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OPTIMISING EXTRACTION OF MICROALGAL OIL USING ACCELERATED SOLVENT EXTRACTION BY RESPONSE SURFACE METHODOLOGY

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Abstract

The extraction of oil from biomass is one of the most important aspects in the harvesting of microalgae for the production of oil. Efficient extraction technique is important for the quantification of oil content in biomass. Solvent extraction is typically employed for the extraction of oil. Accelerated Solvent Extraction (ASE) is an automated pressurised liquid extraction technique that provides rapid and effective extraction process. There are limited studies on the effects of extraction conditions using the ASE technique to achieve optimum oil yield. The aim of this study was to optimise the extraction of oil using the ASE technique by response surface methodology. A face-centred central composite design (CCD) was used to evaluate the effects of static cycle (1 to 6 cycles), static time (2 to 10 min) and temperature (100 to 160 °C) on oil extraction. The optimum condition was found to be at 4 static cycles, static time of 6 min and temperature of 160 °C, with an oil yield of 34.9%. From the ANOVA results, R^2 of the mathematical model is 0.9970. This study showed an improvement in the oil yield using the optimum condition for ASE, where the optimum condition resulted in 1.34-fold increases in oil yield from the control run.

Keywords: Accelerated Solvent Extraction, Microalgae, Oil, Lipid, Response Surface Methodology

1. Introduction

Lipid or microbial oil shows great promise for second generation biodiesel production. Microbial oil is an alternative to the conventional feedstock used for

Nomenclatures

k	The number of factors
x_i	Input variables that influence the response variable Y
x_j	Input variables that influence the response variable Y
X_1	Static cycles
X_2	Static time (min)
X_3	Temperature (°C)
Y	Response variable

Greek Symbols

β_0	Intercept
β_i	Linear coefficient
β_{ii}	Linear-by-linear interaction between x_i and x_j regression coefficients
β_{ij}	Quadratic coefficient
ε	The random error

Abbreviations

ASE	Accelerated Solvent Extraction
CCD	Central composite design
DE	Diatomaceous earth
FAME	Fatty acid methyl ester
PLE	Pressurised liquid extraction
RSM	Response Surface Methodology

biodiesel production, which are plant oils such as soybean oil (edible oil) or Jatropha oil (inedible oil). It is more advantageous to use microbial oil than plant oils for biodiesel production due to several factors such as less labour intensive to cultivate, has short life cycle, is easy to scale up and has no seasonal and climate requirement [1]. Oleaginous microorganisms are microorganisms (e.g., microalgae, yeasts and fungi) that are able to accumulate more than 20% lipids within the cells [2]. Oleaginous microalgae, yeasts and fungi have been reported to be able to produce oil from the cultivation on various carbon sources, including industrial and agricultural wastes [3].

The extraction process is a crucial step in harvesting microalgae from the culture for oil production in order to ensure maximum yield of desired product from microalgae biomass. Solvent extraction is the conventional technique for the extraction of oil or lipid from biomass. The three most commonly used solvent extraction techniques for extracting oil from microbial biomass are the Bligh-Dyer, Folch and Soxhlet extraction techniques [4-6]. However, these extraction methods are usually multi-step procedures and use large amount of solvents for extended periods of time [7, 8]. For instance, Soxhlet extraction requires longer extraction time (8 h) [4], due to slow diffusion and desorption of desired extracts from the sample matrix to the extraction solvents [9]. Folch technique involves two-steps extraction method, which is extraction followed by purification using water [6].

Pressurised liquid extraction (PLE), or known as Accelerated Solvent Extraction (ASE) is an alternative solvent extraction technique which involves extraction at elevated temperatures under high pressures. ASE is an automated

technique consists of stainless-steel cells that hold the samples for liquid extraction at the set up temperature [8]. Temperature, pressure and solvent delivery were electronically controlled by heaters and pumps [8]. In ASE, the high pressure can be used in order to keep the solvents in liquid state at temperatures above their boiling points [10]. The application of higher pressures improves extraction efficiency as the pressure helps diffuse the solvents into desired extracts trapped within the matrix pores of the biomass [10]. Therefore, the mechanical pretreatment may not be necessary prior to the extraction by ASE, unlike the conventional extraction techniques.

