Optimization of Flocculation of Marine *Chlorella* sp. by Response Surface Methodology

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**Abstract** — Harvesting is one of the key processes that determine the feasibility of algal biodiesel production. The cost of algae harvesting can be high, since the mass fractions in culture broth are generally low, while the cells normally carry negative charge and excess algogenic organic matters to keep them suspended in water. One of the most promising harvesting techniques appears to be flocculation. In this work, the ability of two flocculants: aluminum sulphate (alum) and ferric chloride in harvesting of marine *Chlorella* sp. was investigated. The standard jar stirrer test at varying pH, coagulant dosage and flocculation time were operated with the experimental design by response surface methodology (RSM). The empirical models from RSM are in a good agreement with the experimental results. The optimum flocculation efficiency was achieved at a dosage of 120 mg/L at pH 10 for flocculation time of 48 hr for both alum and ferric chloride. Using ferric chloride gave higher efficiency, but also higher cost.

**Keyword:** Microalgae, Flocculation, Harvesting, Alum, Ferric chloride
1. Introduction

Petroleum fuels should be replaced by renewable biofuels due to the limited availability and the contribution to accumulation of carbon dioxide in the environment leading to global warming [1,2]. Biodiesel (monoalkyl esters) is a potential renewable and carbon neutral alternative to petroleum fuels, which is obtained by the transesterification of triglyceride oil with monohydric alcohols [3]. Major sources of triglycerides for biodiesel production are crop oils such as canola, soybean, palm or sunflower oils [4]. However, these edible oils cannot realistically satisfy to replace all uses of petroleum fuels due to their high cost with adversely impacting of supply of food and other agricultural products.

Microalgae are suggested as more sustainable candidates for fuel production because of their advantages of higher photosynthetic efficiency, higher biomass production and faster growth compared to other energy crops [5]. Algae are simple organisms that are mainly aquatic and microscopic. Microalgae are unicellular photosynthetic microorganisms, living in saline or freshwater environments that convert sunlight, water and carbon dioxide to algal biomass [6,7]. If cultivated efficiently with the right conditions, algae can multiply their weight several times in a day. This is beneficial as it would possible for daily harvesting, unlike other biodiesel feedstocks [8]. In biodiesel production, microalgae are needed to be concentrated as much as possible to simplify the lipid extraction step. Increased product concentration decreases the cost of extraction and purification as well as the effective unit cost of the raw biomass. The techniques currently used in microalgae harvesting and recovery are centrifugation, filtration and screening, gravity sedimentation, flotation, electrophoresis techniques and flocculation [9]. Each technique has its disadvantages that affect the overall economics of the process. Centrifugation requires high energy input and high initial capital cost. Filtration and screening require regular replacement of filters, screens and membranes. Gravity sedimentation is a slow process. Electro-flotation requires the replacement of worn electrodes with a high cost of electricity. Flocculation is a low energy process, but can be expensive if the flocculant is costly and the dosage is high. However, coagulation-flocculation is considered as a potential viable microalgae dewatering process if cheap flocculants such as ferric chloride, aluminium sulphate (alum), chitosan and various polymeric flocculants are used [10, 11, 12].

Flocculation efficiency depends upon how precisely flocculant dosage, pH and flocculation time are chosen, which requires a particular set of experiments. Response surface methodology (RSM) is a technique that can help researchers to design experiment, build models, evaluate the effects of several factors and achieve the optimum conditions for desirable responses without running too many experiments.

In this study the flocculation efficiencies of alum and ferric chloride in harvesting *Chlorella* sp. were investigated by varying pH, flocculant dosage and flocculation time using RSM as an optimization tool.

2. Material and Methods

2.1. Algae

Water containing suspended cells of *Chlorella* sp. was obtained from Faculty of Agro Industry, Prince of Songkla University. The suspensions had an original pH of 8.75

2.2. Flocculation Procedure

Alum and ferric chloride used were commercial grade. The complete flocculation procedure is show in Fig.1. pH was adjusted in a range of 6 – 10 with flocculant dosage of 30 - 120 mg/l and flocculation time 1- 48 hr. After a settling period, samples were taken specifically at the 200 ml position for optical density (OD) measurement at 510 nm (OD<sub>510</sub>) with an UV-spectrophotometer. The biomass density and OD are linearly correlated, thus Eq. (1) was used to calculate the harvesting efficiency, H<sub>eff</sub>(%).

\[
H_{eff} (\%) = 1 - \frac{\text{final OD}}{\text{initial OD}}
\]  

Fig. 1. Flocculation procedure by alum or ferric chloride

2.3. Response surface methodology (RSM)

RSM with a Box–Behnken design was used to optimize the three variables: flocculant concentration, pH and flocculation time. The Box–Behnken design contained a total of 15 experiments with the first 12 experiments organized in a factorial design with the experimental trials from 13 to 15 involving the replication of the central point. The independent variable used in this study was coded according to Eq. (2).
Where $X_{\text{coded}}$ is the dimensionless coded value of the independent variable, $X_{\text{actual}}$ is the actual value of the independent variable, $X_0$ and $X_{\text{low}}$ are the high and low independent variables. The behavior of the system is explained by the following empirical second-order polynomial model, Eq. (3).

$$Y_i = a_0 + \sum a_i x_i + \sum a_{ij} x_i x_j + e$$

Where $Y$ is the predicted response, $a_0$ is the constant coefficient, $a_i$ is the $i$th linear coefficient, $a_{ij}$ is the $i$th quadratic coefficient, $a_{ij}$ is the interaction coefficient and $e$ is the error of the model.

