CANDIDA RUGOSA LIPASE IMOBILIZATION ON MODIFIED MESOPOROUS KIT-6 FROM RICE HUSK AS BIOCATALYST IN ESTERIFICATION FOR BUTYL BUTYRATE

NOORULSYAHIDAINI BINTI GOLBAHA

UNIVERSITI TEKNOLOGI MALAYSIA

CANDIDA RUGOSA LIPASE IMMOBILIZATION ON MODIFIED MESOPOROUS KIT-6 FROM RICE HUSK AS BIOCATALYST IN ESTERIFICATION FOR BUTYL BUTYRATE

NOORULSYAHIDAINI BINTI GOLBAHA

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Chemistry)

Faculty of Science
Universiti Teknologi Malaysia

APRIL 2017

Specially dedicated to;

Myself.

My beloved and supportive supervisors;

Prof. Dr. Salasiah Endud and P.M. Dr. Zainab Ramli, who made all this possible.

My dearest husband;

Hilmi Masrur Ab Halim for his endless love and patience.

My daughter;

Nuha Sofea for being my strength to this long journey.

My parents, family and family in-law;

Golbaha Mat & Radziah Md. Noor ; Ab. Halim Mustapha & Sakinah Ismail

for love and endless support.

My labmates & friends.

And

Thank you ALLAH for all this blessing.

ACKNOWLEDGEMENT

First and foremost, I would like to thanks and acknowledge the lessons, support and guidance of my supervisors, Prof. Dr. Salasiah Endud and P.M. Dr. Zainab Ramli. Their continuous commitments to help me in my research studies ensure not only consistent results but also granted her the respect of all her students. I thank her for giving me the opportunity to carry out this research.

My words of gratitude also go to all the lecturers, laboratory officers and research officers from the Department of Chemistry, Ibnu Sina Institute and University Industry Research Laboratory (UIRL UTM) for Fundamental Science Studies, Universiti Teknologi Malaysia (UTM). I am also thankful to UTM for the Research University Grant and Ministry of Higher Education (MyBrain 15-MyPhd).

My sincere appreciation also extends to my husband, Hilmi Masrur Ab. Halim, parents, Golbaha Mat and Radziah Md. Noor, my brothers and sisters for their support, encouragement, care and love. Besides, I would like to thank to all my labmates, especially Ms. Rosliana Rusli, for her valuable experience, time and opinions throughout my study.

Last but not least, for anyone I may have forgotten, that has involved directly or indirectly in completing this project, thank you.

ABSTRACT

Lipases as are widely exploited in various industries, especially in the food processing industries for their ability to act on a variety of substrates, pH and thermal stability, resistance to solvents, and they are also highly specific as chemo-, regio-, and enantioselective biocatalysts. However, enzymes have serious drawbacks such as, often unstable in the long term operation, high cost and non-reusability. Enzyme immobilization is a creative solution to alleviate these problems, whereas the mesoporous silica KIT-6 has great potential as catalyst support due to the high surface area, porosity and stability. This research aimed to explore the potential of APTES-functionalized KIT-6 silica as immobilization matrix for lipase from *Candida rugosa* (CRL). In particular, the immobilized CRL onto KIT-6 silica nanoparticles derived from rice husk ash, is reported here for the first time as a catalyst in the esterification reaction. Additionally, a comparative study of the effectiveness of immobilization methods (physical adsorption and covalent binding) on the biophysical properties of the immobilized CRL, and the hydrolytic activities of free and immobilized enzymes in the esterification of butyric acid with butanol were carried out. The different techniques used for the biophysical characterization of the immobilized CRL onto KIT-6 were SAXS, TEM, FESEM, FTIR, XPS, fluorescence spectra, and TGA. The results showed that both functionalization and immobilization procedures did not disrupt the structural integrity of the immobilized CRL, but found significant differences in the morphology of each of the resultant catalysts. The KIT-6/a/g/Crl catalyst displayed highly dense and more compact morphology than the KIT-6/Crl with a higher enzyme loading. The findings suggest that some of the attached APTES-glutaraldehyde compounds including CRL molecules may be physically adsorbed inside the pores of the KIT-6/a/g/Crl, which resulted in the obvious reduction in surface area and total pore volume when the CRL loading increased. Further, the structure of immobilized CRL in KIT-6 was verified by FTIR spectra and correlated with the XPS analysis. The values of binding constant, K_{ass}, determined by fluorescence quenching studies, confirmed that the direct physical interaction of KIT-6 with CRL was much weaker (5.20×10^2 M^{-1}), compared to the covalent binding (1.20×10^4 M^{-1}). The optimal assay conditions of the CRL catalyzed esterification were identified to be pH 7.0 at 35 °C with 0.10 mg/mL concentration of the CRL in n-hexane as the solvent. The CRL immobilized onto KIT-6 through physical adsorption and covalent binding method is more advantageous compared to the free CRL, as it could withstand extreme reaction conditions and capable to achieve a high activity. Besides, the conformational changes in the CRL were restricted due to entrapment in the porous silica matrix. Overall, Crl immobilized onto silica KIT-6 demonstrated improved properties compared with the free CRL giving excellent results in the thermal stability, storage and reusability studies. Both the immobilized CRL onto KIT-6 by covalent binding method (KIT-6/a/g/Crl) and physical adsorption (KIT-6/Crl) were able to retain more than 50% of the activity after the heat treatment. Moreover, KIT-6/a/g/Crl retained 67.3% of the activity after 85 days of storage, indicating enhanced thermal stability and shelf-life through the immobilization process. Recycling studies showed that the KIT-6/a/g/Crl, which has less loading amount of CRL (2.57 mg/g support versus 3.10 mg/g support in KIT-6/Crl) was reusable up to 20 cycles, with only 75.3% of its activity lost during the first 10 cycles; as compared to KIT-6/Crl which rendered inactive over 7 cycles. Under an optimized reaction condition, even using low CRL loadings, the specific activity of KIT-6/a/g/Crl was shown higher (2144.7 U/mg) than the KIT-6/Crl (1847.6 U/mg) and free CRL (1604.3 U/mg). Optimal yields of the esterification of butyric acid with butanol in hexane using the KIT-6/Crl and KIT-6/a/g/Crl catalysts were 94% and 90% respectively, with 100% selectivity. The kinetic studies revealed that K_m of immobilized CRL was higher than that of free CRL, implying a high affinity towards the substrate. Also, the V_{max}/K_m (catalytic efficiency) value of KIT-6/a/g/Crl was comparable with the free CRL, whereas the value determined for the KIT-6/Crl was lower compared to the KIT-6/a/g/Crl.

ABSTRAK

Lipase dieksploitasikan secara meluas dalam pelbagai industri, terutamanya industri pemprosesan makanan kerana kemampuannya bertindak balas dengan pelbagai substrat, kestabilan pH dan haba, ketahanan terhadap pelarut, dan ia juga sangat spesifik sebagai biomangkin kemo-, regio- dan enantioselektif. Walau bagaimanapun, enzim mempunyai kelemahan yang serius antaranya sering tidak stabil dalam operasi jangka panjang, sangat mahal dan tidak boleh diguna semula. Pemegunan enzim merupakan satu penyelesaian kreatif bagi mengatasi kelemahan tersebut, sedangkan silika mesoliang KIT-6 sangat berpotensi sebagai penyokong mangkin kerana mempunyai luas permukaan, keliangan dan kestabilan yang tinggi. Penyelidikan ini bertujuan meneroka potensi KIT-6 silika berfungsikan-APTES sebagai matriks untuk pemegunan lipase dari *Candida rugosa* (CRL). Khususnya, pemegunan CRL ke atas nanopartikel silika KIT-6 yang diperoleh daripada abu sekam padi, pertama kali dilaporkan di sini sebagai mangkin dalam tindak balas esterifikasi. Tambahan lagi, kajian perbandingan kesan kaedah pemegunan (penjerapan fizikal dan penambatan kovalen) ke atas sifat biofizik CRL, dan aktiviti hidrolisis untuk enzim bebas dan enzim terpegun dalam pengesteran asid butirik dengan butanol telah dijalankan. Teknik berlainan yang digunakan untuk pencirian biofizik CRL terpegun di atas KIT-6 ialah SAXS, TEM, FESEM, FTIR, XPS, spektrum pendafluor, dan TGA. Keputusan kajian menunjukkan bahawa prosedur pemfungsian dan pemegunan tidak menjejaskan integriti struktur CRL terpegun, tetapi terdapat perbezaan morfologi yang ketara bagi setiap mangkin yang dihasilkan. Mangkin KIT-6/a/g/Crl menunjukkan morfologi yang sangat tumpat dan lebih padat berbanding dengan KIT-6/Crl dengan muatan enzim yang lebih tinggi. Keputusan ini mencadangkan sebahagian daripada sebatian APTES-glutaraldehid yang terlekat termasuk molekul CRL, mungkin terjerap di dalam liang KIT-6/a/g/Crl, menyebabkan penurunan luas permukaan dan jumlah isipadu liang KIT-6 yang ketara apabila muatan CRL meningkat. Selanjutnya, struktur CRL terpegun dalam KIT-6 telah dibuktikan oleh spektrum FTIR dan dikorelasikan dengan analisis XPS. Nilai pemalar penambatan, K_{ass}, ditentukan melalui kajian pelindapan pendarfluor, mengesahkan bahawa interaksi fizikal langsung antara KIT-6 dan CRL adalah lebih lemah (5.20×10^2 M^{-1}), berbanding dengan penambatan kovalen (1.20×10^4 M^{-1}). Keadaan optimum assai bagi esterifikasi asid butirik bermangkinkan CRL dikenalpasti sebagai pH 7.0 pada 35 °C dengan kepekatan CRL 0.10 mg/mL dan n-heksana sebagai pelarut. CRL terpegun pada KIT-6 melalui penjerapan fizikal dan kaedah penambatan kovalen adalah lebih menguntungkan berbanding dengan CRL bebas, kerana mampu bertahan dalam keadaan tindak balas yang ekstrim dan berupaya mencapai aktiviti yang tinggi. Lagipun, perubahan konformasi CRL adalah terbatas kerana pemerangkapan dalam matriks silika berliang. Pada keseluruhannya, CRL terpegun ke dalam silika KIT-6 menunjukkan ciri yang lebih baik daripada CRL bebas dengan memberikan keputusan yang cemerlang dalam kajian kestabilan terma, penyimpanan dan kebolehgunaan semula. Kedua-dua CRL terpegun ke atas KIT-6 dengan kaedah penambatan kovalen (KIT-6/a/g/Crl) dan penjerapan fizikal (KIT-6/Crl) mampu mengekalkan lebih 50% aktiviti selepas perawatan terma. Manakala, KIT-6/a/g/Crl mengekalkan 67.3% aktiviti selepas 85 hari penyimpanan, yang menunjukkan peningkatan kestabilan terma dan hayat simpanan melalui proses pemegunan. Kajian kitar semula menunjukkan KIT-6/a/g/Cr yang mempunyai muatan CRL rendah (2.57 mg/g penyokong melawan 3.10 mg/g penyokong untuk KIT-6/Crl), boleh diguna semula sehingga 20 kitaran dengan kehilangan aktiviti sebanyak 75.3% sepanjang 10 kitaran pertama; berbanding dengan KIT-6/Crl yang menjadi tidak aktif selepas 7 kitaran. Di bawah keadaan optimum, walaupun menggunakan muatan CRL yang rendah, aktiviti spesifik KIT-6/a/g/Crl didapati lebih tinggi (2144.7 U/mg) berbanding dengan KIT-6/Crl (1847.6 U/mg) dan CRL bebas (U/mg 1604.3). Hasil optimum esterifikasi asid butirik dengan butanol dalam n-heksana menggunakan mangkin KIT-6/Crl dan KIT-6/a/g/Crl ialah 94% dan 90% masing-masing, dengan selektiviti 100%. Kajian kinetik mendedahkan bahawa K_m untuk CRL terpegun adalah lebih tinggi daripada CRL bebas, mencadangkan afiniti yang kuat terhadap substrat. Juga, nilai V_{max}/K_m (kecekapan pemangkinan) untuk KIT-6/a/g/Crl adalah sebanding dengan CRL bebas, manakala nilai yang ditentukan untuk KIT-6/Crl adalah lebih rendah berbanding dengan KIT-6/a/g/Crl.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	i
	DEDICATION	iii
	ACKNOWLEDGEMENT	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	xi
	LIST OF FIGURES	xii
	LIST OF ABBREVIATIONS	xv
	LIST OF SYMBOLS	xviii
	LIST OF APPENDICES	xxi
1	**INTRODUCTION**	**1**
	1.1 Background of Study	1
	1.2 Statement of Problems	8
	1.3 Objectives of Study	9
	1.4 Scope of Study	9
	1.5 Significant of Study	10
	1.6 Outline of Study	11
2	**LITERATURE REVIEW**	**12**
	2.1 Rice Husk Ash (RHA)	12

2.2 Mesoporous Silica Materials 13

2.2.1 Mesostructure Formation of Silica 15

2.2.2 KIT-6 Mesoporous Silica 18

2.3 Mesoporous Silica as Support 19

2.4 Functionalization of Mesoporous Silica with Organosilanes 20

2.5 Enzymes as Biocatalyst 21

2.5.1 Advantages of Enzyme as Catalyst 24

2.5.2 *Candida Rugosa* Lipase (CRL) 25

2.6 Optimization of Enzyme 28

2.7 Enzyme-Catalyzed Esterification 31

2.8 Immobilization of Enzyme 32

2.8.1 Advantages of Immobilization 33

2.8.2 Properties of Immobilized Enzyme 34

2.8.3 Immobilization Methods 35

2.8.3.1 Immobilization by Physical Adsorption 37

2.8.3.2 Immobilization by Covalent Binding 39

2.8.4 Enzyme Immobilization on Mesoporous Silica 40

2.8.5 Development of Silica as Support for Immobilization of Various Enzymes 41

2.9 Industrial Applications of Lipase 48

3 RESEARCH METHODOLOGY 51

3.1 Chemicals and Materials 51

3.2 Preliminary Study 51

3.3 Synthesis Procedure 52

3.3.1 Synthesis of KIT-6 Silica Material as Support 52

3.3.2 Functionalization of KIT-6 Silica 53

3.3.3 CRL Immobilization 54

3.4 Protein Assay 55

3.4.1 Preparation of BSA Standard Solution 57
3.5 Leaching Analysis 57
3.6 Esterification Reaction 57
3.6.1 CRL-Catalyzed Esterification Reaction 58
3.6.2 Identification of Reaction Product 59
3.7 Optimization Study of Esterification Reaction 59
3.7.1 Effect of pH 60
3.7.2 Effect of Temperature 60
3.7.3 Effect of CRL Concentration 61
3.7.4 Effect of Solvent 61
3.8 Stability Assessment 61
3.8.1 Thermal Stability 61
3.8.2 Storage Stability 62
3.8.3 Reusability 62
3.9 Kinetic Study 63
3.10 Characterization Method 63
3.10.1 Small Angle X-Ray Scattering (SAXS) 64
3.10.2 UV-Visible Spectroscopy (UV-Vis) 65
3.10.3 Transmission Electron Microscopy (TEM) 65
3.10.4 Field Emission Scanning Electron Microscopy (FESEM) 66
3.10.5 Fourier Transform Infrared spectroscopy (FTIR) 67
3.10.6 Nitrogen Adsorption Desorption Analysis 68
3.10.7 X-Ray Photoelectron Spectroscopy (XPS) 68
3.10.8 Fluorescence spectroscopy 69
3.10.9 Thermal Gravimetric Analysis (TGA) 70
3.10.10 Gas Chromatography (GC) 70

4	**RESULTS AND DISCUSSION**	**72**
	4.1 Preliminary Study	72
	4.2 Characterizations	73
	4.3 Optimization Study	86
	4.3.1 Effect of pH	86
	4.3.2 Effect of Temperature	87
	4.3.3 Effect of CRL Concentration	90
	4.3.4 Effect of Solvent	91
	4.3.5 Optimization Study Summary	93
	4.4 Immobilization Properties and Activity	94
	4.4.1 CRL immobilization	94
	4.4.2 CRL Attachment on KIT-6	96
	4.5 Stability Assessment	107
	4.5.1 Thermal Stability	107
	4.5.2 Storage Stability	110
	4.5.3 Reusability	111
	4.6 Kinetic Study	113
5	**CONCLUSION**	**117**
	5.1 Research Conclusions	117
	5.2 Recommendations	120
REFERENCES		**121**
Appendices A – E		159-177

LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	Differences between enzyme and chemical catalyst	23
2.2	Comparison of optimization properties of various sources of lipase	30
2.3	Development of silica as support for immobilization of various enzymes	42
2.4	Industrial applications of lipase	48
3.1	Notations for the catalysts used in this study	55
4.1	Report on CRL loading and yield of esterification catalyst by Crl on different support in preliminary study.	73
4.2	*d*-spacing and unit cell parameter of catalysts	76
4.3	Average particle size of the samples	78
4.4	Physical properties of catalysts	83
4.5	Summary of Optimization Study	94
4.6	CRL immobilized on KIT-6 silica and its properties in esterification and hydrolysis of olive oil	96
4.7	Area ratio of amide group band (CONH) and (Si–O–Si)	99
4.8	Binding constant and Stern–Volmer constant of KIT-6/Crl and KIT-6/a/g/Crl	103
4.9	TGA data of all the samples	106
4.10	Effect of storage duration on the remained activity (%) of free CRL, KIT-6/Crl and KIT-6/a/g/Crl.	111
4.11	Kinetic parameters of free and immobilized CRL	116