In addition, ASE is more advantageous than conventional extraction techniques as it allows higher number of samples loading and uses less amount of solvent with shorter extraction time [5-7]. It has been reported that extraction of persistent organic pollutants (POPs) from soils and sediments using PLE required only 20 min of extraction time and 10 times less solvent than Soxhlet extraction [11]. ASE also has health and safety benefits, as it reduces the potential for contact with chemical solvents. Studies showed that the extraction using ASE resulted either in increased or comparable amounts of oil in comparison to conventional extraction techniques [12, 13]. A study on oil extraction from algae biomass showed that a higher amount of oil was obtained using ASE technique than the Folch method, where the solvents used for both methods were chloroform/methanol (2:1, *v*/*v*) [12]. Higher total fatty acids yields were achieved from the extraction of cereal, egg yolk and chicken breast muscle samples using ASE than a modified Folch method with the use of isopropanol/hexane (2:3, *v*/*v*) for both methods [13].

Previous studies have shown that solvent types and temperature affected oil yields in ASE [12-14]. The extraction of oil from the algae biomass showed higher fatty acid yields were obtained with the use of chloroform/methanol (2:1, *v/v*) compared to the use of isopropanol/hexane (2:3, *v/v*) and hexane [12]. The combination of non-polar and polar solvent (such as chloroform/methanol) was shown to be more effective for extracting neutral lipid (*i.e.* microbial oil) from microbial biomass, in comparison to the use of non-polar solvent (such as hexane) alone [15]. Another extraction study on dry microalgae biomass using ASE reported on the effect of temperature to the oil yield where the study demonstrated that slightly higher amounts of total fatty acid methyl esters (FAMEs) were obtained at 120 °C compared to 110 °C, and significantly higher FAMEs at 120 °C compared to temperatures below 100 °C [16]. Despite these reports, there is no systematic study on optimisation of ASE from microbial biomass that correlates the important extraction parameters such as temperature, the number of process cycles and the process time to the oil yields.

The aim of this study was to optimise the extraction of microbial oil from microalgae *Chlorella protothecoides* using ASE technique by response surface methodology (RSM). The parameters for determining optimum oil yield were the number of static cycles, static time (min) and temperature (°C), with oil yield (%, *w*/*w*) as the response parameter. The optimisation study was conducted through the experimental design, experimental run using microalgal biomass and experimental data analysis for the development of a mathematical model.

2. Experimental Procedures

2.1. Microbial biomass preparation

Chlorella protothecoides ATCC® 30581 (ATCC, USA) was used in this study. Microalgae was maintained in a growth chamber with light intensity from 38 - 47 μmol/m^2/s at 25 °C under a 14 hours light/10 hours dark cycle. Microalgae were subcultured in modified Medium 847 as described in the Product Information Sheet for ATCC® 30581™. The cultivation conditions were similar to inoculum preparation and microalgae cultivation described previously [17], with 10% (*v*/*v*) inoculum was used. Microbial biomass was harvested by centrifugation at 6805 X *g* for 7 min followed by freeze-drying [17].

2.2. Extraction of oil from microalgal biomass using ASE

Dionex ASE 350 (Thermo Fisher Scientific Inc., USA) was used for extraction of oil from microalgal biomass. The biomass samples were prepared by mixing dry microalgal biomass (0.25 g) with 4 g of ASE Prep DE (diatomaceous earth) (Thermo Fisher Scientific Inc., USA) before being loaded into 33 mL cells [12]. The detailed extraction process in Dionex ASE 350 is illustrated in Fig. 1. A static extraction in the cell commences after solvent filling followed by cell heating, up until before the cell is rinsed with fresh solvents. Static time is the period where static extraction occurs, and static cycle is the number of times where static extraction occurs.

