Empirical Modeling Development for Integrated Process Optimization of Poly(3-hydrxybutyrate-*co*-3-hydroxyvalerate) Production

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ABSTRACT: The optimization of P(3HB-*co*-3HV) synthesis was investigated using response surface methodology (RSM). Primary experiments showed different concentrations of 1-pentanol, agitation rates, and incubation times were the main factors influencing the production of this copolymer. To test the influence of the process parameters, two statistical experimental designs were performed. The first step of optimization used two-level factorial designs to determine the significant variables affecting the growth and production. The RSM model was experimentally validated yielding a maximum of 65 wt % PHA content and 10.2 g/L cell dry weight, which represent 12.1% increase in PHA content in comparison to the non-optimized condition using oleic acid and 1-pentanol as the carbon source and carbon precursor, respectively. The experimental results showed that the RSM was a useful tool as the utilization of 1-pentanol by *Cupriavidus* sp. USMAA2-4 was reduced by 33%. A second-order polynomial equation was obtained by multiple regression analysis to explain the combined effect of the three parameters with the coefficient of determination, R^2, greater than 0.9. The optimum process conditions and interactions were determined by analyzing three-dimensional surfaces. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 125: 2155–2162, 2012

Key words: response surface methodology; poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate); *Cupriavidus* sp. USMAA2-4; optimization; 1-pentanol

INTRODUCTION

Polyhydroxyalkanoate (PHA) is an intracellular microbial reserve polymer of 3-hydroxy acids, which are produced by numerous bacterial[1] during the depletion of nutrient such as nitrogen, oxygen, or phosphate in the presence of excess carbon.[2] The intriguing biological polymer has received considerable attention over the last decade because of the fact that PHAs exhibit properties of biodegradable plastics and elastic polymers.[3]

It has been reported that large scale production of poly(3-hydroxybutyrate) P(3HB) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) P(3HB-*co*-3HV) have been attempted. Because P(3HB) is crystalline and brittle, it is unsuitable for many applications. Consequently, the copolymer P(3HB-*co*-3HV) is chosen because of its reduced crystalline and higher flexibility.[4,5]

Improvement in PHA production strategies can lead to reduction in cost of production. Optimizing one-variable-at-a-time has been used traditionally in optimization process. This technique is carried out by monitoring the influence of one factor at a time on an experimental response. The major disadvantage of this single variable optimization is that it does not include the interactive effects among the variables studied. Besides, the increase in number of experiments leads to the increase in time and expenses for experimentation.[6] Therefore, the response surface methodology (RSM) is used for the optimization purpose to optimize all the affecting parameters collectively.

RSM is a statistically designed experimental protocol used for various applications.[7] It is a collection of statistical techniques for building models, designing experiments, evaluating the effects of factors, and searching for optimum conditions of factors for desirable responses.[8] Among these, the central composite design (CCD) is the most popular as it is very efficient and flexible. It provides much information on experimental variable effects and overall experimental error.

Fewer experimental runs are involved, and the interaction between the variables can be identified and quantified.[7] Contour plots and 3D surfaces are generated by linear or quadratic effects of the

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variables. A model equation is derived from the software that fits the experimental data. This equation is used to calculate the optimal response of the system.[9]

In this study, physical optimization of P(3HB-*co*-3HV) production and cell biomass of *Cupriavidus* sp. USMAA2-4 was carried out using a statistical analysis. Three independent parameters were used in statistical strategies to maximize the production of copolymer using two-level factorial design and RSM. The experiments conducted provided basic information to further improve the efficiency of PHA production and supported the analysis of main effect of each parameter.

EXPERIMENTAL

Bacterial strain

Cupriavidus sp. USMAA2-4 (DSM 19379) was isolated from a soil sample collected from Sg. Pinang, Penang, Malaysia. The isolated bacterium was gram negative, nonspore forming, motile, aerobic, and rod shaped.[10] Precultured cells $\sim$ 0.06 g/L of *Cupriavidus* sp. USMAA2-4 was grown at 30°C under aerobic condition in a nutrient broth as previously reported by Amirul et al.[11] For maintenance purpose, *Cupriavidus* sp. USMAA2-4 from the exponential growth phase was stored at −20°C in 20% (v/v) glycerol.

