was chosen as the response variable, while Current intensity ( $X_1$ ), the electrode gap ( $X_2$ ), time for electro-flocculation ( $X_3$ ), stirring speed ( $X_4$ ), material of the electrodes ( $X_5$ ) were chosen as five independent variables.

With the growth of microalgae, the cultivation concentrations are increased and after 10 days the logarithmic growths of microalgae have reached a maximum and after this microalgae growth stage were stopped and their density is practically remained constant and harvesting time was started. At this stage by counting the number of cells by Lam Neubauer and measuring optical density by spectrophotometer device was determined the initial concentration rate in the culture medium based on cells/L. Then the culture medium was transferred into the reactor.

According to the experimental design levels in Table 1, batch tests were conducted in 250 mL glass bottles.

At the end of tests time, was given 30 minutes for the sedimentation of samples and then counting the number of cells was performed by Lam-Neubauer and optical density of by spectrophotometer device. The recovery efficiency was subsequently calculated as (Gao et al., 2010):

$$\text{Microalgae recovery efficiency (\%)} \eta_a = \frac{C_0 - C_t}{C_0} \times 100 \quad (13)$$

Where  $C_0$  is the cell density of microalgae (cell/L) in the initial culture medium, and  $C_t$  is cell density of microalgae (cell/L) after the electro-flocculation.

In this study, for designing an experiment, statistical analysis and modeling was used from the software Design-Expert 8.

**Table 1.** Independent variables and experimental design Levels

Independent variables	Unit	Level	
		-1	+1
Current intensity	mA	300	1000
Electrode gap	cm	1	3
Time	min	5	20
Stirring speed	rpm	0	400
Electrode material	material	Aluminum	Iron

## Results and discussion

### Model development and validation

In order to obtain empirical models for prediction the response, linear equations, interactive, second and third-degree polynomial to the obtained data from the experiments were fitted. Then these models are analyzed statistically to select an appropriate model. Table 2 represents the summary results of statistical analysis models and lack of fit tests, to select an appropriate model in studying the effect of independent variables (current intensity, electrode gap, time, stirring speed, the electrode type) on the recovery efficiency of microalgae in the electric flocculation method. Statistically it is appropriate model the lack of fit test is not significant and has the highest amount of determined, adjusted and predicted coefficients. The test results showed that lack of fit test of quadratic polynomial is not

significant and because of having determined, adjusted and predicted coefficients respectively have more than 98, 96 and 90% power to fit the data. The coefficient of determination as the most commonly factor to well fit is used and is measured the ratio or

percentage of the total changes in the response variable which is explained by the regression model. Thus, this was found suitable for predicting behavior of Response quadratic model.

**Table 2.** Lack of fit tests and model Summary Statistics for selection of an appropriate model in studying the effect of five independent variables on recovery efficiency in EF.

Source	df	Mean Square	F Value	p-value Prob > F	R-Squared	Adjusted R-Squared	Predicted R-Squared
Linear	26	74.8069	9.61803	0.0007	0.859	0.8388	0.8019
2FI	16	27.81036	3.575618	0.0289	0.964	0.9423	0.9073
<u>Quadratic</u>	<u>12</u>	<u>15.61737</u>	<u>2.007947</u>	<u>0.1506*</u>	<u>0.982</u>	<u>0.9657</u>	<u>0.9066</u>
Cubic	0				0.9951	0.9782	
Pure Error	9	7.777778					