The extraction conditions were as follows for the control run and RSM experimental run: rinse volume, 50% of cell volume; purge time, 60 s; with varying static cycles, temperature and static time using chloroform/methanol (2:1, *v*/*v*) as the extraction solvents. The control run was performed based on the optimised condition reported in previous study (ASE with 4 static cycles, static time of 120 ℃ and temperature of 5 min) [12]. The extracted oil was collected in pre-weighed collection bottles. Solvent was later evaporated under a stream of nitrogen. Oil yield (%, *w*/*w*) was calculated as follows,

$$\text{Oil yield } (\%, w/w) = \frac{\text{Dry weight of oil (g)}}{\text{Dry weight of biomass (g)}} \text{ x } 100\% \quad (1)$$

2.3. Design of experiment by response surface methodology (RSM)

A response surface methodology (RSM) with face-centred central composite design (CCD) was applied for designing the experiments of optimising oil yield from the extraction of microalgae biomass by ASE. The parameters (independent variables) selected for optimising the oil yield by ASE are static cycles, static time and temperature. Design of experiments, mathematical modelling and optimisation of process parameters were performed using the Design Expert 7 Trial version software package (Stat-Ease Inc., USA). The independent parameters used in this study were static cycles (X_1), static time (min) (X_2) and temperature (°C) (X_3). The response factor (dependant variable) for optimisation was oil yield (%) (Y). The coded levels for parameters, -1 and 1, indicate the limits of each factor, where the actual values of each factor and its levels for this experimental design are shown in Table 1.

The range of the factor was based on the preliminary study performed previously [18]. A total of 12 experimental runs were conducted in random with 4 factorial points, 6 axial points and a centre point (in duplicate for experimental error calculation).