Biosynthesis of P(3HB-*co*-3HV)

Biosynthesis of P(3HB-*co*-3HV) was carried out as previously described by Amirul et al.[11] Briefly, precultured cells (0.06 g/L) were transferred into 50 mL of mineral salts medium containing (per liter) KH_2PO_4 (3.7), K_2HPO_4 (5.8), $(NH_4)_2SO_4$ (1.1), $MgSO_4.7H_2O$ (0.2), and 1.0 mL microelements solution containing per liter of 0.1*M* HCl: $FeSO_4.7H_2O$ (2.78), $MnCl_2.4H_2O$ (1.98), $CoSO_4.7H_2O$ (2.81), $CaCl_2.2H_2O$ (1.67), $CuCl_2.2H_2O$ (0.17), and $ZnSO_4.7H_2O$ (0.29) in 250 mL flasks. The initial pH of the medium was 7.0. The medium was added with sterilized carbon sources and carbon precursors to promote growth and polymer accumulation. After 48 h of incubation, cell growth was monitored by measuring the optical density of the broth at 540 nm. The broth containing the cells was then centrifuged and rinsed twice with distilled water.

Analytical procedures

PHA content and composition in the lyophilized cells were determined using gas chromatography (Shimadzu GC-2014). A total of 15–20 mg of lyophilized cells was subjected to methanolysis in the presence of 2 mL methanol and 2 mL sulfuric acid [85% : 15% (v/v)]. The mixture was incubated at 100°C for 2 h 20 min.[12] The organic layer containing the reaction products was separated, dried over Na_2SO_4, and analyzed by GC according to a standard method using SPB-1 Fused Silica Capillary Column, 30 m × 0.25 mm × 0.25 μm film thickness (Supelco).

Factorial design

The carbon source and precursor, which had been screened earlier, were used for the optimization (data not shown). For the first phase of the optimization process, two-level factorial designs were chosen to ascertain the effects of independent variable generalizable across all levels or specific to particular level. The ranges of the variables tested were concentrations of 1-pentanol (0.03 to 0.12 wt % C), incubation times (36 to 72 h), and agitation rate (50 to 250 rpm). Factor levels were chosen by considering the operating limits of the experimental system (agitation rate). For a full factorial design, all possible combinations of the two levels of the independent variables were investigated. Three center points were added to estimate the experimental error and to check the adequacy of the model. The cultures were incubated at room temperature in the orbital shaker. The response obtained in these 11 experiments (three factors) was subjected to compatible analysis, which yielded *t*-values. The components giving higher *t*-value were taken up for further studies.

Experimental design of process parameters

Based on the results obtained from the factorial design, the CCD was conducted. Experimental designs of 20 experiments were formulated using Design Expert software 7.1.6 (Stat-Ease Inc). The order in which the runs were made was randomized to avoid systematic errors. Range of variables at different levels for the CCD is shown in Table I. During the analysis, the cell dry weight and PHA content were determined. In developing the regression equation, the test variables were coded according to eq. (1):

$$x_i = \frac{X_i - X_{i*}}{\Delta X_i} \tag{1}$$

where x_i is the dimensionless coded value of an independent variable, X_i is the actual value of an independent variable for the *i*th test, X_{i*} is the actual value of an independent variable at the center point, and ΔX_i is the step change value. The response variable was fitted by a second-order model to correlate the response variable to the independent variable. The general form of the second-degree polynomial equation is given by eq. (2):

TABLE I
Range of Variables at Different Levels for the Central Composite Design

Variables	coded symbol	levels				
		−1.414	−1	0	1	1.414
Concentration of precursor (wt % C)	X_1	0	0.03	0.07	0.12	0.15
Incubation time (h)	X_2	24	36	54	72	84
Agitation rate (rpm)	X_3	0	50	150	250	318

$$Y_i = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i}^{k} \sum_{j}^{k} \beta_{ij} X_i X_j \quad (2)$$

where Y_i is the predicted response, $x_i x_j$ are input variables which influence the response variable Y, β_o is the offset term, β_i is the ith linear coefficient, β_{ii} is the ith quadratic coefficient, and β_{ij} is the ijth interaction coefficient. The second-order polynomial coefficients were calculated using Design Expert software version 7.1.6 (Stat-Ease Inc.). 3D surfaces were generated to visualize graphically the interactions between the factors involved. The statistical analysis of the model was performed in the form of analysis of variance (ANOVA). ANOVA consist of classifying and cross classifying statistical results and testing whether the means of a specified classification differ significantly. This analysis included the Fisher's *F*-test (overall model significance), its associated probability *P(F)*, correlation coefficient *R*, and determination coefficient R^2, which measures the goodness of fit of regression model.

