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Studies on treatment of egg processing industry wastewater using electrocoagulation method: optimization using response surface methodology

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ABSTRACT

The present research analyzes the recovery of protein from egg industry wastewater using electrocoagulation technique under different operating conditions such as current density, initial pH, feed flow rate, and electrode distance. Initial pH value of 6, current density of 25 mA/cm^2, electrode distance of 4.5 cm, and feed flow rate of 60 ml/min were found to be optimal for the maximum protein recovery (89%) with electrical energy consumption of 7.5 kWh/m^3. A Box–Behnken response surface design has been used to evaluate the individual and interactive effects of process conditions on the protein recovery. Recovered protein was analyzed to determine the digestibility of protein and its characteristics were studied using Fourier transform infrared spectroscopy.

Keywords: Egg wastewater; Electrocoagulation; Mathematical modeling; Protein recovery; FT-IR

1. Introduction

During the process of egg powder production in egg processing industries, a large amount of wastewater is generated, which contains a large amount of proteins and some organic substances [1]. If it is discharged without any pre-treatments, the environment will be subjected to serious pollution. Moreover, the discharge of this wastewater will result in abundant loss of valuable substances [2]. Hence, there is an urgent need to design a suitable and low-cost separation method to recover protein from egg processing industry wastewater [3]. Conventionally, proteins were recovered from industrial wastewaters using various separation techniques such as foaming process [4], aqueous two-phase systems [5], heat treatment [6], and membrane separation [7]. But, these separation techniques has disadvantages such as long retention time, higher operating cost, and lower amount of protein recovery.

Nowadays electrocoagulation (EC) has emerged as efficient technique to treat various wastewaters in wastewater treatment [8]. Advantages of EC separation method includes relatively low cost, a compact byproduct recovery, and the possibility of automatic control [9]. EC process is the electrochemical production of destabilization agents, which are produced at the anode and react with the hydroxide ions produced at the cathode to form the metal hydroxides [10]. This metal hydroxides reacts with the organic matters or colloid solids in the wastewater, thus recovery of

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byproducts were obtained (Eqs. (1)–(3)). The electrocoagulation reaction mechanisms are as follows [11]:

$$\text{At anode: } M \rightarrow M^{n+} + ne^- \quad (1)$$

$$\text{At cathode: } nH_2O + ne^- \rightarrow \frac{n}{2}H_2 + nOH^- \quad (2)$$

where M = anode material and n = number of electrons involved in the oxidation or reduction reaction.

$$Mn^+ + nOH^- \rightarrow M(OH)_n \quad (3)$$

In recent years, electrocoagulation method has been applied for the treatment of different industrial wastewaters such as olive mill, distillery industry, dairy industry, pulp and paper industry textile industry, poultry slaughterhouse, and yeast industry. However, there is no research finding was reported regarding the recovery of protein from egg industry wastewater using EC separation method. Thus, the main objective of this study was to find out the effect of process parameters such as initial current density, pH, electrode distance, and feed flow rate to recover the valuable byproduct namely protein from egg processing industry wastewater. Efficiency of EC method was investigated with respect to protein recovery and electrical energy consumption. Then the recovered sludge protein has been investigated to use an animal feed using protein digestibility test and Fourier transform infrared spectroscopy (FT-IR) analysis [12]. Response surface methodology (RSM) coupled with Box–Behnken response surface design (BBD) was used to study the individual and interactive effect of process variables on protein recovery process.

2. Materials and methods

2.1. Raw wastewater and chemicals

The egg processing wastewater sample was collected from the egg processing industry sited near Erode, TamilNadu, India and were stored at 4˚C. The characteristics of wastewater were found to be (American Public Health Association (APHA) method): initial pH of 5.5, protein value of 2,785 mg/l, and COD of 4,000 O_2/l, respectively. Chemicals (HCl and NaOH) used in this experiment were analytical grade and bought from local dealers from Erode, Tamil Nadu.