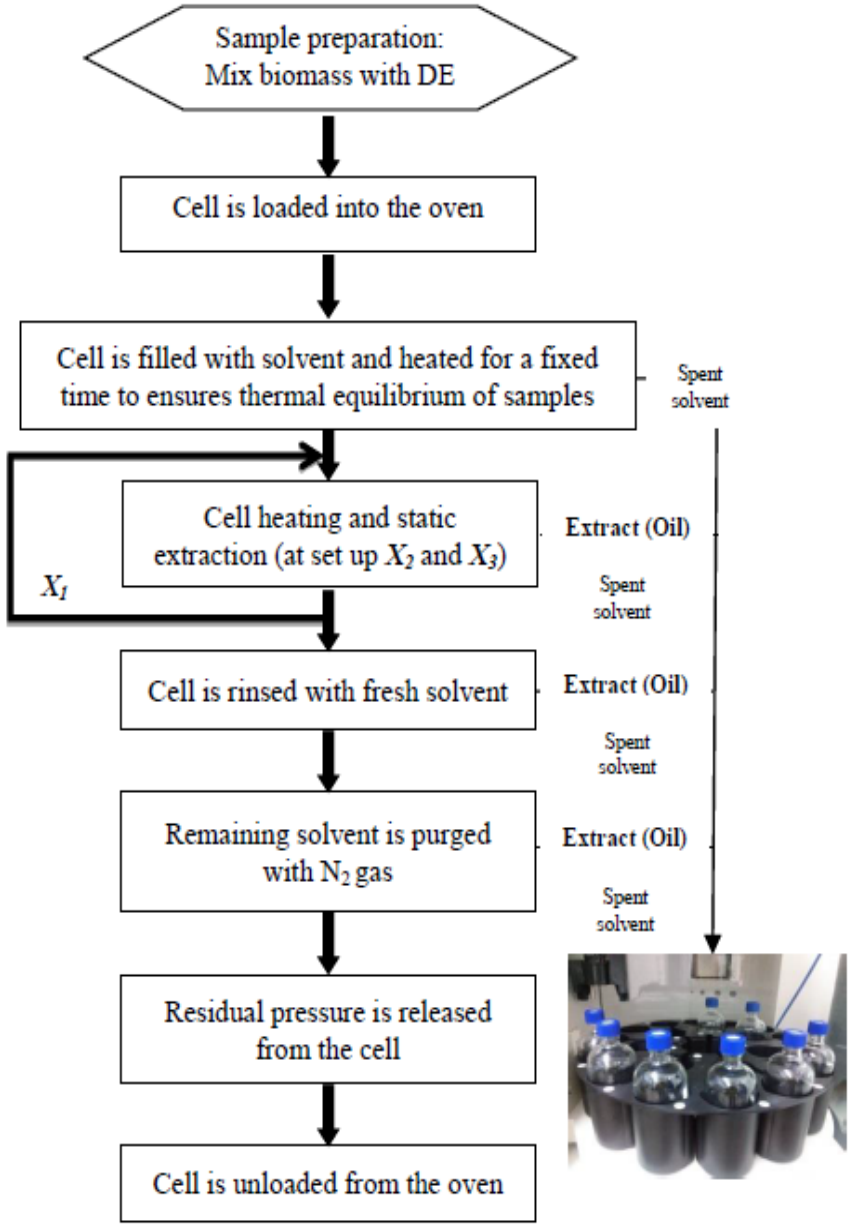

Fig. 1. Flow diagram of Dionex ASE 350 extraction process. X_1 is the number of static cycles, X_2 is static time (min) and X_3 is operating temperature (°C). Cell is the stainless steel sample holder where the extraction process occurs.

2.4. Statistical analysis and modelling

From the experiments that have been performed based on the design by RSM, the oil yield (response variable, *Y*) was fitted by a quadratic model to correlate the response variable to the independent variables. The experimental data obtained were calculated and analysed through an empirical second-order polynomial function:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta x_i + \sum_{i=1}^{k} \beta_{ii} \beta x_i^2 + \sum_{i=1} \sum_{i \neq j=1} \beta_{ij} x_i x_{ij} + \varepsilon \quad (2)$$

where Y is the predicted response; β_0 the intercept, β_i the linear coefficient, β_{ij} the quadratic coefficient, β_{ii} is the linear-by-linear interaction between x_i and x_j regression coefficients, x_i, x_j are input variables that influence the response variable Y, k is the number of factors and ε is the random error [19]. Analysis of variance (ANOVA) was evaluated through statistical analysis of the model. The statistical significance of the model terms was assessed using *P*-value approach.

Table 1. Coded and actual values of the parameters in the experimental design.

Factor	Notation	Units	Coded levels of parameters		
			-1	**0**	**1**
Static cycles	X_1		1	4	6
Static time	X_2	min	2	6	10
Temperature	X_3	°C	100	130	160

3. Results and Discussion

3.1. Mathematical modelling of the experimental data

The second-order model was employed for approximating the relationship between the oil yield and the independent variables, as shown below

$$Y = -44.52 + 11.40X_1 + 5.20X_2 + 0.23X_3 + 0.0043X_1X_2 - 0.043X_1X_3 - 0.014X_2X_3 - 0.56X_1X_1 - 0.25X_2X_2 + 0.00096X_3X_3 \quad (3)$$

where Y is the predicted oil yield and X_1, X_2 and X_3 are static cycles, static time (min), and temperature (°C) respectively. Table 2 shows the experimental designs, the actual oil yield and the predicted oil yield. The actual oil yield was the values from the experimental run, whereas the predicted oil yield was the fitted values of the mathematical model. The mathematical model demonstrates good estimation of the predicted oil yield as standard deviations between the actual and predicted oil values are low.

The analysis of variance (ANOVA) of the model is presented in Table 3(a). F-value is the ratio of the square of mean to the residual. *P-value* is the smallest level of significant that would lead to rejection of the null hypothesis [19]. *F-value* and *P-value* were used to determine the significance of each independent variable and their interactions to the extraction of oil. Values of *Prob>F* (*P-value*) less than 0.05 indicate model terms are significant. The Model *F-value* (74.46)

implies the model is significant (*P-value* < 0.0500). Model terms of X_1, X_3, X_1X_3, X_1X_1 and X_2X_2 are significant. There is only a 1.33% chance that Model *F-Value* could occur due to noise. The lack of fit *F-value* of 0.01 implies the lack of fit is not significant, as there is 94.85% chance that the lack of fit *F-value* occurs due to noise. Noise could be attributed to the properties of samples that could vary due to the duration of storage prior to the extraction process.

Table 2. The experimental results and the predicted values of the oil yield.

