SUPPLEMENTARY INFORMATION

Statistical Optimization of biosurfactant production

Response Surface Methodology (RSM) was used to optimize the most significant parameters for improving the biosurfactant yield using palm oil and coconut oil as substrates. The four independent parameters were studied at three different levels and a set of 30 experiments were carried out.

The analysis was done as per the following model equation

$$Y = \alpha_0 + \sum_{i=1}^{k} \alpha_i x_i + \sum_{i=1}^{k} \alpha_i x_i^2 + \sum_{i<j} \alpha_{ij} x_i x_j$$

Where, Y is the predicted response, k is the number of parameters, α_0 is the design factor of interest, α_i and α_{ij} are coefficients. The second- degree term (x_i^2) gives the optimal values for the response and the interactive term (x_{iXj}) represents the influence of one parameter over the other. Each experimental design was carried out in triplicate, and the mean values were given.

Design expert, version 8.0.7 (Statease Inc., Minneapolis, USA) was used for the experimental designs and regression analysis of the experimental data. Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA).

Results and Discussion

RSM using central composite design was employed to determine the optimal levels of the four significant parameters that affected biosurfactant yield. Three levels with the coded levels for the parameters are shown in Table A1& A2. Based on the regression analysis of the data from the Table A1& A2, effects of the four parameters on biosurfactant yield were predicted by the second order polynomial function as

Biosurfactant yield (g/g of palm oil) = +0.30+0.039 *A-3.333E-003*B+8.333E-004*C-5.000E-003 * D+0.020*A*B-1.250E-003*A*C+0.000*A*D+0.015 * B * C+0.014*B*D-7.500E-003 *C*D-0.027*A^2-0.022*B^2-9.792E-003*C^2-0.012*D^2

where A, B, C, D are time (h), pH, temperature(ºC) and concentration of palm oil (g/L), respectively.

Biosurfactant yield (g/g of coconut oil) =+0.40+0.041 * A-8.333E-004 * B+7.500E-003 *C-0.013 * D-6.250E-003 *A*B-0.017*A*C-5.000E-003*A*D-0.014*B*C+0.021*B*D-5.000E-003*C*D-0.033*A^2-0.036* B^2-0.018*C^2-0.015*D^2

where A, B, C, D are time (h), pH, temperature(ºC) and concentration of coconut oil (g/L), respectively. The second order polynomial equation was developed to fit the experimental data and the coefficients of A, B, C and D can be used to obtain the maximium,minimum or any experimental value obtained at some particular combination of input variables.

ANOVA for response surface quadratic model

The statistical significance of equation was checked by F test and ANOVA for the second order polynomial model. The F test showed that, the second order polynomial model was well adjusted to the experimental data and the coefficient of variation (CV) indicated the degree of precision of the experiment.

In general, higher the value of CV, lower the reliability of the experiment. In the present investigation the CV value of 6.41 and 7.0 was lower with a regression coefficient of 0.9591and 0.9427 indicated a better precision and reliability of experiments for palm oil and coconut oil respectively. Linear and quadratic terms were both significant at the 1% level.

Localization of optimum conditions

The 3D response surface plots (Figures not shown) described by the regression model were drawn to illustrate the effects of the independent parameters and interactive effects of each independent parameter on the response factors.

Table A1: Coded and real values of the factors tested in the RSM experimental design and ANOVA for the second order polynomial model for production of biosurfactant using palm oil

Factor		**Levels of factors**		
		-1	**0**	**+1**
A	Time (h)	72	96	120
B	pH	4	5	6
C	Temperature (°C)	25	30	35
D	Concentration of Palm oil (g/L)	30	40	50

source	**Degree of freedom**	**Mean square**	**F-value**	**P value prob> F**
Model	14	5.918E-003	25.12	<0.0001 (significant)
Residual	15	2.356E-004		
Lack of fit	10	3.200E-004		
Pure error	5	0.000		
Total	29			
R^2				0.9591

Table A2: Coded and real values of the factors tested in the RSM experimental design and ANOVA for the second order polynomial model for production of biosurfactant using coconut oil

Factor		**Levels of factors**		
		-1	**0**	**+1**
A	Time (h)	72	96	120
B	pH	6	7	8
C	Temperature (°C)	35	40	45
D	Concentration of Coconut oil (g/L)	40	50	60

source	**Degree of freedom**	**Mean square**	**F- value**	**P value prob> F**
Model	14	8.755E-003	17.63	<0.0001 (significant)
Residual	15	4.967E-004		
Lack of fit	10	7.450E-004		
Pure error	5	0.000		
Total	29			
R^2				0.9427