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Study on acyl migration in immobilized lipozyme TL-catalyzed transesterification of soybean oil for biodiesel production

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Abstract

During enzymatic transesterification of soybean oils with methanol for biodiesel production, it was supposed that the maximum biodiesel yield was only 66% since lipozyme TL was a typical lipase with a strict 1,3-positional specificity. However, it has been observed that over 90% biodiesel yield could be obtained. It was therefore assumed, and subsequently demonstrated, that acyl migration occurred during the reaction process. Different factors which may influence the acyl migration were explored further and it has been found that the silica gel acting as the immobilized material contributes significantly to the promotion of acyl migration in the transesterification process. The final biodiesel yield was only 66% when 4% lipozyme TL used, while about 90% biodiesel yield could be achieved when combining 6% silica gel with 4% lipozyme TL, almost as high as that of 10% immobilized lipase used for the reaction.

Keywords: Biodiesel; Lipase; Acyl migration; Transesterification

1. Introduction

Biodiesel (fatty acid methyl ester), as a renewable bioenergy, has become more attractive recently because of its environmental benefits. Although biodiesel has been successfully produced chemically at present, there are several associated problems that restrict its development, such as glycerol recovery and removal of inorganic salts [1–3]. The disadvantages caused by chemical catalysts are largely prevented by using lipases as the catalysts. Recently [1–6] interest in the use of lipases with, or without, immobilization in transesterification for the production of biodiesel has gained increasing attention. Extensive reports about lipase-catalyzed transesterification for biodiesel production have focused on the lipase of Novozym435 and lipozyme TL since they have relatively high activity during biodiesel production [7–12].

During lipozyme TL-catalyzed transesterification of renewable oils for biodiesel production, it has been reported, and also observed in our research, that more than 90% biodiesel yield could be obtained [9–12]. Since lipozyme TL is well known as one of the lipases with a strict 1,3-positional specificity, acyl migration should occur during the biodiesel production process. Therefore in this paper the acyl migration phenomenon was explored and demonstrated, based on which different factors including lipase protein, water and the immobilized materials–silica gel that may influence the acyl migration were studied systematically further.

2. Materials and methods

2.1. Materials

Lipase from *Thermomyces lanuginosus* immobilized on silica gel, was a gift from Novo Industries (Denmark). Palmitic acid methyl ester, stearic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester, linolenic acid methyl ester and heptadecanoic acid methyl ester were purchased from Sigma and were chromatographically pure. All other chemicals were obtained commercially and of analytical grade.

2.2. General procedure for lipase-catalyzed transesterification for biodiesel production

The alcoholysis reactions were carried out in a 50 ml shaking flask and heated to 40 °C on a reciprocal shaker. The com-

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positions of the reaction mixtures were 9.65 g soybean oil, a known amount of lipase and three-step addition of methanol with 0.35 g methanol in each step. One hundred microliters of samples were taken from the reaction mixture and centrifuged to obtain the upper layer. An aliquot of the upper layer and heptadecanoic acid methyl ester (as the internal standard) were precisely measured and mixed thoroughly for GC analysis to determine fatty acid methyl ester (FAME) content.

Biodiesel molar yield is defined as the percentage of fatty acid methyl ester (FAME) produced in the reaction mixture versus the total theoretical amount of FAME measured by saponification of soybean oils.

2.3. Thin layer chromatograph analysis for 1,2-(2,3-) and 1,3-diglyceride (DG) intermediates

During the lipase-catalyzed three-step transesterification for biodiesel production, samples (200 μl) were taken from the reaction mixture at 48 h and centrifuged at 10,000 rpm for 5 min to obtain the upper layer. The upper layer was dissolved in acetone and spotted onto a TLC plate. The plate was developed with chloroform/acetone/acetic acid (96:4:1, v/v) and dried thoroughly, sprayed with a mixture of sulphuric acid and methanol (1:1, wt./wt.) and dried again. The spots were visualized by baking the plate at 180 °C on a hot plate.

2.4. GC analysis for fatty acid methyl ester

The methyl ester contents in the reaction mixture were quantified using a gas chromatograph (Shimadzu Corp., Kyoto) equipped with a DB-1 capillary column (0.25 mm × 15 m; J&W Scientific, Folsom, CA). The column temperature was kept at 180 °C for 0.5 min, raised to 370 °C at 15 °C/min and maintained at this temperature for 10 min. The temperatures of the injector and detector were set at 245 and 375 °C, respectively.