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
1.1	Outline of the study	11
2.1	Possible pathways for the liquid crystal templating mechanism	16
2.2	Scheme structures for bicontinuous cubic phases (a) G-surface and the Ia3d network, (b) D-surface and the Pn3m network and (c) P-surface and the Im3m network	17
2.3	(a) KIT-6 with 3D-cubic bicontinuous structure and, (b) KIT-6 structure with two sets of pore in 2-dimensional structure.	18
2.4	Diagrammatic representation of a lipase molecule showing its main features.	22
2.5	The key and lock theory	24
2.6	Induced fit model	24
2.7	(a) X-Ray Diffraction biological molecule of CRL by Protein Data Bank (PDB-PDB id:1CRL) and (b) topology diagram of CRL. The lid forming α-helix of Crl is labelled A-1 by Lipase Engineering Database (LED), 2009	26
2.8	Enzyme immobilization methods	36
2.9	Irreversible methods in enzyme immobilization	37
2.10	Reversible methods in enzyme immobilization	37
2.11	Schematic diagram of adsorption of enzyme on silica surface	38
2.12	Schematic diagram of covalent binding of enzyme on silica surface	39
3.1	Schematic representation of CRL immobilization on to KIT-6	55
3.2	Esterification reaction of butyric acid and butanol	58
4.1	SAXS and scattering two-dimensional patterns (shown in the insect) for (a) KIT-6, (b) KIT-6/Crl, (c) KIT-6/a/g and, (d) KIT-6/a/g/Crl.	75

4.2	TEM image of (a) KIT-6 silica with gyroid structure, (b) KIT-6/Crl and (c) KIT-6/a/g/Crl	76
4.3	FESEM of (a) KIT-6, (b) Free CRL, (c) KIT-6/Crl and (d) KIT-6/a/g/Crl	78
4.4	FTIR spectra of the samples; (a) free CRL, (b) KIT-6, (c) KIT-6/Crl and (d) KIT-6/a/g/Crl.	80
4.5	Suggested amino group-lysine attached on KIT-6 by physical and covalent binding	81
4.6	Schematic diagram of adsorption for the interaction of proteins (positive charge) with the silica surface (negative charge).	82
4.7	Nitrogen adsorption isotherm of (a) KIT-6, (b) KIT-6/Crl, (c) KIT-6/a/g and, (d) KIT-6/a/g/Crl.	85
4.8	Pore size distribution curve of (a) KIT-6, (b) KIT-6/Crl, (c) KIT-6/a/g and, (d) KIT-6/a/g/Crl.	85
4.9	Effect of pH on activity of free CRL, KIT-6/Crl and KIT-6/a/g/Crl	87
4.10	Effect of temperature on activity of free CRL, KIT-6/Crl and KIT-6/a/g/Crl.	89
4.11	Effect of equilibrium concentration of CRL on activity of free CRL, KIT-6/Crl and KIT-6/a/g/Crl	91
4.12	Effect of solvent polarity on activity	92
4.13	Percentage leaching profile of KIT-6/Crl and KIT-6/a/g/Crl	98
4.14	Structure of SBA-15 and KIT-6 showing the molecule in and out from the pore of the structure	98
4.15	Regional FTIR spectra of KIT-6/a/g/Crl with different percentage CRL loading (a) 4 %, (b) 8 %, (c) 12 %, (d) 16 % and, (e) 20 %	100
4.16	XPS of (a) C1s (A-KIT-6/Crl ; B-KIT-6/a/g ; C- KIT-6/a/g/Crl) and (b) O1s (A- KIT-6 ; B- Kit-6/Crl ; C- KIT-6/a/g ; D- KIT-6/a/g/Crl)	100
4.17	Fluorescence spectra of (a)KIT-6, (b) KIT-6/a/g/Crl, (c) KIT-6/Crl and (d) free CRL.	102
4.18	Tryptophan (Trp) amino acid.	102
4.19	Fluorescence spectra of (a) KIT-6/Crl and (b) KIT-6/a/g/Crl at 429 nm with different CRL loading.	103
4.20	Stern-Volmer plot in determining the quenching of the samples	105

4.21	TGA curve of KIT-6, KIT-6/a/g, KIT-6/Crl and KIT-6/a/g/Crl.	107
4.22	Thermal stability of the free CRL, KIT-6/Crl and KIT-6/a/g/Crl (50 °C- dash line; 60 °C-straight line)	109
4.23	Reusability of immobilized CRL on KIT-6 silica	113

LIST OF ABBREVIATIONS

MSU-H	-	Hexagonally Ordered Mesostructured Silicas
SBA-15	-	Santa Barbara Amorphous no-15
MCM-41	-	Mobil Composition Mater no-41
DNA	-	Deoxyribonucleic acid
KIT-6	-	Korean Institute and Technology no.6
KIT-5	-	Korean Institute and Technology no.5
CRL	-	*Candida rugosa* lipase
APTES	-	(3-Aminopropyl) triethoxysilane
SAXS	-	Small Angle X-Ray Scattering
FTIR	-	Fourier Transform Infrared
XPS	-	X-Ray Photoelectron Spectroscopy
TGA	-	Thermal Gravimetric Analysis
FESEM	-	Field Emission Scanning Electron Microscopy
TEM	-	Transmission Electron Microscopy
Uv-Vis	-	Ultraviolet Visible
BSA	-	Bovine Serum Albumin
GC-FID	-	Gas Chromatography – Flame Ionization Detector
GC-MS	-	Gas Chromatography – Mass Spectroscopy
THF	-	Tetrahydrofuran
MSU	-	Michigan State University
FDU	-	Fudan University
HOM	-	Highly Ordered Mesoporous Silica
AMS	-	Anionic-surfactant-templated mesoporous silica
MCM-48	-	Mobil Composition Mater no-48

OMM	-	Ordered Mesoporous Materials
CTMS	-	Chlorotrimethylsilane
MPTMS	-	(3-Mercaptopropyl) triethoxysilane
APTMS	-	(3-Aminopropyl) trimethoxysilane
RNA	-	Ribonucleic acid
PGA	-	Penicillin G Acylase
CLEAs	-	Cross-Linked Enzyme Aggregates
MCFs	-	Mesocellular foams
PMOs	-	Periodic Mesoporous Organosilicas
GOD	-	Glucose Oxidase
HRP	-	Horseradish Peroxidase
PUFA	-	Polyunsaturated fatty acid
RHA	-	Rice husk ash
RT	-	Room temperature
HOMO	-	Highest occupied molecular orbital
LUMO	-	Lowest unoccupied molecular orbital
BET	-	Brunauer-Emmett-Teller
BJH	-	Barrett-Joyner-Halenda
PI	-	Polarity index
RSM	-	Response Surface Methods
STP	-	Standard Temperature and Pressure
BuOH	-	Butanol
sp.	-	Species
IY	-	Immobilization Yield
A	-	Angstrom
a.u	-	Arbitary unit
eV	-	Electron volt
kDa	-	Kilo Dalton

LIST OF SYMBOLS

Fe_2O_3	-	Ferum (III) Oxide
SiO_2	-	Silica
P123	-	Triblock copolymers based on poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol)
CONH	-	Amide/Peptide linkage
Si–O–Si	-	Siloxane
Si–OH	-	Silanol
O–C	-	Carbon-oxygen bond
E_a	-	Activation energy
U	-	Units (µmol substrate converted per min)
K_m	-	Michaelis- Menten constant
V_{max}	-	Maximum velocity
v	-	Velocity
k_{cat}	-	Turnover number/ catalytic constant
V_{max}/K_m	-	Catalytic efficiency
k_{cat}/K_m	-	Specificity constant
a_o	-	Unit cell parameter
d_{211}	-	*d*-spacing at (2 1 1) lattice
A_{SE}	-	Specific activity
A_E	-	Enzyme activity/ Esterification activity
A_{CONH}	-	Area of CONH
$A_{Si–O–Si}$	-	Area of Si–O–Si
P_s	-	Amount of protein adsorbed on the support
V_p	-	Pores Volume

D_p	-	Diameter pores
K_{ass}	-	Binding association constant
K_{sv}	-	Stern-Volmer constant
F_o	-	Initial fluorescence intensity
F	-	Final fluorescence intensity
[R]	-	Equilibrium concentration
[S]	-	Concentration of substrate
[SC]	-	Concentration of Specific compound of interest
η	-	Catalytic coefficient
θ	-	Theta
λ	-	Wavelength

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
A1	Silica's extraction procedure from RHA	160
A2	Calibration curve of BSA standard solution at pH 7 recorded at 595 nm using UV-Vis wavelength program method.	161
A3	Internal standard calibration curve	162
B	Formula involve in this study.	163
C1	FTIR spectra of butyl butyrate (formation of ester bond at 1715.27 cm^{-1})	165
C2	Gas Chromatography spectra of butyl butyrate catalyzed by Free CRL, KIT-6/Crl and KIT-6/a/g/Crl	166
C3	Gas Chromatography Mass Spectroscopy (GC-MS) result for product identification	168
D1	Solvent's polarity chart	170
D2	Si 2p X-ray photoelectron spectroscopy (XPS) spectra of (a)KIT-6, (b)KIT-6/Crl, (c) KIT-6/a/g, and (d) KIT-6/a/g/Crl	171
D3	O 1s XPS spectra of (a)KIT-6, (b)KIT-6/Crl, (c) KIT-6/a/g, and (d) KIT-6/a/g/Crl.	172
D4	C 1s XPS spectra of (a)KIT-6/Crl, (b) KIT-6/a/g, and (c) KIT-6/a/g/Crl.	173
D5	N 1s XPS spectra of (a)KIT-6/Crl, (b) KIT-6/a/g, and (c) KIT-6/a/g/Crl.	174
D6	DSC curve and data of (a) as-synthesized KIT-6, (b) KIT-6, (c) KIT-6/Crl and (d) KIT-6/a/g/Crl	175
E	List of publication/proceeding	176

CHAPTER 1

INTRODUCTION

1.1 Background of Study

It is well known that most isolated enzymes are only stable under its physiological conditions. Therefore, to enhance their stability under severe conditions without altering their structure has become a fundamental question in the enzymatic research. In order to enhance their activity and stability, immobilization of enzymes on different types of supports such as organic polymers (Kallenberg *et al.*, 2005; Katchalski-Katzir and Kraemer, 2000), chitosan (Gilani *et al*., 2016; David *et al.*, 2015), silica gels (Boros *et al.*, 2013), zeolitic (Chang and Chu, 2007; Marthala *et al.*, 2015; Celikbicak *et al.*, 2014) and mesoporous materials (Bautista *et al.*, 2015; Zou *et al.*, 2014) have been investigated. In recent years, nanostructured materials have been widely used as supports for enzyme immobilization owing to their high surface area that can effectively improve the enzyme loading, structural stability and catalytic efficiency of the immobilized enzyme.

In this work, the nanostructured mesoporous materials were synthesized using rice husk ash (RHA) as a silicon source. Rice husk, an agricultural waste, is a major by-product of the rice-processing industries and like most of the other rural biomass materials, for example; sugar, cane leaf and corn leaf are recognized as a potential source for energy generation from gasification or incineration processes (Islam and Mondal, 2013). The burning of rice husk in air results in the formation of RHA with a content in silica (SiO_2) that varies from 85% to 98%, depending on the burning conditions, the furnace type, the rice variety, the rice husk moisture content,

the climate and the geographic area (Lanning, 1963). RHA can be used as an alternative cheap source of amorphous silica for the production of value-added porous silica materials with industrial and technological interests (Prasad and Pandey, 2012; Ziegler *et al.*, 2016; Salama *et al.*, 2016; Cheah *et al.*, 2016). Among the various utilizations of RHA, there is a significant interest in its use in the preparation of silica materials due to the widespread industrial use of silica in separation processes as sorbents (Budnyak *et al.*, 2015; Ebrahimi-Gatkash *et al.*, 2015; Gibson, 2014; Menezes *et al.*, 2012; Kyzas *et al.*, 2013) as well as in catalytic refinery and petrochemical processes (Dubreuil *et al.*, 2017; Vermeiren and Gilson, 2009).

Among different nanostructured materials, mesoporous silica has increasingly proven to be an extremely effective solid support for the immobilization (Singh *et al.*, 2013; Mureseanu *et al.*, 2016) of a wide range of enzymes because a large specific surface area and uniform pore size (Huang *et al.*, 2013). The well-defined pores having a narrow diameter distribution, high surface area and silanol functionalization (Al-Othman *et al.*, 2012; Hoffmann *et al.*, 2006) which can be used as anchoring sites for further organic functionalization (Zhao *et al.*, 1997; Zhao *et al.*, 1998). These features are the key features that allow for the adsorption of molecules through hydrogen bonding and or electrostatic interaction (Hartmann and Kostrov, 2013). The pore diameters of mesoporous materials are important to improving the efficiency of immobilization of different enzymes, as they should be large enough to facilitate the process of mass transfer throughout the pore channels (Misson *et al.*, 2015; Wang *et al.*, 2001; Sheldon and van Pelt, 2013). These materials possess highly ordered pores which can be adjusted between 2 and 50 nm in diameters, allowing them to be employed as hosts to relatively large guest molecules.

Moreover, organic modification of mesoporous silica has been revealed as a key strategy to modulate molecule adsorption and delivery rates (Collila *et al.*, 2008; Al-Qadi *et al.*, 2012). Mesoporous silica materials also have particularly interesting properties for food and pharmaceutical applications, such as safety, hydrophobicity/hydrophilicity of the surface, electrostatic interactions, and

mechanical, chemical and bacterial resistance (Warmuth *et al*., 1995; Pereira *et al*., 2003; Brady *et al*., 1988; Padmini *et al*., 1993; Jeison *et al*., 2003).

Despite the promising properties of the mesoporous silica materials for use as adsorbent for biomolecules (Chaikittisilp *et al.*, 2011), only limited work in this area has been reported. The size of molecules that could be incorporated and loaded inside the pores of mesoporous materials such as MCM-41 and SBA-15 are restricted by the pore sizes of 2-3 nm and ~6 nm, respectively. Consequently, large and bulky molecules such as protein, enzyme and DNA are not able to access to these the pores, thus limiting their application in the field of enzyme immobilization.

More interestingly, mesoporous solids are those structures with three-dimensional (3D) cubic *Ia3d* symmetry, which consist of two interpenetrating continuous networks of chiral channels. These unique 3D channel networks provide highly open porous hosts with easy access for guest species and more resistance to pore blocking, thus facilitating the mass transfer through the pore channels (Kim *et al.*, 2005; Gobin *et al.*, 2007; Fan *et al.*, 2003; Lawrence *et al.*, 2015). However, a simple synthesis using environmental waste (rice husk ash) of such mesoporous solids having cubic *Ia3d* symmetry was remained challenging, limiting their widespread application.

The silica KIT-6 (Korean Institute of Technology-6) synthesized from RHA is composed of two interwoven mesoporous networks similar to that found in MCM-48 silica, but has much larger pore diameters ranging from 8 to 20 nm (Jo *et al.*, 2009). This material has a potential that could be used for the immobilization of enzyme owing to the high surface area, minimum diffusion limitation, and high mass transfer (Kresge *et al*., 1992; Kim *et al.*, 2005). Compared to the MCM-type mesoporous materials, the larger pore sizes of KIT-6 ranging from 8 to 20 nm (Jo *et al.*, 2009), could accommodate a wide variety of biomolecules (Lowe and Baker, 2014; Falahati *et al.*, 2012; Tran and Balkus, 2011; Gustaffson *et al.*, 2012; Guo *et al.*, 2010). The high-quality samples of cubic *Ia3d* KIT-6 materials are obtained in the presence of n-butanol as a co-surfactant at a reduced HCl concentration relative to conventional SBA-15-type mesostructures.

Meanwhile, several silicate materials have also been studied as the supports for biomolecules such as immobilized enzyme on MSU-H (Yu *et al.*, 2013), cation-exchanged functional silica (Zheng *et al.*, 2012), mica (Zaidan *et al.*, 2012) and SBA-15 (Salis *et al.*, 2010; Yang *et al.*, 2013). The biocompatibility, non-toxicity, chemical inertness, and high stability and mechanical strength of these materials make them suitable to be developed as supports for the biomolecules. However, to the best of our knowledge only a few reports are available on the immobilization of biomolecules on KIT-6, and include the following: immobilization of Penicillin G acylase (PGA) on KIT-6 and SBA-15 with different pore sizes (Lu *et al.*, 2008), immobilization of lysozyme on the large-pore KIT-6 (Vinu *et al.*, 2008), and immobilization of lipase in ordered mesoporous materials (focusing on effects of textural and structural parameters) (Serra *et al.*, 2008).

Furthermore, in these immobilization studies, the weakness of interaction between the mesoporous silica surface and the enzyme is revealed by the enzyme being leached off during the washing process. This serious drawback can be overcome by surface modification of the solid support (Takahashi *et al.*, 2000). Both organic (mainly polysaccharides, polyacrylic and polyvinylic materials) and inorganic supports (mainly silica- or other metal-oxide-based) have been described as efficient solid supports for enzyme immobilization (Magner, 2013; Sheldon, 2007; Hudson *et al.*, 2008; Ispas *et al.*, 2009). In particular, the latter are the materials of choice in this field and available with a wide range of porosities and costs. It is possible to chemically modify their surface enabling numerous immobilization techniques. Inorganic supports also present excellent thermal, mechanical and microbial resistance (Magner, 2013).

In comparison with free enzymes which typically work under mild conditions, immobilized enzymes are substantially robust, environmentally-friendly, and often have improved functional properties such as specific activity, retention of the biochemical properties and catalytic activity under appropriate reaction conditions of temperature, pH and organic solvent, allowing for multiple reuse of the enzyme and continuous reaction processes (Wang *et al.*, 2012; Seema and Steven, 2002; Xie and Wang, 2012; Won *et al.*, 2005). In addition, enzyme immobilized into

a support matrix could avoid contamination of the desired product with the enzyme, inhibit the denaturation of the enzyme in organic solvents, and expose active sites of the enzyme for a more efficient bonding with the support (Seema and Steven, 2002; Belhaj *et al.*, 2011; Foresti and Ferreira, 2007; Cabral *et al.*, 2010).

