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Optimization of Poly Lactic Acid (PLA) Plastic Degradation by *Aneurinibacillus Migulanus* Using Response Surface Methodology

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Abstract. Poly Lactic Acid (PLA) is the first commodity polymer produced from renewable resources. It provides environmental benefits since it requires lower energy in polymer production and in turn reduces green house gas release. In recent years, Response Surface Methodology (RSM) is extensively used in optimization study due to its effective and time-saving advantages. It is widely used to predict surface responses and study the interactions among variables. Applying RSM, this study focuses on parameters optimization in microorganism fermentation process using *Aneurinibacillus migulanus*. The 3D response surface plots and 2D contour plots are created by Box-Behnken Design (BBD) and applied to optimize the fermentation process parameters. The results indicate three variables; pH, temperature ($^{\circ}$C) and shaking speed (rpm) have significant effects on PLA degradation. The optimum condition is obtained as: pH 8, 40.5 $^{\circ}$C and 194 rpm. In addition, the strain degraded PLA film significantly at the maximum level of 69.62% within 20 days in liquid medium. The results from this study can be applied to a highly effective and environmental biodegradable plastics production. However, this study is a pioneer in applying *A. migulanus* to PLA film degradation and RSM. Therefore, a well-modified technique to better reflect its optimization is needed for future research.

Keywords: PLA, RSM, BBD, Optimization, *Aneurinibacillus migulanus*

1. Introduction

Poly Lactic Acid (PLA) is the first commodity polymer produced from renewable resources. In addition, different microorganisms can degrade different groups of plastics [1]. Depolymerization is the process of PLA degradation that can be found only in a few bacteria such as *Fusarium monilifore, Penicillium Roquefort*, *Amycolatopsis* sp., *Bacillus brevis*, and *Rhizopus delemer*. After the degradation, mineralization process will be performed and the end products will be CO_2, H_2O, or CH_4. Response Surface Methodology (RSM) is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching optimum conditions of factors for desirable responses. RSM has been used for several decades, in which some of the popular choices include the Box-Behnken design (BBD) [2]. However, few biodegradable plastics have been synthesized and microorganisms capable of degrading them have been slightly identified the optimization [3]. Therefore, the objectives of this study were to screen PLA films degrading microorganisms and determine the optimum conditions of pH, temperature ($^{\circ}$C), and speed shaking (rpm). RSM and BBD are applied to this study.

2. Materials and Methods

2.1. Microorganisms Culture and Media Preparation

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Soil samples in Taiwan were inoculated in this study. Bacterium was identified by 16S rDNA gene sequencing technique and analysed by BLAST using the program available online at Swiss Bioinformatics Institute (http://www.ch.embnet.org/software/aBLAST.html) [4]. The composition of this medium contained 0.1% (w/v) gelatin ; 10 g peptone; 5 g beef extract; 5 g NaCl; and 1000 ml distilled water, followed by sterilization at 121 $^{\circ}$C for 15 mins. The inoculums having a cell density of 10^6 cfu/ml was obtained by growing the culture overnight in liquid broth medium at 30 $^{\circ}$C, 180 rpm. The PLA films sheets (3x3 cm^2) (Benison & Co., LTD Taiwan Company) were sterilized [3] and added to the medium along with the inoculums.

2.2. The Degradation of PLA Films in Liquid Medium

The experiments were conducted in 500 ml Erlenmeyer flasks containing 100 ml of liquid medium with 0.1% (w/v) gelatin (Difco). The flasks were incubated on a rotary shaker (180 rpm) at 30 $^{\circ}$C within 20 days and compared with BBD. After that, PLA film degradation (%) was calculated [5]. The results were recorded and analyzed by SAS system for windows V8.0 and Design Expert Software V. 7.0.0 Trial. The percentage of degradation**:**

$$= \left[\frac{\text{Weight PLA film before degradation (g) - Weight PLA film after degradation (g)}}{\text{Total weight PLA film (g)}} \right] \text{x100}$$