2.2. Experimental setup of electrocoagulation process

Schematic diagram of the electrocoagulation (EC) reactor was presented in Fig. 1. A working volume of 3,000 ml EC reactor was fabricated and used to separate the protein from egg wastewater. Two stainless steel plates with actual surface area of 108 cm^2 each were used as electrodes and the entire electrode assembly was fixed on non-conducting wedges. Temperature was adjusted by automatic controller. Then the setup was connected to DC power supply (DGER-521, India) instrument and desired current density was adjusted. In each test, 1,600 ml of wastewater was used in the reactor and all the experiments were carried out at continuous stirring at 250 rpm. Peristaltic pump (Camon 562) was used to adjust the inlet flow rate of the wastewater and external stirrer was used to agitate the wastewater.

2.3. Analytical method

The initial pH and COD analysis were performed by standard techniques explained by APHA. Open reflux method was used to measure the COD. Protein content in egg wastewater was determined using bovine serum albumin as the standard [13]. Digestibility of recovered sludge protein was analyzed using method described in elsewhere [14]. FT-IR spectra of recovered sludge protein were recorded with a FT-IR spectrometer (Instrument model RX_1, India) in the range of 4,000–400 cm^{-1} using potassium bromide (KBr) pellets [15].

Fig. 1. Schematic diagram of the electrocoagulation (EC) reactors.

2.4. BBD response surface design

In the current study, RSM coupled with BBD was used to find out the individual and interactive effects of process parameters on the protein recovery and electrical energy consumption via Design-Expert 8.0.7.1 (State-Ease Inc., USA) statistical software. Initial pH (*A*), current density (*B*), electrode distance (*C*), and feed flow rate (*D*) were chosen as independent factors, while protein recovery (Y_1) and electrical energy consumption (Y_2) were selected as response functions. Experimental runs were started based on a BBD and the whole design involves of 29 experimental runs (Table 1) with five center points. Then the BBD experimental result was analyzed by multiple regression analysis (sequential sum of squares and model summary statistics) in order to examine the competence of different mathematical models (linear, interactive (2FI), quadratic, and cubic) to describe the protein recovery process efficiently. Regression coefficients of these various models and their effects were analyzed by Pareto analysis of variance (ANOVA) with the help of the *F*-values at probability levels ($p \leq 0.05$). The results were analyzed using different statistical analysis such as determination coefficient (R^2), adjusted determination of coefficient (R^2_a), predicted determination of coefficient (R^2_p), adequate precision (AP), and coefficient of variation (CV) to emulate the statistical significance of the developed polynomial equation. Three-dimensional (3D) response surface graph was created from developed mathematical model to analyze the individual and interactive effects of process factors on response. The detailed methodology used in this study was reported in elsewhere [16].

3. Results and discussions

3.1. Effect of initial pH

Initial pH of wastewater is the crucial parameter in protein recovery process. In order to investigate the

Table 1
BBD experimental design with results

S. no.	Initial pH	Current density	Electrode distance	Feed flow rate	Protein recovery	EEC
1	8	30	5	60	69.24	13.4
2	4	30	5	60	49.58	13.1
3	6	30	5	40	89.52	19.1
4	4	20	6	60	35.48	8.8
5	6	20	5	60	87.25	7.8
6	6	30	5	80	69.25	8.7
7	6	20	5	60	87.25	7.8
8	6	20	4	80	54.29	5.3
9	6	10	6	60	49.35	3.01
10	4	10	5	60	34.25	3.3
11	6	30	4	60	89.65	12.5
12	6	10	5	80	49.35	2.6
13	6	30	6	60	48.75	10.02
14	6	20	5	60	87.25	8.5
15	8	10	5	60	54.21	4.1
16	6	20	6	80	44.65	6.2
17	8	20	5	80	55.86	6.5
18	8	20	4	60	69.81	8.5
19	8	20	6	60	34.28	8.9
20	6	10	4	60	49.68	2.9
21	6	20	4	40	83.52	10.6
22	6	20	5	60	87.25	7.2
23	4	20	4	60	34.98	7.1
24	6	10	5	40	69.25	5.4
25	8	20	5	40	59.39	13
26	6	20	6	40	64.58	11.5
27	4	20	5	40	59.81	13.4
28	4	20	5	80	24.58	6.06
29	6	20	5	60	87.25	7.6