3. Results and Discussion

3.1. Response Surface Models

The application of the response surface methodology indicated an empirical relationship between the response and input variables expressed by Eq. (4) and Eq. (5).

$$Y_1(\%) = 32.22 + 3.19 x_1 + 6.96 x_2 + 11.2 x_3 - 3.567 x_1^2 + 12.47 x_2^2 - 4.377 x_3^2 + 1.115 x_1 x_2 + 3.475 x_1 x_3 + 4.355 x_2 x_3$$

$$Y_2(\%) = 44.33 + 8.91 x_1 + 9.621 x_2 + 7.576 x_3 - 4.381 x_1^2 + 7.026 x_2^2 - 1.229 x_3^2 + 0.915 x_1 x_2 + 2.035 x_1 x_3 + 5.963 x_2 x_3$$

Where $x_1$, $x_2$ and $x_3$ are three independent variables, $Y_1$ and $Y_2$ are flocculation efficiency of alum and ferric chloride, respectively.

From Eq. (4) the first order effect of time ($x_1$) and second order main effect of pH ($x_2^2$) were highly significant. The variable $x_1$ (time) had positive effect on flocculation process and second order effect pH ($x_2^2$) had a significant positive effect on flocculation process by alum. From Eq. (5) the first order effect of pH ($x_2$) and second order main effect of pH ($x_2^2$) were highly significant. The variable $x_2$ (pH) had positive effect on flocculation process and second order effect pH ($x_2^2$) had a significant positive effect on flocculation process by ferric chloride.

3.2. Regression Coefficients and Analysis of Variance (ANOVA)

ANOVA is required to test the significance and adequacy of the model [12]. The ANOVA results are presented in Table 1. The probability values of both predicted models are less than 0.05, indicating that the models are statistically significant. The $R^2$ values are closed to 1 (0.956 for alum and 0.937 for ferric chloride), suggesting a good agreement to the experimental results.

![Fig. 2. Flocculation efficiency when used Alum; $X =$ Actual flocculation efficiency and $Y =$ Predicted flocculation efficiency](image2)

![Fig. 3. Flocculation efficiency when used Ferric chloride; $X =$ Actual flocculation efficiency and $Y =$ Predicted flocculation efficiency](image3)

![Fig. 4. 3D plot showing effect of concentration of alum and pH on flocculation efficiency](image4)

Table 1. ANOVA results for response parameters.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F</th>
<th>F-signif. or p value</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression of alum</td>
<td>2346.3</td>
<td>260.71</td>
<td>11.99</td>
<td>0.00688</td>
<td>9</td>
</tr>
<tr>
<td>Regression of ferric chloride</td>
<td>2276.7</td>
<td>252.97</td>
<td>7.989</td>
<td>0.01701</td>
<td>9</td>
</tr>
<tr>
<td>Residual of alum</td>
<td>108.99</td>
<td>21.80</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Residual of ferric chloride</td>
<td>158.32</td>
<td>31.66</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Total of alum</td>
<td>2455.2</td>
<td></td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Total of ferric chloride</td>
<td>2435.0</td>
<td></td>
<td></td>
<td></td>
<td>14</td>
</tr>
</tbody>
</table>

es024-3
From Figs. 4 show flocculation efficiency using alum decreased with increasing pH from 6 to 8 and then increased with increasing pH and reached maximum at 52.3% at 120 mg/l dosage. With ferric chloride flocculation efficiency increased with increasing pH and reached maximum at 66.3% at 120 mg/l dosage (Fig. 7).

Figs. 5 and 8 represent the effects of concentration and time on the flocculation efficiency using alum and ferric chloride, respectively. The maximum flocculation efficiency was obtained with concentration of 120 mg/l and time of 48 hr for both alum and ferric chloride. Figs. 6 and 9 confirm that the maximum flocculation efficiency was obtained with pH 10 and flocculation of 48 hr.

3.4 Response Optimization and Confirmation

When verifying the models by running further experiments with the optimum condition the observed and predicted flocculation efficiencies are in a good agreement as shown in Table 2. Using of ferric chloride provided higher flocculation efficiency.

Table 2. Flocculation efficiency at optimum values of the process parameters.

<table>
<thead>
<tr>
<th>Concentration (mg flocculant / L algae)</th>
<th>pH</th>
<th>time (hr)</th>
<th>Predicted $H_{ef}$ (%)</th>
<th>Observed $H_{ef}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alum</td>
<td>120</td>
<td>10</td>
<td>66.94</td>
<td>68.22</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>120</td>
<td>10</td>
<td>80.78</td>
<td>83.12</td>
</tr>
</tbody>
</table>

4. Conclusions

RSM can be employed to optimize flocculation of Chlorella sp. using alum and ferric chloride. The optimum flocculation efficiency was achieved at a dosage of 120 mg/L at pH 10 for flocculation time of 48 hr for both alum and ferric chloride. With ferric chloride the flocculation efficiency was higher. However, using of ferric chloride is cost 4.22 baht/L of algae, which is higher than using of alum that cost 2.78 baht/L of algae.

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