2.3. Methodology and Design of Experiments

Response Surface Methodology (RSM) [2; 6; 7] was conducted in this experiment. It is an empirical modeling technique used to estimate the relationship between a set of controllable experimental factors and observed results. In addition, Box-Behnken Design (BBD) was also used to obtain the combination of values that optimizes the response within the region of the three-dimensional observation spaces. Table. 1 illustrated three independent variables focused in this study. The different parameters were chosen as key variables and designated as X_1, X_2 and X_3, respectively. The low, middle, and high levels of each variable were also designated as -1, 0 and +1. According to [7], the variables were coded as Eq. (1):

$$x_i = \frac{X_i - X_0}{\Delta X} \tag{1}$$

Table 1: Code and level of variables chosen for the trials

Factors	Symbols		Level		
	Coded	**Uncoded**	**-1**	**0**	**+1**
pH	x_1	X_1	6	7	8
Temperature ($^{\circ}$C)	x_2	X_2	30	37	44
Shaking speed (rpm)	x_3	X_3	160	180	200

Table. 2 showed the actual design of experiments. The behavior of the system was explained by the following second degree polynomial equation

$$Y = B_0 + \sum_{i=1}^{n} B_i x_i + \sum_{i=j=1}^{n} B_{x_i i j}\, x_i x_j \tag{2}$$

where *Y*= predicted response, it can be observed that in the present study, three variables are involved and hence *n* takes the value 3. Thus, by substituting the value 3 for *n*, Eq. (2) becomes:

$$Y = B_0 + B_1x_1 + B_2x_2 + B_3x_3 + B_{12}x_1x_2 + B_{13}x_1x_3 + B_{23}x_2x_3 + B_{11}x_1^2 + B_{22}x_2^2 + B_{33}x_3^2 \tag{3}$$

where X_1, X_2, and X_3 are variables (pH, temperature, rpm); B_0 is a constant; B_1, B_2, and B_3 are Linear coefficients; B_{12}, B_{13}, and B_{23} are cross-product coefficients. B_{11}, B_{22}, B_{33} are quadratic coefficients. For Eq. (3), a total of 15 runs are necessary for locating the optimal levels and range of selected factors. After that, experiment was carried out by BBD and the percentages of degradation were recorded.

3. Results and Discussion

3.1. Characterization of Bacterium (16S rDNA Sequencing Technique)

The result of 16S rDNA sequence alignment based on BLAST analysis revealed that the isolated bacterium was *Aneurinibacillus migulanus*. This isolate showed 99% identity with an *Aneurinibacillus migulanus* strain, partial sequence, Strain: DSM 2895T. Thus, this was the first report of *Aneurinibacillus migulanus* species that capable for PLA films degradation.

3.2. Regression Models of Response

The values of pH, temperature, and rpm were used in the optimization study. The data was analysed by multiple regression analysis using the Design Expert Software. The following polynomial equation was derived to represent percentage of degradation (Y) as a function of the independent variables tested. The experimental data (Table. 2) were analysed using statistical methods appropriate to the experimental design used. Multiple regression analysis of the experimental data gave the following second order polynomial equation:

$$Y = 9.11 - 0.31x_1 + 0.88x_2 + 1.53x_3 - 0.53\, x_1x_2 - 0.47x_1x_3 + 0.19x_2x_3 - 2.14\, x_1^2 - 4.70\, x_2^2 - 3.41\, x_3^2 \qquad (4)$$

A summary of the analysis of variance (ANOVA) for the selected quadratic model was shown in Table. 3. The correlation measures for testing the goodness of fit of the regression equation were the multiple correlation coefficients R and the determination coefficient R^2. The value of R (0.9545) for Eq. (2) being close to1, indicated a high degree of correlation between the observed and predicted values. The value of the determination coefficient R^2 (0.9110) and adjust R^2 (0.8442) suggested that model may explain 84.42% of the total variation in response. The analysis of variance (ANOVA) for response surface quadratic model was summarized in Table. 3. The model F-value was 13.64 and the F-value for lack of fit was 9.89. The P-value for the model (0.0008) and for lack of fit (0.0947) led to the same conclusion. The coefficient estimate and the corresponding Prob > F-values suggested that all the independent variables studied had significant effects on the percentage of PLA degradation by *Aneurinibacillus migulanus*. The analyses also showed that there were significant interactions between pH, temperature (°C), and speed shaking (rpm) (Fig.1-3).

Table. 2: Box-Behnken Design plan in coded value and the observed response.

