

**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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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), Pembelaan X-ray (XRD) dan Inframerah Transformasi Fourier (FTIR) menunjukkan pemerangkapan biopemangkin ke dalam telah berlaku di dalam filem nipis itu. Pencirian pemegungan 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° hingga 21.424° . 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°C , 0°C , 4°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°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 $V_{\max(\text{Ol})}$ 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 2 θ values of between 19.054° to 21.424°. 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°C, 0°C, 4°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°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 $V_{\max(\text{Ol})}$ 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) المجد 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 أسطح 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 4.144184 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 ، و PVA: CMC-CRL، أظهرت 2-3 أضعاف نتائج أعلى من بوتيل أوليات مقارنة ب 9٪ امن الاستر المنتجة باستخدام CRL الغير معدلة. كما تم تقييم تأثير استقرار التخزين عند درجات حرارة مختلفة (-20 م ، 4 م ، 0 م ودرجة حرارة الغرفة) حيث تم الحصول على أعلى نسبة تحويل (28.9٪) باستخدام PVA-CRL عند درجة حرارة تخزين -20 م. في دراسة استقرار التشغيل، لوحظ أن CMC-CRL ، PVA-CRL ، و PVA:CMC-CRL كانت 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 $V_{max}(O_l)$ اعلى بكثير بالمقارنة مع العينة الغير معدلة في تركيز مختلف من حمض الأوليك . دراسة حركية نتائج واحد من الركائز (PVA:CMC- CRL, CMC-CRL) واثنين من الركائز (الأصلية-CRL) تم تفسيره بوضوح PVA: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.

TABLE OF CONTENTS

CONTENTS	PAGE
AUTHOR DECLARATION	ii
BIODATA OF AUTHOR	iii
ACKNOWLEDGEMENTS	iv
ABSTRAK	v
ABSTRACT	vii
MULAKHKHAS	ix
CONTENT PAGE	xi
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF SCHEMES	xviii
LIST OF APPENDICES	xix
LIST OF EQUATIONS	xx
LIST OF ABBREVIATIONS	xxi

CHAPTER 1: INTRODUCTION

1.1	General Background	1
1.2	Research Scope	6
1.3	Research Objectives	6

CHAPTER 2: LITERATURE REVIEW

2.1	Biocatalysis	8
2.2	Enzymes as Biocatalysts	8
2.3	Behaviours of Enzymes	10
2.4	Enzymes Catalysis Reaction and Kinetics Reaction	10
2.4.1	Enzyme Kinetics	11
2.4.2	Kinetic Modelling and Mechanistic Study	11
2.4.2.1	Michaelis Meten Equation	12
2.4.2.2	Transformation of Michaelis Menten Equation to Lineweaver Burk	16
2.4.2.3	Ping Pong Bi Bi Kinetic Model	17
2.5	Lipases	21
2.5.1	<i>Candida rugosa</i> Lipase	21
2.6	Immobilization of Enzymes	22
2.6.1	Methods of Enzyme Immobilization	24
2.6.1.1	Immobilization through Adsorption	26
2.6.1.2	Immobilization through Covalent Binding	27
2.6.1.3	Immobilization through Cross-Linking	28
2.6.1.4	Immobilization through Entrapment	28
2.6.1.5	Immobilization through Encapsulation	29
2.6.2	Supports for Enzyme Immobilization	29
2.6.2.1	Organic Supports	31
2.6.2.2	Inorganic Supports	36

2.6.3	Advantages of Immobilization	37
2.6.4	Properties of immobilized enzymes	38
2.7	Industrial Applications of Lipases	40
2.7.1	Detergents	40
2.7.2	Food industry	41
2.7.3	Pulp and paper industry	42
2.7.4	Biodiesel Production	43
2.7.5	Crude Oil Refining Process	44
2.7.6	Other Applications of Lipases	45
2.8	Enzymatic Synthesis of Ester	47
2.8.1	Butyl Oleate	48

