EVALUATION OF THIN FILMS PREPARED FROM CARBOXYMETHYL CELLULOSE (CMC) AND POLYVINYL ALCOHOL (PVA) ENTRAPPED WITH *CANDIDA RUGOSA* LIPASE FOR ENHANCED BIOCATALYTIC EFFICACY

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AUTHOR DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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ABSTRAK

PEMERANGKAPAN LIPASE *CANDIDA RUGOSA* DI DALAM FILEM NIPIS KARBOKSIMETIL SELULOSA (CMC) DAN ALKOHOL POLIVINIL (PVA) BAGI MENINGKATKAN KEBERKESANAN BIOPEMANGKIN

Lipase *Candida rugosa* (CRL) yang dipegunkan ke dalam karboksimetil selulosa (CMC) dan/atau alkohol polivinil (PVA) dalam bentuk filem nipis melalui proses pemerangkapan telah dikaji. Pencirian filem-filem nipis yang telah dibuat menggunakan teknik-teknik Mikroskop Pengimbas Elektron (SEM), Sebaran Tenaga X-Ray (EDX), Pembelauan X-ray (XRD) dan Inframerah Transformasi Fourier (FTIR) menunjukkan pemerangkapan biopemangkin ke dalam telah berlaku di dalam filem nipis itu. Pencirian pemegunan CRL di dalam filem nipis polimer dengan menggunakan SEM menunjukkan beberapa jisim berbentuk gelembung-gelembung membuktikan kewujudan CRL di atas permukaan penyokong - penyokong. Analisis terhadap komposisi elemen di dalam semua filem nipis menunjukkan bahawa karbon (C), oksigen (O), dan natrium (Na) merupakan elemen utama seperti yang disebutkan di atas. Dalam analisis menggunakan XRD, kesemua filem nipis menunjukkan "d-spacings" yang hampir sama di antara 4.14418 hingga 4.65414 Å dan nilai 2θ di antara 19.054^{o} hingga 21.424^{o}. Puncak yang sama juga dikesan untuk semua filem nipis di dalam analisis menggunakan FTIR spectrometer. Biopemangkin baru yang disediakan dalam filem nipis kemudiannya diuji keupayaannya dalam memangkinkan pengesteran butil oleate, dalam tindakbalas yang mengandungi butanol dan asid oleik . Filem nipis CMC-CRL, PVA-CRL dan PVA:CMC-CRL, menunjukkan 2-3 ganda lebih tinggi menghasilkan ester butil oleate berbanding 9% ester yang dihasilkan menggunakan CRL dalam bentuk yang bebas. Kesan daripada kestabilan suhu penyimpanan (-20^{0}C, 0^{0}C, 4^{0}C dan suhu bilik) juga telah dinilai, dan peratusan penukaran kepada ester yang paling tinggi (28.9%) telah diperolehi oleh PVA-CRL pada suhu -20^{0}C berbanding CRL pegun yang lain dan tiada CRL. Dalam kajian kestabilan operasi, dapat diperhatikan bahawa CMC-CRL, PVA-CRL dan PVA:CMC-CRL menunjukkan 100% hingga 50% peratusan relatif ester dan ianya mampu untuk mengekalkan aktiviti pemangkinan yang tinggi walaupun selepas 10 kitaran

penggunaan secara berulang. Dalam kajian kinetik native dan terpegun CRL, pemegunan CRL menunjukkan kadar tindakbalas yang tinggi dibandingkan dengan CRL yang bebas. Terdapat 2 jenis keadaan untuk mengkaji faktor kinetik iaitu pada keadaan kepekatan asid oleik yang sama tetapi kepekatan butanol yang berbeza dan sebaliknya. Berdasarkan pemerhatian, filem nipis PVA:CMC –CRL menunjukkan kadar tindak balas yang paling tinggi berbanding CRL yang bebas . Lipase –lipase yang pegun seperti CMC-CRL, PVA-CRL dan PVA:CMC-CRL juga menunjukkan nilai $V_{max(Ol)}$ yang tinggi berbanding dengan Native-CRL dalam tindak balas yang mengandungi kepekatan asid oleik yang berbeza. Kajian terhadap kinetik menemukan bahawa perencatan kompetitif satu substrat (CMC-CRL, PVA-CRL dan PVA:CMC-CRL) dan dua substrat (native-CRL) diterangkan dengan jelas oleh mekanisma model Ping Pong Bi Bi. Keputusan kadar tindakbalas menunjukkan bahawa bahan-bahan polimer yang telah digunakan dalam kajian ini adalah penyokong yang berpotensi dalam mengekalkan kestabilan dan kadar aktiviti CRL yang tinggi untuk diaplikasikan dalam sintesis organik.

