แบบจำลองจลนพลศาสตร์ของปฏิกิริยาทรานส์เอสเทอริฟิเคชั่นของน้ำมันเมล็ดดอกทานตะวัน และเมทานอลที่ถูกเร่งด้วยเอนไซม์ไลเปสที่ถูกตรึง

Kinetic modeling of immobilized lipase catalysed transesterification reactions of sunflower oil and methanol for biodiesel production

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บทคัดย่อ

ในงานวิจัยนี้เป็นการศึกษาแบบจำลองจลนพลศาสตร์ของปฏิกิริยาทรานส์เอสเทอริฟิเคชั่นของน้ำมันเมล็ดดอกทานตะวันและเมทานอลด้วยเอนไซม์ไลเปสที่ถูกตรึงบนเม็ดไคโตซาน ทั้งนี้สภาวะที่เหมาะสมต่อการตรึงเอนไซม์ สามารถหาได้จากการออกแบบการทดลองแบบ two-way ANOVA โดยพบว่า อัตราส่วนโดยน้ำหนักของเอนไซม์ไลเปสจาก *Pseudomonas fluorescens* ต่อน้ำหนักสารละลายไคโตซาน (1.5%wt/vของไคโตซานผงและสารละลายกรดอะซิติก 1.5%v/v) เป็น 1:150 ให้ค่าประสิทธิภาพของเอนไซม์สูงสุดคือ 3,955.6 หน่วยต่อกรัมเอนไซม์ไลเปสที่ถูกตรึง และจากผลการทดลองแสดงให้เห็นว่ากลไกของปฏิกิริยาเป็นแบบปิงปอง บิ บิ โดยสามารถหาค่าคงที่ปฏิกิริยาได้ด้วยวิธีกราฟและวิธีการประเมินค่าด้วยคอมพิวเตอร์แบบทำซ้ำ

ABSTRACT

Immobilization of lipase on chitosan bead catalysed synthesis of fatty acid methyl ester from sunflower oil and methanol was reported in this paper. The optimization conditions for the reaction were received by designing an experiment and used to investigate the kinetic modeling. The lipase enzyme from *Pseudomonas fluorescens* was immobilized on chitosan bead. The best immobilization condition was investigated by two-way ANOVA statistical method. The optimum weight fraction of chitosan powder to lipase was 1:150 with 1.5 % wt/v of chitosan and acetic acid (1.5 %v/v) solution, which gave the highest lipase activity of 3955.6 U/g immobilized lipase. It was found that the kinetic mechanism obey ping pong bi bi mechanism and the kinetic parameters were investigated by graphical and computer iteration methods.

Key word : Lipase; Kinetic modeling ; Ping pong bi bi mechanism ; *Pseudomonas fluorescens;* Immobilization ; Transesterification

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1. Introduction

Due to an increase in energy demand and the limitation of fossil fuel, the research is directed toward alternative renewable fuels. Biodiesel as the alternative fuel, has many advantages because it can derived from a renewable and in domestic resources. Compared to petroleum–based diesel, biodiesel has more environmental favorable combustion emission profile such as low emission of carbon monoxide, particulate matter and unburned hydrocarbons [2]. Biodiesel can be produced from transesterification of fats or utilization of vegetable oils and alcohols with a presence of catalysts such as alkali and acid catalyst. In contrast, biocatalyst especially immobilized lipase allows for synthesis of alkylester from triglyceride and alcohol with mild conditions (nearly room ambient temperature and pressure), uses a lower energy requirement, enhances selectivity and quality of the product ,easy recovery of glycerol, and tranesterification of triglyceride with high free fatty acid.

The kinetic modeling of transesterification of sunflower oil and methanol by immobilized lipase from *Pseudomonas fluorescens* on chitosan bead were investigated in this work. The main objective of this work was to develop an approach that would enable a better understanding of the kinetic modeling for the design of the biodiesel production.

