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THE OPTIMISATION OF LEVELS OF THE VARIABLES pH AND CONCENTRATION OF FERRIC CHLORIDE FOR HARVESTING MARINE MICROALGAE BY FLOCCULATION

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Abstract. Flocculation is one of the effective and inexpensive methods for the large scale harvesting of microalgae. In this work, ferric chloride was chosen as the flocculant agent for treating the microalgae, volume of ferric chloride(X_1) and level of pH(X_2) were chosen as the two independent variables in the 2^2 factorial experiment. The linear approximate equation of the yield response surface is $y= 91.75 + 5.25 X_1 + 1.75 X_2$. Based on the method of the Path of Steepest Ascent, another 5 experiments were tested by moving through the experimental region along a path that yields the maximum increases in the response. In conclusion, sedimentation efficiency would reach to the top 99% when the volume of added ferric chloride solution (concentration 1 mol/L) is 0.44ml per litre and pH value is 8.45.

1. Introduction

The microalgae biomass with high oil content is a significant and sustainable resource for the production of biodiesel which is able to substitute the fossil diesel. Large-scale production of biomass energy could contribute to sustainable development on several fronts; environmentally, socially and economically (Turkenburg, 2000). The harvest of the microalgae biomass is an important process after microalgae cultivation. Recovery of the biomass from the broth has been claimed to contribute 20–30% of the total cost of producing the biomass (Gudin and Therpenier,1986).

There is no single best method for harvesting microalgae. The choice of preferable harvesting technology depends on algae species, growth medium, algae production, end product, and production cost benefit (Shelef e*t al.,*1984).Harvesting of microalgae biomass requires one or more solid–liquid separation steps. Biomass can be harvested by centrifugation, filtration or in some cases, gravity sedimentation. These processes may be preceded by a flocculation step (Molina Grima e*t al.,*2003). Various methods of flocculation can be used to aggregate the microalgae cells to increase the effective 'particle' size and hence ease sedimentation, centrifugal recovery, and filtration (Elmaleh *et al.,*1991). For the large scale, flocculation is preferred for harvesting due to its low costs compared to other methods (Bilanovic e*t al.,*1988).

Flocculation process has been applied in the microalgae biomass recovery (Gualteri *et al.*, 1988; D'Souza *et al.*, 2002; Molina e*t al.,*2003;Knuckey *et al.*, 2006). Multivalent metal salts are effective flocculants or coagulants. The commonly used salts include ferric chloride, aluminum sulfate and ferric sulfate (Molina e*t al.,*2003).Various flocculation methods could be applied in microalgae biomass recovery. However, economic cost and environmental problems should be considered especially in the scale up of the production of microalgae biomass. Some flocculants such as multivalent metal salts would be toxic to the animals when consumed due to the high concentrations and cause water pollution (Buelna *et al,*.1990). Accordingly, the objective of high biomass sedimentation efficiency will have to be achieved by using the least amount of flocculant possible.

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In this work, ferric chloride was chosen as the flocculant. The effect of variables namely $FeCl_3$ concentration (V or X_1) and pH (pH or X_2) on the flocculation were investigated to optimise the sedimentation efficiency.

2. MATERIALS AND METHODS

2.1 Microalgae and Chemicals

The marine microalgae *Nannochloropsis sp* were collected from Universiti Malaysia Terengganu, aerated with filtered air (50L/h) and cultivated with the conditions of temperature 22±2℃, illumination intensity 2000lx, and 12:12 hr photoperiods for 7days.

The flocculant ferric chloride and pH addition of sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). These chemicals used were of analytical grade. Both of these chemicals were prepared as solutions at 1mol/L.

2.2 Measurement of Sedimentation Efficiency

1.0 litre of the microalgae culture in fermentation growth medium was poured into a volumetric cylinder, the microalgae cell density was measured using the hemacytometer as D_1, then $FeCl_3$ solution and NaOH were added, and the mixture was stirred with glass rod, and left to stand for 10h and then the cell density was measured as D_2.

$$\text{Sedimentation Efficiency} = (D_1 - D_2) / D_1 \times 100\% \qquad (1)$$

2.3 The Method of Factorial Experiment and Yates' Method

Based on 2n factorial experiments (Montgomery, 2001) design, Table 1 below shows the levels of the experimental variables used in the 2^2 factorial experiments.

Table 1: Level of Experimental Variables in the 2^2 factorial experiments

Experiment Variables	Levels of Experimental Variables			
	α =-1	α =0	α =+1	Unit
V	0.25	0.3	0.35	ml
pH	6.5	7.5	8.5	pH unit

To be able to use Yates' Method (Yates, 1937), the experiments (consisting of various combinations of the experimental variables each in its upper or lower level) must first be arranged in the particular order as shown in Table 2, The combination of variables can be treated as a full 2^2 factorial using Yates' Method (Yates, 1937) to calculate the main effects and the interactive effects in Table 3:

Table 2: The plan of the 2^2 Factorial Experiments and the Untreated Results

No	V X_1	pH X_2	Sedimentation Efficiency(SE) (%)
1	-1	-1	85
2	+1	-1	95
3	-1	+1	88
4	+1	+1	99

Table 3: The Results of the Calculation of Main Effects and Interactive Effects Using Yates' Method

No	V(X_1)	pH(X_2)	SE (%)	Column 1	Column 2	Effect	Identification
1	-1	-1	85	180	366	183	GT Effect
2	+1	-1	95	186	20	10	V
3	-1	+1	88	10	6	3	pH
4	+1	+1	99	11	1	0.5	V-pH

The main effects and the interactive effect were then tested against the mean square experimental (m.s.e) which was derived by repeating the experiments at the centre point (α=0), the m.s.e = 0.24, for significance at selected confidence level (95% and 99%) using the statistical F-test (Montgomery, 2001). The relevant values of the F-distribution are given in Table 4 and the results of the statistical F-test are given in Table 5.