\*Suggested

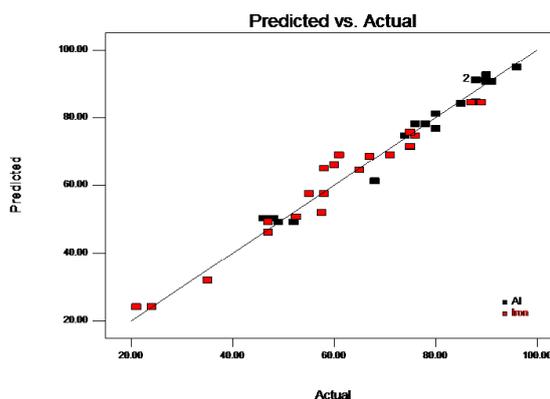
Table 3 indicates the result summarizes of the analysis of variance quadratic reduced response surface model for recovery efficiency. As can be seen after eliminating insignificant phrases, the fitted model to efficient recovery is very significant ( $p < 0.01$ ), whereas there is no significant relation for lack of fit test ( $p > 0.05$ ) and the coefficient of determination, adjusted and predicted respectively 97, 96 and 94 have been modified. In other words, 97% of the variation of recovery efficiency was explained by the modified model. In this study, the recovery efficiency was estimated by using empirical equations 3, then by applying statistical response surface method, by using recovery efficiency equation of the experimental data, based on the Eq. (14) to be predicted.

$$y = 74.3 + 11.61x_1 - 3.71x_2 + 9.98x_3 + 2.24x_4 - 8.18x_5 - 6.50x_1x_2 + 2.65x_1x_3 - 1.99x_2x_3 - 3.93x_2^2 - 3.49x_4^2 \quad (14)$$

where  $x_1$ ,  $x_2$ ,  $x_3$ ,  $x_4$  and  $x_5$  respectively are coded values of current intensity (mA), distance between the electrodes (cm), time (min), and speed of stirring (rpm) and the electrode type.

Comparing the observed values (actual) with predicted values in Fig. 2, and these observations has been mentioned a very good correlation between the obtained results with experimental method in

laboratory and the predicted values with the statistical method, is according to Eq.(14).



**Fig. 2.** Comparing the predicted values with actual (observed)

*Influence of variables on the recovery efficiency*

Table 3 summarizes the analysis of variance (ANOVA) of quadratic decrease model showed that the linear effect of two-independent variables, current intensity, reaction time, electrode gap, stirring speed and electrode material on the recovery efficiency is very significant.

**Table 3.** ANOVA of the fitting model for Microalgae recovery efficiency.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	13875.51	10	1387.55	101.04	< 0.0001
x <sub>1</sub> -Current	4238.5	1	4238.5	308.66	< 0.0001
x <sub>2</sub> B-Gap electrode	408.14	1	408.14	29.72	< 0.0001
X <sub>3</sub> -Time	2926.59	1	2926.59	213.12	< 0.0001
X <sub>4</sub> -Stirrer	159.38	1	159.38	11.61	0.0019
X <sub>5</sub> -Electrode Material	2627.33	1	2627.33	191.33	< 0.0001
x <sub>1</sub> x <sub>3</sub>	1041.06	1	1041.06	75.81	< 0.0001
x <sub>1</sub> x <sub>5</sub>	219.31	1	219.31	15.97	0.0004
x <sub>2</sub> x <sub>3</sub>	100.06	1	100.06	7.29	0.0113
x <sub>2</sub> <sup>2</sup>	87.19	1	87.19	6.35	0.0173
x <sub>4</sub> <sup>2</sup>	51.22	1	51.22	3.73	0.0629
Residual	411.96	30	13.73		
Lack of Fit	341.96	21	16.28	2.09	0.1266
Pure Error	70	9	7.78		
Cor Total	14287.48	40			
C.V. %	5.45				
R-Squared	0.9712				
Adj R-Squared	0.9616				
Pred R-Squared	0.9469				

