CO-IMMOBILIZATION OF CELLULASE AND XYLANASE ON MAGNETICALLY-SEPARABLE HIERARCHICALLY-ORDERED MESOCELLULAR MESOPOROUS SILICA

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I dedicate this thesis to God Almighty my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout these years and on His wings only have I soared. I also dedicate this work to my team; Tazkiyya who have encouraged me all the way and whose encouragement has made sure that I give it all it takes to finish that which I have started. To my family and friends who have been affected in every way possible by this quest. Thank you. My love for you all can never be quantified. God bless you.

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ABSTRACT

Lignocellulosic biomass that exist abundantly in nature is a potential source for producing environmentally sustainable biobased chemicals. Lignocellulosic materials can be converted into fuels and value-added chemicals by the method of enzymatic hydrolysis using cellulase and xylanase enzymes. However, the use of free enzymes is hampered by the low storage stability, difficulty in recovery and non-reusability of the enzymes, which leads to the need for enzyme immobilization. Several inorganic carriers are potentially suitable for enzymatic immobilization, by means of several different techniques. Enzyme immobilization in magnetically-separable hierarchically-ordered mesocellular mesoporous silica (M-HMMS) is an alternative method for producing efficient biocatalyst. In this study, cellulase and xylanase were immobilized using three approaches: enzyme adsorption, enzyme adsorption and cross-linking and enzyme adsorption, precipitation and cross-linking (EAPC). The best precipitant, cross-linker and immobilization method for cellulase and xylanase co-immobilization were tert-butanol, glutaraldehyde and EAPC, respectively. The optimum cellulase and xylanase activity retention were achieved using 2 mL of enzymes, 1 mg: 0.15 mL of magnetic silica-to-enzyme ratio, adsorption temperature of 26 ºC, adsorption time of 40 min, adsorption agitation rate of 162 rpm, 1:11 mL volume of enzyme-to-precipitant ratio, 0.05 % v/v of glutaraldehyde concentration, cross-linking temperature of 37 ºC, 2 hours of cross-linking time and cross-linking agitation rate of 300 rpm. The biocatalysts prepared under optimized condition retained the activity more than 90% with improved storage stability (above 60 % after 14 days). Adsorption study showed that the pseudo-second-order kinetic model and Kolmogorov-Erofeev-Kazeeva-Avrami-Mampel model were the best models to represent the kinetic adsorption process of cellulase and xylanase on M-HMMS. The sorption process was found to be physisorption for cellulase and chemisorption for xylanase, as predicted by the activation energies. The results suggest that co-immobilized cellulase and xylanase in M-HMMS is a promising biocatalyst.

ABSTRAK

Biojisim lignoselulosa yang wujud dengan banyak secara semulajadi adalah sumber yang berpotensi untuk penghasilan bahan kimia yang berasaskan bio secara lestari. Bahan-bahan lignoselulosa boleh ditukar kepada bahan api dan bahan tambah nilai melalui kaedah hidrolisis enzimatik menggunakan selulase dan xilanase. Walau bagaimanapun, penggunaan enzim bebas terhalang oleh kestabilan penyimpanan enzim yang rendah, kesukaran perolehan semula enzim dan enzim tidak boleh digunakan semula, yang menyebabkan perlunya kepada imobilisasi enzim. Beberapa pembawa bukan organik berpotensi untuk imobilisasi enzim, melalui beberapa teknik yang berbeza. Imobilisasi enzim dalam silika mesoporous mesoselular yang tersusun secara hierarki serta boleh diasingkan menggunakan magnet (M-HMMS) adalah kaedah alternatif untuk menghasilkan biomangkin yang cekap. Dalam kajian ini, selulase dan xilanase telah diimobilisasikan menggunakan tiga pendekatan iaitu penjerapan enzim, penjerapan enzim dan pemautsilangan dan penjerapan enzim, pemendakan dan pemautsilangan (EAPC). Agen pemendakan, pemautsilangan dan kaedah imobilisasi yang terbaik untuk imobilisasi bersama selulase dan xilanase masing-masing adalah tert-butanol, glutaraldehid dan EAPC. Pengekalan aktiviti selulase dan xilanase optimum telah dicapai menggunakan 2 mL enzim, 1 mg : 0.15 mL nisbah silika magnet terhadap enzim, suhu penjerapan 26 ºC, masa penjerapan 40 minit, kadar pengadukan penjerapan 162 rpm, 1:11 mL nisbah enzim terhadap agen pemendakan, 0.05 % v/v kepekatan glutaraldehid, suhu pemautsilangan 37 ºC, 2 jam masa pemautsilangan dan kadar pengadukan pemautsilangan 300 rpm. Biomangkin yang disediakan di bawah keadaan optimum dapat mengekalkan lebih 90 % aktiviti enzim dengan kestabilan penyimpanan yang lebih baik (lebih dari 60 % selepas 14 hari). Kajian penjerapan menunjukkan bahawa model kinetik pseudo-tertib kedua dan model Kolmogorov-Erofeev-Kazeeva-Avrami-Mampel adalah model terbaik dalam menerangkan kinetik proses penjerapan selulase dan xilanase pada M-HMMS. Proses penjerapan selulase adalah secara fizikal dan xilanase secara kimia, seperti yang diramalkan oleh tenaga pengaktifan. Keputusan mencadangkan bahawa selulase dan xilanase yang telah diimobilisasi bersama dalam M-HMMS adalah biomangkin yang berpotensi besar.

