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Production of Biodiesel From Unrefined Plants-Derived Oil by *Aspergillus oryzae* Expressing *Bacillus thermocatenulatus* Lipase as a Whole-Cell Biocatalyst

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ABSTRACT
In this study, the enzymatic process reaction using a whole-cell biocatalyst was done for biodiesel production of crude plant oils. This work investigated a streamlined technique to convert crude palm oil that consisted high amount of phosphocholine gums. This reaction by using lipase as a catalyst for biodiesel-fuel has an excellent potential compared with chemical reactions. We explored *Aspergillus oryza*e expressing *Bacillus thermocatenulatus* lipase (BTL) as a biocatalyst in producing fatty acid methyl FAME (95.54%) obtained at 30 ^{o}C, with 5.00 % (w/w) of BTL at 96 h using four molar equivalents of methanol. Therefore, BTL could be useful as whole-cell biocatalyst that can produce biodiesel from feedstock with a high proportion of impurities in industrial application.

Keywords: *biodiesel, Bacillus thermocatenulatus lipase, crude palm oil, enzymatic transesterification*

1. INTRODUCTION

Recently, increasing cost and unavailability of fossil fuel urged the scientist to explore renewable diesel. The high demand for petroleum diesel throughout the world has caused economic, security, and environmental issues. The availabilities of fossil fuels are limited, and the formation of the energy takes million years [1]. Various renewable energy sources have successfully been done to limit consumption. Biodiesel could be an alternative to make some extent for prolonging the decreasing overall consumption of fossil fuel. Biodiesel is described as a long-chain of fatty acid alkyl esters such as methyl or ethyl esters achieved through catalytic trans/esterification of renewable feedstocks such as vegetable oils, animal fats, waste, or microalgal oils with acyl acceptors [2].

Palm oil (*Elaeis guineensis*) is the highest oil crop produced from the processing of fruit called the fresh fruit bunch (FFB) [3]. Palm oil is an economically worthy crop contained a high oil yield per hectare and the cheap cost of palm oil compared to other market oils, such as soybean, rice bran, or olive oil. In this study, one main product of palm oil, crude palm oil (CPO), is utilized as a feedstock for biodiesel synthesis. CPO is obtained from the milling processing steps that implicate sterilization, stripping, digestion, pressing, clarification, and drying of the free fruit bunches (FFB). Trans/esterification reactions are required to perform this synthesis. Alkali or acid catalysts usually use in these reactions. However, some drawbacks appear in free fatty acids (FFAs), for example, saponification [4]. Although acid-catalyzed reactions are possible, there are some problems happened in this process, such as slow reaction rates and the high temperature and pressure for this reaction. To face these problems, various alternative enzyme-catalyzed strategies have been explored as promising candidates for doing both transesterification and esterification reactions. The use of lipases (EC 3.1.1.3) in this case has received significant attention, because both the transesterification of triglycerides and esterification of free fatty acids (FFAs) can be carried out in a single process [5,6]. This study aimed to investigate and determine the ability of lipase to carry its hydrolytic potency out for alkyl ester production. Herein, the effective utilization of an immobilized whole-cell biocatalyst is described in one step processes for biodiesel production.

2. MATERIAL AND METHODS

2.1. Material

Crude palm oil (CPO) was obtained through P.T.Agricinal in Bengkulu, Indonesia. All reagents were bought and supplied from Sigma-Aldrich (Tokyo, Japan) and Nacalai Tesque Inc (Kyoto, Japan). The *A. oryzae* strain was transformed independently with a lipase-encoding gene from *Bacillus thermocatenulatus* (BTL).

2.2. Methanolysis of CPO to FAME

Transesterification was performed with 4 g of the CPO, 5 % (w/v) water, and 200 mg of the immobilized lipase into a screw-capped cylindrical glass tube. The reaction was done by adding an amount of methanol-based on a 1:1 molar ratio of methanol to CPO. Step-wise addition of methanol every 24 h which resulted in a final oil-to-methanol ratio of 1:4 was performed to avoid the denaturation of lipase. The reaction was carried out at 30°C and 35 rpm for 96 h in a Thermoblock rotator (Nissin, Tokyo, Japan).

2.3. Analysis of biodiesel

In analyzing the composition of biodiesel, it was carried out using a gas chromatography instrument equipped with a flame ionization detector (GC-2010, Shimadzu, Tokyo, Japan). Species separation with helium as the carrier gas was in a 15 m × 0.25 µm × 0.10 µm Zebron ™ ZB-5HT column (Phenomenex Inc., 2CA, USA). The fatty acid composition was analyzed separately using the Gas Chromatography Mass Spectrometry (GC-MS) instrument (GCMS-QP2010, Shimadzu, Tokyo, Japan). Except in other statements, all values represented are triple averages.

Within forming functional groups of biodiesel fuel, to analyze total-Fourier-transform infrared spectroscoi (atr-ftir) is by using the infrared microscope of Shimadzu aim-900 with itrraces-100 (Shimadzu corp., Tokyo, Japan) [7].

3. RESULT AND DISCUSSION

3.1. Enzymatic FAME production

Biodiesel was produced via enzymatic transesterification. CPO and BTL were utilized as the sole substrate and the biocatalyst. FAME conversion was measured by GC-FID. FAME was obtained 95.54% after 96 h (Figure 1). It indicated that using BTL as catalyst showed a slow reaction in the first 24 h in which the conversion was 20.20%. However, after the second addition of methanol at 24 h, the reaction going to 56.10% and always increased until the reaction completed. Step-wise addition of methanol is done to avoid the deactivation of lipase due to short-chain carbon (alcohol). Therefore, four times methanol addition was performed every 24 h. This result indicated that CPO is a suitable feedstock for biodiesel production, which could substitute diesel.