Table 4: F-Distribution

d.f. 2 :	d.f. 1	$F_{0.99}$	$F_{0.95}$
1 :	4	21.195	7.7086
SYMBOLS		1	2

Table 5: The results of the F-Test on the Main Effect and Interactive Effect

No	Identification	Effects	df	S^2	S^2/r^2	F- test
1	GT	183	1	33,485	139,537	1
2	V	10	1	100	416	1
3	pH	3	1	9	337	1
4	V-pH	0.5	1	0.25	1.0	-

2.4 The Method of the Path of Steepest Ascent

The method of the Path of Steepest Ascent (Montgomery, 2001) finds the direction (with respect to all the variables considered) in which the increase of yield is steepest. Experimental points can be tested along this direction and the point where the yield differs significantly from that predicted by the linear equation based on the area previously investigated was made the new centre point around which a new set of factorial experiments were constructed. This process was carried on until finally an area was found which satisfied the criterion for the area containing the maximum yield.

The linear equation represent y the response surface of the factorial design was evaluates using SPSS regression Analysis package as follows:

$$y = 91.75 + 5.25\ X_1 + 1.75\ X_2 \quad (1)$$

The method of the Path of Steepest Ascent involves moving through the experimental region along a path that yields the maximum increases in the response. Taking the centre-point of the first factorial experiment as the starting point of the ascent, we need to move towards position P with coordinates($\bar{X}_1$, $\bar{X}_2$, $\bar{X}_3$, $\bar{X}_4$, $\bar{X}_5$) which maximizes the response y($\bar{X}_1$, $\bar{X}_2$, $\bar{X}_3$, $\bar{X}_4$, $\bar{X}_5$). The change in the value of y depends on the distance of the jump J from the starting point to the position P, given by:

$$J = \sqrt{\ }\ (\ \bar{X}_1^2,\ \ \bar{X}_2^2,\ \ \bar{X}_3^2,\ \ \bar{X}_4^2,\ \ \bar{X}_5^2). \quad (2)$$

For a particular value of *J*, the value of x_i which maximizes y is given by:

$$x_i = \ell \frac{\partial y}{\partial x_i} \quad (3)$$

where $\frac{\partial y}{\partial x_i}$ is the partial differential of y with respect to x_i, taken at point P.

ℓ is given by $\ell = \dfrac{J}{\sqrt{\left[\sum(\frac{\partial y}{\partial x_i})^2\right]}}$ (4)

The value of the partial differentials $\frac{\partial y}{\partial x_i}$ can be calculated by differentiating the linear equation

$$y_N = a_0 + a_1 x_1 + a_2 x_2 \quad (5)$$

with response to each x_i in turn.

Suitable values of *J* are chosen, taking care not to make the jump too big lest the peak is missed. For each *J* that is chosen, a new experimental point is found where the coordinates are defined by equation 3:

$$x_i = \ell \frac{\partial y}{\partial x_i} \quad (3)$$

The chosen range of the value of *J* from 1 to 5 and the corresponding ℓ values are shown in the Table 6.

Table 6: Chosen Range of the Values of J from 1 to 5 and the Corresponding ℓ Values

J	ℓ
1	0.181
2	0.361
3	0.542
4	0.723
5	0.904

Table 7: x_i at Different Values of J and The Real Values of Operational Variables

J	ℓ	X_1		X_2		Sedimentation Efficiency (%)
		α	Value(ml)	α	Value(pH)	
1	0.181	0.950	0.35	0.317	7.78	96
2	0.361	1.896	0.40	0.632	8.13	98
3	0.542	2.846	0.44	0.949	8.45	99
4	0.723	3.796	0.49	1.265	8.77	99
5	0.904	4.746	0.54	1.582	9.08	99

x_i was calculated at different values of *J* by using the equation $x_i = \ell \frac{\partial y}{\partial x_i}$, giving values of operational variables as in the Table 7. These new operational points were tested. The results of the new experiments were shown in column 7 of Table 7.

3. EXPERIMENTAL RESULTS, DISCUSSION AND CONCLUSIONS

Table 5 gives the results of the F-test on the main effects and the interactive effect of the experimental variables on the sedimentation efficiency. The main effect of V was significant at 99% confidence level, while the main effect of pH was significant at 95% confidence level.

In the regression coefficients of the linear equation for the response surface of the factorial experiment, a_1 =5.25, a_2 = 1.75, a_0 = 91.75. In this case, the coefficient a_1 is large compared to a_0, thus the area investigated is steep and does not contain the maximum, and the Steepest Ascent Method (Montgomery, 2001) has to be used to find a new centre point.

Table 7 gives the results of the experiments in the Method of the Path of Steepest Ascent. The yield curve of the sedimentation efficiency became flat from the point *J* = 3 onwards to higher values of *J*. Hence the combination of levels of operational variables at *J* = 3 was selected as the best combination. At this contribution, the sedimentation efficiency (%) was 99% and therefore there is no need to build a new 2^2 factorial experiment around it to find a higher maximum.

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