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LIST OF ABBREVIATIONS

MSN	-	Magnetic Silica Nanoparticle
US	-	United States
CAS	-	Chemical Abstracts Service
SBA-15	-	Santa Barbara Amorphous-15
FDU-12	-	Fudan University-12
MCF	-	Mesoporous Cellular Foam
M-HMMS	-	Magnetically-separable Hierarchically-ordered Mesocellular Mesoporous Silica
KEKAM	-	Kolmogorov-Erofeev-Kazeeva-Avrami-Mampel
DME	-	Dimethoxyethane
PEG	-	Polyetylene glycol
EA	-	Enzyme Adsorption
EAC	-	Enzyme Adsorption and Cross-linking
EAPC	-	Enzyme Adsorption, Precipitation and Cross-linking
OPF	-	Oil Palm Frond
OPEFB	-	Oil Palm Empty Fruit Bunch
G	-	Guaiacyl
S	-	Syringyl
H	-	p-Hydroxyphenyl
LCC	-	Lignin Carbohydrate Complex
EG	-	Endo-1,4-beta-D-glucanase
CBH	-	Cellobiohydrolase
EC	-	Enzyme Commission
CRF	-	Clarified Rumen Fluid
CLEA	-	Cross-linked Enzyme Aggregate
APTES	-	3-aminopropyltriethoxysilane
MNP	-	Magnetic Nanoparticle
HMMS	-	Hierarchically-ordered Mesocellular Mesoporous Silica
GA	-	Glutaraldehyde
$(NH_4)_2SO_4$	-	Ammonium sulphate

NH_2	-	Ammonia
Ag(I)	-	Silver (i)
Pb^{2+}	-	Lead ion
DVB-VIM	-	Divinylbenzene-1-vinylimidazole
SEM	-	Scanning Electron Microscope
EDX	-	Energy Dispersive X-ray
FTIR	-	Fourier-Transform Infrared spectroscopy
XRD	-	X-ray Powder Diffraction
$Fe(NO_3)_3.9H_2O$	-	Iron (III) nitrate nonahydrate
$(EO)_{20}(PO)_{70}(EO)_{20}$	-	Pluronic® P-123
SiO_2	-	Silica
NaOH	-	Sodium hydroxide
HCl	-	Hydrochloric acid
DOE	-	Design of Experiment
Cu	-	Copper
FESEM	-	Field Emission Scanning Electron Microscopy
KBr	-	Potassium bromide
UV-Vis	-	Ultraviolet Visible
HPLC	-	High Performance Liquid Chromatography
ANOVA	-	Analysis of Variance
CV	-	Coefficient of Variation
F	-	Fishers's function
P	-	Level of significance
adj	-	adjusted
ICDD	-	International Centre for Diffraction Data
FWHM	-	Full width at half maximum
α-Fe_2O_3	-	Hematite
VSM	-	Vibrating Sample Magnetometer
TRS	-	Total reducing sugar