RESULTS AND DISCUSSION

Screening of parameters using factorial design

Based on the preliminary findings, oleic acid and 1-pentanol were found as the most effective carbon source and carbon precursor, respectively. A two-level factorial design was used to determine the impact of different concentration of carbon precursor (1-pentanol), incubation time, and agitation rate on PHA production and growth. It is also used to eliminate irrelevant factors and as a tool to explore response surfaces. Based on Table II, there was 2^K possible combination of factors or 11 experimental runs for three factors. The two-level factorial design reduced the experimental effort at the expense of reduced ability to identify some higher order interactions among factors. Thus, the 11 experiments proposed by two-level factorial design with three factors and two levels were used for fitting a first order response surface (linear regression model) as noted in Table II. The runs were conducted in randomized order to reduce systematic bias. In the runs noted, the values of the various factors were set close to the optimal settings derived from the preliminary study (Table III). An alternating pattern of high- and low PHA content and cell dry weight were observed. Based on Table II, small amount of PHA content and cell dry weight were obtained when the agitation rate was the lowest (50 rpm), followed by a drastic increase of PHA content and cell dry weight when the agitation rate was high (250 rpm). It was reported that agitation rate plays an important role in mass transfer in a submerged fermentation. Shear rate increases as the agitation rate increase. As a result, the oxygen supply to the bacteria decreases resulting in low growth.[13] Based on the table, when

TABLE II
Experimental Design and Response of Two-Level Factorial Design for the Production of Copolymer[a]

Precursor (wt % C)		Incubation time (h)		Agitation rate (rpm)		Cell dry weight (g/L)	PHA content (wt %)[b]
X_1	Coded	X_2	Coded	X_3	Coded		
0.03	−1	36	−1	50	−1	1.3 ± 0.6	7 ± 1
0.12	1	36	−1	50	−1	0.6 ± 0.0	5 ± 0
0.03	−1	72	1	50	−1	1.5 ± 0.1	8 ± 1
0.12	1	72	1	50	−1	1.3 ± 0.1	9 ± 2
0.03	−1	36	−1	250	1	10.1 ± 0.7	68 ± 7
0.12	1	36	−1	250	1	7.9 ± 0.3	69 ± 6
0.03	−1	72	1	250	1	9.5 ± 1.2	70 ± 2
0.12	1	72	1	250	1	7.3 ± 0.0	46 ± 0
0.07	0	54	0	150	0	8.7 ± 1.2	54 ± 1
0.07	0	54	0	150	0	8.8 ± 0.9	55 ± 1
0.07	0	54	0	150	0	8.1 ± 2.5	56 ± 1

[a] The cells were harvested according to the run. Data is the mean ± standard deviation of triplicate samples.
[b] Calculated from GC analysis.

TABLE III
Range of Variables at Different Levels for the Factorial Design

Variables	Coded symbol	Levels		
		−1	0	1
Concentration of precursor (wt % C)	X_1	0.03	0.07	0.12
Incubation time (h)	X_2	36	54	72
Agitation rate (rpm)	X_3	50	150	250

the concentration of 0.03 wt % C 1-pentanol were used, the cell dry weight and PHA content were higher compared to the usage of 0.12 wt % C 1-pentanol. This was due to the toxicity effect of 1-pentanol at higher concentration. All the three factors were important as *Cupriavidus* sp. USMAA2-4 grew well within certain precursor concentration, agitation speed with a longer incubation time. PHA production was affected greatly by the cell growth and the cell growth was dependent on all the three factors.

Response surface methodology

RSM is used to obtain a second-order model where a maximum production of the PHA and cell biomass can be obtained. RSM is different from two-level factorial in which for each factor, five levels were tested. Once the relevant factors having high *t*-values were selected using factorial design, optimization of this copolymer production was carried out using a CCD. It has been reported that optimization of this copolymer has been carried out previously using *Nostoc muscorum,* and the product (% dcw) increased by 11%.[9] In this study, concentrations of 1-pentanol, incubation time, and agitation rates were selected as independent variables based on the previous studies. The responses were cell dry weight, PHA content, and 3HV monomer composition. Experimental results were fitted to a full quadratic second-order polynomial equation by applying multiple regression analysis, and the regression coefficients were obtained to predict a polynomial model.

Table IV shows the PHA content, biomass, and 3HV monomer for the 20 run in RSM. In RSM, there are three tests needed to be performed for each response, namely test significance of regression model, test for significance of model terms, and the test for insignificance of lack-of-fit. To determine the significance of the quadratic model, ANOVA was conducted.[14] The *P*-values were used to check the significance of each coefficient, which also indicated the interaction strength of each parameter. The smaller the *P*-values are, the bigger the significance of the corresponding coefficient. The significance of fit of the model was examined by the *F*-test. The greater the *F*-value is from unity, the more certain it is that the factors explain adequately the variation around its mean. The residuals were examined to check the adequacy of the model. Table V shows the *F*-value, corresponding *P* values and residuals.

