Production of Biodiesel Using Soybean Oil Catalyzed by Porcine Pancreas Lipase in a Solvent Free System

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Abstract: The aim of present research was to demonstrate the ability of porcine pancreas lipase as catalyst in transesterification reaction of soybean oil with methanol for biodiesel fuel production. The effect of reaction parameters including methanol/oil molar ratio, lipase amount, reaction time, stirring rate and reaction temperature on transesterification was investigated. The biodiesel yield was more than 34.1% in a solvent-free system under optimal reaction conditions. The optimal conditions of reaction including molar ratio of methanol to oil, reaction temperature, the amount of catalyst (based on oil weight) and stirring rate were 3:1, 45°C, 5% and 180 rpm, respectively. After 72 h of transesterification reaction under optimal conditions, maximum oil conversion to methyl esters was obtained.

Key words: Biodiesel % Lipase % Transesterification % Soybean oil % Solvent free

INTRODUCTION

Limitation of common fossil fuel resources and rising price of crude oil as well as concern over environmental restrictions on greenhouse gas emissions have led to an urgent need to develop alternative fuels such as biofuels. As an alternative to fossil fuels, biofuels should be similar to fossil fuels in combustion properties, but essentially environmentally friendly and economically competitive [1].

According to these properties, biodiesel is gaining particular attention as a suitable substitution for petroleum-based diesel fuel.

Biodiesel is a product obtained from transesterification of triglycerides or esterification of fatty acids with mono-alcohols. This reaction is mainly carried out through chemical and enzyme catalytic methods. Recently, enzymatic transesterification using lipases has gained more attention due to some drawbacks of chemical catalysts including: high energy and reactant consumption, complex treatment of glycerol and high amount of wastewater from catalyst [2].

Lipases as biocatalysts have high potential for catalytic transesterification processes. Lipase is able to utilize all mono, di and triglycerides in transesterification reaction. The main benefits of applying lipases are summarized as; low product inhibition, high activity and yield in non-aqueous media, low reaction time, reusability of immobilized enzyme and temperature resistance [3]. Some studies have been conducted using immobilized lipases such as Novozym 435 [4] and Lipozyme TL IM [5] in transesterification reaction. However, application of whole-cell lipase in a solvent free system is not well studied. Lipase of porcine pancreas is one of the low cost and commercially available non-microbial enzymes, which has high thermo stability and activity in anhydrous reaction media which can be used as catalyst in transesterification reaction [4, 6, 7].

In the present research, the effects of various parameters such as reaction time, agitation rate, methanol/oil molar ratio, the amount of lipase and reaction temperature on transesterification of soybean oil catalyzed by porcine pancreas lipase were investigated.

MATERIALS AND METHODS

Materials: Soybean oil (acid number 0.06) was purchased from local supermarket and used as raw material. Table 1 summarized some characterization of soybean oil. Porcine pancreas lipase (31 units/mg protein) was chosen...
Actual amount of methyle esters in the product

Ester conversion = Theoretical amount of methyle esters in the product

Table 1: Soybean oil characteristics

<table>
<thead>
<tr>
<th>Fatty acid composition</th>
<th>Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>0.10</td>
</tr>
<tr>
<td>C16:0</td>
<td>10.75</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.10</td>
</tr>
<tr>
<td>C18:0</td>
<td>9.75</td>
</tr>
<tr>
<td>C18:1</td>
<td>22.85</td>
</tr>
<tr>
<td>C18:2</td>
<td>54.40</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.25</td>
</tr>
</tbody>
</table>

as catalyst. Methanol (purity of 99%, Sigma, USA) was used as the reactant for the enzymatic reaction. Methyl pentadecanoate, methyl oleate, methyl linoleate, methyl stearate and methyl palmitate were provided by Sigma-Aldrich company and served as the standards for GC analysis.

General Procedure for Enzymatic Reaction: In the experimental procedure, all enzymatic reactions were carried out in a 25-ml flask, Stoppard with a plug in a temperature controlled incubator shaker at 180 rpm for 72 h. Methanol was added in a three-step procedure. In each step one equivalent molar of methanol was added at the reaction time of 0, 18 and 36 h.

GC Analysis: The compositions of the product were determined by a gas chromatography analyzer (Agilent 9780) equipped with flame ionization detector (FID) and a 19091J_413 HP5 capillary column (30 m × 0.32 mm × 0.25 μm, Agilent Technologies Inc. USA). For this purpose, at the end of the enzymatic reaction, samples of 100 μl were drawn out from the reaction medium and centrifuged (12000 g, 10 min).