Even though many investigators have reported on immobilized enzyme in the literatures, detailed optimization studies for enzyme immobilization using various methods have been lacking. The optimization of enzymes for particular reactions is necessary as it can significantly reduce the production costs of enzymes used in the industrial processes. Currently, most common methods for lipase immobilization are covalent attachment, entrapment, encapsulation and adsorption to hydrophobic or hydrophilic supports. However, some of these methods have several advantages for examples; the need of very large porous materials/ beads is crucial for encapsulation and cross-linking within the enzyme molecules will decrease the enzyme activity as they could agglomerate and disturb the active site for substrate binding (Radva *et al.*, 2010; Heck *et al.*, 2013).

In this study, two methods are commonly employed for the enzyme immobilized on a solid support, namely the chemical method involving the formation of covalent bonds between the enzyme and the support matrix, and the physical methods; adsorption, which involve weak enzyme-support interactions such as van der Walls force, electrostatic force, hydrophobic interaction, and hydrogen bond (Jian *et al.*, 2006; Chiou and Wu, 2004; Jegannathan *et al.*, 2008). The adsorption method being a simple and economical method, was found to be most suitable for large scale lipase immobilization (Al-Duri and Yong 2000). Besides, enzyme immobilization by the covalent binding method has been so far the most extensively explored (Weetall and Filbert, 1974; Dodor *et al*., 2004; Cho *et al.*, 2008; Datta *et al.*, 2013; Plagemann *et al.*, 2014; Singh *et al.*, 2010; Mateo *et al.*, 2000). It is more favorable because the strong bonding between the enzyme and support provides stable, immobilized enzyme derivatives that do not leach out enzyme into the surrounding solution. Furthermore, the presence of covalent bonds made possible simple and more efficient recovery of the enzyme (Belhaj *et al.*, 2011).

Therefore, in this study, lipase from *Candida rugosa* (CRL) was immobilized on to pure silica KIT-6 synthesized from RHA by physical adsorption method, and subsequently functionalized via covalent binding with 3-aminopropyltriethoxysilane (APTES) and glutaraldehyde as the activating agent. The APTES and glutaraldehyde were demonstrated as one of useful silane derivatives and cross-linking agent, respectively, in protein conjugation (Yiu and Wright, 2005; Chong and Zhou, 2004; Pandya *et al.*, 2005; Yiu *et al.*, 2001). One of the uses of glutaraldehyde is to activate the support for the immobilization of lipase. Consequently, this approach is expected to afford KIT-6 silica particles with high enzyme loading, high enzymatic activity, and improved reaction stability. The activity of the immobilized biocatalyst was tested using esterification of flavor esters.

In addition, esters are important fine chemical and widely used in numerous applications such as, emollients in perfume and cosmetic, emulsifiers in food and beverage, paints and pharmaceutical industries (Brault *et al.*, 2014; Behzad and Nasim, 2014). They are also used as plasticizers, solvents, biological additives, hydraulic fluids and lubricants in machinery industries (Joseph *et al.*, 2005; Mbaraka and Shanks, 2006; Krause *et al.*, 2009; Martinez *et al.*, 2011). Esters can be divided into three main groups which are short, medium and long-chain fatty acid esters. Short- and medium-chain fatty acid esters have a potential interest in the food industry as the flavor compounds. They are commonly used as important flavoring and fragrance molecules because of their typical fruity smell and high volatility (Schrader *et al.*, 2004; Mahapatra *et al.*, 2009).

Direct esterification of citronellol and geraniol with short-chain fatty acids were catalyzed by the free lipase from *Mucor miehie* with high yields were obtained in n-hexane was reported (Laboret and Perrault, 1999). Also, *Mucor miehei* lipase immobilized on the magnetic polysiloxane – polyvinyl alcohol particles by the covalent binding was employed for the synthesis of flavour esters using heptane as solvent. The highest activity of lipase was recovered and the ester synthesis was maximized for the substrates containing excess acyl donar (Bruno *et al.*, 2004). However, there is less report on short chain esters catalyzed by lipase from *Candida*

rugosa (Bezbradica *et al.*, 2006) as they always used for hydrolysis of long- chain fatty acids.

The main advantages of the enzyme catalyzed flavour generation are high selectivity or specificity of the reaction, high reaction rate even at low molar fractions, environmentally compatible and mild reaction conditions, and if viable cells are used there is a possibility to perform the multistep synthesis. Nowadays, this specific application meets the consumer demand for the natural products rather than synthetic products. Hexyl acetate, a short chain ester with fruity odor is a significant green non-flavour compound widely used in food industry (Shieh and Chang, 2001). Hexyl butyrate synthesized by the immobilized lipase (Lipozyme IM-77) from *Rhizomucor miehei* was employed as a flavour and fragrance in the food, beverage and pharmaceutical industry. It was synthesized by lipase catalyzed mild tranesterification of hexanol and trybutyrin. It found enormous interest as the natural flavouring compound rather artificial or synthetic (Chang *et al.*, 2003).

Furthermore, commercially available esters in the industry required strong acid catalyst such as sulfuric acid, which is considered as hazardous materials and can cause detrimental effect to the environment. The commercial esterification also required a high temperature and this may lead to unwanted side products. So, the use of biocatalysts such as enzymes in the esterification reaction was introduced and considered natural as they are biodegradable and safe, especially in the food industry (Mizuki *et al.*, 2013). Enzyme-catalyzed esterification is an effective alternative to the chemical synthesis of short-chain esters. Esterases and lipases (triacylglycerol hydrolases; EC 3.1.1.3) are important industrial enzymes with great potential for the production of flavors and fragrances (Sharma *et al*., 2001; Hasan *et al*., 2006). These biocatalysts generally do not require cofactors and are stable in organic solvents, thus facilitating the synthesis of hydrophobic or water-labile compounds (Klibanov, 2001). In non-aqueous conditions, lipases typically catalyze the esterification, interesterification, and transesterification of alcohols and fatty acids, the three main chemical reactions producing flavoring esters (Hasan *et al*., 2006).

1.2 Statement of Problems

Despite the fact that enzyme immobilization enables easy recovery and repetitive use of enzymes, immobilized enzyme much or less will lose its activity during immobilization regarding the support (Guzik *et al.*, 2014; Norde and Favier, 1992) and methods used for immobilization (Kim *et al*., 2006). A good support requires a large surface area and pore diameters with structures that could trap the enzyme. While, a robust immobilization method requires an easy to fabricate support that affect less on the activity and substrate/product mass transfer to/from the active site of the enzyme. In addition, facile and robust immobilization chemistry that assures respectable loading of the physical and covalently immobilized enzyme that prevents its leaching during repetitive use is required.

Recently, mesoporous silica materials are introduced as promising supports candidates for enzyme immobilization that possess many interesting properties. Among them, KIT-6 showed an exceptional large pore diameter with intertwoven structure and their facile manipulation for enzyme immobilization in comparison with other mesoporous materials (Lee *et al.*, 2013; Vinu *et al.*, 2008). However, the immobilization of lipase on KIT-6 has not been investigated yet.

Other problems are related to the stability of the enzyme in different pH, temperature, enzyme concentration, solvent used and stability in thermal and storage duration. In general, a successful immobilization of the enzyme on a support reduces the dependence of the enzyme performance on exact pH and temperature (makes its activity more stable in wider ranges of pH and temperature), preserves considerable activity over a repeated number of reuses, and increase its storage duration.

1.3 Objectives of Study

The overall goal of this study was to develop a new biocatalyst to economically use in certain industrial reaction by using the immobilized CRL onto KIT-6 mesoporous materials. To reach this goal, the following specific objectives were proposed:

1. Synthesis and characterization of KIT-6 mesoporous silica from rice husk and APTES-functionalized KIT-6 as the support.
2. Immobilization and biophysical characterization of *Candida rugosa* lipase (CRL) onto KIT-6 by the physical adsorption method and APTES-functionalized KIT-6 by covalent binding.
3. Optimization of the reaction conditions of immobilized CRL by using various parameters such as temperature, pH, CRL solution concentration and organic solvents.
4. Evaluation of the activity and kinetic study of the prepared catalysts in the esterification reaction of butyric acid and butanol.

1.4 Scope of Study

In this research, the correlation between the structural and physicochemical properties of the catalyst and catalytic properties of the materials in the esterification of butyric acid are studied. For that reason, the research is divided into four main areas. Firstly, KIT-6 mesoporous silica as support materials for lipase immobilization was synthesized using rice husk ash as a silica source and further modified by functionalization with 3-(Aminopropyl) triethoxysilane (APTES) and glutaraldehyde as the linker. The obtained materials were characterized using small angle X-ray scattering (SAXS), Fourier transform infrared (FTIR) spectroscopy and nitrogen physisorption.

In the second stage, the *Candida rugos*a lipase (CRL) is immobilized onto KIT-6 silica via two methods; physical adsorption and covalent binding. For the preparation method, CRL was immobilized by direct attachment to the KIT-6 (physical adsorption) and covalent binding with functionalized KIT-6 in CRL solution. The obtained materials were characterized by SAXS, FTIR, nitrogen physisorption, X-ray photoelectron spectroscopy (XPS), thermogravimetric analysis (TGA), field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) for the study of the KIT-6 structure before and after immobilization treatments. Lipase loading and leaching was determined by UV-Vis spectroscopy using 3.1 mL assay protocol of Bradford method with Bovine serum albumin (BSA) as standard.

Thirdly, the activity of CRL in the esterification of butyric acid and butanol to produce butyl butyrate and the kinetics parameters of the series of catalysts were evaluated. The activity was determined using titrimetric method of determining residual acid content and gas chromatography (GC) analysis. The product obtained was identified by using gas chromatography-mass spectrometry (GC-MS).

Lastly, the optimization studies were performed involving several parameters such as pH, temperature, lipase concentration, and polarity of solvents. Additionally, storage stability and reusability of the enzyme were determined with respect to esterification reaction.

1.5 Significant of Study

This study developed a high quality KIT-6 mesoporous materials using waste (rice husk ash) and a new catalyst of the enzyme CRL immobilization on mesoporous KIT-6 silica also can be used in many industries, specifically in food industries. Other than that, this study is expected to solve the problems of separation, production of high yields of products and less environmental hazard.

REFERENCES

Abbas, H. and Comeau, L. (2003). Aroma synthesis by immobilized lipase from *Mucor* sp. *Enzyme Microb Technol*. 32, 589–595.

Abo, M. (1990). *World Organization Patent 9,007,606*.

Abu Bakar, B. H., Putrajaya, R. and Abdulaziz, H. (2010). Malaysian rice husk ash-improving the durability and corrosion resistance of concrete: Pre-review. *Concr. Res. Lett*. 1(1), 6–13.

Adachi, S. and Kobayashi, T. (2005). Synthesis of esters by immobilized-lipase-catalyzed condensation reaction of sugars and fatty acids in water-miscible organic solvent. *J. Biosci. Bioeng.* 99(2), 87-94.

Adlercreutz, P. (1992). Effects of the Support Material and the Thermodynamic Water Activity on Enzymatic Synthesis in Organic Media. *Annals of the New York Academy of Sciences*. 672, 314–317.

Aehle, W. (2004). *Enzymes in Industry: Production and Applications*. Wenheim, Germany: Wiley-VCH.

Ahmed, Y. M. Z., Ewaits, E. M. and Zaki Z. I. (2008). Production of porous silica by the combustion of rice husk ash for tundish lining. *J. Univ. Sci. Technol. Beijing.* 5(3), 307.

Akimoto, M., Nagashima, Y.and Sato, D. A. (1999). Kinetic study on lipase-catalyzed interesterificaiton of soybean oil with oleic acid in a continuous packed bed reactor. *Appl. Biochem. Biotechnol.* 81, 131-142.

Akoh, C. C., Chang, S. W., Lee, G. C. and Shaw, J. F. (2007). Enzymatic approach to biodiesel production. *J. Agric. Food Chem.* 55, 8995-9005.

Akova, A. and Ustun, G. (2000). Activity and adsorption of lipase from *Nigella sativa* seeds on celite at different pH values. *Biotechnology Letters*. 22, 355-359.

Al-Duri, B., Yong, Y. P. (2000). Lipase immobilisation: an equilibrium study of lipases immobilised on hydrophobic and hydrophilic/hydrophobic supports. *J. Biochem. Eng*. 4, 207–215.

Alfani, F., Cantarella, L., Cantarella, M., Gallifuoco, A. and Colella, C. (1994). Synthetic zeolites as carrier for enzyme immobilization in laboratory-scale fixedbed columns. *Proceedings of the 10th International Zeolite Conference*. 17-22 July. Garmisch-Partenkirchen, Germany: 1115–1122.

Al-Othman, Z. A. (2012). A Review: Fundamental Aspects of Silicate Mesoporous Materials. *Materials*. 5(12), 2874-2902.

Al-Qadi, S., Grenha, A. and Remuñán-López, C. (2012). Chitosan and its derivatives as nanocarriers for siRNA delivery. *Journal of Drug Delivery Science and Technology*. 22(1), 29-42.

Amirkhani, L., Moghaddas, J. and Malmiri, H. J. (2016). Optimization of *Candida rugosa* Lipase Immobilization Parameters on Magnetic Silica Aerogel Using Adsorption Method. *Iranian Journal of Chemical Engineering*. 13(3), 19-31.

Antczak, M. S., Kubiak, A., Antczak, T. and Bielecki, S. (2009). Enzymatic biodiesel synthesis-key factors affecting efficiency of the process. *Renew. Energy.* 34, 1185-1194.

Arica, M.Y., Kacar, Y., Ergene, A. and Denizli, A. (2001). Reversible Immobilization of Lipase on Phenylalanine Containing Hydrogel Membranes. *Process Biochem.* 36, 847-854.

Atluri, R., Hedin, N. and Garcia-Bennett, E. A. (2008). Hydrothermal Phase Transformation of Bicontinuous Cubic Mesoporous Material AMS-6. *Chemical Materials.* 20(12), 3857-3866.

August, P. (1972). *West Germany Patent 2,064,940*.

Awang, R., Basri, M., Ahmad, S. and Salleh, A. B. (2003). Enzyme-catalyzed synthesis and characterization of octyl dihydroxystearate from palm-based dihydroxystearic acid. *Journal of Oleo Science*. 52(1), 7-14.

Bagshaw, S. A., Prouzet, E. and Pinnavaia, T. J. (1995). Templating of Mesoporous Molecular Sieves by Nonionic Polyethylene Oxide Surfactants. *Science.* 269(5228), 1242-1244.

Bahar, T. and Tuncel, A. (2002). Immobilization of invertase onto crosslinked poly(p-chloromethylstyrene) beads. *Journal of Applied Polymer Science.* 83(6), 1268 -1279.

Bai, Y. X., Li, Y. F., Yang, Y. and Yi, L. X. (2006). Covalent immobilization of triacylglycerol lipase onto functionalized nanoscale SiO_2 spheres. *Process Biochemistry*. 41, 770–777.

Baiker, A. and Blaser, H.U. (1997). *Hanbook of Heterogeneous Catalysis*. Wenheim, Germany: Wiley-VCH.

Bajpai, P. (1999). Application of enzymes in the pulp and paper industry. *Biotechnol. Progr.* 15, 147-157.

Barbero, N., Barni, E., Barolo, C., Quagliotto, P., Viscardi, V., Napione, L., Pavana, S. and Bussolino, F. (2009). A study of the interaction between fluorescein sodium salt and bovine serum albumin by steady-state fluorescence. *Dyes and Pigments*. 80(3), 307–313.

Barbosa, O., Ortiz, C., Berenguer-Murcia, A., Torres, R., Rodrigues, R. C. and Fernandez-Lafuente, R. (2014). Glutaraldehyde in bio-catalysts design: A useful crosslinker and a versatile tool in enzyme immobilization. *RSC Adv*. 4, 1583–1600.

Basri, M., Ampon, K., Wan Yunus, W. M. Z., Razak, C. N. A. and Salleh, A. B. (1994). Immobilisation of hydrophobic lipase derivatives onto organic polymer beads. *J. Chemical Technology Biotechnology*. 59, 37-44.

Basri, M., Rahman, R. N. Z. R. A. and Salleh, A. B. (2013). Specialty oleochemicals from palm oil via enzymatic syntheses. *Journal of Oil Palm Research.* 25(1), 222-235.

Bastida, A., Sabuquillo, P., Armisen, P., Fernández-Lafuente, R., Huguet, J. and Guisán, J. M. (1998). A single step purification, immobilization, and hyperactivation of lipases via interfacial adsorption on strongly hydrophobic supports. *Biotechnol Bioeng.* 58, 486–493.

Bautista, L. F., Vicente, G., Mendoza, A., Gonzalez, S. And Morales, V. (2015). Enzymatic Production of Biodiesel from *Nannochloropsis gaditana* Microalgae Using Immobilized Lipases in Mesoporous Materials. E*nergy Fuels*. *29*(8), 4981–4989.

Bayramoglu, G., Altintas, B., Yilmaz, M. and Arica, M.Y. (2011). Immobilization of chloroperoxidase onto highly hydrophilic polyethylene chains via bio-conjugation: catalytic properties and stabilities. *Bioresource Technology*. 102(2), 475-482.

Bayramoğlu, G., Kacar, Y., Denizli, A. and Arica, M. Y. (2002). Covalent immobilization of lipase onto hydrophobic group incorporated poly (2-hydroxyethyl methacrylate) based hydrophilic membrane matrix. *Journal of food engineering.* 52 (4), 367-374.

Beck, J. S., Vartuli, J. C., Roth, W. J., Leonowicz, M. E., Kresge, C. T., Schmitt, K. D., Chu, C. T. W., Olson, D. H., Sheppard, E. W., McCullen, S. B., Higgins, J. B. and. Schlenkert, J. L. (1992). A New Family of Mesoporous Molecular Sieves Prepared with Liquid Crystal Templates. *J. American Chemical Society*. 114(27), 10834–10843.

Behzad, A. and Nasim, D. (2014) Modified bentonite as catalyst for esterification of oleic acid and ethanol. *Journal of the Taiwan Institute of Chemical Engineers*. 45, 1468–1473.

