UNIVERSITI PUTRA MALAYSIA

TRANSESTERIFICATION OF PALM OIL USING FREE AND IMMOBILIZED LIPASE FROM PSEUDOMONAS SPECIES FOR BIODIESEL PRODUCTION

NIDA JAFRI

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By

NIDA JAFRI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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TRANSESTERIFICATION OF PALM OIL USING FREE AND IMMOBILIZED LIPASE FROM PSEUDOMONAS SPECIES FOR BIODIESEL PRODUCTION

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Chairman: Norhafizah Abdullah, PhD

Faculty: Institute of Bioscience

Refined palm oil (RPO) was used as the feedstock for the synthesis of fatty acid methyl ester (FAME) commonly called as biodiesel by transesterification process. The proximate fatty acid composition of RPO used showed it contained high amount of oleic acid and palmitic acid at 54% and 33%, respectively indicating a good potential of raw feedstock for biodiesel production. The transesterification reaction was done using a mixture containing RPO and methanol in *n*-hexane as a medium with lipase as catalyst in a batch STR. The scouting of lipase was conducted in the same set-up with lipase from *Pseudomonas* species (PS) and *Penicillium camembertii* (PC). The scouting result showed efficient conversion of FAME by lipase PS as compared to lipase PC. Therefore all the subsequent work

used lipase PS. Effects of molar ratio of RPO to alcohol, molar concentration of RPO, amount of lipase dosage and reaction time on the composition of biodiesel were investigated. The optimum conditions obtained were as follows: methanol to RPO molar ratio- 3:1, RPO concentration- 0.9 M, enzyme dosage- 50 units/mg at reaction temperature- 40^{o}C after 72 hours. The composition of FAME was determined by gas chromatography-mass spectrometry (GC-MS) and results showed a total FAME yield of 92%. An eco-friendly immobilized lipase was also designed by entrapment technique in calcium alginate beads in an attempt to further improve the yield of biodiesel and reusability of lipase. Transesterification was performed using the same optimized reaction conditions as in the free lipase experiment. The experimental result showed a maximum biodiesel yield of 97% in STR which was higher than the yield given by free lipase PS in STR at 92%. The reusability and stability of immobilized lipase was tested for consecutive transesterification experiment. A yield of 77% was obtained after the fourth batch cycle. There was no physical deformation in the structure of the beads which indicated a high mechanical stability of the beads even after four batch cycles under continuous mixing at 150 rpm. A preparatory scale-up of transesterification reaction was also done in an oscillatory flow reactor (OFR) using free and immobilized lipase PS. The main purpose of using OFR was to improve mixing condition with higher reaction volume and shortening overall reaction time. The reaction was conducted with 2.5 kg of RPO at molar ratio of 3:1 in *n*-hexane medium. Maximum conversion of 98% and 97% was obtained after a reaction time of 2 hrs from free-suspended lipase and

immobilized lipase, respectively. This study showed lipase PS exhibited excellent catalyst quality for FAME production and a further improvement in productivity when immobilized lipase was used. There was a 37 fold of improvement in FAME productivity when using OFR as compared to STR. The OFR also reduced the reaction time and gave a good biodiesel yield. Results from this study showed a positive indication for the potential use of OFR in industry for biodiesel production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

TRANSESTERIFIKASI MINYAK KELAPA SAWIT DENGAN MENGGUNAKAN LIPASE BEBAS DAN TERPERANGKAP DARIPADA SPESIS PSEUDOMONAS UNTUK PENGHASILAN BIODIESEL

Oleh

Nida Jafri

Februari 2013

Pengerusi: Norhafizah Abdullah, PhD

Fakulti: Institut Biosains

Minyak kelapa sawit bertapis (RPO) telah digunakan sebagai bahan mentah untuk sintesis asid lemak metil ester (FAME) yang biasanya dikenali sebagai biodiesel daripada proses transesterifikasi. Komposisi asid lemak RPO daripada analisis hampiran menunjukkan ia mengandungi jumlah asid oleik dan asid palmitik yang tinggi, iaitu pada 54% dan 33%, masing-masing. Ini menunjukkan potensi yang baik sebagai bahan mentah untuk penghasilan biodiesel. Tindak balas transesterifikasi telah dilakukan dengan menggunakan campuran yang mengandungi RPO dan metanol dalam n-heksana sebagai medium dengan lipase sebagai pemangkin dalam reaktor tangki teraduk (STR) kelompok. Pencarian lipase telah dijalankan dengan