Run	x_1	x_2	x_3	Y Degradation (%)
1	6(-1)	30(-1)	180(0)	0.59
2	8(1)	30(-1)	180(0)	0.99
3	6(-1)	44(1)	180(0)	4.6
4	8(1)	44(1)	180(0)	2.87
5	6(-1)	37(0)	160(-1)	0.41
6	8(1)	37(0)	160(-1)	0.77
7	6(-1)	37(0)	200(1)	7.28
8	6(-1)	37(0)	200(1)	5.75
9	7(0)	30(-1)	160(-1)	0.79
10	7(0)	44(1)	160(-1)	1
11	7(0)	30(-1)	200(1)	0.6
12	7(0)	37(1)	200(1)	1.57
13	7(0)	37(0)	180(0)	11.08
14	7(0)	37(0)	180(0)	11.72
15	7(0)	37(0)	180(0)	10.52

3.3. Localization of Optimum Conditions

The graphical representations of the regression Eq. (1) and (2), called the response surfaces and the contour plots (3D response surface plots and 2D contour plots), were obtained using the Design Expert and were presented in Fig.1-3. These figures were the graphical representation of regression equation for visualizing the relationship between the responses and variables, and the interaction between the variables for deducing the optimum conditions [7].

Table. 3: ANOVA for Response Surface Quadratic Model (Y % Degradation).

Source	Sum of Squares	df	Mean Square	*F*-Value	*P*-value (Prob > *F*)
Model	226.34	6	37.72	13.64	0.0008
Lack of Fit	21.39	6	3.57	9.89	0.0947
Pure Error	0.72	2	0.36		
Cor Total	248.45	14			

R= 0.9545, R^2 = 0.9110, Adj R^2 = 0.8442

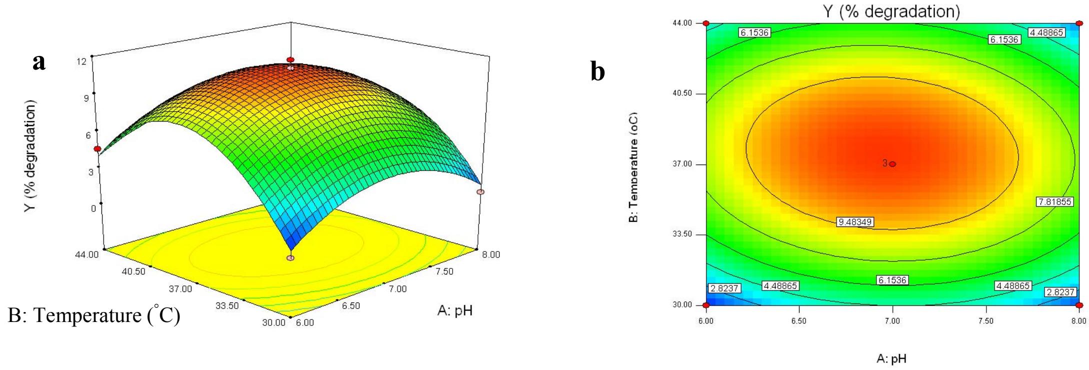

Fig. 1: (**a**) Response surface plot and (**b**) its contour plot of the effects of temperature (°C) and pH and their mutual interactions to determine percentage of PLA degradation by *A. migulanus*.

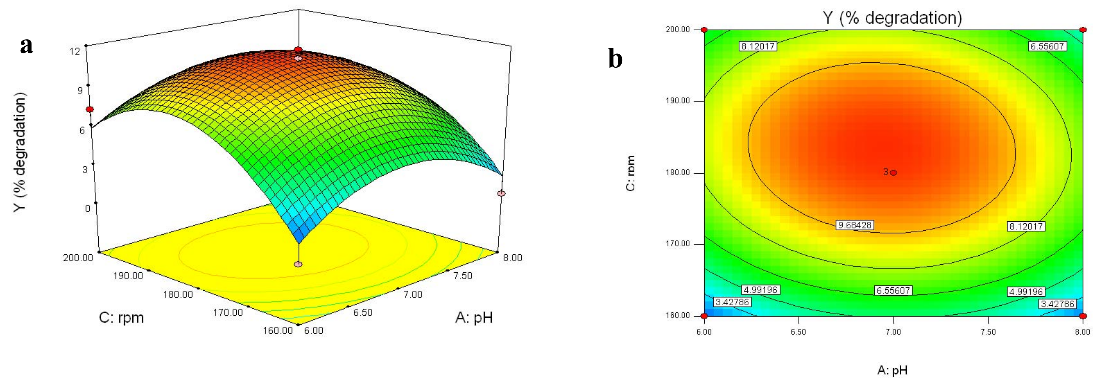

Fig. 2: (**a**) Response surface plot and (**b**) its contour plot of the effects of rpm and pH and their mutual interactions to determine percentage of PLA degradation by *A. migulanus*.