2. Materials and methods

2.1 Materials

Lipase powder from *Pseudomonas fluorescens* containing 2,200 U/g was purchased from Fluka. Chitosan in flake was used as a carrier for lipase immobilization. Purify sunflower oil was purchased from Morakot Co. Ltd. (Thailand).All other chemicals and solvents were of analytical reagent grade.

2.2 Experimental methods

2.2.1 Optimum immobilized lipase condition

Lipase powder was dissolved in 1 ml 1% v/v acetic acid solution and then mixed the lipase solution in chitosan solution (dissolved in 1% , 1.5% and 2% wt/v acetic acid solution). This mixture was dripped through a syringe with a 23 G needle into 100 ml of 0.136 M sodium

tripolyphosphate (TPP) prepared in buffer solution pH 7.2. The beads were cured in the TPP solution for 75 min and then washed twice with 10 ml of phosphate buffer solution pH 7.

The optimum weight fraction was investigated in enzymatic activity by two-way ANOVA (Analysis of variances) statistical method.

2.2.2 The kinetic modeling of biodiesel production

The optimum reaction temperature (30°c) of transesterification of immobilized lipase was used to investigate the kinetic modeling, obtained from the our previous work [5] . The reactions were carried out in a 50 ml elemeyer flask with the amounts of sunflower oil were varied between 5 to 12 mmol/g immobilized lipase (879 g/mol) , methanol concentrations varied between 20 to 200 mmol/g and immobilized lipase (32 g/mol). The mixture was incubated in a shaking incubator at 200 rpm and the samples were withdrawn periodically and analysed by gas chromatography.

2.2.3 Analytical methods

2.2.3.1 Lipase activity assay

Immobilized lipase was assayed using the ratio of substrate A to substrate B, 1:9 by volume. Substrate A, 30 mg of *p*-nitrophenyl palmitate (p-NPP) was dissolved with 10 ml of 2-propanol. Substrate B was prepared by using 0.4 g of Triton-X-100 and 0.1 g of gum Arabic in 90 ml of 0.05 M phosphate buffer solution pH 8. Immobilized lipase of 200 mg was added to a substrate mixture of 2 ml and incubated for 15 min at 30°C. The reaction was terminated by adding 2.9 ml of 0.212 g/l Na_2CO_3. After 5 min, this solution was measured at 410 nm in a UV/VIS spectrophotometer (Secoman, Anthelie Advanced, France). The increase in absorbance at 410 nm caused by the release of *p*-nitrophenol in the hydrolysis of p-NPP , was calculated by comparison with the standard curve of *p*-nitrophenol in range 0.005-0.045 μmol/ml. One unit (U) of enzyme activity was defined as the amount of enzyme,which catalyzed the production of 1 μmol *p*-nitrophenol per minute under the experimental conditions.

2.2.3.2 Percentage of fatty acid methyl ester

The reaction mixture was removed from immobilized lipase by using filter paper and then centrifuged at 3,000 rpm for 20 min. Samples were withdrawed from the top phase and determined %fatty acid methyl ester (%FAME) by gas chromatography (EN14105:2003 standard) . 50 mg of the mixture were added with Tricaprin (8 mg/ml) as an internal standard and 100 microliters MSTFA, mixed the solution severely and stand for 15minutes, and then added n-heptane 8 millilitre. A DB-5HT capillary gas chromatography column (15 m × 2.5 mm i.d.) was used in the sanple analysis. The programming temperatures were maintained at 50°c for 1 min, at 15°c/min up to 180°c , at 7°c/min up to 230°c, at 10°c/min up to 370°c and final temperature held for 5 minute. Temperatures of injection and flame ionize on detector were 280°c.The value of % FAME was calculated as

$$\%FAME = \frac{wt\ of\ methylester}{wt\ of\ biodieselsample} \times 100 \qquad (1)$$

2.2.4 Statistical analysis

A software package (MINITAB) was donated from Department of Industrial Engineering, Srinakarinwirot University and used for statistical analysis for this experiment. Two way ANOVA was used to investigate the optimum of condition for immobilized lipase on chitosan bead.