CHAPTER 3: MATERIALS AND METHODS

3.1	Materials	50
3.2	Methods	52
3.2.1	Protein Loading Assay	52
3.2.2	Partial Purification of Lipases	52
3.2.3	Entrapment of Lipase in Polymeric Thin Film	53
3.2.4	Characterization of Polymeric Thin Films	53
3.2.4.1	Analysis using Scanning Electron Microscopy/ Energy Dispersive X-Ray (SEM/EDX)	53
3.2.4.2	Analysis using X-Ray Diffraction (XRD)	54
3.2.4.3	Analysis using Fourier Transform Infrared (FTIR)	54
3.2.5	Lipase Activity Assay	54
3.2.6	Stability of Native and Immobilized Lipases	56
3.2.6.1	Storage Stability	56
3.2.6.2	Reusability (Operational Stability)	56
3.2.7	Kinetic Study of Native and Immobilized Lipases	56

CHAPTER 4: RESULT AND DISCUSSION

4.1	Screening of Enzymes	58
4.2	Characterization using Scanning Electron Microscopy (SEM)	59
4.3	Characterization using Energy Dispersive X-Ray (EDX)	64
4.4	Cross-Sectional SEM of Supports and Immobilized Lipases	69
4.5	Characterization using X-Ray Diffraction (XRD)	74
4.6	Characterization using Fourier Transform Infrared (FTIR)	78
4.7	Stability Assay of Native and Immobilized Lipases	84
4.7.1	Storage Stability	84
4.7.2	Reusability (Operational Stability)	86
4.8	Kinetic Study of Native and Immobilized Lipases	90
4.8.1	Kinetic Modelling of Michaelis Menten Plots	90
4.8.2	Determination of Kinetic Constants (Lineweaver –Burk Plot)	93

CHAPTER 5: CONCLUSION

5.0 Conclusion	99
5.1 Recommendations for Further Studies	101

REFERENCES	103
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APPENDIX A	114
APPENDIX B	115
APPENDIX C	116
APPENDIX D	117
APPENDIX E	118
APPENDIX F	119
APPENDIX G	120



LIST OF TABLES

Tables	Page
Table 4.1: EDX elemental analysis of free CRL of support and immobilized CRL	68
Table 4.2: Basal spacing of thin films before and after immobilization of CRL	76
Table 4.3: Summary of FTIR analysis result	83
Table 4.4: Kinetic constants obtained for the enzymatic esterification of butyl oleate	95

LIST OF FIGURES

Figures	Page
Figure 2.1: Reaction Velocity Plotted Against Substrate Concentration for a Reaction Obeying Michaelis Menten Kinetics	15
Figure 2.2: A Double Reciprocal or Lineweaver Burk Plot	17
Figure 2.3: Ping pong bi-bi kinetic model with two substrates (A and B) and two products (P and Q)	20
Figure 2.4: Enzymes immobilization in porous carriers and the parameters that were related to the support, the enzyme and the mode of enzyme	23
Figure 2.5: Enzyme immobilization methods	26
Figure 2.6: Chemical structure of CMC	34
Figure 2.7: Chemical structure of PVA	35
Figure 4.1: Graph of percentage Conversion of Ester over the types of polymers	59
Figure 4.2: SEM images of: a) CMC thin film, b) CMC-CRL	61
Figure 4.3: SEM images of: a) PVA thin film, b) PVA-CRL	62
Figure 4.4: SEM images of: a) PVA:CMC thin film, b) PVA:CMC-CRL	63
Figure 4.5: EDX image of a) CMC thin film, b) CMC-CRL, c) PVA thin film, c) PVA-CRL, e) PVA:CMC thin film , f) PVA:CMC – CRL	67
Figure 4.6: SEM cross sectional images of a) CMC thin film (1500x magnifications), b) CMC-CRL (2000x magnifications)	71