Belhaj, I. B. R., Zamen, B. R., Ali, G. and Hafedh, B. (2011). Esterification activity and stability of *Talaromyces thermophiles* lipase immobilized onto chitosan. *Journal of Molecular Catalysis B: Enzymatic*. 68, 230–239.

Berglund, P. and Hutt, K. (2000). Biocatalytic Synthesis of Enantiopure Compounds Using Lipases. In: Patel, R. N. (Ed). *Stereoselective Biocatalysis* (pp. 633-657). New York: Marcel Dekker.

Bernfeld, P. and Wan, J. (1963). Antigens and Enzymes Made Insoluble by Entrapping Them into Lattices of Synthetic Polymers. *Science*. 142(3593),678-679.

Bezbradica, D., Karalazic, I., Ognjanovic, N., Mijin, D., Siler-Marinkovic, S. and Knezevic, Z. (2006). Studies on the specificity of Candida rugosa lipase catalyzed esterification reactions in organic media. *J. Serb. Chem. Soc*. 71 (1), 31–41.

Bhange, P., Sridevi, N., Bhange, D. S., Prabhune, A. and Ramaswamy, V. (2013). Immobilization of bile salt hydrolase enzyme on mesoporous SBA-15 for co-precipitation of cholesterol. *Int J Biol Macromol*. 63, 218-24.

Bhatia, R. P. (1990). *United States Patent No. 4,921,630.*

biology-igcse.weebly.com

Blanco, R. M., Calvete, J. J., and Guisán, J. M. (1989). Immobilization-stabilization of enzymes. Variables that control the intensity of the trypsin (amine)-agarose-(aldehyde) – multipoint attachment. *Enzyme Microb. Technol.* 11, 353-359.

Blanco, R. M., Terreros, P., Fernández-Pérez, M., Otero, C. and Díaz-González, G. (2004). Functionalization of mesoporous silica for lipase immobilization: Characterization of the support and the catalysts. *Journal of Molecular Catalysis B: Enzymatic*. 30(2), 83–93.

Boller, T., Meier, C. and Menzler, S. (2002). Eupergit oxirane acrylicbeads: How to make enzymes fit for biocatalysis. *OrganicProcess Research and Development.* 6, 509–519.

Bornscheuer, U. T. (2003). Immobilizing enzymes: How to create more suitable biocatalysts. *Angew. Chem. Int. Ed.* 42, 3336-3337.

Boros, Z., Weiser, D., Márkus, M., Abaháziová, E., Magyar, A., Tomin, A., Koczka, B., Kovács, P., Poppe, L. (2013). Hydrophobic adsorption and covalent immobilization of *Candida antarctica* lipase B on mixed-function-grafted silica gel supports for continuous-flow. *Biotransformations Process Biochemistry*. 48 (7), 1039–1047.

Borrelli, G. M. and Trono, D. (2015). Recombinant Lipases and Phospholipases and Their Use as Biocatalysts for Industrial Applications. *Int J Mol Sci.* 16(9), 20774-840.

Bosley, J. A. and Peilow, A. D. (1997). Immobilization of lipases on porous polypropylene: reduction in esterification efficiency at low loading. *J. Am. Oil Chem. Soc*. 74,107–111.

Boundy, J. A., Smiley, K. L., Swanson, C. L. and Hofreiter, B.T. (1976). Exoenzymes activity of alpha-amylase immobilized on a phenol-formaldehyde resin. *Carbohyd Res.* 48, 239–244.

Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry.* 72, 248-254.

Brady, C., Metcalfe, L., Slaboszewski, D. and Frank, D. (1988). Lipase immobilized on a hydrophobic microporous supports for the hydrolysis of fats, *J. Am. Oil Chem. Soc.* 65, 917–921.

Brault, G., Shareck, F., Hurtubise, Y., Lépine, F. and Doucet., N. (2014). Short-Chain Flavor Ester Synthesis in Organic Media by an E. coli Whole-Cell Biocatalyst Expressing a Newly Characterized Heterologous Lipase. *PLoS ONE*. 9(3), e91872

Brena, B. M. and Batista-Viera, F. (2006). *Immobilization of enzymes*. In Guisan, J. M. (Ed.). *Immobilization of enzymes and cells.* (pp. 15-30). Totowa, New Jersey: Humana Press Inc.

Brindusa, D. and Emil, D. (2008). Candida antarctica Lipase B Immobilization Onto Hybrid Organic-inorganic Mesoporous Materials. *Acta Chimica Slovenica.* 55(2), 277-285.

Brinker, C. J. and Scherrer, G. W. (1990). *Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing*.USA: Academic Press. Inc.

Bruno, L. M., de Lima Filho, J. L., Melo, E. H. M. and de Castro, H. (2004). Ester synthesis by *Mucor miehei*. Lipase immobilized on magnetic polysiloxane-polyvinyl alcohol particles. *Appl. Biochem. Biotech*. 113(1-3), 189-200.

Budnyak, T. M., Pylypchuk, L. V., Tertykh, V. A., Yanovska, E. S. and Kolodynska, D. (2015). Synthesis and adsorption properties of chitosan-silica nanocomposite prepared by sol-gel method. *Nanoscale Research Letters*. 10(87), 1-10.

Burkett, S. L., Sims, S. D. and Mann, S. (1996). Synthesis of hybrid inorganic-organic mesoporous silica by co-condensation of siloxane and organosiloxane precursors. *Chem. Commun.* 11(11), 1367-1368.

Burleigh, M., Dai, S., Hagaman, E. and Lin, J. (2001). Imprinted Polysilsesquioxanes for the Enhanced recognition of Metals. *Chem Mater.* 13, 2537-2546.

Cabral, P. P., Fonseca, M. M. R. and Dias, S. F. (2010). Esterification activity and operational stability of *Candida rugosa* lipase immobilized in polyurethane foams in the production of ethyl butyrate. *Biochemical Engineering Journal*. 48, 246–252.

Cantone, S., Ferrario, V., Corici, L., Ebert, C., Fattor, D., Spizzo, P and Gardossi, L. (2013). Efficient immobilisation of industrial biocatalysts: criteria and constraints for the selection of organic polymeric carriers and immobilisation methods. *Chem. Soc. Rev*. 42, 6262-6276.

Cao, L. and Schmid, R. D. (2005). *Carrier-Bound Immobilized Enzymes: Principles, Application and Design.* Weinheim, Germany: Wiley-VCH Verlag.

Cao, S. L., Huang, Y. M., Li, X. H., Xu, P., Wu, H., Li, N., Lou, W. Y. and Zong, M. H. (2016). Preparation and Characterization of Immobilized Lipase from Pseudomonas Cepacia onto Magnetic Cellulose Nanocrystals. Scientific Reports. 6(20420), 1-12.

Cardias, H. C. T., Grininger, C. C., Trevisan, H. C., Guisan, J. M. and Giordano, R. L. C. (1999). Influence of Activation on the Multipoint Immobilization of Penicillin G Acylase on Macroporous Silica. *Braz. J. Chem. Eng*. 16(2).

Celikbicak, O., Bayramoglu, G., Yilmaz, M., Ersoy, G., Bicak, N., Salih, B. and Arica, M. Y. (2014). Immobilization of laccase on hairy polymer grafted zeolite particles: Degradation of a model dye and product analysis with MALDI–ToF-MS. *Microporous and Mesoporous Materials*. 199, 57-65.

Chaikittisilp, W., Khunsupat, R., Chen, T. T. and Jones, C. W. (2011). Poly(allylamine)-mesoporous silica composite materials for CO2 capture from simulated flue gas or ambient air. *Ind. Eng. Chem. Res.*, 50, 14203–14210.

Chandra, S. (2007). *Waste Materials Used in Concrete Manufacturing*. Westwood, New Jersey, U.S.A: Noyes Publications.

Chandrasekhar, S., Pramada, P. N. and Majeed, J. (2006). Effect of calcination temperature and heating rate on the optical properties and reactivity of rice husk ash. *J. Mater. Sci.* 41, 7926–7933.

Chang, Q. and Tang, H. (2014). Immobilization of Horseradish Peroxidase on NH_2-Modified Magnetic Fe_3O_4/SiO_2 Particles and Its Application in Removal of 2,4-Dichlorophenol. *Molecules*. 19, 15768-15782.

Chang, S. W., Shaw, J. F. and Shieh, C. J. (2003). Optimization of enzymatically prepared Hexyl butyrate by lipozyme IM-77. Enzymatic synthesis of Hexyl Butyrate. *Food Technol. Biotech.* 41(3), 237-243.

Chang, T.M.S. (1964). Semipermeable Microcapsules. *Science*. 146(3643), 524-525.

Chang, Y. K. and Chu, L. (2007). A simple method for cell disruption by immobilization of lysozyme on the extrudate-shaped Na Y zeolite. *Biochem Eng. J.* 35, 37–47.

Chang, Y. K. and Chu, L. (2007). A simple method for cell disruption by immobilization of lysozyme on the extrudate-shaped Na Y zeolite. *Biochem. Eng. J*. 35, 37–47.

Che, S., Garcia- Bennett, A. E., Yokoi, T., Sakamoto, K., Kunieda, H., Terasaki, O. and Tatsumi, T. (2003). A Novel Anionic Surfactant Templating Route for Synthesizing Mesoporous Silica with Unique Structure. *Nature Materials*. 2(12), 801-805.

Che, S., Liu, Z., Ohsuna, T., Sakamoto, K., Terasaki, O. and Tatsumi, T.(2004). Synthesis and Characterization of Chiral Mesoporous Silica. *Nature*. 429(6989), 281-284.

Cheah, W. K., Ooi, C. H. and Yeoh, F. Y. (2016). Rice husk and rice husk ash reutilization into nanoporous materials for adsorptive biomedical applications: A review. *Mesoporous Biomater*. 3, 27–38.

Chen, B., Hu, J., Miller, E. M., Xie, W., Cai, M. and Gross, A. R. (2008). *Candida antarctica* Lipase B Chemically Immobilized on Epoxy-Activated Micro- and Nanobeads: Catalysts for Polyester Synthesis. *Biomacromolecules*. 9, 463–471.

Chen, F., Zhang, F., Du, F., Wang, A., Gao, W., Wang, Q., Yin, X. and Xie, T. (2012 A novel and efficient method for the immobilization of thermolysin using sodium chloride salting-in and consecutive microwave irradiation. *Bioresour. Technol*. 115, 158-163.

Chibata, I. (1978). *Immobilized Enzymes, Research and Development*. Tokyo and New York : Kondasha Ltd. and John Wiley & Sons Inc.

Chibata, I., Tosa, T., Sato, T., Mori, T. and Matuo, T. (1972). Preparation and Industrial Application of Immobilized Aminoacylases. *Fermentation Technology Today*. Proc. IVth Fermentation Sym.,Kyoto, Japan, Society for Fermentation Technology, Japan, 383-389.

Chiou, S. H. and Wu, W. T. (2004). Immobilization of *Candida rugosa* lipase on chitosan with activation of the hydroxyl groups. *Biomaterials*. 25, 197–204.

Cho, N., Cho, H., Shin, S., Choi, Y., Leonowicz, A. and Ohga, S. (2008). Production of fungal laccase and its immobilization and stability. *J. Fac. Agric. Kyushu Univ*. 53(1), 13–18.

Chong, A. S. M. and Zhou, X. S. (2004). Design of large-pore mesoporous materials for immobilization of penicillin G acylase biocatalyst. *Catalysis Today*. 93-95, 293-299.

Chong, A. S. M. and. Zhao, X. S. (2004). Functionalized nanoporous silicas for the immobilization of penicillin acylase. *Appl. Surf. Sci.* 237, 398-404.

Chuah, T. G., Jumasiah, A., Azni, I, Katayon, S. and Choong, S. Y. T. (2005). Rice husk as a potentially low-cost biosorbent for heavy metal and dye removal: an overview. *Desalination*. 175(3), 305-316.

Clark, J. H. and Macquarrie, D. J. (1998). Catalysis of liquid phase organic reactions using chemically modified mesoporous inorganic solids. *J. Chem. Soc. Chem. Comm.* 8, 853-860.

Clark, S. J., Wagner, L., Schock, M. D. and Piennaar, P. G. (1984). Methyl and ethyl soybean esters as renewable fuels for diesel engines. *J. Am. Oil Chem. Soc.* 61, 1632-1638.

Colilla, M., Manzano, M. and Vallet-Regí, M. (2008). Recent advances in ceramic implants as drug delivery systems for biomedical applications. *Int. J. Nanomedicine*. 3(4), 403–414.

Corriu, R. J. P., Datas, L., Guari, Y., Mehdi, A., Reyé, C. and Thieuleux, C. (2001). Ordered SBA-15 mesoporous silica containing phosphonicacid groups prepared by a direct synthetic approach. Chem. Commun. 8, 763-764.

Creighton, T. E. (1984). *Proteins*. Oxford, UK: Freeman.

Daniel, R. M., Peterson, M. E., Danson, M. J., Price, N. C., Kelly, S. M., Monk, C. R., Weinberg, C. S., Oudshoorn, M. L. and Lee, C. K. (2010). The molecular basis of the effect of temperature on enzyme activity. *Biochemical Journal*. 425(2), 353-360.

Datta, S., Christena, L. R. and Rajaram, Y. R. S. (2013). Enzyme immobilization: An overview on techniques and support materials. *Biotechnology*. 3(1), 1-9.

David, M. Barsan, M. M., Florescu, M., Christopher M. A. B. (2015). Acidic and Basic Functionalized Carbon Nanomaterials as Electrical Bridges in Enzyme Loaded Chitosan/Poly(styrene sulfonate) Self-Assembled Layer-by-Layer Glucose Biosensors. *Electroanalysis*. 27 (9), 2139–2149.

De Vos, D. E., Vankelecom, I. F. J. and Jacobs, P. A. (2000). *Chiral Catalyst Immobilization and Recycling.* New York: Wiley-VCH Verlag GmbH.

Deere, J., Magner, E., Wall, J. G. and Hodnett, B. K. (2001). Adsorption and activity of cytochrome c on mesoporous silicates. *Chem. Commun.* 5, 465.

Deere, J., Magner, E., Wall, J. G. and Hodnett, B. K. (2002). Mechanistic and structural features of protein adsorption onto mesoporous silicates. *J. Phys. Chem. B.* 106, 7340-7347.

Deere, J., Magner, E., Wall, J. G. and Hodnett, B. K. (2002). Mechanistic and Structural Features of Protein Adsorption onto Mesoporous Silicates. *Journal of Chemical Chemistry B*. 106, 7340-7347.

Desai, P. D., Dave, A. M., Devi, S. (2004). Entrapment of lipase into K-carrageenan beads and its use in hydrolysis of olive oil in biphasic system. *J. Mol. Catal. B: Enzym*. 31(4-6), 143–150.

Diaz, J. F. and Balkus, K. J. (1996). Enzyme immobilization in MCM-41 molecular sieve. *Journal of Molecular Catalysis B: Enzymatic*. 2(2-3), 115-126.

Ding, K., Du, H., Yuan, Y. and Long, J. (2004). Combinatorial Chemistry Approach to Chiral Catalyst Engineering and Screening: Rational Design and Serendipity. *Chemistry A European Journal.* 10(12), 2872-2884.

Direnzo, F., Cambon, H. and Dutartre, R. (1997). A 28-year-old Synthesis of Micelle-templated Mesoporous Silica. *Microporous Materials*. 10(4-6), 283-286.

Dodor, D. E., Hwang, H. M. and Ekunwe, S. I. N. (2004). Oxidation of anthracene and benzo[α]pyrene by immobilized laccase from Trametes versicolor. *Enzyme Microb. Technol*. 35, 210–217.

Dong, A. and Huang, P. (1992). Redox-dependent changes in beta-extended chain and turn structures of cytochrome c in water solution determined by second derivative amide I infrared spectra. *Biochemistry*. 31,182–189.

Dordick, J. S. (1989). Enzymatic catalysis in monophasic organic solvents. *Enzyme Microb. Technol.* 11, 194-211.

Dosanjh, N. S. and Kaur, J. (2002). Immobilization, stability and esterification studies of a lipase from a Bacillus sp. *Biotechnol. Appl. Biochem*. 36(1), 7-12.

Dou, Y. Q., Zhai, Y., Zeng, F., Liu, X. X., Tu, B. and Zhao, D. (2010). Encapsulation of polyaniline in 3-D interconnected mesopores of silica KIT-6. *Journal of Colloid and Interface Science*. 341, 353–358.

Dragoi, B. and Dumitriu, E. (2008). Candida antarctica Lipase B Immobilization Onto Hybrid Organic-inorganic Mesoporous Materials. *Acta Chim. Slov*. 55, 277–285.

Dubreuil, A. C., Chainet, F., Bartolomeu, R. M. S., Mota, F. M. M., Janvier, J. and Lienemann, C. P. (2017). Understanding the impact of silicon compounds on metallic catalysts through experiments and multi-technical analysis. *Comptes Rendus Chimie*. 20(1), 55-56.

Dutta, S. and Ray, L. (2009). Production and characterization of an alkaline thermostable crude lipase from an isolated strain of Bacillus cereus C7. *Appl. Biochem. Biotechnol.* 159, 142-154.

Dyal, A., Loos, K., Noto, M., Chang, S. W., Spagnoli, C., Shafi, K. V. P. M, Ulman, A., Cowman, M. and Gross, R. A. (2003). Activity of Candida rugosa Lipase Immobilized on g-Fe_2O_3 Magnetic Nanoparticles. *Journal of the American Chemical Society*. 125(7), 1684-1685.

Ebrahimi-Gatkash, M., Younesi, H., Shahbazi, A. and Heidari, A. (2014). Amino-functionalized mesoporous MCM-41 silica as an efficient adsorbent for water treatment: batch and fixed-bed column adsorption of the nitrate anion. *Applied Water Science.* 1-15

Eed, J. (2013). Factors Affecting Enzyme Activity. *ESSAI.* 10(19), 48-51.