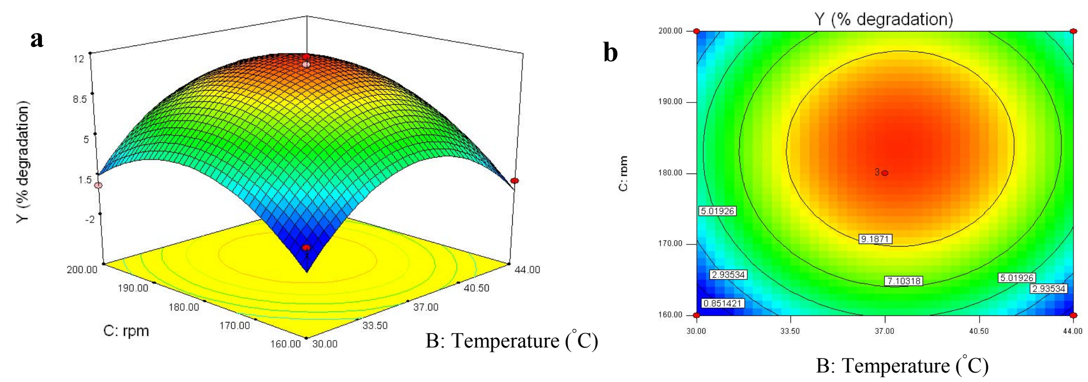

Fig. 3: (**a**) Response surface plot and (**b**) its contour plot of the effects of rpm and temperature (°C) and their mutual interactions to determine percentage of PLA degradation by *A. migulanus*.

To solve the regression equation and analyze the response surface plots, the optimal values of the test variables in coded unit were pH, temperature (°C), and speed shaking (rpm), respectively. The percentage of PLA degradation by *A. migulanus* was x_1= 8, x_2= 40.5, x_3=194, Y= 6.37855, and desirability= 0.735. The optimization results from RSM and BBD illustrated in Fig. 4. The percentage of degradation by *A. migulanus* under the conditions pH 8, 40.5 °C, and 194 rpm within 20 days

(69.62%) was significantly different from one another ($p < 0.0001$) as determined by one-way ANOVA and Duncan's Multiple Range Test. Therefore, this optimum condition by RSM and BBD can be adapted to increase percentage of PLA degradation.

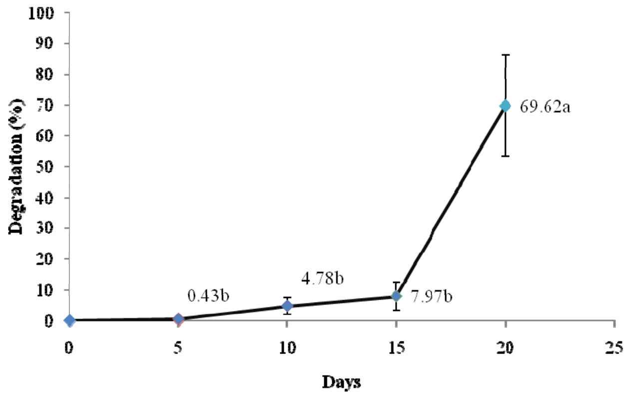

Fig. 4: The percentage of PLA degradation by *A. migulanus* at pH 8, 40.5 °C and 194 rpm within 20 days. Values were expressed as means of three replicate analyses ±SD.

4. Conclusion

This study is a first report of applying *Aneurinibacillus migulanus* to PLA film degradation using Response Surface Methodology (RSM) technique. The results reveal pH, temperature (°C), and speed shaking (rpm) have significant effects on PLA degradation. Furthermore, the optimum condition of *A. migulanus* in PLA degradation is pH 8, 40.5 °C and 194 rpm. The strain significantly degraded PLA films at the maximum rate of 69.62% within 20 days in liquid medium. However, a well-established study on PLA degrading microorganism using RSM technique still requires more research efforts to be generalized and applied to a wider scope of biodegradable plastics production.

5. Acknowledgment

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6. References

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