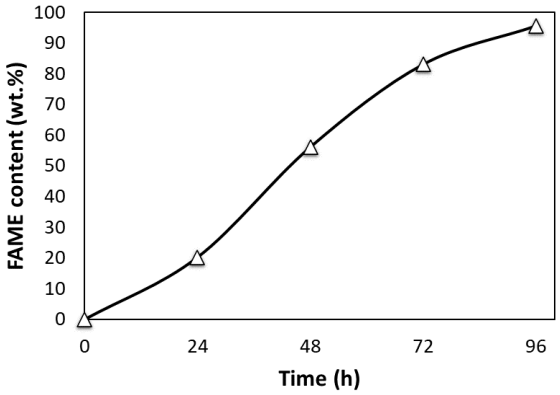

Figure 1. FAME conversion of CPO and methanol by using BTL as the catalyst.

3.2. Biodiesel properties

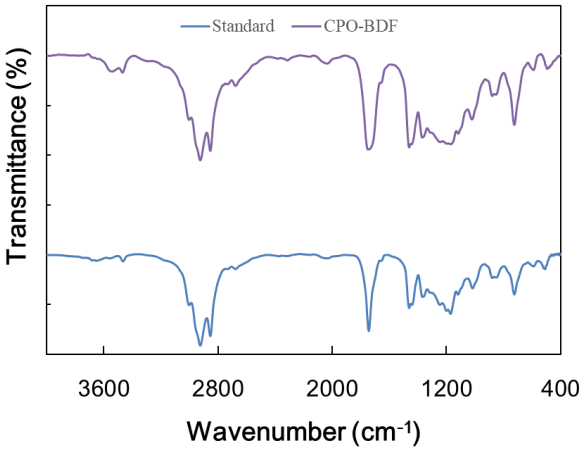

Figure 2. ATR-FTIR spectra from (a) commercial biodiesel and (b) biodiesel from CPO.

In this paper, the mid-infrared spectral data (4000–400 cm^{-1}) was used to identify the functional group of organic and inorganic bonds in biodiesel. The comparison between commercial biodiesel and biodiesel produced from CPO by this research was presented in Figure 2. Both functional groups indicated the same spectra features. The wavenumbers at 3089–2735 cm^{-1} consisted of symmetric and asymmetric stretching vibrations of –C–H alkane groups, at 1816–1639 cm^{-1} attributed to a carbonyl group (–C=O stretching) of the type of ester in biodiesel synthesis, at 1529–1367 cm^{-1} indicated the alkyl ($–CH_3$) groups in the carbon chain, at 1242–1112 cm^{-1} showed the bending vibration of aromatic ester, C–O and $O–CH_3$, and the last at 721 cm^{-1} indicated the methylene functional group (=C–H) in biodiesel [8].

4. CONCLUSION

The concept of CPO, methanol and whole-cell lipase biocatalyst was effectively used for biodiesel production. FAME conversion of 95.54% was achieved within 96 hours when reaction conditions of 30 ºC, 35 rpm and 1:4 oil to methanol molar ratio. This result is almost fulfilled the EN 14214 standard specification of ester content which is 96.5% (w/w) for the minimum required. Optimization of this process is needed due to a crucial contribution to the sustainable production of biodiesel, especially for the palm oil industry.

REFERENCES

[1] S. Firoz, A Review: Advantages and Disadvantages of Biogas, Int. Res. J. Eng. Technol. (2017) 530–535. www.irjet.net.

[2] A. Islam, P. Ravindra, Biodiesel production with green technologies, 2016. https://doi.org/10.1007/978-3-319-45273-9.

[3] C.C. Okolo, E.C. Okolo, A.L. Nnadi, F.E. Obikwelu, S.E. Obalum, C.A. Igwe, The oil palm (*Elaeis guineensis* Jacq): nature's ecological endowment to eastern Nigeria, Agro-Science. 18 (2019) 48. https://doi.org/10.4314/as.v18i3.9.

[4] B. Norjannah, H.C. Ong, H.H. Masjuki, J.C. Juan, W.T. Chong, Enzymatic transesterification for biodiesel production: a comprehensive review, RSC Adv. 6 (2016) 60034–60055. https://doi.org/10.1039/C6RA08062F.

[5] S. Hama, H. Noda, A. Kondo, How lipase technology contributes to evolution of biodiesel production using multiple feedstocks, Curr. Opin. Biotechnol. 50 (2018) 57–64. https://doi.org/10.1016/j.copbio.2017.11.001.

[6] H. Mohamed, I. Arbi, L. El, U. Rashid, S.I. Al-resayes, Lipase / enzyme catalyzed biodiesel production from Prunus mahaleb : A comparative study with base catalyzed biodiesel production, Ind. Crop. Prod. 76 (2015) 1049–1054. https://doi.org/10.1016/j.indcrop.2015.08.023.

[7] N. Rachmadona, J. Amoah, E. Quayson, S. Hama, A. Yoshida, A. Kondo, C. Ogino, Lipase-catalyzed ethanolysis for biodiesel production of untreated palm oil mill effluent, Sustain. Energy Fuels. 4 (2020) 1105–1111. https://doi.org/10.1039/C9SE00457B.

[8] R.M. Silverstein, F.X. Webster, D.J. Kiemle, Spectrometric Identification of Organic Compounds, 7th ed., John Wiley & Sons, Inc, United States of America, 2005.