Preparation and Kinetic Study of Rhizopus oryzae Whole-cell as Biocatalyst for Biodiesel Synthesis through Non-alcohol Route

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Abstract—Direct use of lipase producing whole-cell as biocatalyst for biodiesel synthesis gain extensively attention since it could reduce the catalyst cost. Whole-cell of Rhizopus oryzae was cultivated by single-step and two-step method. At reaction time of 48 h, the single-step cultivated whole-cell produced 23% FAME yield. It is higher comparable with the two-step cultivated whole-cell with 19% FAME yield. Kinetic model based Michaelis-Menten mechanism was found to fit fairly the substrate and product concentration profile of experimental result. \( K_m \) and \( V_{max} \) value for R. oryzae whole-cell are 3 mole L\(^{-1} \), 0.09 mole L\(^{-1} \) h\(^{-1} \) (one-step) and 3 mole L\(^{-1} \), 0.065 mole L\(^{-1} \) h\(^{-1} \) (two-step).

Index Terms—biodiesel, michaelis-menten, whole-cell

I. INTRODUCTION

In recent years, fatty acid methyl esters (FAMEs), commonly referred to biodiesel fuel, have become viewed as promising renewable sources of fuel[1]. Current technology for biodiesel production, using an alkali or acid catalyst, has several important limitations such as requires feedstock free of water, produce salts, generate glycerin as a low grade by-product, etc.[2]. In the other side, utilizing lipase as catalyst for biodiesel production over several advantage, such as require lower heating, works even in the presence of water, produces no salts [2]. Lipases have up to 74 times higher productivity than alkali catalyst [3].

Lipase is easy to be deactivated by alcohol, which is a reactant in biodiesel synthesis reaction. Therefore, we have developed new method to maintain the activity and stability of the biocatalyst during reaction by using methyl acetate as an acyl donor [4].

The price of lipase is expensive due to purification procedures [2]. Haas et. al. reported that catalyst cost in biodiesel production by using enzyme is 0.14US$ per kg per kg ester [5]. It is much higher than using NaOH 0.006 US$ per kg ester [5]. In order to lower the catalyst cost, we used enzyme immobilization method in various support to make the lipase become reusable due to its easy recovery from the reaction mixture [6]–[8]. We have also developed kinetic model based on Michaelis-Menten and Ping Pong Bi Bi Mechanism describing the interesterification behavior of the triglyceride with methyl acetate to produce biodiesel using lipase [4], [6].

Although immobilization make enzyme become reusable, but the process is time and cost consuming [6], [7]. Utilizing whole-cell overproducing intracellular lipase, such as Rhizopus oryzae, as biocatalyst in biodiesel synthesis is a potential way to reduce the biocatalyst cost [9]. The preparation of a biocatalyst requires no complex purification process, and the prepared whole-cells could be directly used as lipase containers [10]. In this research, we have done preparation of R. oryzae whole-cell using two different cultivation methods, i.e. single-step and two-step method.

Works that deal with modeling of enzymatic interesterification for whole-cell catalyzed biodiesel production are not often found in the literature. This works also presents a kinetics study of enzymatic reaction between cooking oil and methyl acetate for biodiesel production using suspended R. oryzae whole-cell as biocatalyst. The kinetic was based on Michaelis-Menten(MM) mechanism.

II. MATERIALS AND METHODS

A. Materials

All experiments were carried out using R. oryzae obtained from School of Life Science and Technology, Bandung Institute of Technology, Indonesia. Potato Dextrose Broth (PDB) was obtained from BD Difco \(^{TM} \) (USA). Trypton, NaNO\(_3\), KH\(_2\)PO\(_4\), MgSO\(_4\).7H\(_2\)O, glutaraldehyde and methyl acetate were purchased from Merck (Darmstadt, Germany). Olive oil and cooking oil were obtained locally.