Then, a 10 μl sample from upper layer of centrifuged solution was mixed with 990 μl of n-hexane and 300 μl of internal standard for GC analysis. The column temperature was programmed, initially held at 180°C for 10 min, heated to 280°C in a rate of 5°C/min and kept for 10 min at 280°C. Nitrogen was used as carrier gas at a flow rate of 7.5 ml/min and the split ratio was 1/20. The injector and the detector temperatures were set at 230 and 280°C, respectively. Methyl pentadecanoate was served as internal standard for GC analysis. A certain amount of methyl oleate, methyl linoleate, methyl stearate and methyl palmitate were mixed together to be an standard solution to prepare the standard curve. For instance, a chromatogram of methyl esters formed in transesterification reaction is illustrated in Fig. 1.

The ester conversion was determined as follows:

\[
\text{Ester conversion} = \frac{\text{Actual amount of methyle esters in the product}}{\text{Theoretical amount of methyle esters in the product}}
\]

RESULTS AND DISCUSSION

Effect of Reaction Time: The effect of reaction time on esterification reaction and methyl esters yield was studied. Samples were taken from the reaction mixture in every eight hours and analyzed with GC. Other parameters were kept constant during the experiment. The reaction conditions such as the amount of lipase (oil weight percent), molar ratio of methanol to oil, reaction temperature and agitation rate were 5%, 3:1, 45°C and 180 rpm, respectively. Fig. 2 shows that the methyl ester
Increasing the agitation rate greater than 180 rpm. This may be due to the structure of the lipase. Shear sensitive biomolecules may be damaged by mechanical collision when the agitation rate exceeded the limit. So, the higher stirring rates could increase the lipase inactivation [8].

**Effect of Methanol/oil Molar Ratio:** In theory, at least three molar equivalents of methanol are necessary in order to complete the transesterification reaction; but when a portion of the alcohol remains insoluble (in excess) it forms droplets which coat the enzyme causing its denaturation [9]. Fig. 4 shows the effect of methanol/oil molar ratio on the transesterification catalyzed by porcine pancreas lipase (PPL). At the beginning of the transesterification, The conversion increased by increasing the molar ratio of methanol to oil, when both the amount of lipase and reaction temperature were kept constant at 5% and 45°C, respectively. On the other hand, when the molar ratio of methanol to oil exceeded a certain value of 3:1, the conversion tendency reversed. Perhaps, high methanol concentrations were found; that caused irreversible denaturation of the lipase. This may be due to that methanol is insoluble in oil at high concentrations.

**Effect of the Amount of Lipase:** The amount of lipase is an important parameter affecting on the transesterification reaction. Considering the cost of industrial production, in this study, the amount of lipase was varied in the range of 1-7% (by the oil weight). The effect of the amount of lipase on the methanolysis of soybean oil for biodiesel production is presented in Fig. 5.
Fig. 5: Effect of lipase amount on transesterification catalyzed by PPL (reaction conditions: soybean oil 5 g, lipase 5%, methanol/oil molar ratio 3:1, 180 rpm)

Fig. 6: Effect of temperature on transesterification catalyzed by PPL (reaction conditions: soybean oil 5 g, lipase 5% (by the oil weight), methanol/oil molar ratio 3:1, 180 rpm)

In general, the methyl ester yield enhanced correspondingly by increasing the lipase concentration in a certain range. When the amount of lipase was more than 5% (by the oil weight) it has approximately remained constant [10]. Perhaps, superfluous amount of enzyme (above the defined value of 5% (w/w)) has caused reduction in catalytic activity of the enzyme.

Effect of Reaction Temperature: Generally, the reaction temperature has an important effect on the activity and stability of biocatalysts. High temperature may denature the lipase; in contrary, it will take a long time to reach to equilibrium at low temperature. The effect of temperature on the methanolysis of oil for biodiesel production is depicted in Fig. 6. As is shown in Fig. 6, high methyl ester yield was achieved with an increment of the temperature up to 45°C. However, increasing the temperature from 45 to 65°C decreased methyl ester yield [11]. High temperatures (greater than 45°C) had a negative impact on the lipase activity and caused enzyme denaturation. Obviously, the optimal temperature of the reaction was 45°C.

Referral to Qin et al. [12] studies which is similar to present research; the maximum methyl ester yield which has been reported in transesterification of soybean oil with porcine pancreas lipase in a solvent free system was 24.1%. However, in present study at 72 h of reaction time, transesterification reaction under optimal conditions, 34.1% of the oil was converted to methyl esters.

CONCLUSION

The reaction parameters including methanol/oil molar ratio, the amount of lipase, reaction time, stirring rate and reaction temperature on transesterification of soybean oil catalyzed by porcine pancreas lipase were investigated. The biodiesel yield was more than 34.1% in a solvent-free system under the optimal reaction conditions.

The optimal conditions were molar ratio of methanol to oil 3:1, reaction temperature of 45°C, the amount of lipase 5% (based on the oil weight) and agitation rate of 180 rpm. For reaction time of 72 h and under optimal conditions, maximum conversion of oil to methyl esters was obtained.

REFERENCES