Considering that based on Faraday's law current intensity and the operation time have same effect on process of coagulation, so in this research was tried to evaluate the results of these two factors together. The coefficients of these two variables at the Eq. (14) showed that by increasing the current intensity and coagulation time, the recovery efficiency has improved (Fig. 3A, Fig. 4A). Also table 3 revealed that the summaries of the analysis of variance in the reduced quadratic response surface model, There is interactive effect between the current intensity and duration of the coagulation on the recovery efficiency and it is in the significant level ( $p < 0.01$ ), in other words the simple Effect of current intensity at different times is not the same response Thus, between the current intensity and the duration of coagulation it is possible the existence of interactive Effect. The ratio of active effect between current intensity and the duration of coagulation indicated based on the Eq. (14), that interactive effect between these two factors has a negative effect on the recovery efficiency. Fig. 3A and Fig. 4A, indicated that the interactive effect between the current intensity variable and period of coagulation on the recovery efficiency. Considering the significant linear effects

quadratic and interaction between current intensity and coagulation time was expected to be curvature on the graph balance and Response procedures. This issue can be attributed to Faraday's law, based on this law by increasing the intensity of the applied current or of electrolysis time, caused increasing corroded ions from sacrificial anode electrode surface, As a result, the concentration of aluminum and iron hydroxide which are produced in the culture medium are increasing as an electrochemical cell. with increasing concentration of aluminum ions in the process, will be increase the surface area of coagulation and the number of the coagulation active places (Zhu *et al.*, 2005) and ion-charged algae particles, depend on the ions interaction which are produced by the dissolution of anode electrode will be neutralize and by absorbing each other, it means that action of coagulation, are separated from the solution. Also hydrogen gas will be free surrounding the cathode electrode, makes some clots floating on the surface of the solute and recovery them. Similar reports is published in this issue by (Kim *et al.* 2012; Vandamme *et al.* 2011; Gao *et al.* 2010).

