UNIVERSITI PUTRA MALAYSIA

ADSORPTION IMMOBILIZATION OF *CANDIDA RUGOSA* LIPASE ONTO ALUMINOSILICATE SUPPORTS AS BIOCATALYSTS FOR FATTY ACID SUGAR ESTER SYNTHESES

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By

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Chairman : Professor Mohd Basyaruddin Abdul Rahman, PhD

Faculty : Science

Nowadays, the industrial applications of the biocatalysts have not yet reached a significant level of use due to the high cost, instability and the inconvenience of separating, recycling and reusing of the enzymes. These deficiencies have motivated researchers to improve their catalytic properties by immobilization of enzymes onto selected supports. The high cost of popular supports such as calcium carbonate, rice husks and rice straw or kaolin causes many to search for cheaper substitutes from the natural environment. Instead of these alternatives, feldspar and mica appear to be most attractive since they are naturally available and have the potential to be used as low cost sorbents.

In this study, aluminosilicates of feldspar and mica originated from the quary area of Tanah Putih, Gua Musang, Kelantan (Malaysia) were used as enzyme supports. They were found to have high surface area, large porosity and good

mesoporous properties. Mica was physically modified either by acid treatment, grafting with Amino-, Octyl-, Vinyl-, Mercapto- and Glycidoxy-triethoxysilanes, and activation of pre-treated support with glutaraldehyde (Glu). The modified mica preparations were used for immobilization of lipase from *Candida rugosa* (CRL) and exhibited improved specific activity as compared to the free enzyme by 1.8 - 2.4 folds. Lipase immobilized on mica showed enhanced protein loading (up to 8.2 mg protein/g support) and immobilization (up to 78%) compared to the free lipase (Free-CRL) and those immobilized on unmodified mica. However, another two new approaches of enzyme-aggregate-coating (EAC-CRL) and nanoscale-enzyme-reactor (NER-CRL) were found to perform higher specific activities of about 2.6 folds.

The selected immobilized lipases were then used in the esterification reaction to optimize the reaction conditions factors. Optimal production conditions (83%) for the synthesis of fatty acid sugar esters (FASEs) were achieved after 48 hours with the use of capric acid (C10) and lactose sugar as substrates at temperature of 55 °C. In addition, substrates molar ratio of 1:2 (capric acid to lactose) exhibited the highest ester conversion in acetone which was the best solvent used. On the other hand, the operational stability with half lives of over 13, 10, 7 and 6 reaction cycles for NER-CRL, Amino-CRL, EAC-CRL and GluAmino-CRL, respectively, indicated the efficiency of the immobilization process. The best performance by the immobilized lipase, in terms of both activity and stability was achieved by NER-CRL, which was prepared through

immobilization using the combination of physical adsorption and cross-linking approaches of lipase on unmodified mica support.

The formation of lactose caprate from lactose sugar (Lac) and capric acid (CA) using biocatalysts were also evaluated through a kinetic study. Determination of the apparent kinetic parameters of K_m and V_{max} by means of Michaelis-Menten kinetic model was used. The Ping-Pong Bi-Bi mechanism with one substrate inhibition was adopted as it best explained the experimental findings. The kinetic results showed lower K_m values (CA = 14.5 mmol/L.mg); Lac = 9308 mmol/L.mg) of the NER-CRL when compared to the Free-CRL, indicating a higher affinity of the NER-CRL towards the both substrates ($K_{m,app\ (CA,\ Lac)} < K_{m\ (CA,\ Lac)}$), thus yielding an increase in reaction rate. The kinetic parameters deduced from this model were used to simulate the initial rate data, which was in excellent agreement with the experimental values.

The physicochemical properties of synthesized FASEs product of lactose caprate have been studied in order to meet their potential as high value-added biosurfactants. The present study had shown that *Candida rugosa* lipase immobilized on natural and low cost mica support was successfully employed as potential biocatalyst for FASEs syntheses. Furthermore, immobilized lipase preparations were able to enhance the productivity and stability, suggesting that the preparations are more versatile and adaptable for FASEs production as potential biosurfactant in industrial applications.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENYEKATGERAKAN LIPASE DARIPADA *CANDIDA RUGOSA* KE ATAS PENYOKONG ALUMINOSILIKAT SEBAGAI BIOPEMANGKIN DALAM SINTESIS ESTER GULA ASID LEMAK

Oleh

USWATUN HASANAH BT. ZAIDAN

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Pengerusi : Profesor Dr. Mohd Basyaruddin Abdul Rahman, PhD

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Pada masa ini, aplikasi industri terhadap biokatalis belum mencapai tahap yang signifikan disebabkan oleh kos enzim yang tinggi, ketidakstabilan serta kesukaran dalam pemisahan dan penggunaan semula enzim. Semua kekurangan ini telah menggalakkan penyelidik meningkatkan ciri-ciri pemangkinan melalui penyekatgerakan enzim ke atas bahan penyokong terpilih. Kos bahan penyokong yang tinggi seperti kalsium karbonat, sekam padi dan jerami padi atau kaolin menyebabkan ramai mencari pilihan yang lebih murah dari sumber semulajadi. Selain alternatif ini, felspar dan mika menonjol dan menarik kerana wujud secara semulajadi dan berpotensi untuk digunakan sebagai penyokong kos rendah.