Eftink, M. R. (1999). *Fluorescence quenching. Theory and applications*. In Lakowicz, J. R. (Ed.). *Principles of Fluorescence Spectroscopy* (pp. 53-127). New York, USA: Kluwer Academic /Plenum Publishers.

El- Safty, S. A. and Evans, J. (2002). Formation of Highly Ordered Mesoporous Silica Materials Adopting Lyotropic Liquid Crystal Mesophases. *J. Materials Chemistry*. 12(1), 117-123.

Ettalibi, M. and Baratti, J.C. (2001). Sucrose hydrolysis by thermostable immobilized inulinases from *aspergillus ficuum. Enzymatic Microbial Technology*. 28, 596-601.

Falahati, M., Saboury, A. A., Ma'mani, L., Shafiee, A. and Rafieepour, H. A. (2012). The effect of functionalization of mesoporous silica nanoparticles on the interaction and stability of confined enzyme. *International Journal of Biological Macromolecules*. 50, 1048–1054.

Fan, J., Lei, L. J., Wang, L. M., Yu, C. Z., Tu, B. and Zhao, D. Y. (2003). Rapid and high-capacity immobilization of enzymes based on mesoporous silicas with controlled morphologies. *Chem. Commun.* 2140-2141.

Fan, J., Yu, C., Gao, F., Lei, J., Tian, B., Wang, L., Luo, Q., Tu, B., Zhou, W. and Zhao, D. (2003). Cubic mesoporous silica with large controllable entrance sizes and advanced adsorption properties. *Angew. Chem. Int. Edn. Engl.* 42(27), 3146-3150.

Fernández-Lafuente, R., Armisen, P., Sabuquillo, P., Fernández-Lorente, G. and Guisán, J. M. (1998). Immobilization of lipases by selective adsorption on hydrophobic supports. *Chem Phys Lipids*. 93, 185–197.

Fernandez-Lorente, G., Terreni, M., Mateo, C., Bastida, A., Fernandez-Lafuente, R., Dalmases, P., Huguet, J. and Guisan, J. M. (2001). Modulation of lipase properties in macro-aqueous systems by controlled enzyme immobilisation: enantioselective hydrolysis of a chiral ester by immobilised Pseudomonas lipase. *Enzyme Microb. Technol.* 28, 389-396.

Ferreira, L., Ramos, M. A., Dordick, J. S. and Gil, M. H. (2003). Influence of different silica derivatives in the immobilization and stabilization of a Bacillus licheniformis protease (Subtilisin Carlsberg). *J. Mol. Catal. B: Enzymatic*. 21, 189–199.

Finnefrock, A. C., Ulrich, R., Gilman, E. S., Gruner, S. M. and Wiesner, U. (2003). The Plumber's Nightmare: A New Morphology in Block Copolymer-Ceramic Nanocomposites and Mesoporous Aluminosilicates. *J. American Chemical Society*. 125, 13084 -13093.

Fischer, E. (1894). Einfluss der Configuration auf die Wirkung der Enzyme. *Ber. Dtsch. Chem. Ges*. 27, 2985–2993.

Foresti, M. L. and Ferreira, M. L. (2007). Chitosan-immobilized lipases for the catalysis of fatty acid esterifications. *Enzyme and Microbial Technology*. 40, 769–777.

Fowler, C. E., Burkett, S. L. and Mann, S. (1997). Synthesis and characterization of ordered organo-silica-surfactant mesophases with functionalized MCM-41-type architecture. *J. Chem. Soc. Chem. Commun*. 1769-1770.

Fowler, C. E., Mann, S. and Lebeau, B. (1998). Covalent coupling of an organic chromophore into functionalized MCM-41 mesophases by template-directed co-condensation. *Chem. Commun.* 17, 1825-1826.

Fukuda, S., Hayashi, S. Ochiai, H., Iiizumi, T. and Nakamura, K. (1990). *Japanese Patent 2, 229-290.*

Gabel, D., Steinberg, I. Z. and Katchalski, E. (1971). Changes in conformation of insolubilized trypsin and chymotrypsin, followed by fluorescence. *Biochemistry*. 10, 4661–4665.

Galarneau, A., Mureseanu, M., Atger, S., Renard, G. and Fajula, F. (2006). Immobilization of lipase on silicas. Relevance of textural and interfacial properties on activity and selectivity. *New J. Chem*. 30(4), 562-571.

Gao, C., Qiu, H., Zeng, W., Yasuhiro, S., Osamu, T., Kazutami, S., Chen, Q. and Che, S. (2006). Formation mechanism of anionic surfactant-templated mesoporous silica. *Chemistry of materials*. 18(16), 3904-3914.

Gao, S., Wang, Y., Diao, X., Luo, G. and Dai, Y. (2010). Effect of pore diameter and cross-linking method on the immobilization efficiency of Candida rugosa lipase in SBA-15. *Bioresource Technology.* 101(11), 3830–3837.

Gao, S., Wang, Y., Wang, W., Luo, G. and Dai, Y. (2010). Enhancing performance of lipase immobilized on methyl-modified silica aerogels at the adsorption and catalysis processes: Effect of co-solvents. *Journal of Molecular Catalysis B: Enzymatic*. 62, 218–224.

Garcia, R., Martinez, M. and Aracil, J. (2002). Enzymatic esterification of an acid with an epoxide using an immobilized lipase from *Mucor miehei* as catalyst: Optimization of the yield and isomeric excess of ester by statistical analysis. *J. Ind. Microb. Biotech.* 28(3), 173-179.

Gemeiner, P. (1992). *Enzyme Engineering: Immobilized Biosystems.* Chichester: Ellis Horwood, Bratislava: Alfa publishers.

Gibson, L. T. (2014). Mesosilica materials and organic pollutant adsorption: part A removal from air. *Chem. Soc. Rev*. 43(15), 5163-5172.

Gilani, S. L., Najafpour, G. D., Moghadamnia, A. and Kamaruddin, A. H. (2016). Kinetics and Isotherm Studies of the Immobilized Lipase on Chitosan Support. *IJE Transaction A: Basics*. 29(10), 1319-1331.

Gillies, B., Yamazaki, H. and Armstrong, D. W. (1987). Production of flavour esters by immobilised lipase. *Biotechnology Letters*. 9, 709-719.

Glatter, O. and Kratky, O. (Ed.) (1982). *Small Angle X-ray Scattering* O. London: Academic Press.

Gobin, O. C., Wan, Y., Zhao, D., Kleitz, F. and Kaliaguine, S. (2007). Mesostructured Silica SBA-16 with Tailored Intrawall Porosity Part 1: Synthesis and Characterization. *J. Phys. Chem.* 111(7), 3053-3058.

Gole, A., Thakar, G. and Sastry, M. (2003). Protein diffusion into thermally evaporated lipid films: role of protein charge/mass ratio. *Colloids Surface B.* 28, 209–214.

Grochulski, P., Li, Y., Schrag, J. D., Bouthillier, F., Smith, P., Harrison, D., Rubin, B. and Cygler, M. (1993) Insights into Interfacial Activation from An Open Structure of Candida Rugosa Lipase. *J. Biological Chemistry*. 268(17),12843-12847.

Grubhofer, N. and Schleith, L. (1953).Modified Ion-exchange Resin as Specific adsorbent. *Naturwissenschaften*. 40(19), 508.

Grunwald, P. (2009). *Biocatalysis: Biochemical Fundmental and Applications*. London, Imperial College Press.

Guillén, M.; Benaiges, M. D. and Valero, F. (2012). Biosynthesis of ethyl butyrate by immobilized recombinant *Rhizopus oryzae* lipase expressed in *Pichia pastoris. J. Biochemical Engineering*. 65, 1-9.

Guisan, J. M., Bastida, A., Blanco, R. M. et al (1997). *Immobilization of enzymes on glyoxyl supports: strategies for enzyme stabilization by multipoint covalent attachment.* In Bickerstaff, G. (Ed.). *Immobilization of enzymes and cells Serie. Methods Biotechnol. Vol 1* (pp. 277-288). New Jersey: The Humana Press Inc.

Guo, W., Kleitz, F., Cho, K. and Ryo, R. (2010). Large pore phenylene-bridged mesoporous organosilica with bicontinuous cubic Ia3d (KIT-6) mesostructure. *Journal of Materials Chemistry*. 20(38), 8175-8418.

Gupta, M. and Mattiasson, B. (1992). *Unique applications of immobilized proteins in bioanalytical systems*. In Suelter, C. H. (Ed.). *Methods of Biochemical Analysis, Volume 36*. (pp. 1-34). New York: Wiley.

Gustafsson, H., Johansson, E. M.. Barrabino, A., Odén, M. and Holmberg, K. (2012). Immobilization of lipase from mucor miehei and rhizopus oryzae into mesoporous silica-the effect of varied particle size and morphology. *Colloids Surf. B. Biointerfaces*. 100, 22–30.

Gustafsson, H., Küchler, A., Holmberg, K. and Walde, P. (2015). Co-immobilization of enzymes with the help of a dendronized polymer and mesoporous silica nanoparticles. *J. Mater. Chem. B.* 3, 6174-6184.

Han, Y. J., Watson, J. T., Stucky, G. D., and Butler, A. (2002). Catalytic Activity of Mesoporous Silicate-Immobilized Chloroperoxidease. *Journal of Molecular Catalysis. B:Enzymatic.* 17, 1-8.

Handayani, N., Loos, K., Wahyuningrum, D., Buchari, and Zulfikar, M. A. (2012). Immobilization of Mucor miehei Lipase onto Macroporous Aminated Polyethersulfone Membrane for Enzymatic Reactions. *Membranes*. 2, 198-213.

Hanefeld, U., Gardossi, L. and Magner, E. (2009). Understanding enzyme immobilization. *Chem. Soc. Rev*. 38, 453-468.

Hartmann, M. and Kostrov, X. (2013). Immobilization of enzymes on porous silicas--benefits and challenges. *Chem. Soc. Rev.* 42, 6277-6289.

Hartmeier, W. (1988). *Immobilized Biocatalysts*. Berlin: Springer-Verlag.

Hasan, F., Shah, A. A. and Hameed, A. (2009). Methods for detection and characterization of lipases: A comprehensive review. *Biotechnol. Adv.* 27, 782-798.

He Z, Zhang Z, and He M. (2000). Kinetic study of thermal inactivation for nativeand methoxypolyethylene glycol modified trypsin. *Process Biochem*. 35, 1235–1240.

He, J., Li, X., Evans, D. G., Duan, X. and Li, C. (2000). A new support for the immobilization of penicillin acylase. *Journal of Molecular Catalysis B: Enzymatic*. 11, 45-53.

Heck, T., Faccio, G., Ritcher, M. and Thony-Meyer, L. (2013). Enzyme-catalyzed protein crosslinking. *Appl. Microbiol. Biotechnol*. 97(2), 461–475.

Ho, L. F., Li, S. Y., Lin, S. C. and Hsu, W. H. (2004). Integrated enzyme purification and immobilization processes with immobilized metal affinity adsorbents. *Process Biochem*. 39, 1573-1581.

Hoffmann, F., Cornelius, M., Morell, J. and Froba, M. (2006). Silica-Based Mesoporous Organic–Inorganic Hybrid Materials. Angew. Chem. Int. Ed. 45, 3216 – 3251.

Hong, J., Xu, D., Gong, P., Yu, J., Ma, H. and Yao, S. (2008). Covalent bonded immobilization of enzyme on hydrophilic polymer covering magnetic nanogels. *Microporous Mesoporous Mater*. 109,470-477.

Huang, X., Young, N. P. and Helen, E. T (2015). Characterization and Comparison of Mesoporous Silica Particles for Optimized Drug Delivery. *Nanomaterials and Nanotechnology*. 4(2), 1-15.

Hudson, S., Cooney, J. and Magner, E. (2008). Proteins in mesoporous silicates. *Angew. Chem. Int. Ed.* 47, 8582–8594.

Hung, B. Y., Kuthati, Y., Kankala, R. K., Kankala, S., Deng, J. P., Liu, C. L. and Lee, C. H. (2015). Utilization of Enzyme-Immobilized Mesoporous Silica Nanocontainers (IBN-4) in Prodrug-Activated Cancer Theranostics. *Nanomaterials.* 5, 2169- 2191.

Huo, Q., Margolese, D. I., Ciesla, U., Feng, P., Gier, T. E., Sieger, P., Leon, R., Petroff, P. M., Schüth, F. and Stucky, G. D. (1994). Generalized Synthesis of Periodic Surfactant/ Inorganic Composite Materials. *Nature*. 368, 317-321.

Hwang, C. L. and Wu, D. S. (1989). *Properties of cement paste containing rice husk ash*. In Malhotra, V. N. (Ed.). *ACI SP-114: Fly Ash, Silica Fume, Slag, and Natural Pozzolans in Concrete*. (pp. 733-765). U. S. A.: Farmington Hills.

ibguides.com

Isabel, D. M. and Otero, C. (1998). Kinetic and spectroscopic behaviour of a lipase–microgel derivative in aqueous and micellar media. *Journal of Molecular Catalysis B: Enzymatic*. 4, 137–147.

Islam, M. S. and Mondal, T. (2013). Potentiality of Biomass Energy for Electricity Generation in Bangladesh. *Asian Journal of Applied Science and Engineering (AJASE)*. 2(2), 103-110.

Ison, A. P., Dunnill, P. and Lilly, M. D. (1988). Effect of solvent concentration on enzyme catalysed interesterification of fats. *Enzyme and Microbial Technology*. 10(1), 47-51.

Ispas, C., Sokolov, I. and Andreescu, S. (2009). Enzyme-functionalized mesoporous silica for bioanalytical applications. *Anal. Bioanal. Chem*. 393, 543–554.

Ito, S., Kobayashi, T., Ara, K., Ozaki, K., Kawai, S. and Hatada, Y. (1998). Alkaline detergent enzymes from alkaliphiles: Enzymatic properties, genetics and structures. *Extremophiles*. 2, 185-190.

Jaeger, K. E. and Eggert, T. (2002). Lipases for biotechnology. *Current Opinion in Biotechnology*. 13(4), 390-397.

Jaeger, K. E. and Reetz, M. T. (1998). Microbial lipases from versatile tools for biotechnology. *Trends. Biotechnol*. 16,396-403.

Jain, A., Toombes, G. E. S., Hall, L. M., Mahajan, S., Garcia, C. B. W., Probst, W., Gruner, S. M. and Wiesner, U. (2005). Direct Access to Bicontinuous Skeletal Inorganic Plumber's Nightmare Network from Block Copolymers. *Angew. Chem. Int. Ed*. 44(8), 1226-1229.

James, M. and Lord, M. P. (1992). *Macmillan's Chemical and Physical Data*. London and Basingstoke: The Macmillan Press Ltd.

Jang, H. T., Park, Y., Ko, Y. S., Lee, J. Y. and Margandan, B. (2009). Highly siliceous MCM-48 from rice husk ash for CO_2 adsorption. *Int. J. Greenhouse Gas Control*. 3, 545–549.

Jegannathan, K. R., Abang, S., Poncelet, D., Chan, E. S. and Ravindra, P. (2008). Production of biodiesel using immobilized lipase-A critical review. *Critical Reviews in Biotechnology*. 28(4), 253–264.

Jeison, D., Ruiz, G., Avecedo, F. and Illanes, A. (2003). Process Simulation of the effect of intrinsic reaction kinetics and particle size on the behaviour of immobilised enzymes under internal diffusional restrictions and steady state operation. *Process Biochemistry*. 39, 393-399.

Jian, X., Wang, Y. J., Yu, H., Luo, G. S. and Dai, Y. Y. (2006). *Candida rugosa* lipase immobilized by a specially designed microstructure in the PVA/PTFE composite membrane. *Journal of Membrane Science*. 281, 410–416.

Jo, C., Kim, K. and Ryoo, R. (2009). Synthesis of High Quality SBA-15 and KIT-6 Mesoporous Silicas Using Low-cost Water Glass, Through Rapid Quenching of Silicate Structure in Acidic Solution. *Microporous Mesoporous Materials*. 124(1-3), 45-51.

Joseph, B., Ramteke, P. W. and Thomas, G. (2008). Cold active microbial lipases: Some hot issues and recent developments. *Biotechnol. Adv.* 26, 457-470.

Joseph, T., Sahoo, S. and Halligudi, S. B. (2005). Bronsted acidic ionic liquids: A green, efficient and reusable catalyst system and reaction medium for Fischer esterification. *Journal of Molecular Catalysis A Chemical*. 234(1-2), 107-110.

Kallenberg, A. I., van Rantwijk, F. and Sheldon, R. A. (2005). Immobilization of penicillin G acylase: the key to optimum performance. *Adv. Synth. Catal*. 347 (7/8), 905 – 926

Kang, Y., He, J., Guo, X. D., Guo, X. and Song, Z. H. (2007). Influence of Pore Diameters on the Immobilization of Lipase in SBA-15. *Industrial and Engineering Chemistry Research*. 46, 4474–4479.

Kareem, S. O., Adio, O. Q. and Osho, M. B. (2014). Immobilization of Aspergillus niger F7-02 Lipase in Polysaccharide Hydrogel Beads of Irvingia gabonensis Matrix. *Enzyme Research*. 2014, 1-7.

Karimi, B., Emadi, S., Safaria, A. A. and Kermaniana, M. (2014). Immobilization, stability and enzymatic activity of albumin and trypsin adsorbed onto nanostructured mesoporous SBA-15 with compatible pore sizes. *RSC Advance*. 4, 4387-4394.

Katchalski, E., Silman, I. and Goldman, R. (1971). Effect of The Microenvironment on The Mode of Action of Immobilized Enzymes. *Advanced in Enzymology*. 34, 445-536.

Katchalski-Katzir, E. and Kraemer, D. M. (2000). Eupergit® C, a carrier for immobilization of enzymes of industrial potential. *J. Mol. Catalysis B: Enzymatic*, 10, 157-176.

