UNIVERISITI TEKNOLOGI MARA

ULTRASONIC ASSISTED TRANSETSERIFICATION OF USED COOKING OIL WITH METHYL ACETATE USING IMMOBILISED *CANDIDA ANTACTICA* LIPASE TYPE A (CaLA)

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of the requirement for degree of
Master of Science
(Chemical Engineering)

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Postgraduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

This work is about the production of biodiesel through ultrasonic-assisted enzymatic transesterification of used cooking oil with methyl acetate. Used cooking oil was chosen due to its abundance and availability, as well as low cost. The ultrasonic-assisted enzymatic reaction functions to increase enzymatic activity. The application of methyl acetate (MA) as acyl acceptor instead of methanol could resist enzyme inhibition and immobilised lipase, making it recyclable. The objectives of this research are to study the activity, kinetic and stability of the immobilised lipase through hydrolysis process, determine the effect of molar ratio of the acyl acceptor (1:3 - 1:15), reaction time (2 h - 3 h) and immobilised enzyme loading (0.2% (w/v)– 2.0% (w/v)) towards biodiesel production, and evaluate the activity of lipase in the reusability of ultrasonic-assisted enzymatic transesterification process and the properties of biodiesel. In this study, the activity of three different commercial immobilised lipases, which are *Pseudomonas cepacia* (PcL), *Thermomyces lanuginosus* (TLIM), and *Candida Antarctica* A (CaLA), were measured at different temperatures (30–50°C) and immobilised lipase loadings (1% (w/v) – 5% (w/v)). The benefits of ultrasonic assisted in enzymatic activity were studied through kinetics and stability. It was found that the enzymatic activity of immobilised lipase increased with the increased temperature and immobilised lipase loading. The optimum temperature for all types of immobilised lipase was at 40 °C. The optimum immobilised lipase loading was 4% (w/v) of for immobilised PcL and immobilised CaLA, and 3% (w/v) for immobilised TLIM. However, immobilised CaLA showed the highest activity of 1.89×10^{-3} mM/min. Furthermore, the ultrasonic-assisted enzymatic hydrolysis gives benefits towards the enzymatic activity. The affinity of substrate towards the immobilised enzyme active site was increased due to the application of ultrasonic assisted in the reaction medium. The K_m values of immobilised CaLA were 2.2156 mM and 57.412 mM for reactions with and without ultrasonic assisted, respectively. The production of biodiesel was found increased with the increased of the molar ratio used cooking oil with methyl acetate, reaction time and the immobilised CaLA loading. The optimum ultrasonic assisted enzymatic transesterification reaction was recorded at 96.73% of biodiesel conversion using molar ratio of 1:9, 2.5 h of reaction time and 1.8% (w/v) of immobilised CaLA loading. The ultrasonic parameters were 580 W and 20 kHz. The reusability of immobilised CaLA with high biodiesel conversion was obtained up until the third reaction cycle. The properties of biodiesel were followed the European Biodiesel Standard (EN 14214) requirement, where the ester content, viscosity, acid value, heat value, and cloud point were 96.73%, 4.4 mm^2/s, 0.47 mg KOH/g, 36.5 MJ/kg, and 9.50°C, respectively. Therefore, the ultrasonic-assisted enzymatic reaction was beneficial for the enzymatic activity, as the immobilised CaLA gave the highest biodiesel in short reaction time. Moreover, the application of MA as acyl acceptor makes the immobilised CaLA reusable and helps the biodiesel meet the quality standards and requirements.

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TABLE OF CONTENTS