3. Results and discussion

3.1 Optimum immobilized lipase condition using two-way ANOVA

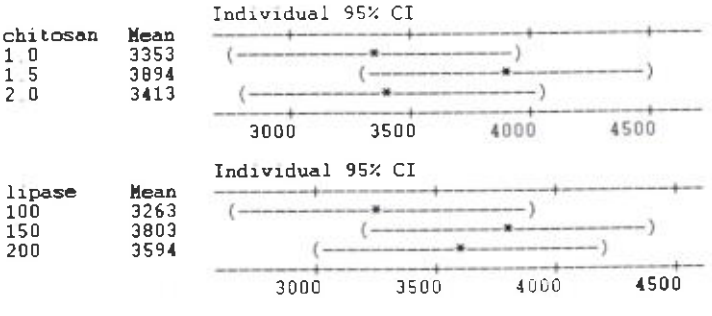

Fig. 1. Two way ANOVA results on activity of immobilized lipase from *Pseudomonas fluorescens* on chitosan bead from MINITAB software program.

In the preliminary experiments, the optimum fraction of chitosan powder and lipase were investigated by two-way ANOVA statistical method.

The results from MINITAB statistical software showed that, the different means of the activity were not significant at 95% confidential level ($F_{critical} < F_0(0.01,4,9)$). However, the percentage of the weight of the lipase powder to chitosan solution (dissolved in 1.5% wt/v acetic acid) at 1:150 gave the highest activity value (3955.6 U/g) and the immobilized lipase gave more activity compared to the free lipase (2,200 U/g). Therefore, the following experiments were conducted at this condition for the kinetic modeling.

3.2 Kinetic model analysis

The initial rates of transesterification were observed to increase with increasing of the amounts of sunflower oil (TG) and methanol (M). The double-reciprocal plots of initial rate by many researchers [9,10] were reported. The mechanism of biodiesel production from immobilized lipase obey the ping-pong bi bi mechanism with methanol inhibition. The reaction mechanism may be depicted as followed:

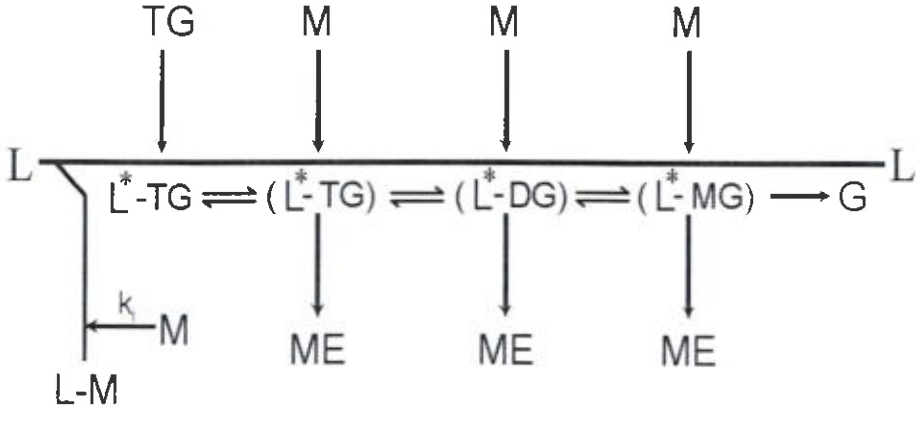

Fig. 2. Kinetic modeling of the tranesterification of Triglyceride and Methanol by immobilized lipase .

where, L, lipase; M, methanol ; TG, Triglyceride in sunflower oil; ME, Methyl ester ; G, glycerol ; L^*-TG , lipase triglyceride complex ; L^*-DG , lipase diglyceride complex ; L^*-MG , lipase monoglyceride complex ;

