Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular Catalysis B: Enzymatic 30 (2004) 125–129
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors

Wei Du*, Yuanyuan Xu, Dehua Liu*, Jing Zeng

Department of Chemical Engineering, Tsinghua University, Beijing 100084, PR China

Received 21 August 2003; received in revised form 19 April 2004; accepted 21 April 2004

Available online 1 June 2004

Abstract

Methyl acetate, a novel acyl acceptor for biodiesel production has been developed, and a comparative study on Novozym 435-catalyzed transesterification of soybean oil for biodiesel production with different acyl acceptors was conducted and reported in this paper. Methanol has a serious negative effect on enzymatic activity. A molar ratio of methanol to oil of above 1:1 leads to serious inactivation of the enzyme. However, when methyl acetate was used as the acyl acceptor, a yield of 92% of methyl ester could be obtained with a molar ratio of methyl acetate to oil of 12:1, and methyl acetate showed no negative effect on enzymatic activity. Additionally, with crude soybean oil as the oil source and methanol as acyl acceptor, a much lower methyl ester yield was obtained than that with refined soybean oil, while with methyl acetate as acyl acceptor, an equally high yield of methyl ester (92%) was achieved for both soybean oils. Lipase loses its activity very rapidly during repeated experiments with methanol as the acyl acceptor, while there is almost no detected loss in lipase activity, even after being continuously used for 100 batches, when methyl acetate was used for biodiesel production. Moreover, the by-product triacetylglycerol is an important chemical with a higher value than glycerol, and this novel acyl acceptor seems very promising for lipase-catalyzed large-scale production of biodiesel.

Keywords: Biodiesel; Lipase; Methyl acetate; Transesterification; Interesterification

1. Introduction

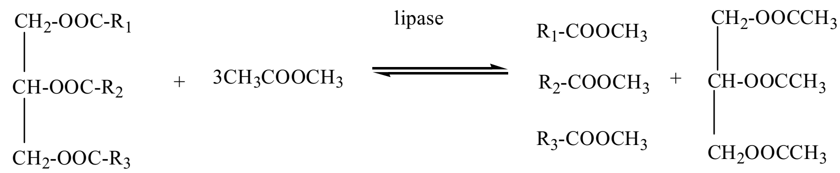
Biodiesel (fatty acid methyl esters), which is produced by transesterification of triglycerides with methanol (i.e. methanolysis), has become increasingly important due to diminishing petroleum reserves and the environmental consequences of exhaust gases from petroleum-fueled engines [1,2]. The attractive features of biodiesel fuel are: it is plant-derived, not a fossil fuel, and as such, its combustion does not increase current net atmospheric levels of carbon dioxide, a greenhouse gas. Additionally, it can be domestically produced, offering the possibility of reducing petroleum imports; it is biodegradable and relative to conventional diesel fuel, its combustion products have reduced levels of particulates, carbon monoxide, sulfur oxides, hydrocarbons, soot and, under some conditions, nitrogen oxides [3–7].

Utilization of lipase as a catalyst for biodiesel fuel production has a great potential compared with chemical methods using alkaline catalyst because no complex operations are needed not only for the recovery of glycerol but also in the elimination of catalyst and salt [1–7]. Usually some short-chain alcohols like methanol are adopted as the acyl acceptor for biodiesel production. However, excess methanol would lead to inactivation of enzyme and glycerol, as a major by-product, could block the immobilized enzyme resulting in low enzymatic activity. These problems could be the limitations for industrial production of biodiesel with enzymes as catalyst [4–12].

A novel acyl acceptor for biodiesel production, methyl acetate, has been developed, and with this novel acyl acceptor, no glycerol is produced in the process (Scheme 1). A comparative study was carried out on the Novozym 435-catalyzed transesterification of soybean oil with methanol and interesterification with methyl acetate.

* Corresponding author. Tel.: +86-10-62772130; fax: +86-10-62794742.
E-mail address: duwei@tsinghua.edu.cn (W. Du).

1381-1177/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2004.04.004



Scheme 1. Biodiesel production by interesterification with methyl acetate.

2. Materials and methods

2.1. Materials

Refined and crude soybean oil sources were obtained locally and *Candida antarctica*B lipase, immobilized on acrylic resin (Novozym 435), 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 were of analytical grade.

