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Optimization of PET Hydrolysis Using Response Surface Methodology

Abstract

Response Surface Methodology (RSM) was used to model and optimize the enzymatic activity of *TiCut2*, a thermostable cutinase enzyme (Barth et al. 2015). Under varying temperature and pH conditions, nano absorbance at 260 nm (A260), the response variable, was recorded to evaluate catalytic performance during PET film hydrolysis. Using a second-order polynomial model, the effects of the factors temperature and pH were analyzed, and 3D response surfaces were generated using RStudio to visualize the effects caused by the factors. The RSM model predicted an optimal pH of approximately 7.5 and an optimal temperature of 51°C, showing that *TiCut2* exhibits maximal catalytic efficiency under moderately basic and moderate heat conditions. This study focuses on the mathematical theory behind RSM, applied to optimizing biocatalytic parameters while minimizing experimental trials.

Introduction

Enzymatic hydrolysis offers a sustainable approach for degrading PET (polyethylene terephthalate), a major contributor to plastic pollution. *TiCut2* from *Thermobifida fusca* can depolymerize PET into environmentally monomers TPA and EG (Barth et al. 2015). However, the catalytic efficiency of the enzyme depends on several factors, specifically temperature and pH.

RSM (Response Surface Methodology) gives a mathematical and statistical framework for modeling. It also optimizes responses influenced by multiple quantitative factors (Khuri and Mukhopadhyay 2010). By fitting low-order polynomial models to experimental data, RSM identifies the most efficient combination of conditions. This study applies RSM to quantify the effect of temperature and pH on *TiCut2* activity during PET hydrolysis, using absorbance at 260 nm (A260) as the response variable.

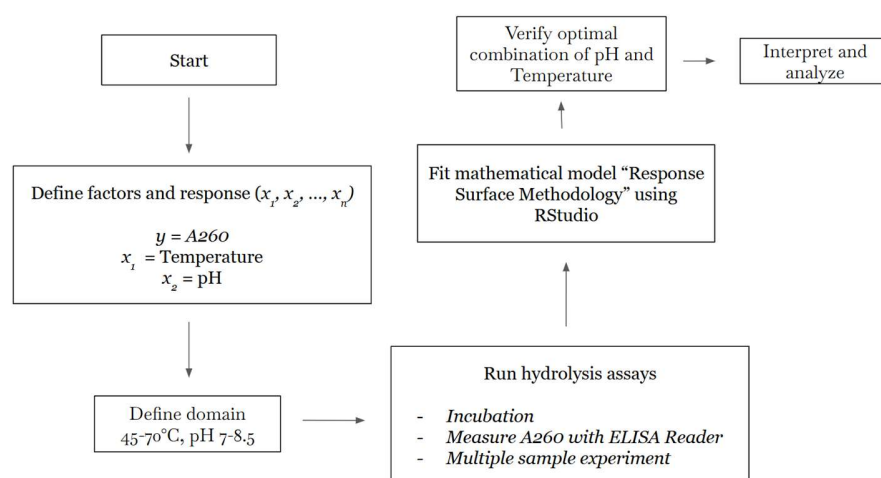


Fig. 1: Experimental workflow for response surface modeling of PET hydrolysis.

Mathematical Model

The A260, y , is modeled as a function of independent variables temperature x_1 and pH x_2 .

$$y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 + \varepsilon$$

- Linear Terms $[b_1x_1, b_2x_2]$ capture the direct effect of each factor
- Quadratic Terms $[b_{11}x_1^2 + b_{22}x_2^2]$ allows the curve to bend, which is important when computing optima
- Interaction Terms $[b_{12}x_1x_2]$ show how factors interact together to influence the dependent variable y

R-studio computes the coefficients by using ordinary least squares, which minimizes the sum of squared residuals:

$$\mathbf{b}_L = (\mathbf{X}^T\mathbf{X})^{-1}\mathbf{X}^T\mathbf{y}$$

$$SSR = \sum (y_{observed} - y_{predicted})^2$$

Once the algorithm determines the coefficients, the optimas can be found using the gradient. By taking the partial derivatives and solving for critical points, we are able to obtain the candidates for optimal temperature and pH.

$$\frac{\partial y}{\partial x_1} = b_1 + 2b_{11}x_1 + b_{12}x_2 = 0$$

$$\frac{\partial y}{\partial x_2} = b_2 + 2b_{22}x_2 + b_{12}x_1 = 0$$

Then, by using the 2 variable determinant test, the algorithm is able to compute the optimal temperature and pH. If there are at least 3 independent variables, the Hessian is required to obtain the optima.

$$D = f_{xx}f_{yy} - (f_{xy})^2$$

$$D > 0, f_{xx} > 0 \rightarrow \text{Local Minimum}$$

$$D > 0, f_{xx} < 0 \rightarrow \text{Local Maximum}$$

$$D < 0 \rightarrow \text{Saddle}$$

$$D = 0 \rightarrow \text{Inconclusive}$$

Method

PET film fragments were incubated in buffered mixtures under varied temperatures (35-70°C) and pH (7.0-8.5). Enzymatic activity was quantified by measuring the absorbance at 260 nm (A₂₆₀) of soluble hydrolysis products using an ELISA reader (UV-vis). The absorbance values were obtained for the TfCut2 wild-type and two mutated variants (Variant 3 and 4), which were previously found to exhibit enhanced performance (Barth et al. 2015).

