DEVELOPMENT OF OPTIMALITY CRITERION FOR THE ENZYME CATALYSED BIO-POLYMERIZATION

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Abstract - This study highlights the optimisation of parameters for the synthesis of a biodegradable polymer – polycaprolactone, using enzyme as a catalyst. Process parameters namely temperature, time, mixing speed and solvent volume are optimised using optimal design in design of experiment software. Furthermore, polymer yield was considered as response and later monomer conversion percentage was calculated using gravimetric analysis. The results obtained display a good agreement with 3.4% percent deviation between predicted values and actual values.

Keywords - Optimisation, biodegradable polymer, Enzyme catalysis, Design of experiment, polycaprolactone, lipase.

I. INTRODUCTION

As in today's events, implementing environmentally friendly products is becoming more and more essential in order to maintain greener and safer world. In this context, biodegradable polymers are one such product, whose demand has been increased phenomenally in recent days. [1]. Plastic regulations and bans led to the increased demand and market value of the biodegradable polymers. Besides, their potential applications in industrial, medical and agronomical fields adds to their high demand. Among many biodegradable polymers, polycaprolactone was one among them with wide range of applications[2, 3].Conventional synthesis of polycaprolactone involves ring-opening polymerization using chemicals and metals as catalyst. In recent advances, enzyme catalysis is more predominant in usage, to avoid toxic residues.

Additionally, their activity in mild conditions, high specificity for substrates and natural origin makes them prudent [4]. Nevertheless, biodegradable polymers synthesised using enzymes reaching the market still remains a problem. Moreover, optimisation of process parameters in any production is important endeavor for the economy and feasibility of the process. Besides, they do play a prominent role in determining the product yield and quality. In order to improve the yield, specific conditions for parameters are need to be selected. However, this process will be taunting when it comes to industrial scale. Regular optimization methods like single factor analysis is tiresome with less accuracy [5]. Furthermore, enzyme catalyzed polymerization does notallow any wastage of materials. Therefore, it's important to choose a powerful technique to predict the optimized conditions with good accuracy. Given that, design of experiments (DOE), a new statistical approach was touted to be the efficient to evaluate the relation between independent process variables and chosen responses along with determining the optimised conditions. In this study, the process parameters were optimized for synthesis of polycaprolactone via enzyme catalysis. The objective of this study is to predict the optimised conditions to achieve maximum yield by performing minimum number of experimental runs. Also, to understand the process i.e. the interaction of each parameters and their effect on polymer yield.

II. MATERIALS AND METHODS

A. Materials

Caprolactone was used as monomer, Solvent opted for this bio-polymerisation process was toluene. Lipase B from Candida Antarctica(Novozyme 435) has been used as a catalyst, to convert monomer to polymer. Other solvents like chloroform and methanol were used to terminate the process and precipitate the polymer respectively. Monomer and solvents have been obtained from Merck Sdn Bhd, Malaysia. Enzyme catalyst was purchased from Sigma Aldrich Sdn Bhd, Malaysia. All the materials were used as received. Solvents purchased are all of analytical grade.

B. Methods

1. Procedure for synthesis of polycaprolactone using Novozyme 435

Bio polymerisation reaction was performed in a 250 ml conical flask with constant stirring using magnetic hot plate. Initially 10 ml of monomer and 1g of enzyme are added to the flask. Throughout the process, the amount of monomer and enzyme were kept constant. Whereas, the temperature, reaction time, mixing speed (RPM) and solvent volume have been operated according to the DOE table obtained from the software. Later, when the reaction reached

the specified time, termination of the reaction was done by adding excess amounts of cold chloroform. Furthermore, the enzyme filtration was done using filter paper, followed by rotatory evaporation inorder to get rid of excess chloroform. Finally, methanol was added to precipitate the polymer and later dried in oven for overnight, maintained at 50°C. Final dry polymer obtained was weighed and further monomer conversion percentage was calculated using gravimetric analysis.

2. D- Optimal Experimental Design

Stat- ease software version 9 was used to develop the model. D-optimal design, one among the other designs in response surface methodology was opted to optimise the synthesis process to achieve maximum polymer yield.Four process parameters considered for optimisation are temperature (50 – 110 °C), reaction time (1-7 hours), mixing speed (50 – 500 rpm) and solvent volume (10 – 60 ml). Polymer yield was chosen as response and rangeof parameters were chosen from previous literature[6]. Based on the 25 experimental runs generated by the optimal design, the effect of process parameters on response is studied and determined statistically. Four process parameters have been considered as continuous factors from low to high ranges.