Kavardi, S. S. S., Alemzadeh, I. and Kazemi, A. (2012). Optimization of Lipase Immobilization. *IJE Transactions C: Aspects*. 25(1), 1-9.

Kawamura, Y., Tanibe, H., Imamura, S. and Harada, J. (1996). *United States Patent. 5508185.* Retrieved on April 16, 1996, from http://www.google.com.gh/patents/US5508185

Kazlauskas, R. J. (1994). Elucidating structure-mechanism relationships in lipases: Prospects for predicting and engineering catalytic properties. *Trends Biotechnol.* 12, 464-472.

Keng, P. S., Basri, M., Rahman, M. B. A., Salleh, A. B. Rahman, R. N. Z. A. and Ariff, A. (2005). Optimisation of palm-based wax esters production using statistical experimental designs. *J. Oleo Science.* 54(10), 519-528.

Kennedy, J. F. (1987). *Enzyme technology*. In Rehm, H. J. and Reed, G. (Eds). *Biotechnology*, Vol 7a (pp. 347-404). Weinheim, Germany: VCH.

Kenneny, J. F. and Cabral, J. M.S. (1985). *Immobilization of biocatalysts by metal-link/chelation process*. In Woodward, J. (Ed.). *Immobilized enzymes and cells: a practical approach.* (19-38). Oxford: IRL Press.

Khmelnitsky, Y. L. and Rich, J. O. (1999). Biocatalysis in nonaqueous solvents. *Curr. Opin. Chem. Biol.* 3(1), 47-53.

Kim, T. W., Kleitz, F., Paul, B. and Ryoo, R. J. (2005). MCM-48-like Large Mesoporous Silicas with Tailored Pore Structure: Facile Synthesis Domain in A Ternary Triblock Copolymer-butanol-water System. *J. American Chemical Society*. 127(20), 7601-7610.

Kim, Y. S. and Jurng. J. (2011). Gold nanoparticle-based homogeneous fluorescent aptasensor for multiplex detection. *Analyst*. 136, 3720-3724.

Kiran, K. R. and Divakar, S. (2001). Lipase catalyzed synthesis of organic esters of lactic acid in nonaqueous media. *J. Biotech*. 87, 109-121.

Kiran, K. R., Krishna, S. H., Suresh, B. C. V., Karanth, N. G. and Divakar, S. (2000). An esterification method for determination of lipase activity. *Biotechnology Letters.* 22, 1511–1514.

Klabunovskii, E.I. (1991). Assymetric Hydrogenation on Metals. *Russian Chemical Review*. 60(9), 980-995.

Klein, M. P., Scheeren, C. W., Lorenzoni, A. S. G., Dupont, J. and Frazzon, H. P.F. (2011). Ionic liquid-cellulose film for enzyme immobilization. *Process Biochem*. 46, 1375–1379.

Kleitz, F., Choi, S. H. and Ryoo, R. (2003). Cubic Ia3d Large Mesoporous Silica: Synthesis and Replication to Platinum Nanowires, Carbon Nanorods and Carbon Nanotubes. *Chemical Communications.* 9(17), 2136-2137.

Klibanov, A. M. (2001). Improving enzymes by using them in non-aqueous solvents. *J Biol Chem.* 409(11), 241 –246.

Knezevic, Z. D., Marinkovic, S. S. S. and Mojovic, L. V. (2004). Immobilized lipases as practical catalysts. *APTEFF*. 35, 151-164.

Kobayashi, J., Mori, Y. and Kobayashi, S. (2006). Novel immobilization method of enzymes using a hydrophilic polymer support. *Chem. Commun*. 40, 4227-4229.

Koch-Schmidt, A. and Mosbach, K. (1977). Studies on conformation of soluble and immobilized enzymes using differential scanning calorimetry. 1. Thermal stability of nicotinamide adenine dinucleotide dependent dehydrogenases. *Biochemistry*. 16(10), 2101-2105.

Koshland, D. E. Jr., (1958) Application of a Theory of Enzyme Specificity to Protein Synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 44(2), 98-104.

Krause, P., Hilterhaus, L., Fieg, G., Liese, A. and Bornscheuer, U. (2009). Chemically and enzymatically catalyzed synthesis of C6–C10 alkyl benzoates. *Eur. J. Lipid Sci. Technol.* 111, 194–201.

Kresge, C. T., Leonowicz, M. E., Roth, W. J., Vartuli, J. C. and Beck, J. S. (1992). Ordered Mesoporous Molecular Sieves Synthesized by A Liquid-crystal Template Mechanism. *Nature*. 359(6397), 710-712.

Krishna, H. S., Sattur, A. P. and Karanth, N.G. (2001). Lipase-catalyzed synthesis of isoamyl isobutyrate-optimisation using a central composite rotatable design. *Process Biochemistry*. 37(1), 9-16.

Krishnarao, R. V., Subrahmanyam, J. and Jagadish, K. T. (2001). Studies on the formation of black particles in rice husk silica ash. *J. Eur. Ceram. Soc.* 21, 99–104.

Kyzas, G. Z., Fu, J. and Matis, K. A. (2013). The Change from Past to Future for Adsorbent Materials in Treatment of Dyeing Wastewater. *Materials*. 6, 5131-5158.

Laane, C., Boeren, S., Vos, K. and Veeger, C. B. (1987). Rules for optimization of biocatalysis in organic solvents. *Biotechnol. Bioeng.* 30, 81-87.

Laboret, F. and Perraud, R. (1999). Lipase-catalyzed production of short-chain acids terpenyl esters of interest to the food industry. Appl. Biochem. Biotechnol. 82(3), 185-98.

Lakshmi, U. R., Vimal Chandra, S., Indra Deo, M. and Lataye, D. H. (2009). Rice husk ash as an effective adsorbent: Evaluation of adsorptive characteristics for Indigo Carmine dye. *J. Environ. Manage.* 90, 710–720.

Lanning, F. C. (1963). Silicon in rice. *Journal of Agricultural and Food Chemistry*. 11 (5), 435-437.

Lawrence, G., Baskar, A. V., El-Newehy, M. H., Cha, W. S., Al-Deyab, S. S. and Vinu, A. (2015). Quick high-temperature hydrothermal synthesis of mesoporous materials with 3D cubic structure for the adsorption of lysozyme. *Sci. Technol. Adv. Mater.* 16(024806), 1-11.

Leffingwell & Associates, 2015. http://www.leffingwell.com/top_10.htm.

Li, W. and Zhao, D. (2013). An overview of the synthesis of ordered mesoporous materials. *Chem. Commun.* 49, 943-946.

Li, Y., Gao, F., Wei, W., Qu, J. B., Ma, G. H., Zhou, W. (2010). Pore size of macroporous polystyrene microspheres affects lipase immobilization. *J Mol Catal B Enzym*. 66, 182–189.

Lim, M. H. and Stein, A. (1999). Comparative Studies of Grafting and Direct Syntheses of Inorganic-Organic Hybrid Mesoporous Materials. *Chem. Mater*. 11, 3285-3295.

Lim, M. H., Blanford, C. F. and Stein, A. (1997). Synthesis and Characterization of a Reactive Vinyl-Functionalized MCM-41: Probing the Internal Pore Structure by a Bromination Reaction. *J. Am. Chem. Soc*. 119, 4090-4091.

Lima, F. V., Pyle, D. L. and Asenjo, J. A. (1997). Reaction Kinetics of the esterification of lauric acid in Iso-octane using an immobilized biocatalyst. *Appl. Biochem. Biotechnol.* 61, 411-422.

Lima, L. N., Aragon, C. C., Mateo, C., Palomo, J. M., Giordano, R. L. C., Tardioli, P. W., Guisán, J. M. and Fernández-Lorente, G. (2013). Immobilization and stabilization of a bimolecular aggregate of the lipase from Pseudomonas fluorescens by multipoint covalent attachment. *Process Biochem.* 48, 118–123.

Lin, K. M. (1975). *The study on the manufacture of particle-board made of China fir flakes and hulls.* Master thesis. National Chung-Hsing University,China.

Liu, X., Tian, B., Yu, C., Gao, F., Xie, S., Tu, B., Che, R., Peng, L. and Zhao, D. (2002). Room temperature synthesis in acidic media of large-pore-three-dimensional bicontinuous mesoporous silica with *Ia3d* symmetry. *Angew. Chem. Int. Engl.* 41(20), 3876-3878.

Liu, Y. L., Hsu, C. Y. and Hsu, K. Y. (2005). Poly(methylmethacrylate)-silica nanocomposites films from surface-functionalized silica nanoparticles. *Polymer*. 46, 1851–1856.

Ller, R. K. (1979). *The chemistry of silica: solubility, polymerization, colloid and surface properties, and biochemistry*. United States: John Wiley & Sons Inc.

Lomako, O. V., Menyailova, I. I. Nakhapetyan, L. A., Nikitin, Y. and Kiselev, A. V. (1982). Immobilization of Glucoamylase on Porous Silicas. *Enzyme Microb. Technol.* 4, 89 92.

Longo, M. A. and Sanroman, M. A. (2006). Production of food aroma compounds: microbial and enzymatic methodologies. *Food Technol. Biotechnol*. 44, 335–353.

Lowe, J. B. and Baker, R. T. (2014). Deformation of Ordered Mesoporous Silica Structures on Exposure to High Temperatures. *Journal of Nanomaterials*. 754076, 1-13.

Lu, Y., Guo, Y., Wang, Y., Liu, X., Wang, Y., Guo, Y., Zhang, Z. and Lu, G. (2008). Immobilized penicillin G acylase on mesoporous silica: The influence of pore size, pore volume and mesophases. *Microporous and Mesoporous Materials*. 114(1–3), 507–510.

Luckarift, H. R., Spain, J. C., Naik, R. R. and Stone, M. O. (2004). Enzyme immobilization in a biomimetic silica support. *Nature Biotechnology*. 22, 211–213.

Lue, B-M., Karboune, S., Yeboah, F. and Kermasha, S. (2005). Lipase-catalyzed esterification of cinnamic acid and oleyl alcohol in organic solvent media. *J. Chem. Technol. Biotechnol.* 80(4), 462 – 468.

Macedo, G. A., Lozano, M. M. S. and Pastore, G. M. (2003). Enzymatic synthesis of short chain citronellyl esters by a new lipase from Rhizopus sp. *Journal Biotechnol.* 6, 72-75.

Macquarrie, D. J. (1996). Direct Preparation of Organically Modified MCM-type materials. Preparation and characterisation of aminopropyl-MCM and 2-cyanoethyl-MCM. *Chem. Comm*. 16, 1961-1962.

Macrae, A. R. (1983). Lipase – catalyzed interesterification of oils and fats. *J. Am. Oil. Chem. Soc.* 60, 243-246.

Magner, E. (2013). Immobilisation of enzymes on mesoporous silicate materials. Chem. Soc. Rev. 42, 6213–6222.

Mahapatra, P., Kumari, A., Garlapati, V. K., Banerjee, R. and Nag, A. (2009). Enzymatic synthesis of fruit flavor esters by immobilized lipase from *Rhizopus oligosporus* optimized with response surface methodology. *Journal of Molecular Catalysis B: Enzymatic*. 60(1-2), 57-63.

Makino, K., Maruo, S. I., Morita, Y. and Takeuchi, Y. (2004). A study on the glutaraldehyde activation of hydrophilic gels for immobilized enzymes. *Biotechnology. Bioengeneering.* 31(6), 617-619.

Mandake, M. B., Anekar, S. V. and Walke, S. M. (2013). Kinetic Study of Catalyzed and Uncatalyzed Esterification Reaction of Acetic acid with Methanol. *American International Journal of Research in Formal, Applied & Natural Sciences*. 3(1), 114-121.

Marangoni, A. G. (2002). *Lipases: Structure, Function and Properties*. In Kuo, T. M. and Gardner, H. W. (Ed.) *Lipid Biotechnology* (pp. 360- 386). New York: Marcel Dekker Inc.

Marlot, C., Lanagrand, G., Triantaphylides, C. and Baratti, J. (1985). Ester synthesis in organic solvent catalyzed by lipases immobilized on hydrophilic supports. *Biotechnology Letters*. 7, 647-650.

Marthala, V. R. R., Friedrich, M., Zhou, Z., Distaso, M., Reuss, S., Al-Thabaiti, S. A., Peukert, W., Schwieger, W. and Hartmann, M. (2015). Zeolite-Coated Porous Arrays: A Novel Strategy for Enzyme Encapsulation. *Advanced Functional Materials.* 25 (12), 1832–1836.

Martinez, M., Oliveros, R. N. and Aracil, J. (2011). Synthesis of biosurfactants: Enzymatic esterification of diglycerol and oleic acid. 1. Kinetic modeling. *Ind Eng Chem Res.* 50, 6609–6614.

Marwa, N. E-N., Shaaban, M.H., Fayed, A.T. and Hassan, M. F. (2014). Optical Nanosensor Design for The Detection of Heavy Metals Using Chalcone Isothiocyanate Embedded into Mesoporous Silicates. *Int. J. Chemical & Applied Biological Sciences*. 1(5), 40-47.

Mateo, C., Abian, O., Fernández-Lafuente, R. and Guisan, J. M. (2000). Increase in conformational stability of enzymes immobilized on epoxy-activated supports by favoring additional multipoint covalent attachment. *Enzyme Microb. Technol*. 26, 509–515.

Mathe, C., Devineau, S., Aude, J. C., Lagniel, G., Chedin, S., Legros, V., Mathon, M. H., Renault, J. P., Pin, S., Boulard, Y. and Labarre, J. (2013). Structural Determinants for Protein adsorption/nonadsorption to Silica Surface. 8(11), 1-13.

Mattiasson, B. and Kaul, R. (1991). *Determination of coupling yields and handling of labile proteins in immobilization technology*. In: Taylor, R. F. (Ed.). *Protein immobilization. Fundamentals and Applications*. (pp. 161-179). New York: Marcel Dekker.

Maugard, T., Rejasse, B. and Legoy, M. D. (2002). Synthesis of water-soluble retinol derivatives by enzymatic method. *Biotechnol. Prog*. 18, 424-428.

Mbaraka, I. K. and Shanks, B. H. (2006). Conversion of oils and fats using advanced mesoporous heterogeneous catalysts. *JAOCS*, 83, 79–91.

Means, G. A. and Feeney, R. E. (1971). *Chemical Modification of Proteins* . San Francisco, Cambridge, London, Amsterdam: Holden Day, Inc.

Melde, B. J. and Johnson, B. J. (2010). Mesoporous Materials in Sensing: Morphology and Functionality at The Meso-interface. *Analytical and Bioanalytical Chemistry*. 398(4), 1565-1573.

Melero, J. A., Stucky, G., van Grieken, R. and Morales, G. (2002). Direct Syntheses of Ordered SBA-15 Mesoporous Materials Containing Arenesulfonic Acid Groups. *Journal of Materials Chemistry*. 12(6), 1664-1670.

Melo, L. L. M. M., Pastore, G. M. and Macedo, G. A. (2005). Optimized synthesis of citronellyl flavour esters using free and immobilized lipase from *Rhizopus* sp. *Process Biochemistry*. 40(10), 3181-3185.

Menaa, B., Herrero, M., Rives, V., Lavrenko, M. and Eggers, D. K. (2008). Favourable influence of hydrophobic surfaces on protein structure in porous organically-modified silica glasses. *Biomaterials*. 29(18), 2710–2718.

Mendes, A. A., Oliveira, P. C. and Castro, H. F. (2012) Properties and bio - technological applications of porcine pancreatic lipase. *J Mol Catal B Enzym*. 78, 119–134.

Mendes, A., Giordano, R. C., Giordano, R. L. C. and Castro, H. (2011). Immobilization and stabilization of microbial lipases by multipoint covalent attachment on aldehyde-resin affinity: Application of the biocatalysts in biodiesel synthesis. *J. Mol. Catal. B: Enzymatic*. 68, 109–115.

Menezes, E. W., Lima, E. C., Royer, B., Souza, F. E., Santos, B. D., Gregorio, J. R., Costa, T. M. H., Gushikem, Y. and Benvenutti, E. V. (2012). Ionic silica based hybrid material containing the pyridinium group used as an adsorbent for textile dye. *Journal of Colloid and Interface Science*. 378, 10-20.

Messing, R. A. (1976). *Adsorption and inorganic bridge formations*. In: Mosbach, K. (Ed.). *Methods in Enzymology, Volume XLIV*. (pp. 148-169). New York: Academic Press.

Mihaela, M., Anne, G., Gilbert, R. and Francüois, F. (2005). A New Mesoporous Micelle-Templated Silica Route for Enzyme Encapsulation. *Langmuir*. 21, 4648-4655.

Milasinovic, N., Knezevic-Jugovic, Z., Jakovljevic, Z., Filipovic, J. and Kalagasidis Krušić, M. K. (2012). Synthesis of n-amyl isobutyrate catalyzed by Candida rugosa lipase immobilized into poly(N-isopropylacrylamide-co-itaconic acid) hydrogels. *Chemical Engineering Journal*. 181-182, 614–623.

Minovska, V., Winkelhausen, E., and Kuzmanova, S. (2005). Lipase immobilized by different techniques on various support materials applied in oil hydrolysis. *J. Serb. Chem. Soc*. 70 (4), 609–624.

Miranda, M., Pardo, J. P. and Petrov, V. V. (2011). Structure-function relationships in membrane segment 6 of the yeast plasma membrane Pma1 H(+)-ATPase. *Biochim. Biophys. Acta.* 1808(7), 1781-1789.

Misson, M., Zhang, H. and Jin, B. (2015). Nanobiocatalyst advancements and bioprocessing applications. *J. R. Soc. Interface*. 12, 1-20.

Mitz, M.A. (1956).New Soluble Active Derivatives of An Enzyme as A Model for Study of Cellular Matabolism. *Science*. 123,1076-1077.