2.2. General procedure with methanol for biodiesel production

The enzymatic transesterification reactions were carried out in a 50-ml shaking flask and were heated to 40 °C on a reciprocal shaker. The reaction mixtures consisted of 9.65 g soybean oil, 4% Novozym 435 (based on oil weight) and 1 molar equivalent of methanol. Fifty-microliter samples were taken from the reaction mixture at specified times and centrifuged to obtain the upper layer. Five microliters of the upper layer and 300 μl of 1.4 mM heptadecanoic acid methyl ester (served as the internal standard), were precisely measured and mixed thoroughly for gas chromatography analysis. The residual activity of the lipase was defined as percentage of methyl ester yield at given time compared to the maximum methyl ester yield, which was 100% when 3 molar equivalent methanol (three-step addition) was used for biodiesel production.

2.3. General procedure with methyl acetate for biodiesel production

The enzymatic interesterification reactions were carried out in a 50-ml shaking flask and were heated to 40 °C on a reciprocal shaker. The reaction mixtures consisted of 9.65 g soybean oil, 30% Novozym 435 (based on oil weight) and 12 molar equivalent of methyl acetate. Fifty-microliter samples were taken from the reaction mixture at specified times and centrifuged to obtain the upper layer. Five microliters of the upper layer and 300 μl of 1.4 mM heptadecanoic acid methyl ester (served as the internal standard) were precisely measured and mixed thoroughly for gas chromatography analysis. The residual activity of the lipase was defined as percentage of methyl ester yield at given time compared to the maximum methyl ester yield, which was 100% when methyl acetate was used for biodiesel production.

2.4. Effect of methyl acetate on enzymatic activity

Effect of methyl acetate on enzymatic activity has been realized by keeping the lipase within methyl acetate for 100 h and then the interesterification has been carried out using the pretreated lipase. The change in enzymatic activity was measured by comparing the methyl ester yield given by the lipases before pretreatment and after pretreatment in methyl acetate.

2.5. Analytical procedure

The methyl ester contents in the reaction mixture were quantified using a GC-14B gas chromatograph (Shimadzu Corp., Kyoto) connected to a HP-5 capillary column (0.1 mm × 10 m; Hewlett-Packard, Waldron, Germany). The column temperature was kept at 180 °C for 0.5 min, heated to 300 °C at 10 °C/min, and then maintained for 10 min. The temperatures of the injector and detector were set at 245 and 305 °C, respectively.

3. Results and discussion

3.1. Effect of different acyl acceptors on biodiesel production

A lot of studies on alcoholysis of triacylglycerides (TAGs) with lipases have been reported, and it has been found that too much methanol would lead to serious inactivation of lipase [5–13]. Indeed, when the methanolysis of vegetable oil was conducted with Novozym 435, the lipase was inactivated seriously in the presence of 1 equivalent molar of methanol in the reaction medium. This was thought to be caused by the contact between lipase and insoluble methanol, which existed as drops in the oil (Fig. 1).

Methyl acetate was chosen as a novel acyl acceptor for biodiesel production and the effect of substrate ratio on biodiesel production was investigated (Fig. 2). The highest methyl ester yield of 92% could be obtained at a 12:1 molar ratio of methyl acetate to oil. Either higher or lower methyl acetate content decreased methyl ester yield to some degree.

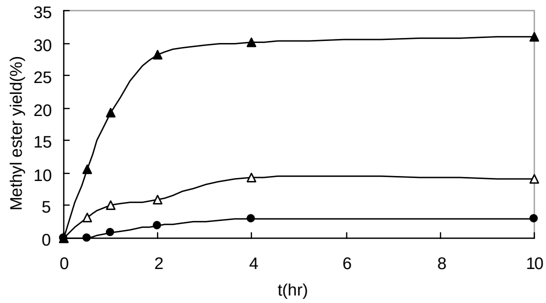


Fig. 1. Effect of short-chain alcohols on the transesterification of soybean oil. Reaction conditions: 150 rpm; 40 °C; 4% Novozym 435 based on oil weight; methanol/oil molar ratio: 3:1 (●), 2:1 (△), 1:1 (▲).