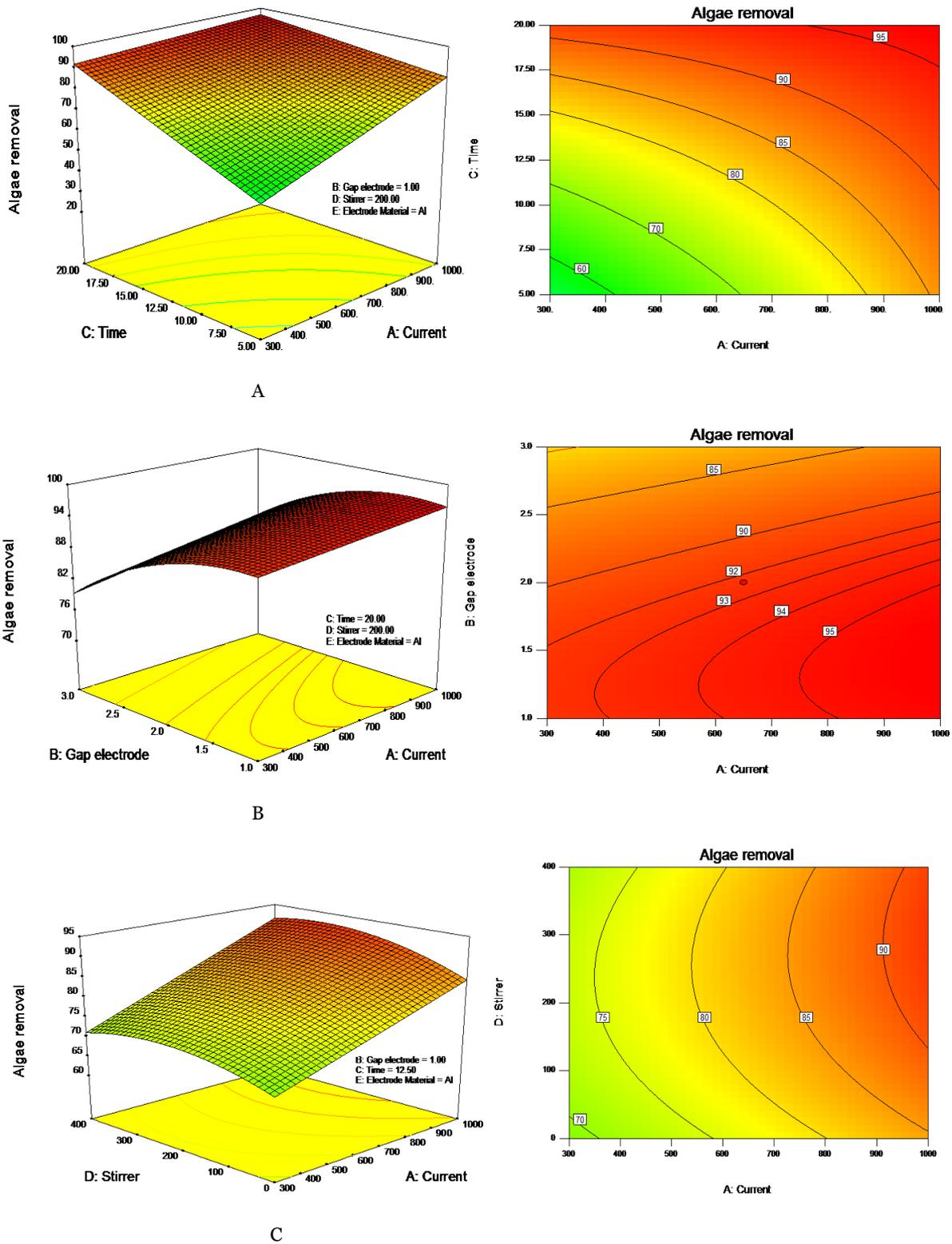


Fig. 3. Response surface plot and corresponding contour plot recovery of microalgae with Aluminum electrodes.