Run	***Static cycle (X_1)***	***Static time (min) (X_2)***	***Temperature (°C) (X_3)***	***Oil yield (%, w/w) (Y)***		
				Experimental	**Predicted**	**Standard deviation**
1	1	6	130	18.32	18.35	0.02
2	4	6	130	26.01	26.97	0.68
3	6	6	130	27.84	27.87	0.02
4	4	10	130	24.19	24.22	0.02
5	4	6	160	34.89	34.92	0.02
6	4	6	130	28.06	26.97	0.77
7	4	6	100	20.04	20.07	0.02
8	4	2	130	21.10	21.13	0.02
9	1	10	160	25.88	25.86	0.01
10	1	2	100	1.59	1.57	0.01
11	6	10	100	24.07	24.05	0.01
12	6	2	160	25.85	25.83	0.01

Table 3(b) shows the value of R^2 for this model. Montgomery defined R^2 as the variability in the data explained by the model, where larger values of R^2 ($0 \leq R^2 \leq 1$) is more desirable [19]. R^2 value of the model (0.9970) indicates that the total variation of 99.7% for the oil yield was attributed to the independent variables and only about 0.3% of the total variation could not be explained by the model [20]. Predicted R^2 is a measure of predictive capacity of the model, whereas Adjusted R^2 measures the amount of variation about the mean explained by the model adjusted for the number of parameters in the model [21].

The model shows that the Predicted R^2 (0.9885) is in good agreement with the Adjusted R^2 (0.9836). From Predicted R^2, 98.85% of the variability of new data attributed to the independent variables, where only 1.15% of the total variation of the new data cannot be explained by the model.

The mathematical model was further evaluated by plotting the predicted oil yield against the actual oil yield as shown in Fig. 2(a). The plot demonstrated a

good agreement of the predicted oil yield to the actual oil yield. The model was also evaluated through the plot of residuals versus fitted values, Fig. 2(b).

The plot showed that the residuals are structureless and do not display any obvious pattern. The undesirable pattern in the plot can appear in the form of megaphone or outward-opening funnel due to the increase of variance, as the magnitude of the predicted values increases [19]. Therefore, this analysis demonstrated that the mathematical model is correct and the assumptions are satisfied [19]. The model is reliable for predicting the extraction of oil microalgae biomass using ASE technique.

Table 3. (a) ANOVA results for the response surface analysis of the quadratic model of the extraction of oil, (b) Regression model diagnostics from ANOVA of the model.

(a)

Source	Sum of squares	Degrees of freedom	Mean square	*F-value*	*P-value* (*Prob>F*)	Significance
Model	708.70	9	78.74	74.46	0.0133	Significant
X_1	45.32	1	45.32	42.85	0.0226	Significant
X_2	2.44	1	2.44	2.31	0.2679	Not significant
X_3	120.09	1	120.09	113.55	0.0087	Significant
X_1X_2	3.81	1	3.81	3.60	0.1981	Not significant
X_1X_3	23.31	1	23.31	22.04	0.0425	Significant
X_1X_1	20.19	1	20.19	19.09	0.0486	Significant
X_2X_2	46.20	1	46.20	43.69	0.0221	Significant
X_3X_3	0.68	1	0.68	0.64	0.68	Not significant
Residual	2.12	2	1.06			
Lack of fit	0.01	1	0.01	0.01	0.9485	
Pure error	2.10	1	2.10			
Corrected Total Sum of Squares	710.82	11				

(b)

	Value
Standard deviation	1.03
Mean	23.15
Coefficient of variance (%)	4.44
PRESS	8.14
R^2	0.9970
Adjusted R^2	0.9836
Predicted R^2	0.9885

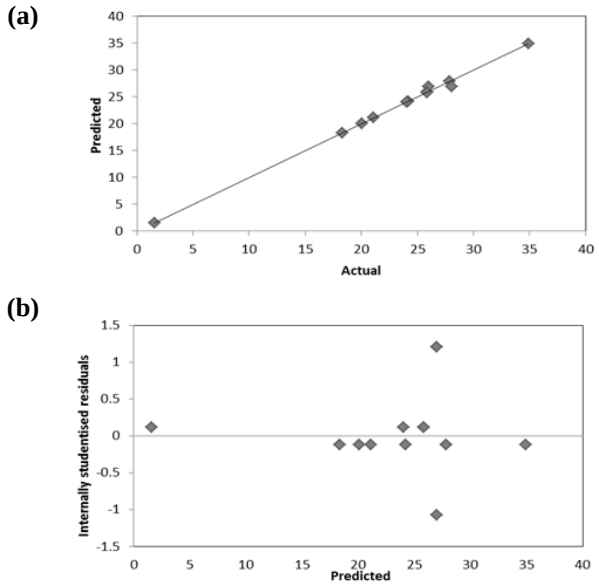

Fig. 2. (a) Predicted versus actual plot of oil yield.
(b) Internally studentised residuals versus predicted plot of oil yield.