Mizuki, T., Sawai, M., Nagaoka, Y., Morimoto, H. and Maekawa, T. (2013). Activity of Lipase and Chitinase Immobilized on Superparamagnetic Particles in a Rotational Magnetic Field. *PLoS ONE*. 8(6), e66528.

Mobarak-Qamsari, E., Kasra-Kermanshahi, R. and Moosavi-nejad, Z. (2011). Isolation and identification of a novel, lipase-producing bacterium, Pseudomnas aeruginosa KM110. *Iran J. Microbiol*. 3(2), 92–98.

Moller, K. and Bein, T. (1998). Inclusion Chemistry in Periodic Mesoporous Hosts. *Chem. Mater*. 10(10), 2950-2963.

Moon, I. K., Kim, J., Lee, J., Shina, S., Na, H. B., Hyeon, T., Park, H.G. and Chang, H. N. (2008). One-dimensional crosslinked enzyme aggregates in SBA-15: Superior catalytic behavior to conventional enzyme immobilization. *Microporous and Mesoporous Materials*. 111(1–3), 18–23.

Moriguchi, H., Hirata, J. and Watanabe, T. (1990). *Microorganism based agent for treatment of organic wastes*. Japanese Patent 2105899.

Mureseanu, M., Trandafir, I., Babeanu, C., Parvulescu, V. and Paun, G. (2016). Laccase Immobilized on Mesoporous Silica Supports as an Efficient System for Wastewater Bioremediation. *Environment Protection Engineering*. 42(2), 81-95.

Murty, V. R., Bhat, J. and Muniswaran, P. K. A. (2002). Hydrolysis of Oils by Using Immobilized Lipase Enzyme: A Review. *Biotechnol. Bioprocess Eng*. 7, 57-66.

Nakajima, M., Snape, J. and Khare, S. K.(2000). *Method in Non-Aqueous Enzymology*. In Gupta, M. N. (Ed.). *Biochemistry* (pp. 52-69). Basel, Switzerland: Birkhauser Verlag.

Nakamura, K. and Nasu, T. (1990). *Japanese Patent 2,208,400.90.*

Naoe, K., Ohsa, T., Kawagoe, M. and Imai, M. (2001). Esterification by Rhizopus delemar lipase in organic solvent using sugar ester reverse micelles. *Biochemical Enginerering Journal*. 9(1), 67-72.

Neerupma. N., Singh, R. and Kaur, J. (2006). Immobilization and stability studies of a lipase from thermophilic Bacillus *sp*: The effect of process parameters on immobilization of enzyme. *J. Biotechnol.* 9, 559-565.

Nelson, J.M. and Griffin, E.G. (1916). Adsorption of Invertase. *J. American Chemical Society.* 38(5),1109-1115.

O'Driscoll, K. F. (1976). *Techniques of enzyme entrapment in gels*. In: Mosbach, K. (Ed.). *Methods in Enzymology, Volume XLIV*. (pp. 169-183). New York: Academic Press.

Orrego, C. E., Salgado, N., Valencia, J. S., Giraldo, G. I., Giraldod, O. H. and Cardonae, C. A. (2010). Novel chitosan membranes as support for lipases immobilization: characterization aspects. *Carbohydrate Polymers*. 79, 9–16.

Ozyilmaz, G. and Gezer, E. (2010). Production of aroma esters by immobilized Candida rugosa and porcine pancreatic lipase into calcium alginate gel. *Journal of Molecular Catalysis B: Enzymatic*. 64(3-4), 140–145.

Padmini, P., Rakshit, S. K. and Baradarajan, A. (1993). Lipase catalysed hydrolysis of ricebran oil by free and immobilised enzyme system in batch stirred reactor. *Bioprocess Engineering*. 9, 103-106.

Palomo, J. M., Munoz, G., Fernández-Lorente, G., Mateo, C., Fernández-Lafuente, R. and Guisán, J. M. (2002). Interfacial adsorption of lipases on very hydrophobic support (Octadecyl-Sepabeads): immobilization, hyperactivation and stabilization of the open form of lipases. *J. Mol. Catal. B: Enzym.* 19–20, 279–286.

Pandey, A., Benjamin, S., Soccol, C. R., Nigam, P., Krieger, N. and Soccol, V. T. (1999). The realm of microbial lipases in biotechnology. *Biotechnol Appl Biochem*. 2, 119-131.

Pandey, A., Benjamin, S., Soccol, C. R., Nigam, P., Krieger, N. and Soccol, V. T. (1999). The Realm of Microbial Lipases in Biotechnology. *Biotechnological Applied Biochemistry.* 29, 119–131.

Pandey, A., Selvakumar, P., Soccol, C. R. and. Nigam, P. (1999). Solid state fermentation for the production of industrial enzymes. *Curr. Sci.* 77, 149-162.

Pandya, P. H., Jasra,R. V., Newalkar, B. L. and Bhatt, P. N. (2005). Studies on the activity and stability of immobilized α-amylase in ordered mesoporous silicas. *Microporous and Mesoporous Materials*. 77(1), 67–77.

Park, E. Y., Sato, M. and Kojima, S. (2006). Fatty acid methyl ester production using lipase-immobilizing silica particles with different particle sizes and different specific surface areas. *Enzyme and Microbial Technology*. 39 (4), 889-896.

Park, M., Park, S. S., Selvaraj, M., Kim, I. and Ha, C-S. (2010). Hydrophobic periodic mesoporous organosilicas for the adsorption of cytochrome c. *J. Porous Mater.* 18(2) 217-223.

Park, M., Park, S. S., Selvaraj, M., Zhao, D. and Ha, C-S. (2009). Hydrophobic mesoporous materials for immobilization of enzymes. *Microporous and Mesoporous Mater.* 124, 76-83.

Park, S. W., Kim, Y. I., Chung, K. H. and Kim, S. W. (2001). Improvement of stability of immobilized GL-7-ACA acylase through modification with glutaraldehyde. *Proc. Biochem.* 37(2), 153-163.

Paul, C. S. (2002). *The HPLC Solvent Guide.* (2nd Ed.). New York: Wiley-Interscience.

Pedersen, N. R., Wimmer, R., Emmersen, J., Degn. P. and Pedersen, L. H. (2002). Effect of fatty acid chain length on initial reaction rates and regioselectivity of lipase–catalyzed esterification of disaccharides. *Carbohyd. Res.* 337(13), 1179-1184.

Pencreaćh, G., Leullier, M. and Baratti, J. C. (1997). Properties of free and immobilized lipase from Pseudomonas cepacia. *Biotechnol. Bioeng.* 56(20), 181-189.

Pereira, E. B., Zanin, G. M. and Castro, H. F. (2003). Immobilization and catalytic properties of lipase on chitosan for hydrolysis and esterification reactions. *Brazilian Journal of Chemical Engineering*. 20, 343-355.

Petersen, S. B. (2005). Protein Engineering: Design and Engineering on the Nano Scale . In Straathof , A. J. J. and Adlercreutz, P. (Eds.) Applied Biocatalysis, 2nd Edition. (pp. 263-310). Amsterdam: Harwood Academic Publications.

Pifferi, P. G., Tramontini, M. and Malacarne, A. (1989). Immobilization of endo-polygalacturonase from Aspergillus niger on various types of macromolecular supports. Biotechnol. Bioeng., 33(10), 1258-1266.

Pires, E. L., Miranda, E. A. and Valença, G.P. (2002). Gas-phase enzymatic esterification on immobilized lipases in MCM-41 molecular sieves. *Appl Biochem Biotechnol.* 98-100, 963-76.

Pitcher W.H. (1980). *Immobilized enzymes for food processing*. Florida: CRC Press.

Plagemann, R., von Langermann, J. and Kragl, U. (2014). Microwave-assisted covalent immobilization of enzymes on inorganic surfaces. *Engineering in Life Scien*ces. 14(5), 493-499.

Poppe, L. and Lajos, N. (1992). *Selective Biocatalysis: A Synthetic Approach*. Wenheim, Germany: VCH.

Porath, J. and Axén, R. (1976*). Immobilization of enzymes to agar, agarose, and Sephadex supports*. In: Mosbach, K. (Ed.). *Methods in Enzymology, Volume XLIV*. (pp. 19-45). New York: Academic Press.

Prasad, R. and Pandey, M. (2012). Rice Husk Ash as a Renewable Source for the Production of Value Added Silica Gel and its Application: An Overview. *Bulletin of Chemical Reaction Engineering & Catalysis*. 7(1), 1–25.

Pronk, W., Boswinkel, G. and Riet, K. (1992). Parameters influencing hydrolysis kinetics of lipase in ahydrophilic membrane bioreactor. *Enzyme Microb. Technol.* 14, 214-220.

Protein data Bank : http://www.rcsb.org/pdb/home/home.do (PDB ID:1CRL)

Queiroz, J. A., Tomaz, C. T. and Cabral, J. M. S. (2001). Hydrophobic interaction chromatography of proteins. *Journal of Biotechnology*. 87, 143-159.

Quiocho, F.A. and Richards, F.M. (1964). Intermolecular Cross Linking of a Protein in The Crystalline State: Carboxypeptidase-A. *Proc. Natl. Acad. Sci.U.S.A.* 52(3), 833-839.

Ra. S. (2008). *Waste Materials and By-Products in Concrete*. London: Springer.

Rabolt, J. F., Burns, F. C., Schlotter, N. E. and Swalen, J. D. (1983). Anisotropic orientation in molecular monolayers by infrared spectroscopy. *J. Chem. Phys.* 78, 946–952.

Radva, D., Spanyol, J. and Kosary, J. (2011). Testing of the Effect of Reaction Parameters on the Enzyme Immobilization by Adsorption and Cross-Linking Processes with Kinetic Desorption Method. *Food Technol. Biotechnol*. 49(2), 257–262.

Rahman, M. B. A., Yong, K. C., Basri, M., Rahman, R. N. Z. A., Razak, C. N. A. and Salleh, A. B. (2001). Synthesis of oleyl oleate, a wax ester using lipozyme. *Malaysian Journal of Chemistry.* 3, 46-50.

Rahman, R. N. Z. R. A., Baharum, S. N., Basri, M. and Salleh, A. B. (2005). High-yield purification of an organic solvent-tolerant lipase from Pseudomonas sp. strain S5. *Anal. Biochem*. 341.267–74.

Ranjbakhsh, E., Bordbar, A. K., Abbasi, M., Khosropour, A. R. and Shams, E. (2012). Enhancement of stability and catalytic activity of immobilized lipase on silica-coated modified magnetite nanoparticles. *Chem. Eng. J.* 179, 272–276.

Ravindra, R., Zhao, S., Gies, H. and Winter, R. (2004). Protein encapsulation in mesoporous silicate: The effects of confinement on protein stability, hydration, and volumetric properties. *J. Am. Chem. Soc.* 126, 12224-12225.

Real, C., Alcala, D., Maria, C. and Jose, M. (2008). Preparation of silica from rice husks. *J. Am. Ceram. Soc.* 79(8), 2012–2016.

Romero, M. D., Calvo, L., Alba, C., Habulin, M., Primozic, M. and Knez, Z. (2005). Enzymatic synthesis of isoamyl acetate with immobilized *Candida antarctica* lipase in supercritical carbon dioxide. *Journal of Supercritical Fluids*. 33(1), 77–84.

Ruren, X., Wenqin, P., Jihong, Y., Qisheng, H. and Jiesheng, C. (2007). *Chemistry of Zeolites and Related Porous Materials:Synthesis and Structure*. Clementi Loop, Singapore : John Wiley & Sons (Asia).

Ryoo, R., Kim, J. M., Ko, C. H. and Shin, C. H. (1996). Disordered Molecular Sieve with Branched Mesoporous Channel Network. *J. Physical Chemistry*. 100(45), 17718-17721.

Sabularse, V. C., Tud, M. T., Lacsamana, M. S. and Solivas, J. L. (2005). Black and white lahar as inorganic support for the immobilization of yeast invertase. *ASEAN J. Sci. Technol. Dev.* 22, 331–344.

Sahare, P., Ayala, M., Vazquez-Duhalt, R. and Agrawal, V. (2014). Immobilization of peroxidase enzyme onto the porous silicon structure for enhancing its activity and stability. Nanoscale Res Lett. 9(1), 409.

Sakamoto, Y., Gao, C., Che, S. and Terasaki, O. (2006). TEM Studies of Bicontinuous Cubic Mesoporous Crystals. *5th International Mesostructured Materials Symposium (IMMS2006)*. August 5-7 2006. Shanghai, P.R.China, 207-210.

Salah, B. R., Ghamghui, H., Miled, N., Mejdoub, H. and Gargouri Y. (2007). Production of butyl acetate by lipase from novel of *Rhizopus oryzae. J. Biosci. Bioeng*. 104, 268-372.

Salama, T. M., Ali, I. O., Gumaa, H. A., Lateef, M. A. and Bakr, M. F. (2016). Novel Synthesis of Nay Zeolite from Rice Husk Silica: Modification with Zno and Zns for Antibacterial Application. *Chem. Sci. J.* 7(1), 1-9.

Salis, A., Casula, M. F., Bhattacharyya, M. S., Pinna, M., Solinas, V. and Monduzzi, M. (2010). Physical and chemical lipase adsorption on SBA-15: effect of different interactions on enzyme loading and catalytic performance. *Chem. Cat. Chem*. 2, 322–329.

Sanchez, A., Rio, J. L., Valero, F., Lafuente, Faus, I. and Sola, C. (2000). Continuous enantioselective esterification of trans-2-phenyl-1-cyclohexanol using a new *Candida rugosa* lipase in a packed bed bioreactor. *J. Biotech*. 84, 1-12.

Sanjay, G. and Sugunan, S. (2006). Enhanced pH and thermal stabilities of invertase immobilized on montmorillonite K-10. *Food Chemistry*. 94, 573–579.

Saphir, J. (1967). *West Germany Patent 1, 242,794.*

Saponjic, S., Knezevic-Jugovic, Z. D., Bezbradica, D. I., Zuza, M. G., Saied, O. A., · Boskovic-Vragolovic, N. and Mijin, D. Z. (2010). Use of *Candida rugosa* lipase immobilized on sepabeads for the amyl caprylate synthesis: Batch and fluidized bed reactor study. *Process Biotechnology*. 13(6), 1-8.

Saxena, R. K., Ghosh, P. K., Gupta, R., Sheba, D. W., Bradoo, S. and Gulati, R. (1999). Microbial lipases, potential biocatalysts for the future industry. *Curr. Sci.* 77, 101-115.

Schlenker, J. L. (1992). A new family of mesoporous molecular sieves prepared with liquid crystal templates. *Journal of the American Chemical Society*. 114,10834-10843.

Schrader, J., Etschmann, M. M. W., Sell, D., Hilmer, J.-M. and Rabenhorst, J. (2004). Applied biocatalysis for the synthesis of natural flavour compounds – current industrial processes and future prospects. *Biotechnol. Lett.* 26, 463–472.

Seema, S. B. and Steven, H. N. (2002). Immobilization of lipase using hydrophilic polymers in the form of hydrogel beads. *Biomaterials*. 23, 3627–3636.

Serra, E., Mayoral, A., Sakamoto, Y., Blanco, R. M. and Diaz, I. (2008). Immobilization of lipase in ordered mesoporous materials, Effect of textural and structural parameters. *Microporous Mesoporous Mater*. 114, 201–213.

Serra, E., Mayoral., A., Sakamoto, Y., Blanco, R. M. and Diaz, I. (2008). Immobilization of lipase in ordered mesoporous materials: Effect of textural and structural parameters *Microporous and Mesoporous Materials*. 114(1–3), 201–213.

Serri, A., Kamaruddin, A. H. and Long, S. (2006). Studies of reaction parameters on synthesis of Citronellyl laurate ester via immobilized Candida rugosa lipase in organic media. *Bioprocess Biosyst. Eng.* 29, 253-260.

Sharma, R., Chisti, Y. and Banerjee, U. C. (2001). Production, purification, characterization and applications of lipases. *Biotechnol. Adv*. 19, 627-662.

Sharma, R., Chisti, Y. and Banerjee, U. C. (2001). Production, purification, characterization, and applications of lipases. *Biotechnology Advances*. 19(8), 627–662.

Sharma, S., Kaur, P., Jain, A., Rajeswari, M. R. and Gupta, M. N. (2003). A smart bioconjugate of chymotrypsin. *Biomacromolecules*.4, 330–336.

Sheldon, R. A. (2007). Enzyme immobilization: The quest for optimum performance. *Adv. Synth. Catal.* 349, 1289–1307.

Sheldon, R. A. (2007). Enzyme immobilization: The quest for optimum performance. *Adv. Synth. Catal*. 349, 1289–1307.

Sheldon, R. A. and van Pelt, S. (2013). Enzyme immobilisation in biocatalysis: why, what and how. *Chem. Soc. Rev*. 42(15), 6223-6235.

Shelke, V. R., Bhagade, S. S., Mandavgene, S. A. (2010). Mesoporous silica from rice husk ash. *Bull. Chem. React. Eng. Catal.* 5(2), 63–67.

Shieh, C. J. and Chang, S. W. (2001). Optimized synthesis of lipase–catalyzed hexyl acetate in n-hexane by response surface methodology. *J. Agric. Food Chem*. 49(3), 1203-1207.

Shin, Y., Lee, D., Lee, K., Ahn, K. H. and Kim, B. (2008). Surface properties of silica nanoparticles modified with polymers for polymer nanocomposite applications. *J. Ind. Eng. Chem.* 14, 515–519.

Shuler, M. L. and Kargi, F. (1992). Bioprocess engineering: basic concepts. (6^{th} ed.). Englewood Cliffs, N.J: Prentice Hall.

Silva, C. J. S. M., Sousa, F., Gubitz, G. and Paulo, A. C. (2004). Crosslinking of Proteins with Glutaraldehyde. *Food Technol. Biotechnol.* 42 (1), 51–56.