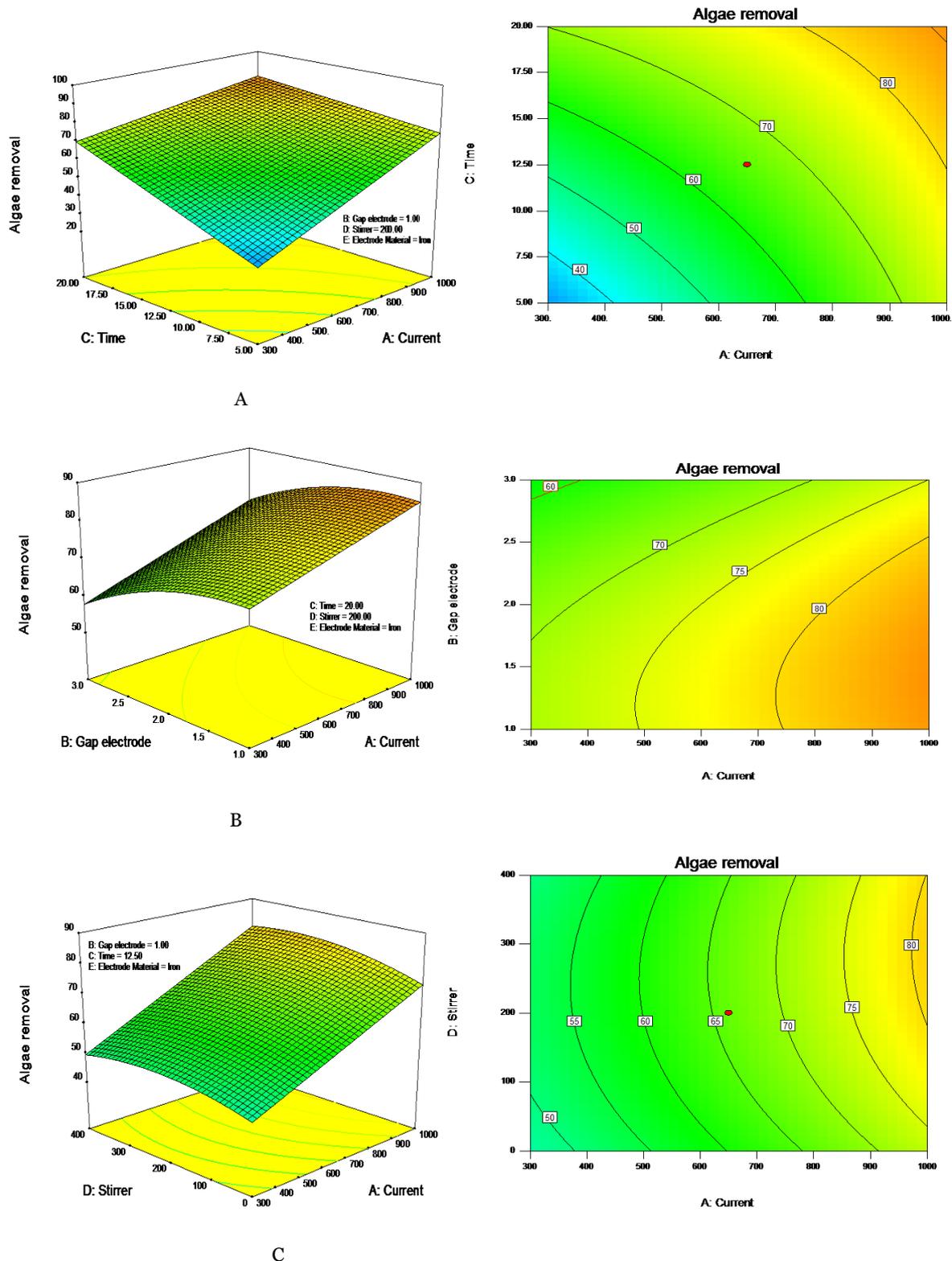


Fig. 4. Response surface plot and corresponding contour plot recovery of microalgae with Iron electrodes

Also the coefficients of the independent variables of Eq. (14) be inferred that the electrode distance has positive linear effect and negative quadratic effects on the recovery efficiency (Fig. 3B, Fig. 4B). Previous

research results on electric coagulation for other applications also indicates that in the electrochemical process and in the same time, when the distance between the electrodes is increased the coagulation

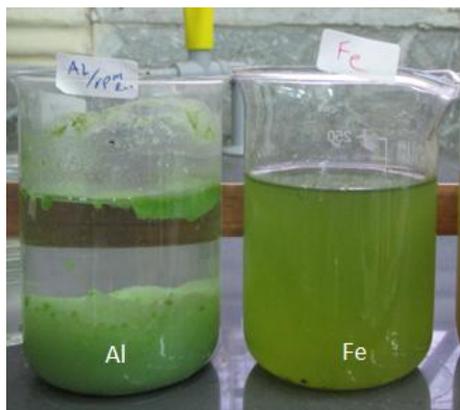
efficiency is reduced (Kim *et al.*, 2002). This may be due to very small displacements of ions which are formed during the electrolysis process (Nanseau-njiki *et al.*, 2009).

The coefficients of the independent variables in Eq. (14) will be inferred that linear of stirring rate is positive and negative quadratic effects are on the recovery efficiency. Considering the significant linear effects, quadratic of this variable, were expected the existence of curvature in the graph surface and the balance (Fig. 3C., Fig. 4C). The results are consistent with the research report of (Canizares *et al.*, 2006; Mollah *et al.*, 2011; Vandamme *et al.*, 2011).

Electrode type for electric-flocculation process is an extremely vital factor. So we can say that the heart of the electrical flocculation system is electrode type. Aluminum and iron are both widely have been used by many researchers as electrodes in other applied industries such as water and wastewater treatment and wastewater management and removing the pollutants (Alaton *et al.* 2008; Zaied and Bellakhal 2009). However, there is very little information about comparing electrode type in the recovery of microalgae. In this study were made comparison under equal conditions between aluminum and iron electrodes. Table 3 summarizes the analysis of reduced variance quadratic response surface model, is showed that linear effect of electrode type on the recovery efficiency is very meaningful.