The three-dimensional (3D) response surfaces were generated to study the interaction among the

TABLE IV
Experimental Design as Given by Response Surface Methodology[a]

Precursor (wt % C)		Incubation time (h)		Agitation rate (rpm)		Biomass (g/L)	PHA content (wt %)[b]	3HV monomer (mol %)
X_1	Coded	X_2	Coded	X_3	Coded			
0.03	−1	36	−1	50	−1	1.3 ± 0.6	7 ± 1	45 ± 6
0.12	1	36	−1	50	−1	0.6 ± 0.0	5 ± 0	65 ± 1
0.03	−1	72	1	50	−1	1.5 ± 0.1	8 ± 1	21 ± 1
0.12	1	72	1	50	−1	1.3 ± 0.1	9 ± 2	36 ± 4
0.03	−1	36	−1	250	1	10.1 ± 0.7	68 ± 7	2 ± 1
0.12	1	36	−1	250	1	7.9 ± 0.3	69 ± 6	6 ± 1
0.03	−1	72	1	250	1	9.5 ± 1.2	70 ± 2	2 ± 1
0.12	1	72	1	250	1	7.3 ± 0.0	46 ± 0	9 ± 0
0	−1.414	54	0	150	0	4.8 ± 0.4	20 ± 11	0
0.15	1.414	54	0	150	0	0.4 ± 0.1	7 ± 4	75 ± 18
0.07	0	24	−1.414	150	0	4.3 ± 0.3	17 ± 10	36 ± 1
0.07	0	84	1.414	150	0	10.3 ± 1.4	50 ± 14	10 ± 4
0.07	0	54	0	0	−1.414	0.4 ± 0.1	6 ± 4	72 ± 0
0.07	0	54	0	318	1.414	9.5 ± 2.0	60 ± 3	4 ± 1
0.07	0	54	0	150	0	6.2 ± 1.0	26 ± 1	11 ± 1
0.07	0	54	0	150	0	6.2 ± 1.0	26 ± 1	11 ± 1
0.07	0	54	0	150	0	6.2 ± 1.0	26 ± 1	11 ± 1
0.07	0	54	0	150	0	7.9 ± 1.0	16 ± 1	17 ± 1
0.07	0	54	0	150	0	7.9 ± 1.0	16 ± 1	17 ± 1
0.07	0	54	0	150	0	7.9 ± 1.0	16 ± 1	17 ± 1

[a] The cells were harvested according to the run. Data is the mean ± standard deviation of triplicate samples.
[b] Calculated from GC analysis.

TABLE V
Analysis of Variance and Regression Analysis for PHA Content, Cell Dry Weight, and 3HV Monomer

Response	Source	Sum of squares	DF	Mean square	*F* value	Prob > *F*
	Model	8332.08	9	925.79	10.81	0.0005
PHA content	Residual	856.52	10	85.65		
	Lack of fit	703.50	5	140.70	4.60	0.0598
	Pure error	153.02	5	30.60		
	Total	9188.60	19			
	Model	211.70	9	23.52	11.56	0.0003
Cell dry weight	Residual	20.35	10	2.03		
	Lack of fit	16.01	5	3.20	3.69	0.0889
	Pure error	4.34	5	0.87		
	Total	232.05	19			
	Model	7983.46	9	887.05	27.95	<0.0001
3HV	Residual	317.34	10	31.73		
	Lack of fit	263.34	5	52.67	4.88	0.0535
	Pure error	54.00	5	10.80		
	Total	8300.80	19			

R^2 (PHA content) = 0.907.
R^2 (cell dry weight) = 0.912.
R^2 (3HV monomer) = 0.962.
DF, degrees of freedom; F, variance ratio; P, probability.

three factors tested and the combined effects of factors keeping one factor constant at a time. In the response of PHA content, the model shows prob>*F* less than 0.05, which indicated that the model was significant (Table V). In the response, the "Lack-of-fit F-value" of 4.6 implied that the lack of fit was not significant in relative to the pure error. There was a 5.98% chance that a "Lack-of-fit *F*-value" this large could occur due to the noise. These ANOVA data clearly confirmed that the estimated models fitted the experimental data adequately.