menggunakan eksperimen yang sama daripada spesies *Pseudomonas* (PS) dan *Penicillium camembertii* (PC). Keputusan kajian menunjukkan kecekapan penukaran FAME yang efisien oleh lipase PS berbanding dengan lipase PC. Oleh itu kesemua eksperimen yang berikut akan menggunakan lipase PS. Kesan nisbah molar PO kepada kepekatan alkohol, kepekatan molar RPO, jumlah dos lipase dan masa tindak balas terhadap komposisi biodiesel telah diselidik. Keadaan optimum yang diperolehi adalah seperti berikut: nisbah molar metanol kepada RPO-3:1, kepekatan molar RPO- 0.9 M, dos lipase -50 unit/mg pada tindak balas selepas 72 jam dan suhu pada 40^{o}C. Komposisi FAME telah ditentu mengguna alatan oleh kromatografi gas-spektrometri jisim (GC-MS) dan keputusan menunjukkan hasil FAME keseluruhan sebanyak 92%. Lipase terperangkap yang mesra alam turut direka oleh teknik perangkap dalam manik kalsium alginat dalam usaha untuk meningkatkan lagi hasil biodiesel dan kebolehgunaan semula enzim lipase. Transesterikasi dilakukan menggunakan keadaan tindakbalas optimum yang sama seperti dalam eksperimen lipase bebas di atas. Hasil kajian menunjukkan penghasilan biodiesel maksimum yang lebih tinggi pada 97% yang lebih tinggi oleh lipase PS terperangkap berbanding dengan lipase bebas pada 92%. Kebolehgunaan semula dan kestabilan lipase terperangkap telah diuji dalam empat eksperimen transesterifikasi secara berterusan. Hasil sebanyak 77% telah diperolehi selepas empat kitaran kelompok. Tiada perubahan dalam bentuk fizikal dan struktur manik alginat yang mengandungi lipase dan ini menunjukkan kestabilan mekanikal yang bagus walaupun selepas empat kitaran proses transesterifikasi di bawah keadaan

adukan berterusan pada 150 rpm. Eksperimen penskalaan tindak balas transesterifikasi juga dilakukan dengan menggunakan reaktor aliran ayunan (OFR) bagi kedua-dua jenis lipase PS bebas dan lipase PS terperangkap. Tujuan utama OFR digunakan adalah untuk meningkatkan lagi keefisienan pencampuran dalam isipadu tindak balas yang lebih tinggi dan memendekkan masa tindak balas secara keseluruhan. Tindak balas telah dijalankan dengan menggunakan 2.5 kg RPO, nisbah molar metanol kepada minyak sebanyak 3:1 dan *n*-heksana sebagai medium. Penukaran maksimum sebanyak 98% dan 97% telah diperolehi selepas masa tindak balas 2 jam dihasilkan oleh lipase PS bebas dan lipase PS terperangkap, masing-masing. Kajian ini menunjukkan lipase PS mempunyai kualiti pemangkin yang sangat baik untuk penghasilan biodiesel dan penambahbaikan dalam produktiviti apabila lipase terperangkap digunakan. Peningkatan penghasilkan biodiesel merekodkan gandaan produktiviti sebanyak 37 kali apabila OFR digunakan, berbanding dengan STR. OFR juga dapat mengurangkan masa tindak balas dan memberikan hasil biodiesel yang baik. Hasil kajian ini boleh digunakan sebagai satu indikasi yang positif dalam potensi penggunaan OFR sebagai reaktor diperingkat industri bagi pengeluaran biodiesel.

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May Allah help us in following Islam according to Quran and Sunnah. *(Aameen*)

I certify that a Thesis Examination Committee has met on the 15th February, 2013 to conduct the final examination of Nida Jafri on her M.Sc thesis entitled "Transesterification of palm oil using free and immobilized lipase from *Pseudomonas species* for biodiesel production" in accordance with Universities and University Colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U. (A) 106]. The committee recommends that the student be awarded the Master of Science degree.

Members of the Thesis Examination Committee were as follows:

Assoc. Prof. Dr. Siti Aslina Hussain, PhD
(Chairman)

Assoc. Prof. Dr. Rosfarizan binti Mohamad, PhD
(Internal Examiner)

Dr. Dayang Radiah binti Awang Biak, PhD
(Internal Examiner)

Assoc. Prof. Dr. Mashitah Mat Don, PhD
(External Examiner)

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory committee were as follows:

Norhafizah Abdullah, PhD
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

Tinia Idaty Md. Ghazi, PhD
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

_____

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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NIDA JAFRI

Date: 15th February 2013

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