The polynomial equation developed by the optimal design software enunciates the relation between process parameters and their chosen responses. Represented in equation 1

$$Y = \beta_0 + \sum \beta i\, Xi + \sum \beta ij\, Xi\, Xj + \sum \beta i Xi^2$$

(Eq 1)

Wheredenotes the response, β0 is coefficient of independent constant, βi is the individual coefficient, Xi stands for independent parameters (i = A, B, C, D), βij is for interaction coefficient effects (i = A, B, C, D and j = A, B, C, D)

Significance of the model was given by Analysis of variance and data was analysed through analysis of variance i.e. ANOVA, to investigate the importance of each process parameters along with their interactions. Statistical significance and the quality of the model fit were expressed by F-test and the p value along with few more terms explained in later sections.

C. Gravimetric analysis

The monomer conversion percentage was reported using gravimetric analysis. The conversion rate was calculated gravimetrically as shown in equation 2.

$$\text{Monomer conversion (\%)} = \frac{WP}{WM} \times 100 \text{ (Eq 2)}$$

Where, WP is the weight of the polymer and WM is the weight of the monomer.

III. RESULTS AND DISCUSSION

A. ANOVA

ANOVA evaluates the regression analysis of the model developed[7]. It consists of set of evaluation terms such ascoefficient of determination (R^2), adjusted R^2, predicted R^2, coefficient of variance (C.V.%), adeq precision, F value and p value to explain the significance of the model. Table 1 presents all the values related to the developed model. Table 2 presents the experimental runs with actual and predicted values of the polymer yield. The polynomial equation regenerated for the model given below (Equation 3)

$$R1= +5.86 +0.4834*A -0.0184*B -0.0153*C +0.8526+D -0.1779*AC -0.2986 *AD -0.6345*BC -0.5309 -0.4687*CD +1.17*A^2 +0.4192*B^2 +1.05*C^2 -0.9685*D^2 \text{(Eq 3)}$$

Whereas R1 represents the response that is monomer conversion percentage and A, B, C& D represents temperature, reaction time and solvent volume respectively.

TABLE 1: MODEL ANALYSIS BY ANOVA

ANOVA terms	Values
R2	0.9914
Adj R2	0.9774
Pre R2	0.9589
F value	70.82
P value	<0.0001
CV	1.50
Adequate precision	26.9

From the Table 1, the coefficient of determination (R^2)for response is 0.99 respectively. The R^2 values near to ≈1, indicates the model significance and also the realconnection between the process parameters and responses[8].

The values of predicted R^2 and adjusted $R^2$are 0.94 and 0.97 respectively, they represent the high degree variations of the model which will help in predicting the optimised conditions with good accuracy and also controlling the predictions to avoid any overfitting of the data. For the significant statistical model, the value of the difference between adjusted R^2 and predicted R^2 will be lesser than 0.2.

From the Table 1, the difference between these two terms is less than 0.02, presenting the significance of the model. It also explains the consistency of the variations considered throughout the model development. Low values of C.V.% indicates the great interrelationship and accuracy in-between the actual values and the predicted values. The CV value obtained from the developed model is 1.50 indicatinghigh accuracyamong the actual and predicted values of the polymer yield.

TABLE 2: EXPERIMENTAL RUNS WITH ACTUAL ANDPREICTED VALUES

S no	P1 (°C)	P2 (hr)	P3 (rpm)	P4 (ml)	R1 (gm)	R2 (gm)
1.	110	7	50	10	8.3	8.33
2.	85	4	50	30	6.9	6.88
3.	101	1	500	60	8.4	8.49
4.	79	1	219	10	5.7	5.59
5.	50	1	500	20	7.9	7.75
6.	50	7	500	10	6.4	6.40
7.	110	7	405	10	7.1	7.06
8.	50	6	500	60	7	6.98
9.	110	7	500	60	6.7	6.72
10.	110	1	500	10	7.8	7.85
11.	50	4	50	10	4.1	4.30
12.	50	1	151	30	6.4	6.38
13.	80	7	50	60	7.8	7.78
14.	110	4	208	60	7.5	7.40
15.	50	7	500	10	6.4	6.40
16.	101	1	500	60	8.6	8.49
17.	110	4	208	60	7.3	7.40
18.	87	5	50	30	6.8	6.83
19.	50	7	223	40	7.2	7.21
20.	50	1	50	60	8.4	8.49
21.	110	1	50	30	4.3	4.50
22.	110	1	500	10	7.9	7.85
23.	99	7	259	40	7.1	7.15
24.	50	1	500	20	7.6	7.75
25.	70	3	311	60	6.1	6.13