B. Preparation of R. Oryzae Whole-cell

There are two different methods used for fungi propagation, i.e. single-step and two-step method. One-step cultivation was carried out using 50ml of PDB
medium at room temperature (25°C) for 24 hours on reciprocal shaker at 60 oscillations. Two-step method was worked following method of Jin and Bierma[2]. The method consisted of pre-incubation and incubation step. For pre-incubation, Erlenmeyer flask (100ml) containing 50ml of PDB were inoculated by aseptically transferring spores from a potato dextrose agar plate. For the incubation step, the filamentous fungi were aseptically transferred to other Erlenmeyer flask (100ml) containing 50ml of basal medium. The basal medium contained, in 1L of distilled water: 70g trypton, 1g NaNO$_3$, 1g KH$_2$PO$_4$, 0.5g MgSO$_4$.7H$_2$O and 30g olive oil. Each step in two-step method was conducted using the same operating condition as in single-step cultivation method.

C. Biodiesel Synthesis Through Non-alcohol Route

The interesterification reaction was carried out at 37°C in 250ml screw-cap bottle with incubation on a reciprocal shaker 150rpm. Cooking oil and methyl acetate were used with 1:12 mole ratio. The whole-cell biocatalyst added was 10% of total reactant mass.

D. Analysis

Reactant and product were analyzed using a HPLC system (L-7100, Hitachi, Ltd., Tokyo, Japan) equipped with an Inertsil ODS column (particle size 5.0×10$^{-4}$ cm, i.d. 0.46 cm, length 25 cm, GL Science, Inc., Tokyo, Japan) and a UV detector (L-7400, Hitachi,Ltd., Tokyo, Japan) at 210 nm. Temperature of the column oven was 313 K. The mobile phase composition was methanol: acetone = 100:0 (v/v) for 20 min and then the acetone ratio increased up to 20% (v/v) and the value was maintained from 21 min to 35 min. The flow rate of the mobile phase was 1.0 cm$^2$ min$^{-1}$.

E. Kinetic Modelling

Michaelis-Menten kinetic based model was used to predict substrate and product concentration along reaction time.

III. RESULTS AND DISCUSSION

A. Catalytic Performance of R. oryzae Whole-cell

To investigate the effect of cultivation method to catalytic performance of suspended whole-cell, we tried to use two different cultivation methods for fungi propagation. Figure 1 shows the time course of FAME yield produced by reaction using suspended whole-cell cultivated by single-step and two-step method. When R. oryzae cells were cultivated by two-step method, the reaction produced lower FAME yield (19%) than when the cells were cultivated by single-step method (23%). Hama et al. reported that [11] R. oryzae produces two type lipase bound to the cell wall and membrane. In suspended cells, the amount of membrane-bound lipase decreased sharply with cultivation time. Furthermore, the basal medium used in two-step cultivation method contained 30% olive oil which is consisted of 55-83% oleic acid [12]. The unsaturated fatty acid existence in medium broth altered cell membrane become more permeable [13] inducing lipase secretion.

B. Model Verification

Fig. 2 shows that the model fits fairly with the experimental data. The one-step cultivated whole-cell has higher $V_{max}$ (Table I). Thus, it is predicted that the one-step cultivated whole-cell biocatalyst could achieve higher maximum interesterification rate than the two-step cultivated whole-cell. However, the one-step cultivated whole-cell needs more substrate to achieve half of its maximum reaction rate. This is indicated by $K_m$ value (Table I).
experimental results. This work could predict the substrate and product concentration profile in whole cell-catalyzed biodiesel synthesis along the reaction time since it fits fairly to the concentration profile in whole cell-catalyzed biodiesel.

We have demonstrated the cultivation of *R. oryzae* cells using two different methods. The two-step method proposed here seems to be a promising alternative to single-step method, where such method increases the catalysis performance of whole-cell.

Michaelis-Menten based kinetic model proposed in this work could predict the substrate and product concentration profile in whole cell-catalyzed biodiesel synthesis along the reaction time since it fits fairly to the experimental results.

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REFERENCES


IV. CONCLUSION

This table shows the kinetic parameters for the single-step and two-step methods.

<table>
<thead>
<tr>
<th>Kinetic Parameter</th>
<th>MM model</th>
<th>Single-step</th>
<th>Two-step</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_i ) (mole(^{-1}) L(^{-1}))</td>
<td>3.000</td>
<td>3.000</td>
<td></td>
</tr>
<tr>
<td>( V_{\text{max}} ) (mole(^{-1}) L(^{-1}) h(^{-1}))</td>
<td>0.090</td>
<td>0.065</td>
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</tr>
<tr>
<td>Error</td>
<td>0.469</td>
<td>0.205</td>
<td></td>
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</tbody>
</table>

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