ABSTRACT

ENTRAPMENT OF *CANDIDA RUGOSA* LIPASE WITHIN CARBOXYMETHYL CELLULOSE (CMC) AND POLYVINYL ALCOHOL (PVA) THIN FILM FOR ENHANCED BIOCATALYTIC EFFICACY

Candida rugosa lipase (CRL) immobilized into the carboxymethyl cellulose (CMC) and polyvinyl alcohol (PVA) thin films via the entrapment method was studied. Characterization of the thin films which was done using Scanning electron microscope (SEM) coupled with Electron dispersive X-ray (EDX) elemental analyzer, X-ray diffractometer (XRD) and Fourier transform infrared (FTIR) spectrometer showed that entrapment of the biocatalyst had occurred within the thin films. Characterization of the immobilized CRL within the polymeric thin films using SEM showed a mass of globular patterns which confirmed the existence of CRL on the surfaces of the supports. The analysis on the elemental compositions of all thin films showed that carbon (C), oxygen (O) and sodium (Na) were major elements in the thin films, while thin films containing CRL showed the existence of potassium (K) in addition to the major elements mentioned above. In the analysis using XRD, all of thin films exhibited almost similar d-spacings of between 4.14418 to 4.65414 Å and 2θ values of between 19.054^{o} to 21.424^{o}. Similar peaks were also detected for all thin films in the analysis using FTIR spectrometer. The newly prepared biocatalyst thin films were then tested for their abilities in catalyzing the esterification of butyl oleate, in reactions which consisted of butanol and oleic acid. Thin films of CMC-CRL, PVA-CRL and PVA:CMC-CRL, showed 2-3 folds higher yields of butyl oleate compared to 9% yield of ester produced without CRL. The effect of storage stability at different temperatures (-20^{o}C, 0^{o}C, 4^{o}C and room temperature) was also evaluated where the highest percent of conversion (28.9%) was obtained using PVA-CRL at storage temperature of -20^{o}C. In the operational stability study, it was observed that the CMC-CRL, PVA-CRL and PVA:CMC-CRL were capable of retaining 50% of their catalytic activities even after 10 cycles of repeated uses. A kinetic study was also done to evaluate the efficacy of the free CRL and immobilized CRL. In the study, all immobilized CRL generally showed higher reaction rates than the free CRL. There were 2 types of conditions whereas at constant concentration of butanol and varying

concentration of oleic acid and vice versa. PVA:CMC-CRL thin film showed the highest reaction rate compared to the free CRL . The immobilised lipases such as CMC-CRL, PVA-CRL and PVA:CMC –CRL have also showed considerably higher $V_{max(Ol)}$ compared to the native one in reactions of different concentration of oleic acid. The study of kinetics findings of one substrate (CMC-CRL, PVA-CRL and PVA:CMC-CRL) and two substrates (native-CRL) competitive were clearly explained by Bi Bi Ping Pong Model mechanism. These results indicated that the polymeric materials used for the entrapment of lipase in this study are potential supports for maintaining stability and activity of CRL for application in organic syntheses.

الملخص

تم دراسة (كانديدا روجوسا ليبياز)(CRL) المجمد في كاربوكسي ميثيل سيليلوز (CMC) و / أو كحول البولي فينيل (PVA) رقيقة الأفلام عبر طريقة الفخ. من العديد من التشكيلات. تم توصيف العينات المحضرة باستخدام المسح المجهر الإلكتروني (SEM) إلى جانب الإلكترون متبدد للأشعة السينية (EDX) المحلل العنصري، الأشعة السينية (XRD)، تحليل الأشعة تحت الحمراء (FTIR) أظهر أن الفخ من المحفز الحيوي قد حدث داخل رقيقة الأفلام.