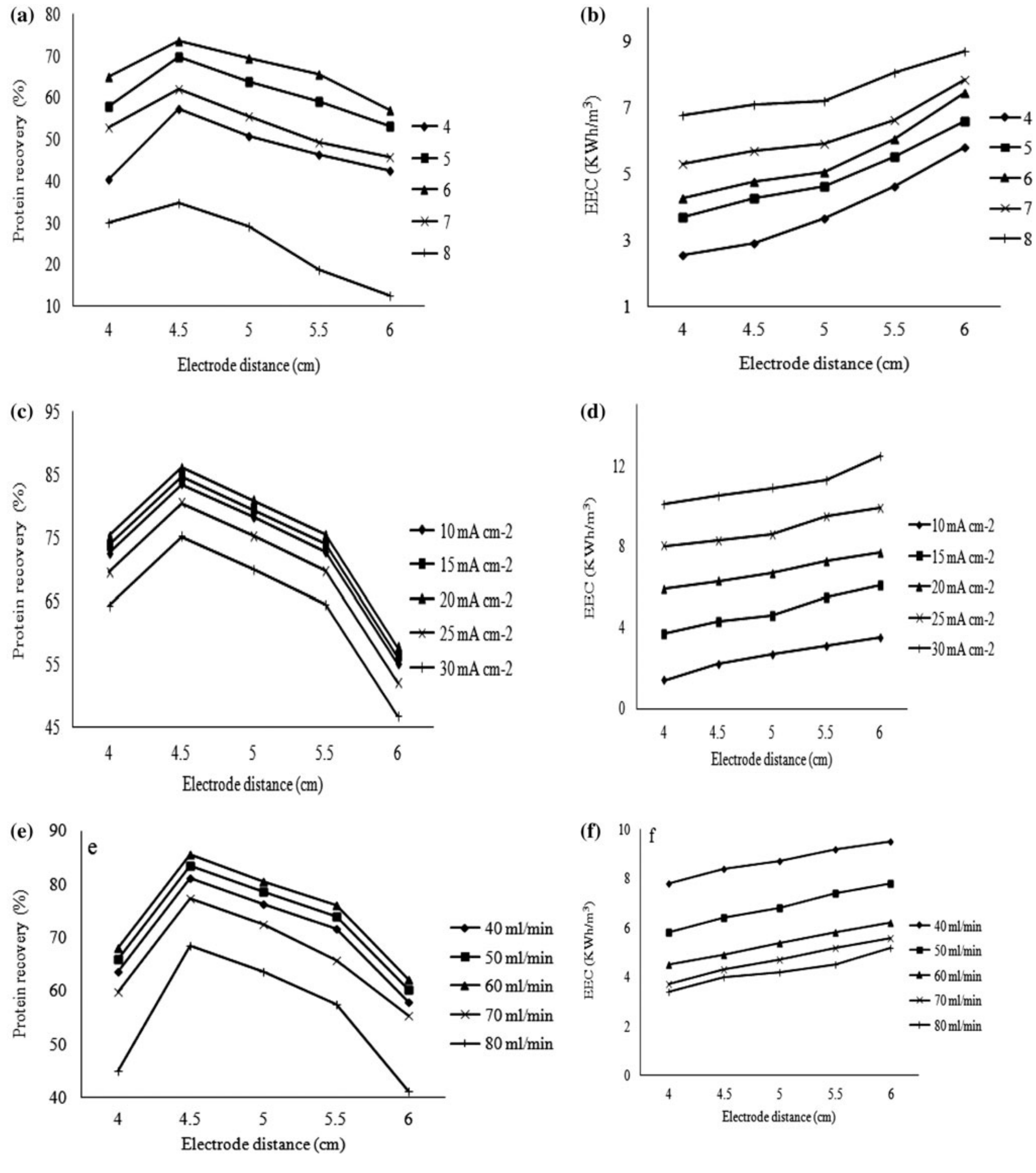

Fig. 2. Effect of process variables on protein recovery (a, c, e) and electrical energy consumption (b, d, f).