Sindorf, D.W. and Maciel, G.E. (1983). Solid-state NMR Studies of The Reactions of Silica Surfaces with Polyfunctional Chloromethylsilanes and Ethoxymethylsilanes. J. *American Chemical Society*. 105, 3767-3776.

Singh, R. K., Tiwari, M. K., Singh, R. and Lee, J. K. (2013). From Protein Engineering to Immobilization: Promising Strategies for the Upgrade of Industrial Enzymes. *Int. J. Mol. Sci*. 14(1), 1232-1277.

Singh, R. K., Zhang, Y. W., Nguyen, N. P., Jeya, M. and Lee, J. K. (2010). Covalent immobilization of β -1,4-glucosidase from Agaricus arvensis onto functionalized silicon oxide nanoparticles. *Appl. Microbiol. Biotechnol*. 89, 337–344.

Smythe, C. V. (1951). Microbiological production of enzymes and their industrial application. *Econ. Bot.* 5, 126-144.

Snyder, R. L. and Lifshin, E (Ed.) (1999). *X-Ray Diffraction, in X-ray Characterization of Materials.* Weinheim, Germany: Wiley-VCH Verlag GmbH.

Soo, E. L., Salleh, A. B., Basri, M., Rahman, R. N. Z. A. and Kamaruddin, K. (2003). Optimisation of enzyme-catalysed synthesis of amino acid-based surfactants from palm oil fractions. *J. Bioscience and Bioengineering*. 95, 361-367.

Srivastava, A. and Prasad, R. (2000). Triglycerides-based diesel fuels. *Renewable Sustainable Energy Rev.* 4, 111-133.

Stan, D., Mihailescu, C., Savin, M. and Matei, I. (2013). 2-(2-Hydroxy-5-nitrobenzylidene)-1,3-indanedione versus Fluorescein Isothiocyanate in Interaction with Anti-hFABP Immunoglobulin G1: Fluorescence Quenching, Secondary Structure Alteration and Binding Sites Localization. *Int. J. Mol. Sci.* 14, 3011-3025.

Stergiou, P. Y., Foukis, A., Filippou, M., Koukouritaki, M., Parapouli, M.,Theodorou, L. G., Hatziloukas, E., Afendra, A., Pandey, A. and Papamichael, E. M. (2013). Advances in lipase-catalyzed esterification reactions. *Biotechnology Advances*. 31(8), 1846–1859.

Stinson, S. C. (1995). Fine and intermediate chemical markers emphasize new products and process. *Chem. Eng. News*. 73, 10-26.

Stryer, L. (1995). *Biochemistry*. New York: Freeman.

Sumner, J. B. and Somers, G. F. (2014). *Chemistry and methods of enzymes*. (3rd ed.). New York: Academic Press.

Svendsen, A. (1994). *Sequence comparisons within the lipase family*. In Woolley, P. Y. and Petersen, S. B. (Eds.). *Lipases: Their structure, biochemistry and applications*. (pp. 1-21). Cambridge: Cambridge University Press.

Svensson, I., Aldlercreutz, P., and Mattiasson, B. (1990). Interesterification of phosphatidylcholine with lipase in organic media. *Applied Microbiology and Biotechnology*. 33(3), 255-258.

Tai, A., Harada, T. and Iwasawa, Y (Ed.). (1986). *Taylored Metal Catalysts*. Dorrecht: D. Reidel.

Takahashi, H., Li, B., Sasaki, T., Miyazaki, C., Kajino, T. and Inagaki, S. (2001). Immobilized enzymes in ordered mesoporous silica materials and improvement of their stability and catalytic activity in an organic solvent. *Micropor. Mesopor. Mater.* 44–45, 755–762.

Takahashi, H., Li, B., Sasaki, T., Miyazaki, C., Kajino, T. and Inagaki, S. (2000). Catalytic Activity in Organic Solvents and Stability of Immobilized Enzymes Depend on the Pore Size and Surface Characteristics of Mesoporous Silica. *Chem. Mater.* 12(11), 3301-3305.

Tanev, P. T. and Pinnavaia, T. J. (1995). A Neutral Templating Route to Mesoporous Molecular Sieves. *Science*. 267(5199), 865-867.

Torres, C. and Otero, C. (2001). Part III. Direct enzymatic esterification of lactic acid with fatty acids. *Enzyme and Microbial Technology*. 29(1), 3-12.

Tosa, T., Mori, T., Fuse, N., and Chibata, I. (1967). Studies on continuous enzyme reactions. Screening of carriers for preparation of water insoluble aminoacylase. *Enzymologia*. 31, 214–224.

Tran, D. N. and Balkus, K. J. (2011). Perspective of recent progress in immobilization of enzymes. *ACS Catal*. 1, 956–968.

Trevan, M. (1980) Techniques of Immobilization. In Trevan, M. (Ed.) *Immobilized Enzymes: An Introduction and Applications in Biotechnology* (pp. 1-9). Chichester-New York : John Wiley & Sons.

Tsuneo, Y., Toshio, S., Kazuyuki, K. and Chuzo, K. (1990). The Preparation of Alkyltrimethylammonium-kanemite Complexes and Their Conversion to Microporous Materials. *Bulletin of the Chemical Society of Japan*. 63(4): 988-992

UNDP. 2002. Malaysia Biomass-based power generation and cogeneration the palm oil industry. Malaysia. United Nation Development Programme

Undurraga, D., Markovits, A. and Erazo, S. (2001). Cocoa butter equivalent through enzymic interesterification of palm oil mid fraction. *Process Biochem.* 36, 933-939.

Vandamme, E. J. and Soetaert, W. (2002). Bioflavours and fragrances via fermentation and biocatalysis. *J Chem Technol Biotechnol*. 77, 1323–1332.

Vermeiren, W. and Gilson, J. P. (2009). Impact of Zeolites on the Petroleum and Petrochemical Industry. *Top Catal*. 52, 1131-1161.

Vianello, F., Zennaro, L., Di Paolo, M. L., Rigo, A., Malacarne, C. and Scarpa, C. (2000). Preparation, morphological characterization, and activity of thin films of horseradish peroxidase. *Biotechnol. Bioeng.* 68, 488-495.

Vicente, G., Martinez, M. and Aracil, J. (2004). Integrated biodiesel production: A comparison of different homogeneous catalysts systems. *Bioresour. Technol.* 92, 297-305.

Vieth, W.R. (1994). *Bioprocess engineering*. New York: John Wiley & Sons.

Villeneuve, P. (2003). Plant lipases and their applications in oils and fats modification. *Eur. J. Lipid Sci. Technol.* 105, 308–317.

Vinu, A., Gokulakrishnan, N., Balasubramanian, V. V., Alam, S., Kapoor, M. P., Ariga, K. and Mori, T. (2008). Three dimensional ultra large pore Ia3d mesoporous silica with various pore diameters and their application in biomolecule immobilization. *Chem. Eur. J.* 14(36), 11529-11538.

Vulfson, E. N. (1994). *Industrial Applications of Lipases*. In: Woolley, P. and Peterson, S. B. (Eds). *Lipases-Their Structure Biochemistry and Application* (pp. 271-288). United Kingdom, UK: Cambridge University Press.

Wadiack, D. T. and Carbonell, R. G. (1975). Kinetic behavior of microencapsulated β -galactosidase. *Biotechnol. Bioeng.* 17, 1157–1181.

Wang, A., Gao, W., Zhang, F., Chen, F., Du, F. and Yin, X. (2012). Amino acid-mediated aldolase immobilisation for enhanced catalysis and thermostability. *Bioproc. Biosyst. Eng*. 35(5), 857-863.

Wang, J., Meng, G., Tao, K., Feng, M., Zhao, X., Li, Z., Xu, H., Xia, D. and Lu, J. R. (2012). Immobilization of Lipases on Alkyl Silane Modified Magnetic Nanoparticles: Effect of Alkyl Chain Length on Enzyme Activity. *PLoS ONE*. 7(8), e43478.

Wang, P., Dai, S., Waezsada, S. D., Tsao, A. Y. and Davison, B. H. (2001). Enzyme stabilization by covalent binding in nanoporous sol-gel glass for nonaqueous biocatalysis. *Biotechnol. Bioeng.* 74, 249-255.

Wang, Y. and Caruso, F. (2005). Mesoporous Silica Spheres as Supports for Enzyme Immobilization and Encapsulation. *Chem. Mater.* 17 (5), 953–961.

Wang, Y., Xin, J., Shi, J., Wu, W. and Xia, C. (2014). A kinetic study of starch palmitate synthesis by immobilized lipase-catalyzed esterification in solvent free system. *Journal of Molecular Catalysis B: Enzymatic*. 101, 73–79.

Warmuth, W., Wenzig, E. and Mersmann, A. (1995). Selection of a support for immobilization of a microbial lipase for the hydrolysis of triglycerides. *Bioprocess Engineering.* 12 (1), 87-93.

Washmon-Kriel, L., Jimenez, V. L. and Balkus, K. J. Jr. (2000). Cytochrome c immobilization into mesoporous molecular sieves. *J. Mol. Catal. B: Enzymatic.* 10, 453–469.

Webb, G. and Wells, P.B. (1992). Assymetric Hydrogenation. *Catalysis Today*. 12(2-3), 319-337.

Weetall, H. H. (1993). Preparation of immobilized proteins covalently coupled through silane coupling agents to inorganic supports. *Appl. Biochem. Biotechnol*. 41, 157–188.

Weetall, H. H. and Filbert, A. M. (1974). *Methods in Enzymology*. New York: Academic Press.

Widmann, M., Juhl P. B. and Pleiss J. (2010) Structural Classification by the Lipase Engineering Database: A Case Study of *Candida Antarctica* Lipase A. *BMC Genomics*. 11,123.

Won, K. H., Kim, S. B., Kim, K. J., Park, H. W. and Moon, S. J. (2005). Optimization of lipase entrapment in Ca-alginate gel beads. *Process Biochemistry* 40, 2149–2154.

Wongjunda, J. and Saueprasearsit, P. (2010). Biosorption of chromium (VI) sing rice husk ask and modified rice husk ash. *Environ. Res. J*. 4(3), 244–250.

Woodward, J. (1985). *Immobilized enzymes: Adsorption and covalent coupling*. In Woodward, J. (Ed.). *Immobilized Cells and Enzymes: A Practical Approach*. (pp. 3-17). Oxford, UK: IRL.

Wu, C., G. Zhou, X. Jiang, J. Ma, H. Zhang, H. Song, Process Biochem., (2010). Active biocatalysts based on Candida rugosa lipase immobilized in vesicular silica. *Process Biochemistry.* 47(6), 953-959.

www.dosm.gov.my

Xie, W. L. and Wang, J. L. (2012). Immobilized lipase on magnetic chitosan microspheres for transesterification of soybean oil. *Biomass and Bioenergy*. 36, 373–380.

Xue, P., Xu, F. and Xu, L. (2008). Epoxy-functionalized mesostructure cellular foams as effective support for covalent immobilization of penicillin G acylase. *Applied Surface Science*. 255, 1625-1630.

Yadav, G. D. and Devendran, S. (2012). Lipase catalyzed synthesis of cinnamyl acetate via transestrification in non-aqueous medium. *Process Biochemistry*. 47(3), 496-502

Yadav, G. D. and Shinde, S. D. (2012). Kinetic Modeling and Optimization of Immobilized Candida antarctica Lipase B Catalysed Synthesis of Butyl-4-Methyl-3-Oxopentanoate using Response Surface Methodology. *Int. J. Chem. Reac. Eng*. 10, 1-21.

Yalcin. N. and Sevinc, V. (2001). Studies on silica obtained from rice husk. *Ceram. Int.* 27, 219–224.

Yang, G., Wu, J., Xu, G. and Yang, L. (2009). Improvement of catalytic properties of lipase from Arthrobacter sp. by encapsulation in hydrophobic sol-gel materials. *Bioresource Technology.* 100(19), 4311–4316.

Yang, J., Hu, Y., Jiang, L., Zou, B., Jia, R. and Huang, H. (2013). Enhancing the catalytic properties of porcine pancreatic lipase by immobiliza-tion on SBA-15 modified by functionalized ionic liquid. *J. Biochem. Eng*. 70, 46–54.

Yasutaka, K., Takato, Y., Takashi, K., Kohsuke, M. and Hiromi, Y. (2011). Enhancement in Adsorption and Catalytic Activity of Enzymes Immobilized on Phosphorus- and Calcium-Modified MCM-41. *J. Phys. Chem. B.* 115 (34), 10335–10345.

Yiu, H. H. P. and Wright, P. A. (2005). Enzymes supported on ordered mesoporous solids: A special case of an inorganic-organic hybrid. *Journal of Materials Chemistry*. 15(35-36), 3690-3700.

Yiu, H. H. P., Wright, P. A. and Botting, N. P. (2001). Enzyme immobilisation using SBA-15 mesoporous molecular sieves with functionalised surfaces. *Journal of Molecular Catalysis B: Enzymatic.* 15(1–3), 81–92.

Yiu, H. H. P., Wright, P. A. and Botting, N. P. (2001). Enzyme immobilization using siliceous molecular sieves. *Microporous Mesoporous Mater.* 4, 763-768.

Yu, C. Y., Huang, L. Y., Kuan, I. C. and Lee, S. L. (2013). Optimized production of biodiesel from waste cooking oil by lipase immobilized on magnetic nanoparticles. *Int. J. Mol. Sci*. 14, 24074–24086.

Yu, C. Z., Yu, Y. H. and Zhao, D. Y. (2000). Highly Ordered Large Caged Cubic Mesoporous Silica Structures Templated by Triblock PEO-PBO -PEO Copolymer. *Chemical. Communications*. 7, 575-576.

Yu, W. H., Tong, D. S., Fang, M., Shao, P. and Zhou, C. H. (2015). Immobilization of Candida rugosa lipase on MSU-H type mesoporous silica for selective esterification of conjugated linoleic acid isomers with ethanol. *Journal of Molecular Catalysis B: Enzymatic*. 111, 43–50.

Yu, W. H., Zhao, H. B., Tong, D. S., Zhou, C. H. and Shao, P. (2015). Immobilization of lipase onto aminopropyl-functionalized MSU-H type mesoporous silica and esterification. *Korean Journal of Chemical Engineering*. 32 (8), 1694-1697.

Zaidan, U. H., Rahman, M. B. A., Othman, S. S., Basri, M., Abdulmalek, E., Rahman, N. Z. R. A. and Salleh, A. (2012). Biocatalytic production of lactose ester catalysed by mica-based immobilised lipase. *Food Chem*. 131, 199–205.

Zaidi, A., Gainer, J. L. and Carta, G. (1995). Fatty acids esterification using Nylon-immobilized lipase. *Biotechnol. Bioeng.* 48, 601-605.

Zhang, D-H., Yuwen, L-X. and Peng, L-J. (2013). Parameters Affecting the Performance of Immobilized Enzyme. *Journal of Chemistry*. 2013, 1-7.

Zhang, Q., Han, X. and Tang, B. (2013). Preparation of a magnetically recoverable biocatalyst support on monodisperse Fe_3O_4 nanoparticles. *RSC Advances*. 3, 9924–9931.

Zhang, S., Gao, S. and Gao, G. (2010). Immobilization of β-Galactosidase onto magnetic beads. *Appl. Biochem. Biotechnol.* 160, 1386–1393.

Zhao, D., Feng, J., Huo, Q., Melosh, N., Fredrickson, G. H., Chmelka, B. F. and Stucky, G. D. (1998). Triblock Copolymer Syntheses of Mesoporous Silica with Periodic 50 to 300 Angstrom Pores. *Science*. 279(5350), 548-552.

Zhao, J. P., Cundy, C. and Dwyer, J. (1997). *Synthesis*. In Chon, H., Ihm, S.-K, and Uh, Y. S. (Eds.). *Progress in Zeolites and Microporous Materials* (pp. 181). Amsterdam: Elsevier.

Zhao, X. S., Bao, X. Y., Guo, W. and Lee, F. Y. (2006). Immobilizing catalysts on porous materials. *Materials Today*. 9(3), 32-39.

Zhao, X. S., Lu, G. Q., Whittaker, A. K., Millar, G. J. and Zhu, H. Y.(1997). Comprehensive Study of Surface Chemistry of MCM-41 Using 29Si CP/MAS NMR, FTIR, Pyridine-TPD, and TGA. *J. Phys. Chem. B.* 101(33), 6525-6531.

Zheng, M. M., Lu, Y., Dong, L., Guo, P. M., Deng, Q. C., Li, W. L., Feng, Y. Q. and Huang, F. H. (2012). Immobilization of Candida rugosa lipase on hydrophobic/strong cation-exchange functional silica particles for biocatalytic synthesis of phytosterol esters. *Bioresour. Technol*. 115, 141–146.

Ziegler, D., Formia, A., Tulliani, J-M. and Palmero, P. (2016). Environmentally-Friendly Dense and Porous Geopolymers Using Fly Ash and Rice Husk Ash as Raw Materials. *Materials*. 9(6), 1-22.

Zlateski, V., Keller, T. C., Ramíreza, J. P. and Grass, R. N. (2015). Immobilizing and de-immobilizing enzymes on mesoporous silica. *RSC Adv.* 5, 87706-87712.

Zou, B., Song, C., Xua, X., Xia, J., Huo, S., Cui, F. (2014). Enhancing stabilities of lipase by enzyme aggregate coating immobilized onto ionic liquid modified mesoporous materials. Applied Surface Science. 311, 62–67.

APPENDIX A1

Silica Extraction

Raw material used for the experiment was rice husk ash (RHA). RHA was boiled in 1 M HCl at 80 °C for 1 h. After acid pretreatment, the ash was rinsed with distilled water, dried in an oven at 110 °C overnight and then calcined in a furnace at 650 °C for 4 h. The extracted silica obtained was examined by FTIR and XRD techniques, respectively. Then, the extracted silica was utilized as a siliceous raw material for MCM-41, MCM-48, KIT-6 and SBA-15 preparation.