وصيف عينات CRL داخل بوليمر رقيقة الأفلام باستخدام SEM أظهرت أنماط كروية التي

ت أكدت وجود CRL على سطوح العينات المحضرة. تحليل العناصر لجميع العينات أظهرت أن الكربون (C)، الأوكسجين (O) و الصوديوم (Na) كانت العناصر الرئيسية في العينات ، في حين ان رقيقة الأفلام التي تحتوي على CRL أظهرت وجود البوتاسيوم (k) بالإضافة إلى العناصر الرئيسية المذكورة أعلاه. التحليل باستخدام XRD جميع العينات أظهرت قيم متماثلة للمسافة d بين 144184. 4 إلى 4.6541 ، وقيم 2 مابين 19.054 الى 21.424، قمم مماثلة تم الكشف عنها باستخدام مطياف FTIR.

م اختبار العينات المحضرة كمحفزات في تفاعل استرة البيوتيل أوليات، في التفاعل الذي يتألف ت من بيوتانول و حمض الأوليك. العينات CMC-CRL,- ، PVA-CRL وPVA: CMC-CRL، اظهرت 2-3 أضعاف نتائج أعلى من بوتيل أوليئات مقارنة ب 9٪ امن الاسترالمنتجة باستخدام CRL الغير معدلة. كما تم تقييم تأثير استقرار التخزين عند درجات حرارة مختلفة (-20م ، 4م ، 0م ودرجة حرارة الغرفة) حيث تم الحصول على أعلى نسبة تحويل (28.9٪) باستخدام PVA-CRL عند درجة حرارة تخزين -20م. في دراسة استقرار التشغيل، لوحظ أن CMC-CRL, PVA-CRL و-PVA:CMC CRLكانت قادرة على الاحتفاظ 50٪ من نشاطها الحفزي حتى بعد 10 دورات من الاستخدامات المتكررة تمت الدراسة الحركية أيضا لتقييم فعالية CRL الغير معدلة والعينات المعدلة. في هذه الدراسة، جميع العينات المعدلة عموما أظهرت أعلى معدلات تفاعل من CRL الغير معدل. كانت هناك شرطين في تركيز ثابت من البيوتانول و تركيز متفاوت تركيز من حمض الأوليك والعكس صحيح.PVA:CMC-CRL أظهرت أعلى معدلات

تفاعل بالمقارنة مع CRL الغير معدلة. العينات المعدلة مثل CMC-CRL, -PVA CRL و PVA:CMC- CRL أظهرت(Ol)Vmax اعلى بكثير بالمقارنة مع العينة الغير معدلة في تركيز مختلف من حمض الأوليك . دراسة حركية نتائج واحد من الركائز (CMC-CRL،, PVA:CMC- CRL) واثنين من الركائز (الأصلية–CRL) تم تفسيره بوضوحPVA:CMC- CRL باستخدام آلية بي بي بينغ بونغ النموذجية. وأشارت هذه النتائج التي تم الحصول عليها أن المواد البوليمرية المستخدمة لتعديل CRL في هذه الدراسة هي الدعامات المحتملة للحفاظ على الاستقرار والنشاط CRL لتطبيقها في التخليقات العضوية.

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LIST OF ABBREVIATIONS

CMC	Carboxymethyl Cellulose
PVA	Polyvinyl Alcohol
CRL	*Candida Rugose* Lipase
XRD	X-Ray Diffraction
SEM	Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
T_g	Transition Temperature
^oC	Degree Celcius
V_{max}	Maximum Velocity
Min	Minutes
PVAc	Vinyl Acetate
RNA	Ribonucleic Acid
CALB	*Candida Antartica* Lipase B
PGA	Penicilin G Acylase
MC	Methyl Cellulose
HPMC	Hydroxyl Propyl Methyl Cellulose
HPC	Hydroxyl Propyl Cellulose
K	Michaelis Menten Constant
K_i	Inhibition Contant