effect of pH, experiments were conducted in various pH values (4, 5, 6, 7, and 8) with constant conditions as follows: current density of 20 mA/cm^2 and feed flow rate of 60 ml/min. The results presented in Fig. 2(a) and (b). It is depicted that protein recovery and electrical energy consumption raised with higher

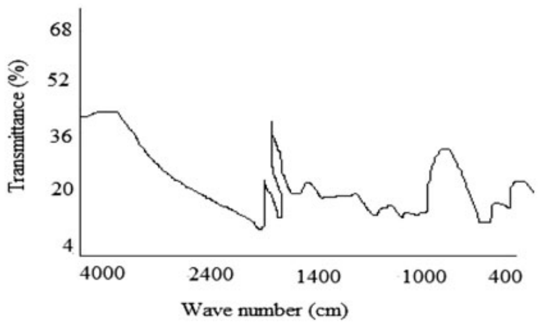

Fig. 3. FT-IR spectrum of recovered sludge protein.

initial pH values. This result revealed that the acidic pH affects the coagulation mechanism and leads to the formation of effective metal hydroxide species. This has the strong affinity towards organic matters present in the wastewater [17]. However, beyond pH of 6 shows negative effect on protein recovery and electrical energy consumption. Therefore, considering protein recovery and economic viability, pH of 6 was selected as an optimal for further experiments.

3.2. Effect of current density

Current density is an important factor which affects the recovery of protein from egg industry wastewater using EC technique. In order to evaluate the effect of current density on protein recovery, various current densities (10, 15, 20, 25, and 30 mA/cm^2) were investigated with constant conditions as follows: pH of 6 and feed flow rate of 60 ml/min. From the results (Fig. 2(c) and (d)), it is found that protein recovery is increased with increasing current density up to 20 mA/cm^2. This behavior can be explained by the fact that the higher current density produces the large number of metal hydroxides, which enhances the protein recovery [18]. However, beyond 20 mA/cm^2 of current density shows negative effect on protein recovery. This is due to the fact that higher current produces rise in temperature, thus treatment efficiency decreased. Whereas, electrical energy consumption is raised with higher current density during the experiments. Therefore, taking into account both of energy consumed as well as protein recovery, a current density of 20 mA/cm^2 and pH of 6 was considered as an optimal for further experiments.

3.3. Effect of electrode distance

Electrode distance affects the amount of electrical energy lead into the EC reactor which significantly affects the protein recovery process. To find out its effect, different electrode distances (4, 4.5, 5, 5.5, and 6 cm) were investigated and the results were presented in Fig. 2(c) and (d). The result shows that higher protein recovery is obtained at the electrode distance of 4.5 cm. Moreover, increase in electrode distance shows increase in electrical energy consumption and reduction in protein recovery. This can explained by the fact that short distance between each electrode requires lesser electrical energy for motion of ions due to shorter travel path that reduce the resistance of motion. But the condition was inverse for the case of large distance between each electrode and decreased the protein recovery [19]. Considering protein recovery and electrical energy consumption; an electrode distance of 4.5 cm, pH of 6 and current density of 20 mA/cm^2 were considered as an optimal for further experiments.

3.4. Effect of feed flow rate

Feed flow rate is one of the critical variables which affect the protein recovery process significantly. For studying the effect of the feed flow rate, experiments were performed with the different feed flow rates (40, 50, 60, 70, and 80 ml/min) with constant conditions as follows: pH of 6 and current density of 25 mA/cm^2. Results were shown in Fig. 2(e) and (f) and it indicate the protein recovery higher than 80% under optimum feed flow rate of 60 ml/min. From the results, it is clearly known that increasing feed flow rate decreases the protein recovery as well as electrical energy consumption. This can described by the fact that increased feed flow rate leads to ineffective contact between organic matters present in the wastewater and the metal hydroxides produced in the electrochemical reactor [20]. Finally, feed flow rate of 60 ml/min, pH of 6, and electrode distance of 4.5 cm and current density of 25 mA/cm^2 were considered as an optimal experimental conditions. Under these conditions, 89% of protein recovery with electrical energy consumption of 7.5 kWh/m^3 was achieved.