The A₂₆₀ values were analyzed using RStudio, applying the second-order polynomial model described in the math model (Dean, Voss, and Draguljić 2017). The algorithm generated 3D response surface plots and determination of the optimal pH-temperature combination for maximum enzyme activity.

Data and Results

5ZOA	7	7.5	8	8.5	Variant 3	7	7.5	8	8.5	Variant 4	7	7.5	8	8.5
70	-0.01967	-0.02133	-0.011	-0.015	70	0.006333	-0.02633	0.011	0.104	70	0.068333	0.149333	0.020333	0.074
65	-0.01617	0.005833	0.068833	0.080167	65	0.207833	0.095167	0.528833	0.446833	65	0.0685	0.0105	0.2835	0.074833
60	0.27625	0.267917	0.40125	0.201917	60	1.475917	1.684583	2.058917	1.202917	60	1.24725	0.758583	1.782583	0.747917
55	0.2995	0.205167	0.802167	0.536167	55	2.551167	2.816833	2.8455	2.7795	55	2.4825	2.795833	1.295167	0.9495
50	1.140917	1.11025	0.88925	0.25325	50	3.022917	2.83225	3.066583	2.79325	50	2.79425	2.981917	2.574917	0.93425
45	0.081485	0.034818	0.370485	-0.03185	45	2.225818	2.401818	2.727152	2.360485	45	-0.09052	-0.10152	-0.08652	-0.07585
35	-0.02708	-0.02075	-0.00775	-0.00442										

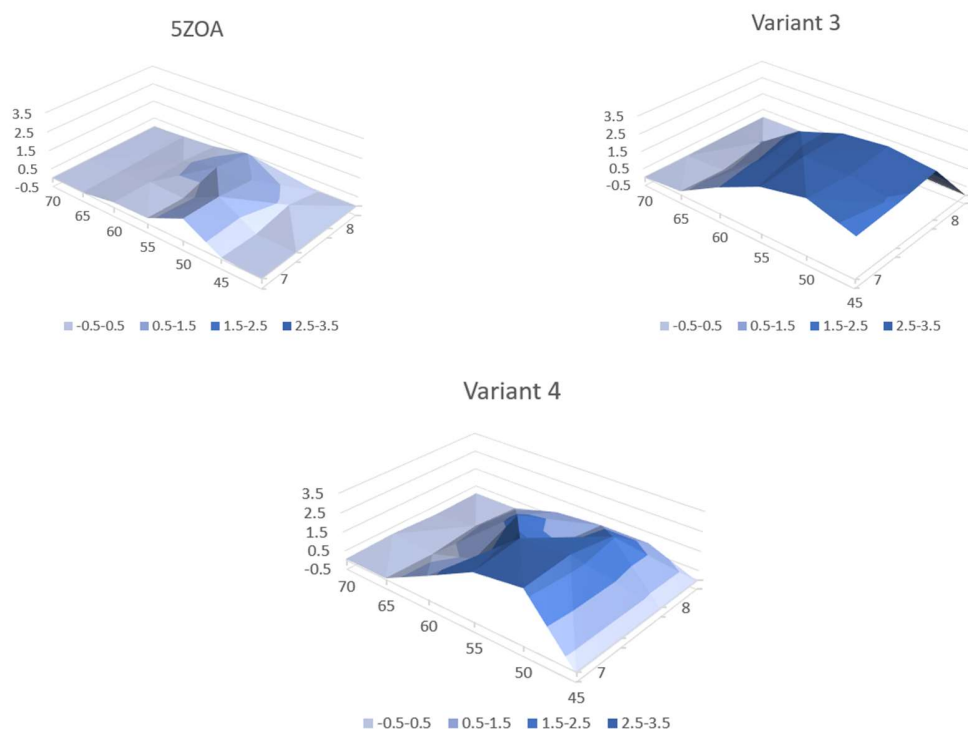


Fig. 2: Response surface plots showing the combined effect of temperature and pH on A₂₆₀ absorbance for wild-type (TfCut2) and its engineered variants.

Across all the data, the average optimal pH was approximately 7.5, and the average optimal temperature was 51°C. The resulting 3D surfaces revealed a distinct peak near these conditions. These results confirm that moderate temperature and slightly basic pH favor the degradation of PET by *TfCut2*, mirroring the previously reported enzymatic behavior for the engineered enzymes (Barth et al. 2015).

References

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3. Barth, M., T. Oeser, R. Wei, J. Then, J. Schmidt, and W. Zimmermann. 2015. “Effect of Hydrolysis Products on the Enzymatic Degradation of Polyethylene Terephthalate Nanoparticles by a Polyester Hydrolase from *Thermobifida fusca*.” *Biochemical Engineering Journal* 93: 222–28. <https://doi.org/10.1016/j.bej.2014.10.012>