3.2. Optimising the parameters of the extraction of oil from microbial biomass

The response surface analysis through 3-dimensional response surface plot (Fig. 3) was performed for optimising the yield of oil from microbial biomass using ASE. Figure 3(a) shows that at 130 ℃ and 6 min static time, the amount of oil extracted increased with increasing static cycles, up until approximately 4-5 static cycles. This result showed that oil could be extracted from even after 4 static cycles, which is in accordance to other study on oil extraction from macroalgae biomass (*Rhizoclonium hieroglyphicum*) using ASE [12]. From Fig. 3(b), oil yield significantly increased with increasing temperature. The impact of static cycle on the oil yield was more apparent at lower temperature than higher temperature. Figure 3(c) shows that at 4 static cycles for all extraction temperatures, the oil yields increased with increasing static time up until an optimum time at approximately 6 min. The extraction at 4 static cycles in Fig. 3(c) demonstrated that at a very high extraction temperature such as at 160 °C, the oil yield was gradually decreasing when the static time was more than 6 min. This is because that there is the possibility for the samples to degrade during prolonged extraction process at a very high temperature. High extraction temperature has been suspected to cause thermal degradation of lipids [14]. Even though there was a small decrease in oil yield at lower temperature (100 °C) for the extraction at more than 6 min, the drop in oil yield was too low.

The parameters of optimisation of extraction of oil by ASE were determined based the numerical optimisation according to the criteria showed in Table 4. Numerical optimisation was performed based on an objective function called desirability [21]. At desirability (0 to 1) of 1 from the numerical optimisation (Fig. 4), the parameters of optimum oil yield selected were 4 static cycles, 6 min and 160 ℃ for the extraction using ASE, with the maximum oil yield of 34.9% (*w/w*).