Dalam kajian ini, aluminosilikat felspar dan mika yang berasal dari tapak kuari di Tanah Putih, Gua Musang, Kelantan (Malaysia) telah digunakan sebagai

penyokong enzim. Mereka didapati mempunyai luas permukaan dan porositi yang tinggi serta memiliki ciri-ciri bahan mesoporos. Mika telah diubahsuai melalui rawatan asid, penambahan kumpulan Amino-, Oktil-, Vinil-, Merkapto- dan Glisidoksi-trietoksisilan, dan pengaktifan menggunakan glutaraldehid (Glu). Mika terubahsuai telah digunakan untuk penyekatgerakan lipase daripada *Candida rugosa* (CRL) dan menunjukkan peningkatan aktiviti spesifik berbanding enzim bebas iaitu pada 1.8 - 2.4 kali ganda. Lipase tersekatgerak pada mika menunjukkan penjerapan protein (sehingga 8.2 mg protein/g) dan penyekatgerakan (sehingga 78%) berbanding dengan lipase bebas (Free-CRL) dan lipase pada mika takterubahsuai. Namun, dua pendekatan baru enzim-agregasi-lapisan (EAC-CRL) dan nano-enzim-reaktor (NER-CRL) masing-masing didapati mempamerkan aktiviti lebih tinggi iaitu sehingga 2.6 kali ganda.

Lipase tersekatgerak terpilih telah digunakan dalam sintesis ester untuk mengkaji faktor-faktor tindakbalas. Keadaan optimum (83%) sintesis ester gula asid lemak (FASEs) telah dicapai pada 48 jam menggunakan asid kaprik (C10) dan laktosa sebagai substrat pada suhu 55 °C. Selain itu, nisbah molar substrat 1:2 (asid kaprik kepada laktosa) telah mempamerkan penukaran ester tertinggi dalam aseton sebagai pelarut terbaik. Malah, kestabilan operasi separuh hayat melebihi 13, 10, 7 dan 6 kitaran tindakbalas bagi NER-CRL, Amino-CRL, EAC-CRL dan GluAmino-CRL, masing-masing menunjukkan kecekapan proses penyekatgerakan. Prestasi terbaik lipase tersekatgerak, baik dari segi aktiviti dan kestabilan telah dicapai oleh NER-CRL, yang telah disediakan melalui

penyekatgerakan menggunakan kombinasi penjerapan fizikal dan pendekatan interaksi lipase pada sokongan mika takterubahsuai.

Sintesis laktosa kaprat daripada laktosa (Lac) dan asid kaprik (CA) menggunakan biokatalis juga telah dinilai melalui kajian kinetik. Penentuan parameter kinetik K_m and V_{max} berdasarkan model Michaelis-Menten telah digunakan. Mekanisma Ping-Pong Bi-Bi dengan satu perencatan substrat adalah yang terbaik menjelaskan penemuan eksperimen. Keputusan kinetik menunjukkan nilai K_m (CA = 14.5 mmol/L.mg); Lac = 9308 mmol/L.mg) bagi NER-CRL lebih rendah berbanding dengan Free-CRL, dan menunjukkan bahawa NER-CRL mempunyai afiniti yang lebih tinggi terhadap kedua-dua substrat ($K_{m,app\ (CA,\ Lac)} < K_{m\ (CA,\ Lac)}$), seterusnya meningkatan kadar tindakbalas. Parameter kinetik dari model ini telah digunakan untuk mensimulasikan data kadar awal tindakbalas, yang sangat menepati dengan nilai-nilai eksperimental.

Sifat-sifat fizik-kimia produk laktosa kaprat telah dikaji dalam rangka memenuhi potensi produk sebagai biosurfaktan dengan nilai tambah yang tinggi. Penyelidikan ini telah menunjukkan bahawa lipase tersekatgerak dari bahan semulajadi dan berkos rendah telah berjaya digunakan sebagai suatu biokatalis yang berpotensi untuk sintesis FASEs. Lebih-lebih lagi, lipase tersekatgerak berupaya meningkatkan produktiviti dan kestabilan, seterusnya membuktikan bahawa penyediaannya adalah lebih fleksibel dan sesuai untuk penghasilan FASEs sebagai biosurfaktan yang berpotensi dalam aplikasi industri.

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I certify that a Thesis Examination Committee has met on 24 June 2011 to conduct the final examination of Uswatun Hasanah bt. Zaidan on her thesis entitled "Adsorption immobilization of *Candida rugosa* lipase onto aluminosilicate supports as biocatalysts for fatty acid sugar ester syntheses" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

Members of the Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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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.

USWATUN HASANAH BT. ZAIDAN

Date: 24 June 2011

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