LIST OF SYMBOLS

rpm	-	Rotation per minute
q_{eq}	-	Amounts of enzymes adsorbed at equilibrium
q_t	-	Amounts of enzymes adsorbed at time t
t	-	Time
k_1	-	Pseudo-first-order rate constant
k_2	-	Pseudo-second-order rate constant
U/g	-	Unit activity per gram
g/U.min	-	Gram per unit activity minute
α	-	Initial sorption rate
U/g.min	-	Unit activity per gram minute
β	-	Desorption constant
g/U	-	Gram per unit activity
q_m	-	Optimum protein adsorbed on the adsorbent
k_{AV}	-	Overall adsorption rate constant
n_{AV}	-	Specific kinetic parameter
k	-	Kinetic parameter
A	-	Variable of pre-exponential
E_a	-	Energy of activation
R	-	Gas constant (8.314 kJ/mol.K)
T	-	Temperature in Kelvin
k_{id}	-	Intraparticle diffusion rate constant
C	-	Effect of the boundary layer on molecule diffusion
ΔG^o	-	Gibbs Free Energy
ΔH^o	-	Enthalpy change
ΔS^o	-	Entropy change
kJ/mol.K	-	Kilojoule per mole Kelvin
K	-	Thermodynamic equilibrium constant
V_{max}	-	Maximum reaction rate
K_m	-	Michaelis constant
v/v	-	Volume per volume

c_E	-	Concentration of the enzymes
c_i	-	Initial concentration of enzymes
c_t	-	Final concentration of enzymes
U/mL	-	Unit activity per milliliter
q	-	Number of enzymes adsorbed onto a unit mass
V_s	-	Volume of enzyme solution
m	-	Over-dry mass
w/v	-	Weight per volume
dF	-	Degree of freedom
R^2	-	Coefficient
U	-	Unit activity
q_e^*	-	Experimental q_e
C_e	-	Equilibrium concentration
θ	-	Theta
Ms	-	Saturation magnetization
Mr	-	Remanent magnetization
Hc	-	Coercive force
emu/g	-	Electromagnetic unit per gram
G	-	Gauss

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Lignocellulosic biomass is a renewable raw material that is abundantly available throughout the year and is sustainable. It can be hydrolyzed into simple sugars (glucose and xylose) as it is rich with celluloses and hemicelluloses (Halim et al., 2018). Maximal utilization of lignocellulose will increase the value of the by-products generated and promote sustainability to the sugar-derived industry. However, the pretreatment process of lignocellulose is considered one of the costly steps in the conversion of lignocellulose feedstocks into fermentable sugars. Amongst the most significant approach is by the application of cellulase and xylanase, which has been ascribed infeasible due to the high cost of the enzymes (Goh et al., 2010). Furthermore, the unsatisfactory biocatalyst recovery, sluggish enzyme-catalyzed reaction rates, and low biocatalyst stability under industrial procedure conditions have limited the economically viable scale-up of enzyme-catalyzed cellulose transformation to glucose (Chapman et al., 2018). Therefore, highly stable, efficient, and economical cellulase and xylanase for industrial use have attracted the attention of many scientists (Yin et al., 2010).

Cellulases are proteins that have been conventionally divided into three major groups: endoglucanase, which attacks low crystallinity areas in the cellulose materials by endoaction, creating free chain-ends; exoglucanases or cellobiohydrolases which hydrolyze the 1, 4-glycocidyl linkages to form cellobiose; and β-glucosidase which converts cello-oligosaccharides and disaccharide cellobiose into sugar elements. Besides the three main groups of cellulose enzymes, several other hydrolysis enzymes that attack hemicelluloses are glucuronide, acetylesterase, xylanase, β-xylosidase, galactomannase, and glucomannase. These enzymes work synergistically to attack cellulose and hemicellulose (Verardi et al., 2012).