Methyl acetate had no negative effect on enzyme activity, which has been demonstrated by measuring the methyl ester yield given by the lipase, which has been kept in methyl acetate for 100 h and there was no difference in the reaction rate between the lipases before pretreatment and after pretreatment in methyl acetate. Excess methyl acetate (over 16:1) led to an excessive dilution of oil resulting in a reduced methyl ester yield.

3.2. Effect of different acyl acceptors on biodiesel production from crude soybean oil

The cost of oil sources accounts for a large part in the production of biodiesel, and a crude soybean oil source was also explored for biodiesel production with different acyl acceptors. When methanol was used as the acyl acceptor, the reaction rate of crude soybean oil was much lower than that with the refined one just as shown in Fig. 3. This phenomenon has also been found by some other researchers and it was thought to be due to the inhibitive effect on the enzymatic activity caused by lipids existing in crude oil sources [13].

However, it has been found in our research that when methyl acetate is used as the acyl acceptor for biodiesel production, crude soybean oil could give methyl ester yield

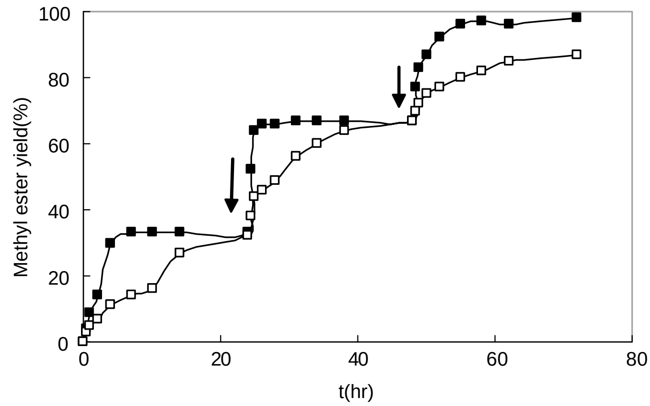


Fig. 3. Biodiesel production from refined and crude soybean oil by three-step addition of methanol. Reaction conditions: 40 °C, 150 rpm, molar ratio of methanol to oil 1:1 (three-step addition just as arrows indicating), 4% Novozym 435, refined soybean oil (■), crude soybean oil (□).

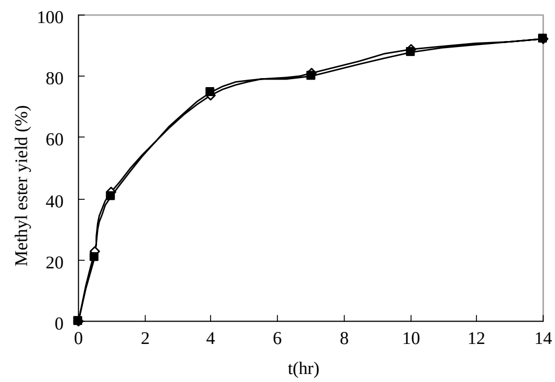


Fig. 4. Biodiesel production from refined and crude soybean oil with methyl acetate as the acyl acceptor. Reaction conditions: 40 °C, 150 rpm, methyl acetate/oil 12:1, 30% Novozym 435, refined soybean oil (■), crude soybean oil (□).

of 92% just as high as that with the refined soybean oil (Fig. 4). It might be due to more methyl acetate present in the reaction medium resulting in dilution effect of lipids in crude oil sources, and less concentration of lipids could contribute to less negative effect of the lipids on enzymatic activity.

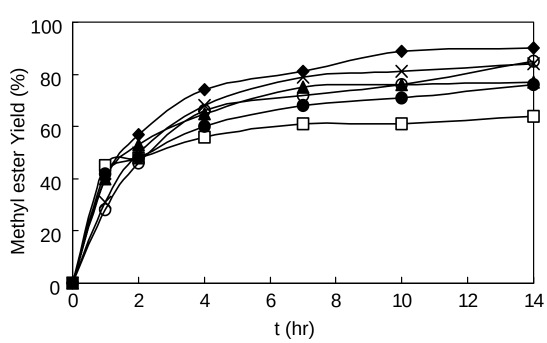


Fig. 2. Effect of substrate ratio of methyl acetate to oil on biodiesel production. Reaction conditions: 40 °C, 150 rpm, 30% Novozym 435 based on oil weight; methyl acetate/oil molar ratio: 6:1 (□), 8:1 (●), 10:1 (▲), 12:1 (◆), 16:1 (□), 24:1 (○).