The model was significant for the response of cell dry weight as the value from the Fisher's *F*-test (F_{model}, mean square regression/mean square residual = 11.56) with a very low probability value ($P_{\text{model}} > F$) = 0.0003 (Table V). The "Lack-of-fit F-value" of 3.69 implied that the lack of fit was not significant in relative to the pure error. There was an 8.89% chance that a "Lack-of-fit *F*-value" this large could occur due to the noise. The model for 3HV monomer also showed the significant value with the lack of fit not significant (Table V). The goodness of fit of the model was checked by determination coefficient (R^2). For all the responses, the high value of these coefficients indicates a better precision and reliability of the experiments carried out.

The application of RSM yielded the following regression equation between the responses and the test variables in coded unit regardless of their significance

$$\text{PHA content} = 20.87 - 4.73X_1 + 4.25X_2 + 21.96X_3 - 0.62X_1X_2 - 5.10X_1X_3 - 0.97X_2X_3 - 1.62X1^2 + 5.69X_2^2 + 5.84X_3^2$$

$$\text{Cell dry weight} = 7.05 - 0.92X_1 + 0.72X_2 + 3.32X_3 - 0.063X_1X_2 - 0.44X_1X_3 - 0.26X_2X_3 - 1.54X_1^2 + 0.11X_2^2 - 0.72X_3^2$$

$$\text{3HV monomer} = 14.15 + 9.82X_1 - 7.52X_2 3 - 18.55X_3 - 1.37X_1X_2 - 1.87X_1X_3 + 5.88X_2X_3 + 2.38X_1^2 + 2.20X_2^2 + 7.50X_3^2$$

The term X_1, X_2, and X_3 represent the coded values of concentration of precursor, incubation time, and agitation rate. Based on the regression equation, different composition of PHA content, cell dry weight, and 3HV molar fraction could be obtained.

A better understanding on the relationship between the factors and responses could be done by investigating a series of 3D surface. Each contour curve represented an infinitive number of combinations of two test variables with the other one variable maintained at zero level.[15] Figure 1 shows the 3D surface for the interaction between precursor concentrations, agitation rate, and incubation time on PHA content. Referring to the plot, it can be seen that the PHA content increased with the incubation time and agitation rate but decreased with increasing 1-pentanol concentration. The contour plots were not perfectly elliptical. These indicated that there were fewer interactions among the independent variables.

It can be inferred from the graph that PHA content increased as the agitation rate increased up to 250 rpm. This was supported by the fact that PHA accumulation was favored by an increase in culture volume to flask ratio (low oxygen). Poorer agitation in larger culture volumes and the oxygen limitation