Biotechnology of cellulases and xylanases in animal feed, food, textile, laundry and paper industries had already begun in the early 1980s (Das et al., 2012). Juices, oils, and agar can be extracted using cellulase alone or in combination with xylanase (Illanes, 2008). The cellulase can also be used along with xylanase for recognition of turbidity levels, production of fluids and juices from plant materials, preparation of dextran as food thickeners, and clarification of juices (Mishra et al., 2017). The depolymerization of xylan gives off xylose, which can be converted to xylitol for used as a sweetener in medicine and food industries (Selvarajan & Veena, 2017). Other applications of cellulase and xylanase are in energy generation and waste management (Ali et al., 2013).

Cellulases, together with xylanases, account for 20% of the global industrial enzyme market (Pastor et al., 2007). The global market value of cellulase is US$1500 million in 2017, and it is estimated to reach US$2300 million in 2025 according to Global Cellulase (CAS 9012-54-8) Market Research Report 2018 (Tamilanban et al., 2018). Given the commercial importance of cellulase and xylanase, it is necessary to find an effective method which not only stabilizes but also improves the enzyme activity. Therefore, much effort has already been focused on the recycling of catalysts through immobilization to develop efficient catalytic systems (Lee et al., 2006).

The potential use of cellulase and xylanase for biodiesel production has been illustrated by previous works on the immobilization of cellulase and xylanase. For instance, Lima et al. (2017) demonstrated that immobilized cellulase had improved thermostability than the free cellulase and sustained almost 70% from the initial activity after eight cycles of cellulosic biomass conversion to glucose. Besides, Khorshidi et al. (2016) reported that under lower pH conditions and higher temperatures, immobilized cellulase had significantly higher activity compared to free cellulase, indicating that immobilization techniques can functionalize biocatalyst at industrial settings. Significant activity retention of immobilized cellulase through efficient reuse of biocatalyst implies that the immobilization method enhances process economics. Apart from reusability, immobilization hinders undesirable conformational changes of the enzyme in unfavorable conditions, thus improving its chemical stability (Kumar et al., 2014). Cellulase that covalently immobilized on magnetic silica nanoparticles (MSNs) has reportedly produced a constant yield of more than 80 % from the hydrolysis of pretreated cellulose to sugar(Chang et al., 2011). In addition, the xylanase adsorbed on silica and encapsulated in calcium alginate beads have been reported to increase juice clarity (Sharma et al., 2012, Bhushan et al., 2015). However, the reduction in activity is still inevitable even when the immobilized cellulase and xylanase have shown improved stability and reuse capacity in the cyclic catalytic process (Xu et al., 2018). On that account, developing a new reliable carrier for cellulase and xylanase immobilization with preserved catalytic activity remains a significant challenge.

Biocatalytic degradation of cellulosic substrates using cellulases and xylanases has been extensively studied, in which cellulases and xylanases from different sources have been immobilized on several types of inert carriers (Khorshidi et al., 2015). Khoshnevisan et al. (2011), for instance, reported a successful adsorption of cellulase on superparamagnetic nanoparticles where the adsorption capacity and binding efficiency was 31 mg/g and 95 %, respectively. Xu et al. (2011) for the first time reported successful cellulase immobilization on nanomagnetic iron oxide particles via glutaraldehyde binding. Cellulase immobilization by physisorption and chemical bonding on SBA-15, organo-functionalized FDU-12, MCFs, and MSNs have also been reported (Zhou & Hartmann, 2013). Nevertheless, no studies have been reported to date on the immobilization of cellulase and xylanase on magnetically-separable hierarchically-ordered mesocellular mesoporous silica (M-HMMS). According to Song et al. (2016), cellulases and xylanases immobilized on magnetic nanocarriers offer the benefits of magnetic separation and repeated use for continuous hydrolysis.