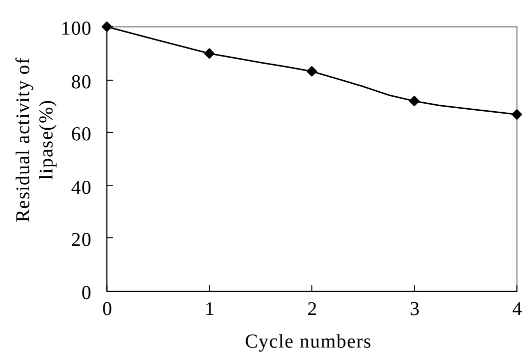


Fig. 5. Operational stability of lipase with methanol as the acyl acceptor. Reaction conditions: three-step addition of methanol, 150 rpm, 40 °C, 4% Novozym 435.

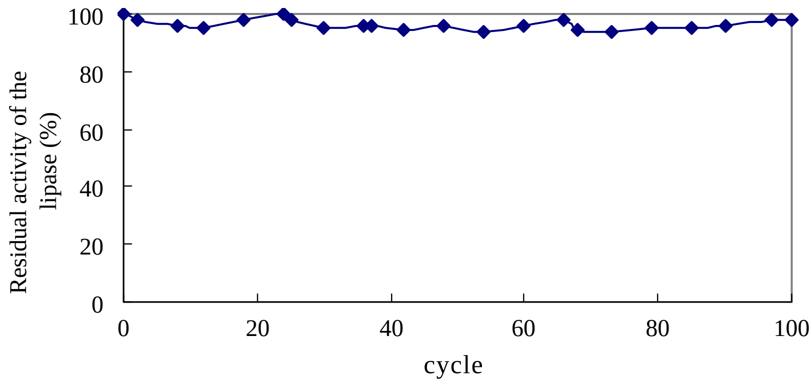


Fig. 6. Operational stability of lipase with methyl acetate as the acyl acceptor. Reaction conditions: methyl acetate/oil molar ratio 12:1, 150 rpm, 40 °C, 30% Novozym 435.

3.3. *Effect of different acyl acceptors on lipase activity during repeated experiments*

It has been demonstrated that there were several advantages with methyl acetate used for biodiesel production compared to methanol. However, as it could be noticed that the reaction rate was much lower with methyl acetate than that with methanol and accordingly more lipase used just as shown in the above studies. From an economic point of view, the cost of lipase accounts for a large part in the cost of biodiesel production. So, the stability and activity of the lipase during the repeated experiments are of great significance.

When methanol was adopted for biodiesel production, it has been found that lipase exhibited poor activity during the repeated experiments, which might be due to the inactivation effect caused by methanol and the negative effect caused by by-product glycerol absorbed on the surface of the immobilized lipase. This phenomenon has also been observed by some other researchers [13–15] (Fig. 5).

However, when methyl acetate was used as the acyl acceptor, there was no glycerol produced in the reaction system and lipase was recycled directly for the repeated reaction without any additional treatment. Continuous operation with methyl acetate as the acyl acceptor for biodiesel production within 0.5 L bioreactor has been carried out and it has been found that there was no observable loss in enzymatic activity even after 100-cycle reaction (Fig. 6).

Chemical processes have been industrialized for large-scale production of biodiesel; however, there are some unavoidable drawbacks associated with chemical processes such as being energy intensive, the need for removal of alkaline catalyst from the product and treatment of alkaline wastewater (usually alkaline used most in chemical processes). Especially when waste oils were used for biodiesel production, relatively high content of free fatty acid and water contained in waste oils would cause serious side reaction such as saponification and this would have some serious negative effect on biodiesel production. To solve this problem, usually some complicated measures were taken for the pretreatment of waste oils. However, with enzymatic methods for biodiesel production, the above-mentioned problems could be avoided.