The rate equation for the reaction was as followed: [9]

$$V_i = \frac{V_{max} \cdot [Trigly] \cdot [MeOH]}{K_{m(trigly)} \cdot [MeOH](1 + \frac{[MeOH]}{K_{i(MeOH)}}) + K_{m(MeOH)} \cdot [Trigly] + [Trigly][MeOH]} \quad (2)$$

where, V_i is the rate of reaction, V_{max} is the maximum rate of reaction. $[Trigly]$ is the initial concentration of sunflower oil . $[MeOH]$ is the initial concentration of methanol. $K_{m(trigly)}$ is Michaelis constant for sunflower oil. $K_{m(MeOH)}$ is Michaelis constant for methanol. $K_{i(MeOH)}$ is inhibition constant for methanol.

The kinetic constants were obtained by the double-reciprocal plots of initial rate versus methyl ester concentration. The plot in fig.2 was shown at low concentration of methanol (25 – 62.5 mmol of methanol) and high concentration of methanol (100 – 200 mmol of methanol). The results showed that plot was followed the ping pong bi bi mechanism with high concentration of methanol . To determine all the kinetic parameters, equation (2) can be rewritten as :

$$y - \text{int}ercept = k_{m,MeOH} \frac{1}{[MeOH]} + \frac{1}{V_{max}} \quad (3)$$

$$Slope = \frac{k_{m,Tri}}{V_{max} K_{i,MeOH}} [MeOH] + \frac{k_{m,Tri}}{V_{max}} \quad (4)$$

The value of kinetic parameters ($k_{m,Tri}$, $k_{m,MeOH}$, $k_{i,MeOH}$ and V_{max}) are presented in table 1 :

Kinetic parameters			
V_{max} ($\mu mol \cdot h^{-1}$)	$k_{m,Tri}$ (mmol)	$k_{m,MeOH}$ (mmol)	$k_{i,MeOH}$ (mmol)
69.45	12.81	3.212	16.18

Table 1. The values of kinetic parameters from the double-reciprocal plots of initial rates versus methyl ester concentrations

The rate equation can be rewritten as :

$$V_i = \frac{V_{max} \cdot [Trigly] \cdot [MeOH]}{12.81 \cdot [MeOH](1 + \frac{[MeOH]}{16.18}) + 3.212 \cdot [Trigly] + [Trigly][MeOH]} \quad (5)$$

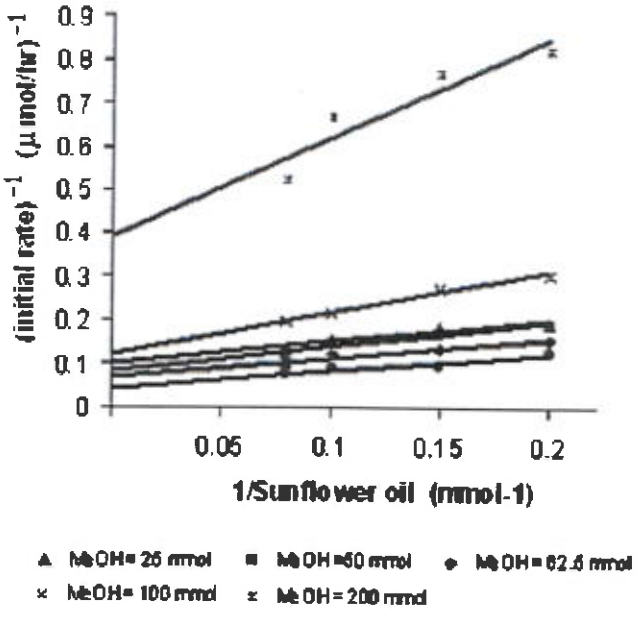

Fig.3 Reciprocal initial velocity of the reaction versus reciprocal sunflower oil concentration