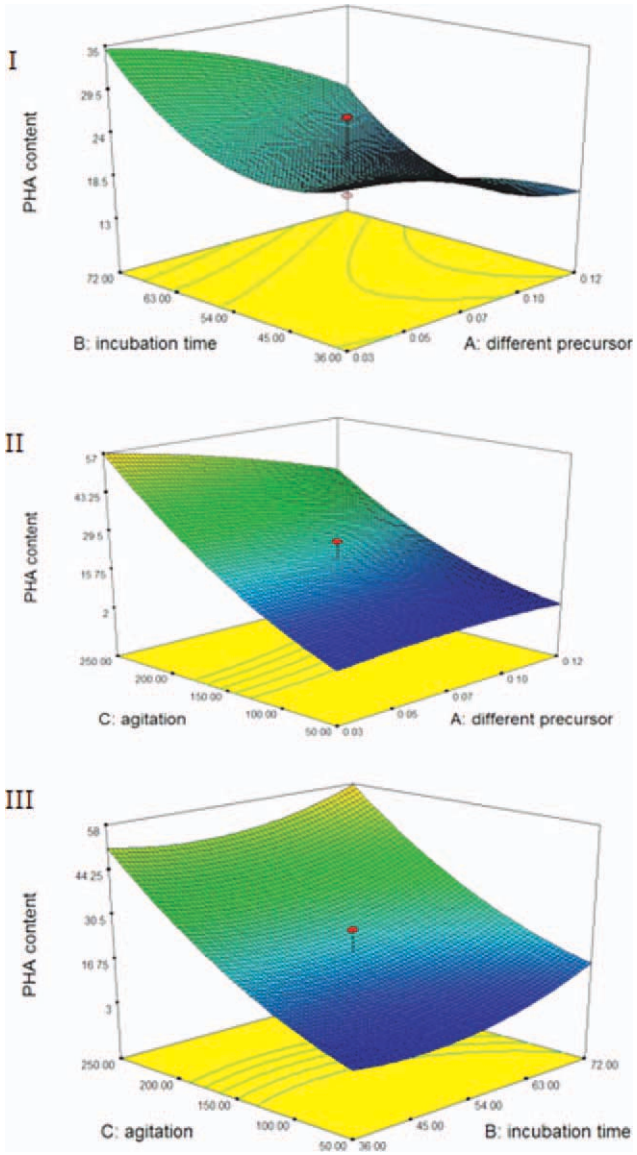

Figure 1 3D response surface: Interactive effects of (I) varied incubation time and different concentrations of precursor at 150 rpm, (II) varied agitations and different concentrations of precursor at incubation time of 54 h, (III) varied agitations and incubation time at 0.07 wt % C precursor. (Response toward PHA content). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

to the culture were likely to contribute to a higher P(3HB) yield due to higher NADH/NAD^+. Biosynthesis of P(3HB) was stimulated by high concentration of NADH. Key enzymes related to TCA cycle, such as citrate synthase and isocitrate dehydrogenase, were inhibited by high level of NADH. This ensured the availability of acetyl-CoA for the polymer production.[16]

Figure 2 shows the 3D surface for the interaction between different concentrations of precursor, agitation rates, and incubation times on cell dry weight (CDW). CDW increased with incubation time, agitation rate, and concentration of 1-pentanol until 0.07 wt % C. The 3D plots were slightly inclined to the horizontal showing that there was appreciable interaction between two parameters.

Referring to the plot, as the concentration of 1-pentanol was increased, the CDW were high. However, increasing further the concentration of 1-pentanol caused the amount of CDW formed decreased due to inhibitory effect of 1-pentanol on the cell growth.[17,18] This was reported by Bhubalan et al.[19] that increasing the concentration of carbon precursors above certain limit would decrease the amount of PHA content and CDW.

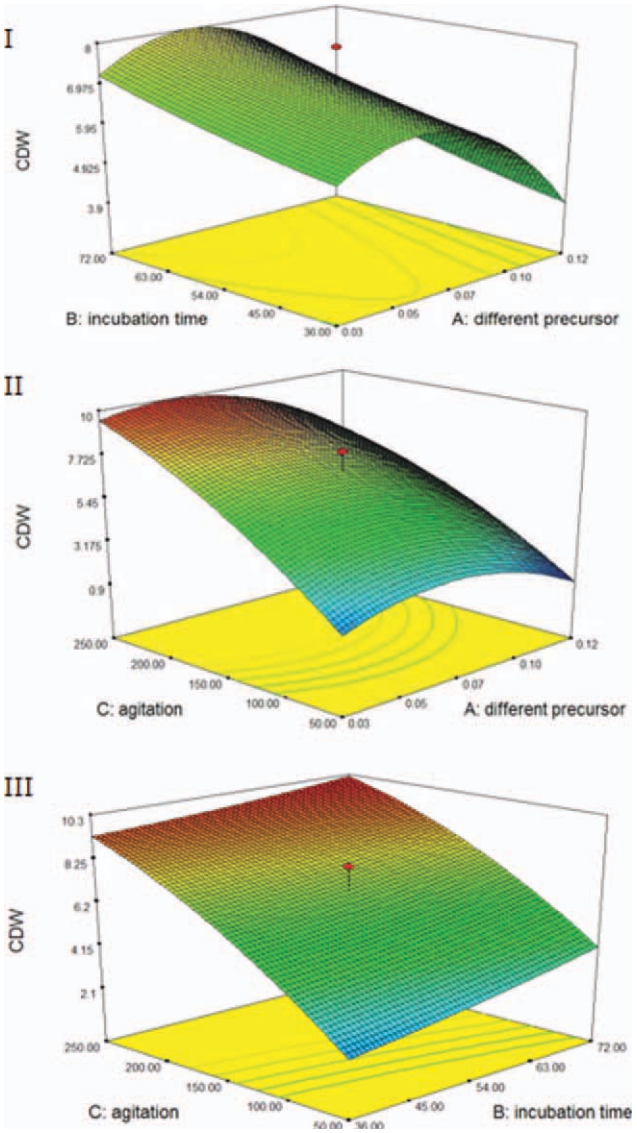

Figure 2 3D response surface: Interactive effects of (I) varied incubation time and different concentrations of precursor at 150 rpm, (II) varied agitations and different concentrations of precursor at incubation time of 54 h, (III) varied agitations and incubation time at 0.07 wt % C precursor. (Response toward cell dry weight). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