In all EF experiments, Microalgae recovery efficiency increased with time following. This observation is in accordance with a model in which metal ions such as  $Al^{3+}$  or  $Fe^{2+}/Fe^{3+}$  are continuously released from the anode during the ECF treatment. These aluminum and iron ions react with water to form metal hydroxides (Duan and Gregory, 1996). Positively charged soluble metal hydroxides bind to the negative surface of the microalgae cells and destabilize the microalgae suspension by charge neutralization. Insoluble metal hydroxides can destabilize the microalgae suspension through a mechanism known

as sweeping flocculation, resulting in enmeshment of microalgae and insoluble precipitates (Duan and Gregory, 2003). For both mechanisms, the inflection point of the sigmoidal curve corresponds to the time required to produce a sufficient amount of aluminum or iron hydroxides to destabilize the microalgae dispersion (Mollah *et al.*, 2001, 2004). Iron electrodes were observed to be less efficient as compared with aluminum electrodes, as demonstrated by the difference between the removal efficiencies (Fig. 3, Fig. 4). The reason for this could be due to differences in material structure of aluminum and iron, and probably due to the much higher current efficiency generated by aluminum electrodes than that by iron electrodes. It is also possible under the pH experiment 7.5; the amount of aluminum hydroxide was much higher than that of ferric/ferrous hydroxide, which may be another reason accounting for the better recovery of microalgae with aluminum electrodes (Duan and Gregory, 2003). Similar reports by (Vandamme *et al.*, 2011; Zongo *et al.*, 2009; Caizares *et al.*, 2005; Gao *et al.*, 2010) has been established in other applied industries that are consistent with the results of this research. In addition, in the case of aluminum electrodes, it was observed that a layer of greenish flocs floated at the surface of water, which might be composed of aluminum hydroxide and algae cells. While in the case of iron electrodes, the water in the whole ECF reactor only appeared yellowish green at the beginning, and turned reddish-brown gradually, due to the presence of Fe (II) and Fe (III) species (Fig. 5) After 20 min of settlement, tiny flocs produced by iron electrodes still remained in the water. Overall, aluminum was considered as the better one for the microalgae recovery.



**Fig. 5.** Comparison of the effect of two types of electrode aluminum and iron on microalgae separation in the same test conditions.

### Conclusion

Effect of current intensity, distance between electrodes, time of electro-flocculation duration, stirring speed and electrode material on harvesting and recovery microalgae was investigated in batch test, and the modeling of microalgae recovery process was conducted by response surface methodology with combining categorical and numeric factors based on the D- optimal design. The modified quadratic model was used to fit the microalgae recovery efficiency data obtained from each batch test. The experimental data and the predicted data by the models was highly correlated ( $R^2 > 0.98$ ). The results indicated that the linear effect of independent variable on recovery efficiency is very statistically significant ( $p < 0.01$ ), as with increasing the electric current intensity and time of electro-flocculation, or reduce the distance between the electrodes, the recovery efficiency has increased significantly. Also by increasing stirring from 0 to 200 rpm the amount of recovery efficiency is increased and the recovery efficiency round has decreased from 200 to 400 rounds. Although both aluminum and iron anodes achieved destabilization of the microalgae suspensions, aluminum anodes proved to be more efficient. During electro-flocculation,  $Al^{3+}$  and  $Fe^{2+}$  are released from the sacrificial anode and form metal hydroxides in the solution. Destabilization of the microalgae suspension was probably achieved through a combination of charge neutralization by positively

charged metal hydroxides and sweeping coagulation–flocculation by insoluble metal hydroxides. The results showed that aluminum electrodes on the recovery of microalgae from the culture medium are more efficient than iron electrodes.

### Acknowledgment

This paper is a fruit of the research plan called “Optimization and development of the most appropriate of harvest and recovery process of biomass from the culture medium in production cycle of microalgae-to-biodiesel” is performed in the Department of Agricultural Mechanization, Science and Research Branch, Islamic Azad University, Tehran, Iran.

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