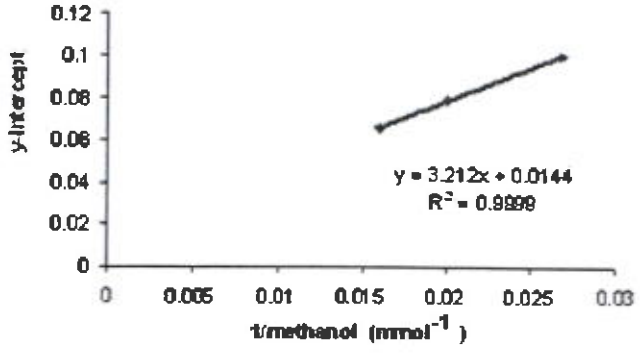

Fig.4 Plot of the y-intercept determined in Fig 3. and reciprocal of methanol concentration

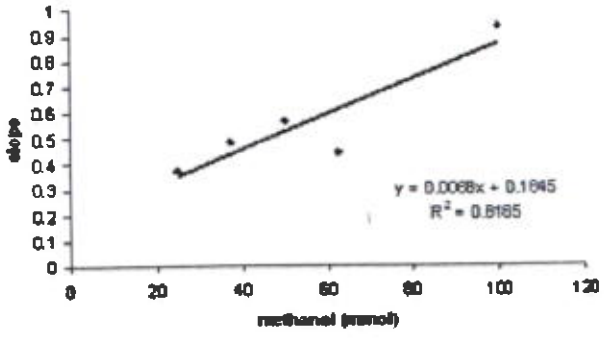

Fig.5 Plot of the slope determined in Fig 3. and methanol concentration

The kinetic parameters determined from graphical method appears rather inaccurate. Because the value of $k_{m,MeOH}$ is less than $k_{m,Tri}$, this result is different from other works [9,10]. To solve this problem, the Excel computer program was used as reported by [9]. The problem was solved by minimize total error:

$$Total\ \ error \quad = \quad \sum_{i} \left(V_i^{\exp} - V_i^{\mathrm{model}}\right)^2 \tag{6}$$

The kinetic parameters from solver in the Excel program were shown in the Table1 and the rate equation was rewritten as:

$$V_i \quad = \quad \frac{V_{max} \cdot [Trigly] \cdot [MeOH]}{12.81 \cdot [MeOH](1 + \frac{[MeOH]}{13.84}) + 68.36 \cdot [Trigly] + [Trigly][MeOH]} \tag{7}$$

The values of $K_{m(MeOH)}$ and $K_{i(MeOH)}$ from computer program were comparable to other works indicating the same inhibit concentrations of different types of alcohols [4,9] . The values of $k_{m,Tri}$ and $k_{m,MeOH}$ indicated that the half value of sunflower oil and methanol concentration were obtained for the maximum initial rate (V_{max}). It was found that the value of $k_{m,MeOH}$ was greater than $k_{m,Tri}$. The ratio of $k_{m,MeOH}$ to $k_{m,Tri}$ was about 6:1 corresponding to the optimum molar ratio of methanol to oil of 6:1, the result from previous work [5] compared with other works, the value of $k_{m,Tri}$ was higher than the others. It was indicated that the reaction occurred either at high concentration of sunflower oil to react with methanol or because of the

low solubility of methanol in sunflower oil and the insoluble glycerol is adsorbed onto the active site of immobilized lipase to reduce the activity of lipase.

4. Conclusion

Two way ANOVA was used to find the best condition for an activity of immobilized lipasee on chitosan bead at the weight fraction of chitosan solution (dissolved in 1.5 % wt/v acetic acid). The result shows that lipase enzyme was not statistically different between the immobilized conditions. The percentage of weight of the lipase powder to chitosan solution (dissolved in 1.5% wt/v acetic acid) at 1:150 was gave the activity of enzyme lipase of 3955.6 U/g.

The transesterification of sunflower oil by immobilizes lipase from *Pseudomonas fluorescens* was used to investigate the kinetic parameters from graphical and computer numerical method. The reciprocal plots show good agreement of the initial velocity and the substrate concentration is follow the Ping Pong bi bi with methanol inhibition.

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