Figure 4.7:	Images of SEM cross sectional of a) PVA thin film (2000x magnifications), b) PVA-CRL (2000x magnifications)	72
Figure 4.8:	Images of SEM cross sectional of a) PVA:CMC thin film (1500x magnifications) , b) PVA:CMC-CRL (2000x magnifications)	73
Figure 4.9:	XRD spectra of CMC thin film and CMC-CRL.	76
Figure 4.10:	XRD spectra of PVA thin film and PVA-CRL.	77
Figure 4.11:	XRD spectra of PVA:CMC thin film and PVA:CMC-CRL.	77
Figure 4.12:	FTIR Spectrum of CMC thin film and CMC-CRL	80
Figure 4.13:	FTIR Spectrum of PVA thin film and PVA-CRL	81
Figure 4.14:	FTIR Spectrum of PVA:CMC thin film and PVA:CMC-CRL	82
Figure 4.15:	Percentage conversion of ester (%) vs storage condition °C for native CRL and immobilized CRL	84
Figure 4.16:	Relative activity (%) vs storage condition °C for native CRL and immobilized CRL.	85
Figure 4.17:	Relative activities of CMC, PVA and PVA:CMC-immobilized lipases as affected by repeated use in the esterification of butyl oleate in hexane, at 37 °C for 6 hours.	89
Figure 4.18:	Michaelis Menten plots on the reaction rates of butyl oleate synthesis at various concentration of butanol for CRL-CMC, CRL-PVA and CRL-PVA:CMC	92

Figure 4.19:	Michaelis Menten plots on the reaction rates of butyl oleate synthesis at various concentration of butanol for Native CRL, CMC-CRL, PVA-CRL, and PVA:CMC-CRL.	92
Figure 4.20:	Double reciprocal plots of initial rates of butyl oleate synthesis at different Butanol concentration and constant Oleic acid concentration for Native, CMC-CRL, PVA-CRL and PVA:CMC-CRL.	96
Figure 4.21:	Double reciprocal plots of initial rates of butyl oleate synthesis at different Oleic acid concentration and constant Butanol concentration for Native, CMC-CRL, PVA-CRL and PVA:CMC-CRL	96

LIST OF SCHEMES

Scheme	Page
Scheme 2.1: Basic Model of Enzyme-Catalyzed Reaction	13
Scheme 2.2: Ping Pong mechanism with inhibition by both the substrates, A and B	19
Scheme 4.1: Ping Pong Bi Bi mechanism pattern with one substrate (Butanol)	98
Scheme 4.2: Ping Pong Bi Bi mechanism pattern with one substrate (oleic acid).	98
Scheme 4.3: Ping Pong Bi Bi mechanism pattern with two substrates	99

LIST OF APPENDICES

Appendices	Page
Appendix A: The Standard Curve of Absorbance Versus Concentration of Bovine Serum Albumin (BSA)	114
Appendix B: Preparation of Bradford Reagent, Bovine Serum Albumin (BSA) Standard Solution and Distilled Water in Protein Assay Procedure	115
Appendix C: Calculation of Specific Activity	116
Appendix D: Calculation of Relative Activity	117
Appendix E: Calculation The Percentage Conversion of Ester	118
Appendix F: Calculation The Percentage of Immobilization and Protein Loading	119
Appendix G: Calculation of The Reaction Rate	120

LIST OF EQUATIONS

Equations	Page
2.1	19
4.1	93
4.2	93
4.3	93
4.4	94
4.5	97

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LIST OF ABBREVIATIONS

CMC	Carboxymethyl Cellulose
PVA	Polyvinyl Alcohol
CRL	<i>Candida Rugose</i> Lipase
XRD	X-Ray Diffraction
SEM	Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
T _g	Transition Temperature
°C	Degree Celcius
V _{max}	Maximum Velocity
Min	Minutes
PVAc	Vinyl Acetate
RNA	Ribonucleic Acid
CALB	<i>Candida Antartica</i> 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