3.5. Digestibility of recovered protein

Protein concentration in recovered solid was analyzed and it found to be 48%. Then, digestibility of recover protein (112%) is determined and compared with proteins derived from corn meal (54%). From the results, it was found that digestibility of recovered protein was considerably higher than corn meal protein. This can be explained by the fact that denaturation of proteins in recovered egg solid, making them

Table 2
Sequential model sum of squares for responses

Source	Sum of squares	df	Mean square	F-value	Prob. > F	Remarks
Sequential model sum of squares for protein recovery						
Mean	109,201.17	1.00	109,201.17			
Linear	4,192.95	4.00	1,048.24	3.68	0.0179	
2FI	1,008.92	6.00	168.15	0.52	0.7862	
Quadratic	5,746.00	4.00	1,436.50	243.45	<0.0001	Suggested
Cubic	60.78	8.00	7.60	2.09	0.1926	Aliased
Residual	21.83	6.00	3.64			
Total	120,231.64	29.00	4,145.92			
Sequential model sum of squares for electrical energy consumption						
Mean	2,034.33	1.00	2,034.33			
Linear	375.62	4.00	93.91	55.00	<0.0001	
2FI	16.78	6.00	2.80	2.08	0.1068	
Quadratic	16.80	4.00	4.20	7.94	0.0015	Suggested
Cubic	6.28	8.00	0.79	4.22	0.0481	Aliased
Residual	1.12	6.00	0.19			
Total	2,450.92	29.00	84.51			

Table 3
Model summary statistics for responses

Source	Std. dev.	R^2	Adjusted R^2	Predicted R^2	Press	Remarks
Model summary statistics for protein recovery						
Linear	16.8789	0.3801	0.2768	0.1788	9,058.3253	
2FI	17.9948	0.4716	0.1780	−0.0716	11820.7826	
Quadratic	2.4291	0.9925	0.9850	0.9569	475.8252	Suggested
Cubic	1.9073	0.9980	0.9908	0.7151	3,143.0700	Aliased
Model summary statistics for electrical energy consumption						
Linear	1.3066	0.9016	0.8853	0.8490	62.89	
2FI	1.1594	0.9419	0.9097	0.8215	74.34	
Quadratic	0.7271	0.9822	0.9645	0.9066	38.90	Suggested
Cubic	0.4315	0.9973	0.9875	0.9175	34.36	Aliased

more susceptible to enzymatic digestion, thus digestibility of recovered protein is increased [21].

3.6. FT-IR spectra analysis

To examine the functional groups present in the recovered protein, Fourier transform infrared spectroscopy (FTIR) was used and it was shown in Fig. 3. From Fig. 3, it is found that FTIR spectrum of recovered sludge has two prominent peaks at 1,650 cm^{-1} (Amide I) and 1,540 cm^{-1} (Amide II). This is mainly due to the fact that, C–O stretching vibration and the latter is attributed to the N–H bending as well as C–N stretching vibrations [22]. This result confirmed that the presence of secondary structure of protein functional groups in recovered sludge protein.

3.7. Mathematical modeling

RSM coupled with BBD was used to study the individual and interactive effect of process parameters on protein recovery process. According to BBD, 29 statically designed experimental runs were performed and their results were shown in Table 1. Then the BBD experimental data were analyzed by two different analysis namely the sequential model sum of