3. Results and discussion

3.1. Immobilized lipozyme TL-catalyzed transesterification for biodiesel production

During enzymatic transesterification for biodiesel production, it has been demonstrated that excessive methanol present in the reaction medium would cause significant deactivation of the lipase. Therefore a three-step addition of methanol in solvent-free medium was adopted for biodiesel production. This stepwise addition for the methanolysis could reduce the negative effect of methanol on lipase activity to some extent [6–12]. Hencein the following study, three-step addition of methanol was used for the enzymatic transesterification in biodiesel production.

Lipozyme TL is well known as the lipase with a strict 1,3-positional specificity in many reactions, therefore the theoretical biodiesel yield should be only 66% with such 1,3-specificity

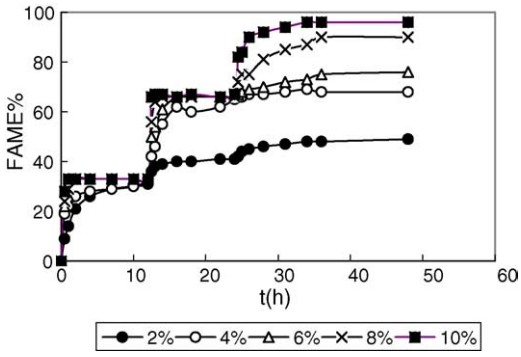

Fig. 1. Effect of lipase quantity on biodiesel yield during three-step methanolysis. Reaction conditions: stepwise addition of methanol, molar ratio of methanol to oil 1:1 in each step, 40 °C, 150 rpm.

lipase as the catalyst. However, as over 90% biodiesel yield was obtained, it was proposed that acyl migration occurred during the reaction process [9–12]. During our study, we found that the more lipase was used for the transformation, the higher the biodiesel yield that could be obtained. As can be seen from Fig. 1, during three-step methanolysis for lipozyme TL-catalyzed biodiesel production, when 10% lipase was used for the transesterification, about 92% biodiesel yield was achieved. However, the reaction hardly proceeded in the third step when only 4% (wt./wt., based on oil weight) lipase was used. Based on the above research it was thought the acyl migration might be correlated with the lipase quantity.

3.2. Comparative study on acyl migration with immobilized and free lipase as the catalyst

From the above study it can be seen that the acyl migration seems to correlate with the amount of the lipase. To study whether the enzyme protein itself accelerates the acyl migration during the reaction process, a comparative study on the acyl migration with the immobilized and free lipase was carried out further. During the free lipase-catalyzed transesterification of soybean oil with methanol for biodiesel production, it has been found that with the same lipase activity as that of 10% immobilized lipase, the biodiesel yield was only 63% even after 72 h reaction.

To further explore the factors influencing the acyl migration during the transesterification process, thin layer chromatograph was used to analyze the intermediates of the reaction. If no acyl migration occurred during the reaction process, then only 1,2-(2,3-) diglycerides would be formed. Conversely, if acyl migration does occur, then not only 1,2-(2,3-), but (1,3)-diglycerides should be formed. It can be seen from Fig. 2 that when free lipase was used for biodiesel production, no 1,3-diglycerides were detected, while in the immobilized lipase-catalyzed three-step transesterification for biodiesel production, 1,3-diglycerides were detected in each step. From this result it can be assumed that some ingredients contained in the immobilized lipase, apart from the protein itself, contribute to the acyl migration.

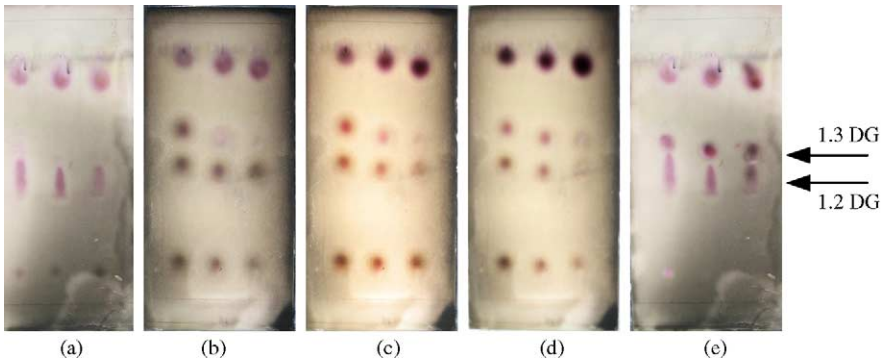

Fig. 2. Intermediates analysis of immobilized and free lipase-catalyzed three-step transesterification for biodiesel production using thin layer chromatograph. Note: From left to right, the three lanes are the samples from first-step, second-step and third-step methanolysis in each plate; (a) 30% free lipase; (b) 4% immobilized lipase; (c) 6% immobilized lipase; (d) 8% immobilized lipase; (e) 10% immobilized lipase.

