

THE EFFECT OF LIPASE-CATALYSED TRANSESTERIFICATION  
OF FATTY ACIDS USING *PSEUDOMONAS*  
SP. STRAIN WITH PALM OIL AS SUBSTRATE

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**THE EFFECT OF LIPASE-CATALYSED TRANSESTERIFICATION OF  
ALKYL ESTERS USING *PSEUDOMONAS* SP. LIPASE  
WITH PALM OLEIN AS SUBSTRATE**

**By**

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**PENGAKUAN DAN PENGESAHAN LAPORAN  
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Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: THE EFFECT OF LIPASE-CATALYSED TRANSESTERIFICATION OF ALKYL ESTERS USING PSEUDOMONAS SP. LIPASE WITH PALM OLEIN AS SUBSTRATE oleh Farah Dhaniah binti Abdul Rahman, no. matrik: UK 8096 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh Ijazah Sarjana Muda Sains – Sains Biologi, Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

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## LIST OF ABBREVIATIONS

g - gram

ml - milliliter

M - molar

° C - celcius

% - percentage

NaOH - sodium hydroxide

rpm - rotation per minute

RBD - refined, bleached and deodorized

## ABSTRACT

Biodiesel (fatty acid alkyl esters), which is derived from triglycerides has attracted considerable attention during the past decade as a renewable, biodegradable, and nontoxic fuel. Transesterification using alkali-catalysis which gives high levels of conversion of triglycerides has been widely utilized for biodiesel fuel. However, the reaction has several drawbacks and therefore, recently, enzymatic transesterification using lipase has become more attractive for biodiesel fuel production, since it offer many advantages over chemical reactions. In this study, the synthesis of alkyl esters by immobilized *Pseudomonas* sp. lipase using RBD palm olein as substrate was investigated. The production of alkyl esters involved a two-steps process: hydrolysis of palm olein to release fatty acids from the triglycerides backbones, followed by alcoholysis of the free fatty acids to produce esters. Several reaction parameters were studied to obtain the optimum conditions for lipase activity: incubation time, temperature, amount of palm olein as substrate and amount of enzyme for hydrolysis; type of alcohol, substrate ratio and type of solvent for alcoholysis/transesterification. It was found that optimum fatty acids were released after four hours of incubation, at 37°C, with 12 g palm olein as substrate and 0.6 g of lipase. As for transesterification, the amount of alkyl esters formed was optimum with methanol as reactant, at palm olein:methanol ratio of 1:2 (w/v) and in isooctane as solvent. These results indicate that *Pseudomonas* sp. lipase is capable of catalyzing the alkyl ester production from palm olein. Further investigation involving several other parameters is recommended to ensure highest yield of alkyl esters for use as biodiesel.

# KESAN TINDAKBALAS TRANSESTERIFIKASI ALKIL ESTER YANG DIMANGKINKAN OLEH LIPASE *PSEUDOMONAS* SP. MENGGUNAKAN PALM OLEIN SEBAGAI SUBSTRAT

## ABSTRAK

Biodisel (asid lemak alkil ester), terbitan daripada trigliserida telah menarik perhatian yang besar sejak sedekad yang lalu sebagai bahan yang boleh diperbaharui, diurai secara biologi dan bahan api yang tidak bertoksik. Transesterifikasi menggunakan katalisis alkali yang menghasilkan tahap penukaran trigliserida yang tinggi telah digunakan secara meluas dalam bahan api biodisel. Walau bagaimanapun, tindakbalas tersebut mempunyai beberapa kekurangan dan oleh sebab itu, transesterifikasi secara enzimatik menggunakan lipase telah menjadi tarikan dalam penghasilan bahan api biodisel, memandangkan biodisel memberikan banyak kelebihan berbanding tindakbalas secara kimia. Dalam kajian ini, sintesis alkil ester menggunakan *Pseudomonas* sp. dan RBD minyak sawit olein sebagai substrat telah dikaji. Penghasilan alkil ester melibatkan dua proses: hidrolisis minyak sawit olein untuk membebaskan asid lemak bebas daripada tulang belakang trigliserida, diikuti dengan alkoholisis asid lemak bebas untuk menghasilkan ester. Beberapa tindakbalas parameter telah dikaji untuk mendapatkan keadaan yang optimum bagi aktiviti lipase: masa pengeraman, suhu, kuantiti minyak sawit olein sebagai substrat dan kuantiti enzim bagi hidrolisis; jenis alkohol, nisbah substrat dan jenis pelarut untuk alkoholisis/transesterifikasi. Didapati bahawa asid lemak yang dibebaskan adalah optimum selepas 4 jam masa pengeraman, pada suhu 37°C, dengan 12 g palm olein dan 0.6 g lipase. Untuk transesterifikasi, kuantiti alkil ester yang optimum

dihasilkan dengan menggunakan metanol sebagai reaktan, minyak sawit olein:metanol pada nisbah 1:2 (w/v) dan isooktana sebagai pelarut. Keputusan ini menunjukkan bahawa *Pseudomonas* sp. lipase berkebolehan dalam memangkinan penghasilan alkil ester daripada minyak sawit olein. Kajian selanjutnya yang melibatkan beberapa parameter yang lain adalah dicadangkan untuk memastikan hasil alkil ester yang tinggi bagi kegunaan biodisel.