*P1=Temperature, P2=Reaction time, P3= mixing speed, P4=Solvent volume,R1= actual conversionand R2 = predicted conversion.

Other variables such as F and p values have been used to explain the model significance. F value is measure of scattered data from that of the mean data, indicating the two variance ratio. On the other hand, p value stands for the statistic indicating the possibility of observed F value are under acceptance of null hypothesis [9]. And the p values less than 0.05 indicates the denial of null hypothesis through the complete model [10]. F and p value for the response are7.82 and <0.0001 respectively, indicating the model terms are significant. Subsequently, observed huge F value could not occur due to noise. Besides, this is well supported by the non-significant value (0.94) obtained for the lack of fit.

Additionally apart from ANOVA results and their validation, software generates diagnostic plots to validate the model significance. Figure 1 depicts the normal plot of the residual data, as all the points falls near to the line almost in straight line following the normal distribution.
Figure 2 shows residual experimental runs are assigned along the regression line representing good compliance between the predicted and actual values.

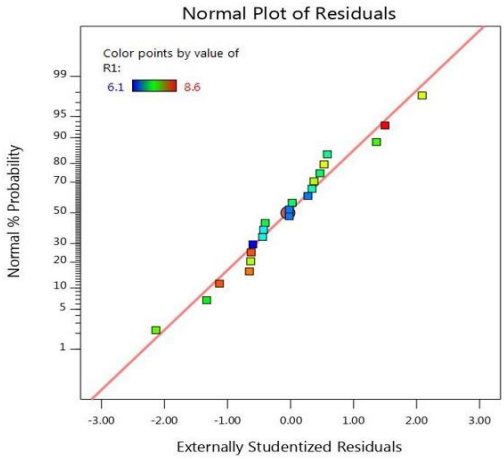


Fig 1. Normal plot for the response (polymer yield)

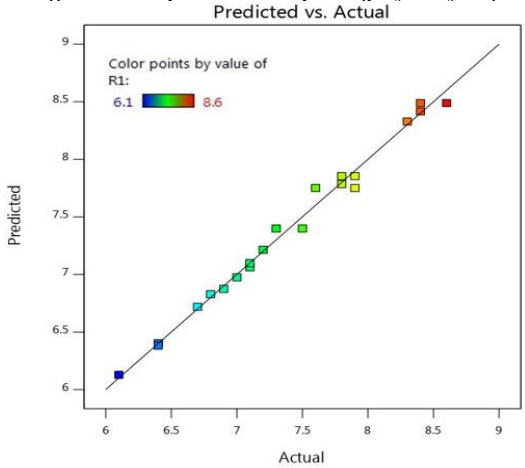


Fig 2. Plot for Residual vs predicted runs

Furthermore, Figure 3 and 4 illustrates the externally studentized residuals versus predicted and residuals vs predicted for the selected response respectively. Both of the plot are exhibiting the scattered residual runs within the brim of ± 4, supporting 95% of the confidence limits relyingon the run number. Consequently, the irregular trend of the scattered runs, explains that the values predicted by the model, has both lower and higher values (than the actual runs) with the equal prospect [11].