3.3. Effect of water on the acyl migration during lipase-catalyzed three-step transesterification for biodiesel production

The above analysis demonstrated that the enzyme protein itself did not contribute to the acyl migration in this reaction process. So some other ingredients involved in the immobilized lipase such as water, the immobilized material-silica gel might be the driving force for the acyl migration during the immobilized lipase-catalyzed transesterification for biodiesel production. It has been reported that water accelerates acyl migration [13–16]. Since there is always some water in the immobilized lipase, the effect of water on the acyl migration was studied and is reported below.

Different amounts of water were added to the reaction medium in which 4% immobilized lipase was used for the three-step methanolysis for biodiesel production. From Fig. 3 it can be seen that when water content was more than 0.2% (based on oil weight) in the medium, the reaction rate decreased to some extent, which could result from the hydrolysis side reaction occurring. It can therefore be concluded that that too much water in the reaction medium has some negative effect on the transesterification for biodiesel production.

The amount of water contained in 10% immobilized lipase was calculated to be 0.3% (based on oil weight). From Fig. 1, it has been found that over 90% biodiesel yield could be achieved with 10% immobilized lipase for biodiesel production. While adding some amount of water directly into the medium containing 4% immobilized lipase (the total amount of water in the system is 0.3%, just as much as that contained in 10% immobilized lipase), biodiesel yield was just as high as that of 4% immobilized lipase. Therefore, it can be concluded that water contained in the immobilized lipase did not accelerate the acyl migration during immobilized lipozyme TL-catalyzed three-step methanolysis for biodiesel production.

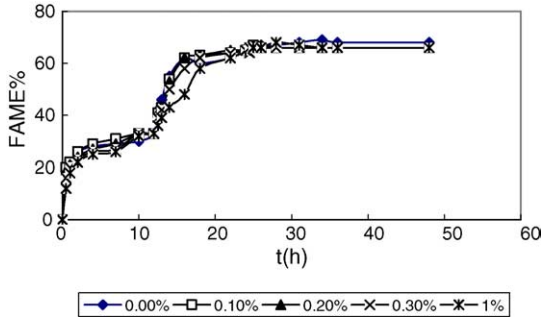

Fig. 3. Effect of water on acyl migration in immobilized lipozyme TL-catalyzed transesterification for biodiesel production. Reaction conditions: 0.4 g immobilized lipozyme TL, stepwise addition of methanol, methanol/oil 1:1 in each step, 40 °C, 150 rpm.

3.4. Effect of silica gel on acyl migration in immobilized lipozyme TL-catalyzed three-step methanolysis for biodiesel production

Another ingredient associated with the immobilized lipase is silica gel, which acted as the immobilization material. Therefore the effect of silica gel on the acyl migration was explored further.

From Fig. 1 it can be seen that about 92% biodiesel yield could be achieved with 10% immobilized lipase for the three-step methanolysis in biodiesel production, while the reaction hardly proceeded in the third-step if only 4% immobilized lipozyme TL was used. To study the effect of silica gel on the acyl migration during the transesterification, 6% silica gel (based on oil weight) was added to the reaction medium containing 4% immobilized lipase. It was found that not only the reaction rate improved significantly, but biodiesel yield was enhanced significantly compared to that with 4% immobilized lipase only (Fig. 4). When combining 6% silica gel with 4% immobilized

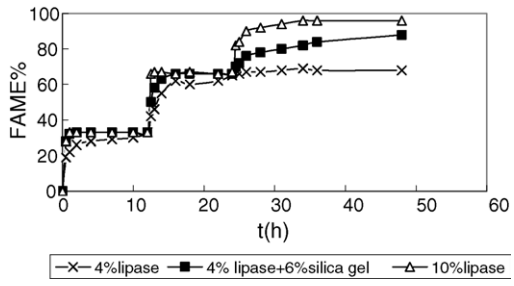

Fig. 4. Effect of silica gel on acyl migration in lipase-catalyzed three-step transesterification for biodiesel production. Reaction conditions: stepwise addition of methanol, methanol/oil 1:1, 40 °C, 150 rpm.

lipase for the three-step methanolysis for biodiesel production, the final biodiesel yield was almost as high as that with 10% immobilized lipase as the catalyst.

4. Conclusion

During immobilized lipozyme TL-catalyzed transesterification for biodiesel production, it has been demonstrated that acyl migration occurred and over 90% biodiesel yield could be achieved with 10% immobilized lipase for the methanolysis. The immobilized material, silica gel was the main factor accelerating the acyl migration and over 90% biodiesel yield could be given when 6% silica gel combined with 4% immobilized lipase for the transformation.

Acknowledgement

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