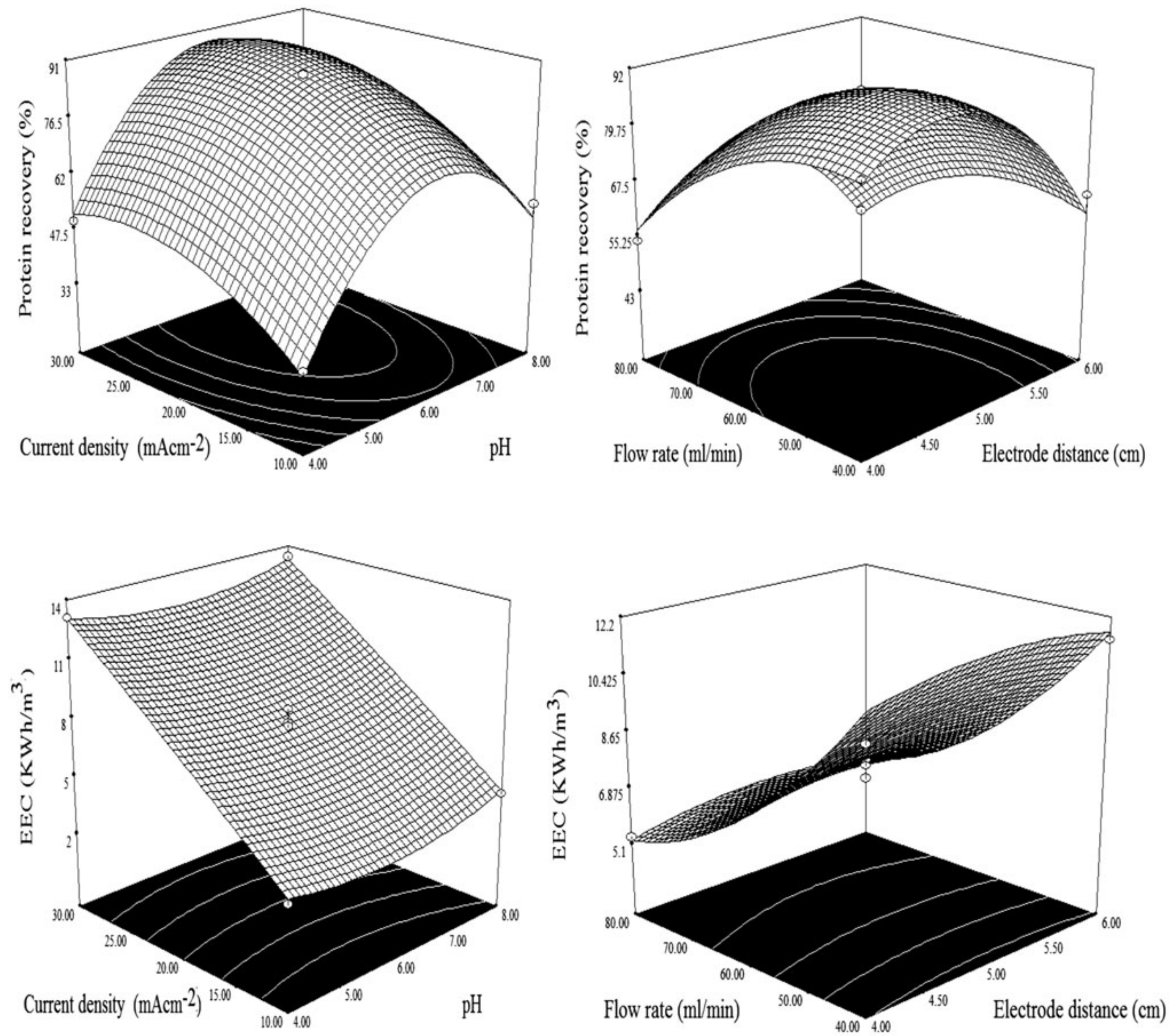

Fig. 4. 3D response surface plots representing the effect of process variables on responses.

squares and model summary statistics so as to choose effective mathematical model to explain the protein recovery process (Tables 2 and 3). From the results, it was found that, quadratic model shows high R^2, adjusted-R^2, predicted-R^2, F-value, and low p-value, when compared with other models (linear and interactive (2FI)). Cubic model was found to be aliased [23]. So that quadratic model was chosen to signify the effects of process parameters on the protein recovery process (Table 4). To study the interactive relationship between the responses and process factors, two empirical models were developed and final developed mathematical models obtained in terms of coded factors are given below:

$$\begin{aligned}\text{Protein recovery } (\%) = {} & 87.25 + 8.68A + 9.16B - 8.74C \\ & - 10.67D - 0.075AB - 9.01AC \\ & + 7.92AD - 10.14BC - 0.092BD \\ & + 2.33CD - 26.91A^2 - 9.34B^2 \\ & - 17.22C^2 - 9.09D^2\end{aligned} \tag{4}$$