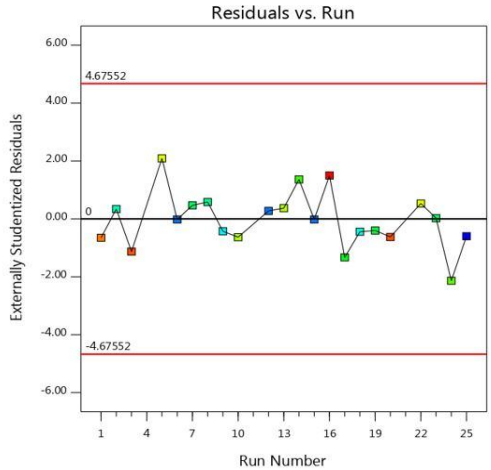


Fig 3. Plot for Residual vs run

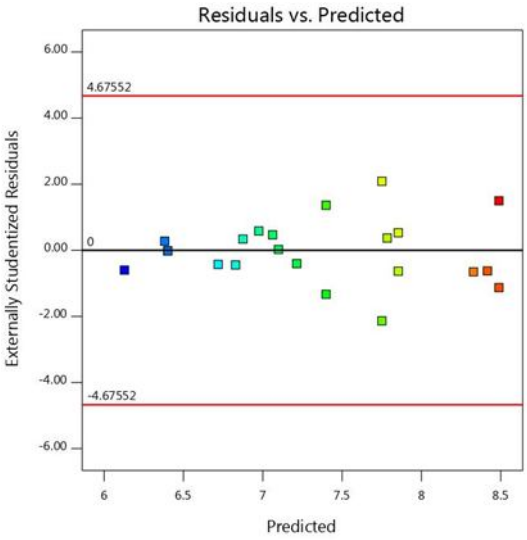


Fig4. Plot for Residuals vs Predicted

Finally there is perturbation plot depicted in Figure 5 explaining the effects of the four process parameters i.e. temperature, reaction time, mixing speed and solvent volume on the polymer yield. Slopes or steep curves in the perturbation curve speak for susceptibility of the chosen parameters on the polymer yield. Conversely, a flat line shows insensitivity to change in that particular factor. Thus, the perturbation plot could be used to find those factors that most affect the response [12, 13]. From the Figure 5, the slopes or steep deviations occurred depicts the sensitivity and profound effect of all the four process parameters on the polymer yield.

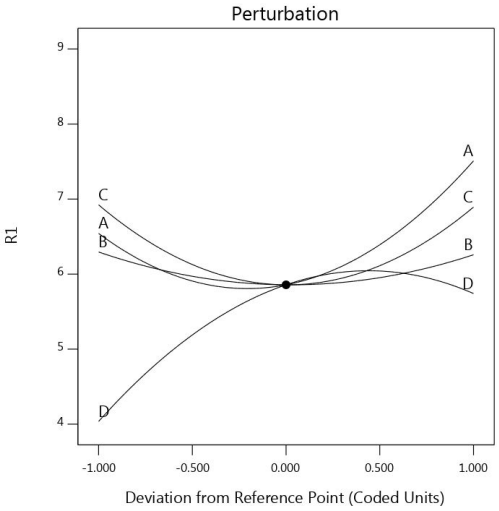


Fig5. Perturbation curve

B. Validation of the developed model

Validation was performed by conducting the predicted optimised experimental run. Table 3 presents the predicted optimised values of process parameters and the observed experimental response with the deviation percentage.Up to date, most of the researchers reported the polycaprolactone synthesis using the single variate approach, where it takes 60 – 70 runs to optimise the process parameters. Thus, this study attempts to achieve the optimised conditions to achieve the maximum polymer yield by performing only 25 runs. Additionally, this developed model not only simplifies the process by reducing the number of trials but also reduces the usage of chemicals, which in turn reducing the production cost.

Therefore to perform the validation, the polycaprolactone was synthesised by employing the predicted conditions developed by the D-optimal design. From Table 3 it is evident that predicted and observed values of the experiemntal run are almost equivalent and were very much within the accepted error of 3.4%.

TABLE 3:VALIDATION OF THE MODEL

	P1	P2	P3	P4	R1
Predicted	75.9	4	295	15	8.7
Observed	75	4	295	15	8.4
Error percentage					3.4%

*P1=Temperature, P2=Reaction time, P3= mixing speed, P4=Solvent volume, R1= response (polymer yield).

CONCLUSION

Successful implementation of D-optimal for polycaprolactone synthesis was achieved. 75°C, 4hrs, 295rpm and 15ml of solvent volume were identified as optimised conditions to achieve the maximum polymer yield with 84% monomer conversion percentage. Also, all the four parameters chosen have a profound effect on the response. Furthermore, error percentage is only 3.4% representing the robustness of the DOE.

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