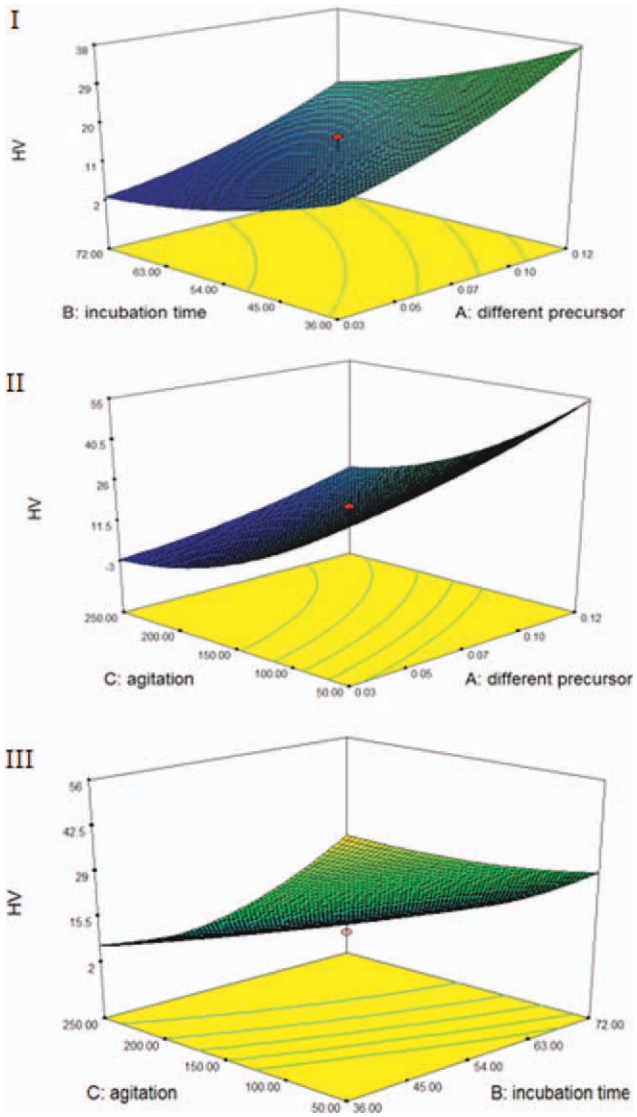

Figure 3 3D response surface: Interactive effects of (I) varied incubation time and different concentrations of precursor at 150 rpm, (II) varied agitations and different concentrations of precursor at incubation time of 54 h, (III) varied agitations and incubation time at 0.07 wt % C precursor. (Response toward 3HV monomer). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 3 shows the interaction between different concentrations of precursor, agitation rate, and incubation time on 3HV molar fraction. By referring to the plot, 3HV molar fraction decreased with incubation time within the experimental limit, remained almost constant with agitation rate and increased with different concentrations of 1-pentanol. The contour plots were not perfectly elliptical showing less interaction between the parameters.

From the plot, it can be seen that as the incubation time increased, the 3HV monomer decreased. This was most probably because at the beginning of incubation time, ketothiolase enzyme activity was high.

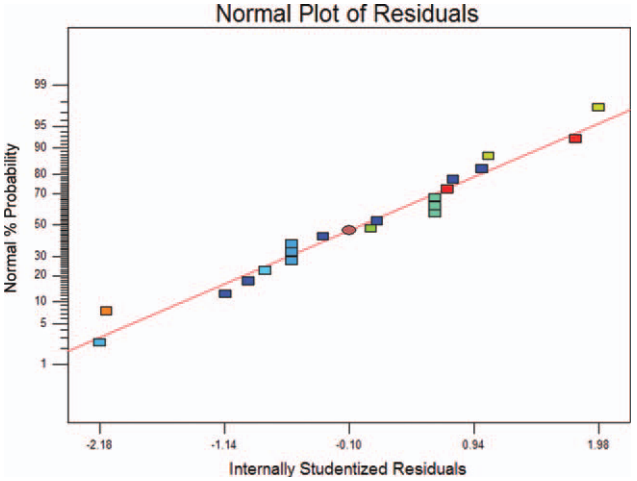

Figure 4 Normal (%) probability plot of the "studentized" residuals for PHA content. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