Table 4
ANOVA results for responses

	Protein recovery				Electrical energy consumption			
Source	Sum of squares	Mean square	*F*-value	*p*-value	Sum of squares	Mean square	*F*-value	*p*-value
Model	10,947.87	781.99	132.53	<0.0001	409.19	29.23	55.29	<0.0001
A	903.24	903.24	153.08	<0.0001	0.58	0.58	1.10	0.3123
B	1,006.50	1,006.50	170.58	<0.0001	256.78	256.78	485.73	<0.0001
C	915.95	915.95	155.23	<0.0001	0.20	0.20	0.37	0.5533
D	1,367.25	1,367.25	231.71	<0.0001	118.06	118.06	223.33	<0.0001
AB	0.02	0.02	0.00	0.9516	0.06	0.06	0.12	0.7361
AC	324.54	324.54	55.00	<0.0001	0.42	0.42	0.80	0.3864
AD	251.22	251.22	42.58	<0.0001	0.18	0.18	0.33	0.5727
BC	411.48	411.48	69.74	<0.0001	1.68	1.68	3.17	0.0966
BD	0.03	0.03	0.01	0.9404	14.44	14.44	27.32	0.0001
CD	21.62	21.62	3.66	0.0762	0.00	0.00	0.00	1.0000
A^2	4,698.05	4,698.05	796.20	<0.0001	5.03	5.03	9.51	0.0081
B^2	565.40	565.40	95.82	<0.0001	0.10	0.10	0.19	0.6723
C^2	1,923.15	1,923.15	325.92	<0.0001	1.45	1.45	2.75	0.1195
D^2	535.97	535.97	90.83	<0.0001	8.66	8.66	16.38	0.0012

Notes: Initial pH (*A*); current density (*B*); electrode distance (*C*); feed flow rate (*D*).

$$\begin{aligned}\text{EEC (kWh/m}^3) = {} & 7.78 + 0.22A + 4.63B + 0.13C \\ & - 3.14D - 0.12AB - 0.33AC \\ & + 0.21AD - 0.65BC - 1.90BD \\ & + 0.000CD + 0.88A^2 - 0.12B^2 \\ & - 0.47C^2 + 1.16D^2 \end{aligned} \quad (5)$$

Then the acceptability of developed mathematical models to represent the protein recovery process was assessed by creating diagnostic plots such as predicted vs. actual plot. The data points align very near to the diagonal line for both the graphs and it depicts a good relationship between experimental and predicted data. Also, Pareto ANOVA was used to analyze the experimental result and predicted value equations were evaluated by their corresponding *F*-value and *p*-values (Table S1).

As can be seen in ANOVA, *F*-value was <25 and implied that the quadratic model was significant. Moreover, each term in the model was also tested for significance. A *p*-value smaller than 0.05 implies that the corresponding model term is significant. The *p*-values <0.0001 show that there is only a 0.01% chance that a model *F*-value this large is the product of noise in the experiment [24]. The 3D response surface plots were created using the developed mathematical model to analyze the relation between independent and dependent factors and it was shown in Fig. 4. The result revealed that all the combined process parameters shows the major effect on the electrocoagulation-based separation process and the trends obtained in the 3D response contour plots are closely agreed with Fig. 2. These results indicate that the developed mathematical models have the ability to describe the EC process to recover the protein from egg wastewater very robustly (Table 4).

4. Conclusion

This study described the recovery of protein from egg industry wastewater by EC technique. Under the optimum operating conditions such as pH of 6, current density of 25 mA/cm^2, electrode distance of 4.5 cm, and feed flow rate of 60 ml/min shows the 89% of protein recovery with electrical energy consumption of 7.5 kWh m^3. FT-IR analysis and digestibility of recovered protein confirmed that the protein recovered may serve as an excellent livestock feed ingredient. Mathematical model of the current experiment was designed by RSM-coupled BBD with four variables at three levels. Second-order polynomial models were created from the observed result in order to predict the responses with high correlation coefficient values. These results revealed that, EC is found to be an appropriate separation method to recover the protein from egg industry wastewater with reasonable operating cost.

Supplementary material

The supplementary material for this paper is available online at http://dx.doi.10.1080/19443994.2015.1129504.

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