From an economic point of view, enzymatic approaches have not been industrialized, which is mostly due to the relatively high cost of the lipase. Therefore reducing the cost of catalyst or improving the operational life of the lipase is the key issue. With traditional enzymatic methods for biodiesel production in which short-chain alcohols such as methanol are used as the acyl acceptor, the lipase is rapidly deactivated during repeated experiments and this short operational life of the lipase leads to high cost of the catalyst in the production of biodiesel. However, with methyl acetate as the acyl acceptor, there is no loss detected in enzymatic activity even after being continuously used for 100 cycles and this significant improvement in lipase operational life could contribute a lot to the reduction of biodiesel production cost. Additionally, when methyl acetate is used as the acyl acceptor for biodiesel production, triacetylglycerol instead of glycerol would be produced as one of the by-products in the process and it has been demonstrated that triacetylglycerol had no negative effect on the reaction (while glycerol would block the active site of the lipase resulting in a serious negative effect on lipase activity and stability). Moreover, triacetylglycerol has a higher value than glycerol and 25% triacetylglycerol (based on oil weight) would be produced in the process, which could contribute a lot to the reduction of production cost.

4. Conclusion

In the production of biodiesel, enzymatic activities were influenced seriously by traditional acyl acceptors such as methanol and lipase expressed poor activity during the repeated experiments; however, methyl acetate, a novel acyl acceptor, showed no negative effect on enzymatic activity and lipase could be reused directly without any additional treatment. There is no loss detected in enzymatic activity even after being continuously used for 100 batches, and this significant improvement in lipase operational life would reduce the cost of the catalyst dramatically. Additionally, with crude soybean oil as the oil sources, methanol gave much lower methyl ester yield than that with the refined soybean oil sources, while methyl acetate could give methyl ester yield of 92% just as high as that with the refined one. What's more, with methyl acetate for biodiesel production, by-product triacetylglycerol would be produced instead of glycerol, which has much higher value than glycerol. Therefore, it could be concluded that this novel acceptor seems very promising for lipase-catalyzed large-scale production of biodiesel.

Acknowledgements

This work is sponsored by Chinese National 863 Project (Project No. 2003AA214061).

References

[1] H. Noureddini, D. Zhu, J. Am. Oil Chem. Soc. 74 (1997) 1457–1463.
[2] M. Mattelbach, J. Am. Oil Chem. Soc. 67 (1990) 168–170.
[3] Y. Shimada, Y. Watanabe, A. Sugihara, Y. Tominaga, J. Mol. Catal. B: Enzym. 17 (2002) 133–142.
[4] Ö. Köse, M. Tüter, A.H. Aksoy, Bioresour. Technol. 83 (2002) 125–129.
[5] M. Iso, B.X. Chen, M. Eguchi, T. Kudo, S. Shrestha, J. Mol. Catal. B: Enzym. 16 (2001) 53–58.
[6] L.A. Nelson, T.A. Foglia, W.N. Marner, J. Am. Oil Chem. Soc. 73 (1996) 1191–1195.
[7] V. Dossat, D. Combes, A. Marty, Enzyme Microb. Technol. 25 (1999) 194–200.
[8] Y. Shimada, Y. Watanabe, T. Samukawa, A. Sugihara, H. Noda, H. Fukuda, Y. Tominaga, J. Am. Oil Chem. Soc. 76 (1999) 789–793.
[9] Y. Watanabe, Y. Shimada, A. Sugihara, H. Noda, H. Fukuda, Y. Tominaga, J. Am. Oil Chem. Soc. 77 (2000) 355–360.
[10] B. Selmi, D. Thomas, J. Am. Oil Chem. Soc. 75 (1998) 691–695.
[11] G. Steinke, R. Kirchhoff, K.D. Mukherjee, J. Am. Oil Chem. Soc. 77 (2000) 361–366.
[12] M. Kaieda, T. Samukawa, T. Matsumoto, K. Ban, A. Kondo, Y. Shimada, H. Noda, F. Nomoto, K. Ohtsuka, E. Izumoto, H. Fududa, J. Biosci. Bioeng. 88 (1999) 627–631.
[13] Y. Watanabe, Y. Shimada, A. Sugihara, Y. Tominaga, J. Mol. Catal. B: Enzym. 17 (2002) 151–155.
[14] D. Wei, X. Yuanyuan, L. Dehua, Z. Jing, Biotechnol. Appl. Biochem. 38 (2) (2003) 103–106.
[15] X. Yuanyuan, D. Wei, L. Dehua, Z. Jing, Biotechnol. Lett. 25 (15) (2003) 1239–1241.