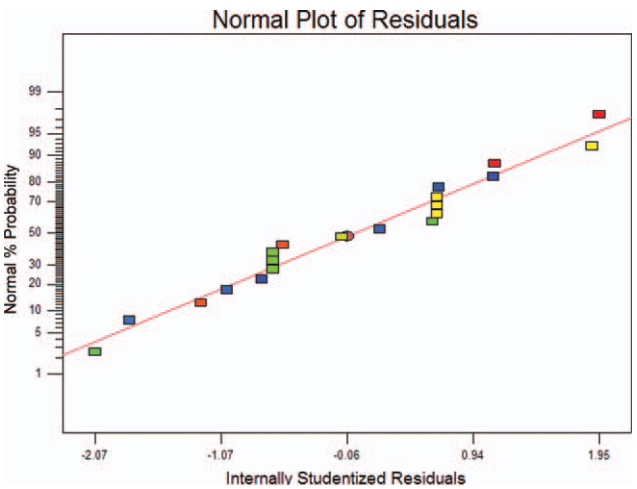

Figure 5 Normal (%) probability plot of the "studentized" residuals for cell dry weight. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

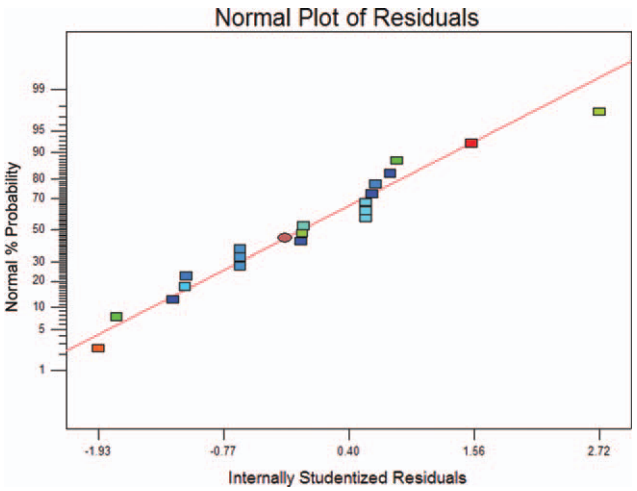

Figure 6 Normal (%) probability plot of the "studentized" residuals for 3HV monomer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE VI
PHA Content and Cell Dry Weight Before and After Optimization

Variable	PHA content (wt %)					Cell dry weight (g/L)		
	Before	After	Before	After		Before	After	
				Predicted[a]	Actual[b]		Predicted[a]	Actual[b]
Concentration of precursor (wt % C)	0.06	0.04	58	65	64	6.7	10.2	9.6
Agitation rate (rpm)	200	250						
Incubation time (h)	48	72						

[a] Predicted value generated by software.
[b] Actual value,

This caused the valeryl-CoA that was produced by 1-pentanol was used completely for 3HV monomer production. As the incubation time increased, some of the valeryl-CoA was converted to acetyl-CoA to produce 3HB monomer and also for cell metabolism.

Figures 4–6 shows the normal (%) probability plot of "studentized" residuals. It could be seen that for all the response the points were all almost near to the straight line indicating that no violation of the assumption in the analysis. This means that the actual and predicted values were almost near to each other for all the three response.

To verify the model, experiments were performed in triplicates using the optimized condition given by the software as shown in Table VI. PHA content of 64 wt % and CDW of 9.6 g/L were recorded compared to the predicted PHA content with 65 wt % and CDW with 10.2 g/L. Thus after optimization, the copolymer was obtained at the lower concentration of 1-pentanol namely 0.04 wt % C when compared with preoptimized condition where the PHA content and the biomass density were 58 wt % and 6.7 g/L, respectively, with the concentration of carbon precursor of 0.06 wt % C. It was obvious that the predicted and experimental values for PHA content and biomass density were in agreement. Upon optimization, the product (PHA content) increased by 12.1% and the requirement of 1-pentanol reduced by 33%.

CONCLUSIONS

The major goal of the research is to study the effect of external factors in the production of PHA. Therefore, it was our intention to advance the production of PHA by including crucial factors. Statistical analysis using RSM is a valuable tool for optimizing this copolymer production as it resulted in a production protocol which maximized production at a reduced cost and time. Based on the statistical analysis, the significant interaction between different concentrations of 1-pentanol, incubation times, and agitation rates on PHA content, CDW, and 3HV monomer were also determined. The methodologies of two-level factorial design were very useful in the screening process. A significantly higher maximum biomass of 10.2 g/L and PHA content of 65 wt % were obtained after optimization. Accurate prediction of the maximum value of the experimental response and the constant variance of residuals indicated that the quadratic model was adequately selected to describe the response surface within the experimental region. Finally, RSM proved to be an effective tool in optimizing and improving the production of PHA.

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