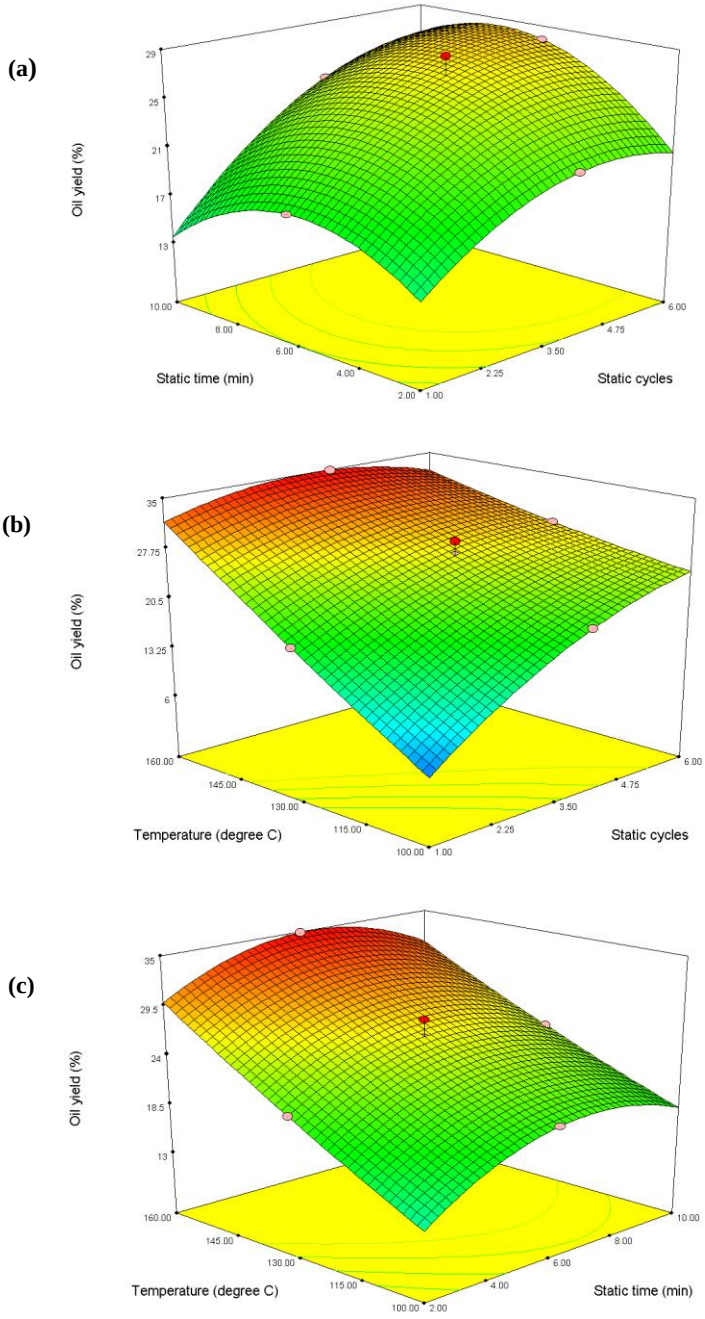

Fig. 3. 3-Dimensional surface plots of binary interaction between different variables to the oil yield: (a) static cycles and static time at 130 °C, (b) static cycles and temperature at 6 min and (c) static time and temperature at 4 static cycles.

Table 4. Criteria for numerical optimisation of maximum oil yield.

Criteria	Goal	Lower limit	Upper limit
Static cycles	In range	1	6
Static time (min)	In range	2	10
Temperature (°C)	In range	100	160
Oil yield	Maximise	1.59	34.89

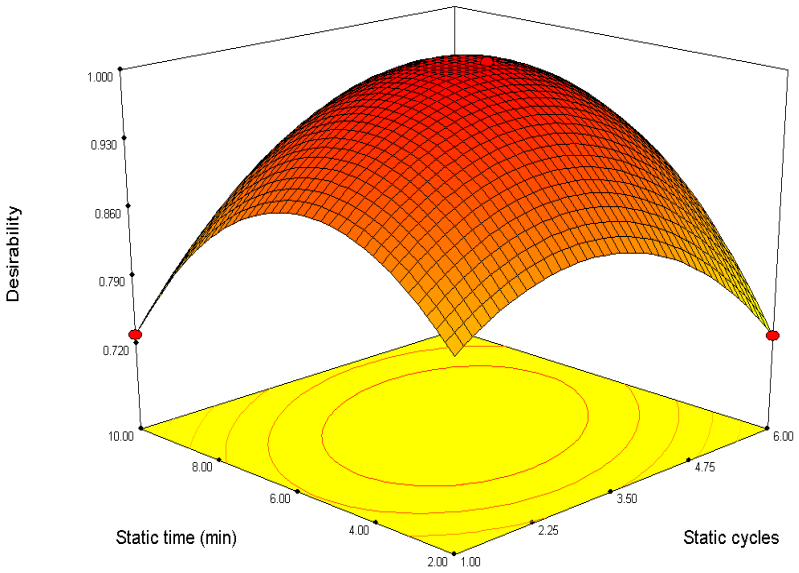

Fig. 4. 3-Dimensional surface plot of the binary interaction at 160 °C between static time and static cycle to the desirability value.

In this study, the control run on microalgal biomass resulted in an oil yield of 26.0% (*w/w*), which is lower than the maximum oil yield obtained in this study. Therefore, the extraction using optimised conditions in this study showed 1.34-fold increases in oil yield from the control run. The optimised ASE conditions in this study demonstrated an improvement in extraction technique for quantifying the amount of oil in the biomass.

4. Conclusions

The RSM was utilised for optimising the oil yield from the extraction on microalgal biomass using ASE technique. The mathematical model developed from the response surface analysis was reliable to predict the oil yield. The results showed a good agreement of the predicted oil yield to the actual oil yield from the experimental run. Based on the surface response analysis, the optimised ASE conditions determined were 4 static cycles, static time of 6 min and temperature of 160 °C, with the maximum oil yield of 34.9% (*w/w*). The optimised oil yield also resulted in significant improvement of oil yield in comparison to the oil yield from the control run, with 1.34-fold increases in oil yield.

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