1.2 Problem Statement

Enzymes can be immobilized onto carriers by different methods, such as physical adsorption, entrapment, and covalent binding (Kumar et al., 2018). Enzymes can be more tightly bound to the carrier through covalent bonding than through physical adsorption. The tight binding is required in the preparation of the immobilized enzymes as they have a tendency to leave the carrier to be adsorbed strongly on the surface of the cellulosic biomass particle (Oh, 1982). Although covalent immobilization inhibits enzyme desorption, it also contributes to low enzyme activity (Yurekli, 2010). The activity of covalently linked hydrolyze enzymes is 50–70% less than physically adsorbed enzymes (Sutarlie & Yang, 2013). In addition, the substrate entry into the active sites is usually prevented by the binding of surface functional groups to active sites of enzymes, resulting in loss of activity (Jordan et al., 2011). On the other hand, polymer matrixes such as poly(vinyl alcohol) (Imai et al., 1986) or acrylate-based polymer can also be used to bind hydrolyze enzymes (Fang et al., 2011). However, massive mass transfer opposition from the matrix often faced by the enzymes entrapment in the polymer matrixes (Guzik et al., 2014). Hence, investigating a suitable method for co-immobilizing cellulase and xylanase on M-HMMS becomes the main focus and considered as a critical factor in producing the optimum immobilization yield.

To the best of knowledge, only three studies had been reported on enzyme immobilization on M-HMMS by Kim et al. (2005), Lee et al. (2009) and Chang et al. (2012), each using different immobilization methods . However, these methods along with optimization of preparation condition of the immobilization have not been fully explored. Therefore, throughout this study, different immobilization methods were investigated to determine the best immobilization method for cellulase and xylanase on M-HMMS, and the preparation condition of that method was optimized to obtain optimum enzyme activity retention.

Furthermore, adsorption study of the co-immobilization of cellulase and xylanase on M-HMMS is also critical to determine the adsorption efficiency and to conclude the mechanism of thadsorption. The knowledge of the adsorption mechanism can help determine the durability, cause of enzyme behavior after immobilization, and improvement for the enzyme immobilization. Thus, different adsorption kinetic models, including pseudo-first-order, pseudo-second-order, Elovich and Kolmogorov-Erofeev-Kazeeva-Avrami-Mampel (KEKAM) were considered for this study. Moreover, the lack of information regarding the utilization of M-HMMS in enzyme immobilization was the main factor that leads to this study.

1.3 Objectives of Research

1. To screen the best precipitant and cross-linker for cellulase and xylanase immobilization.

2. To screen the best immobilization method for optimum enzyme activity retention in cellulase and xylanase co-immobilization on M-HMMS.

3. To synthesize and optimize the preparation condition of the best immobilization method for optimum yield of cellulase and xylanase activity retention co-immobilized on M-HMMS.

4. To determine cellulase and xylanase adsorption kinetics, mechanism and thermodynamic on M-HMMS

5. To evaluate the structure, property, stability and catalytic performance of co-immobilized cellulase and xylanase on M-HMMS.

1.4 Scope of the Study

The objectives of this study can be achieved through several scopes:

1) Screening of the best precipitant and cross-linker for cellulase and xylanase before co-immobilization in M-HMMS. The precipitants chosen include acetone, ammonium sulphate, dimethoxyethane (DME), n-propanol, polyethylene glycol (PEG), and tert-butanol. The cross-linkers chosen were glutaraldehyde and dextran polyaldehyde. The best precipitant and cross-linker were used throughout the study.

2) Investigation on the best immobilization method for cellulase and xylanase on M-HMMS. The methods are enzyme adsorption (EA), enzyme adsorption and cross-linking (EAC) and enzyme adsorption, precipitation and cross-linking (EAPC). The best method was used in the optimization process.

3) Optimization of preparation condition, using two-level fractional factorial design for optimum cellulase and xylanase activity retention on M-HMMS. The parameters involved include enzyme amount, M-HMMS-to-enzyme ratio, adsorption condition (temperature, time and agitation rate), enzyme-to-precipitant ratio, cross-linker concentration and cross-linking condition (temperature, time and agitation rate).

4) Study on the effect of temperature on adsorption of cellulase and xylanase on M-HMMS. The kinetic models that were used are pseudo-first-order, pseudo-second-order, Elovich and KEKAM. The mechanism was studied using the intraparticle diffusion model and the thermodynamic properties were also determined.

5) Evaluation in terms of structure, property, stability and catalytic performance of the developed co-immobilized cellulase and xylanase on M-HMMS was evaluated. Assessment on enzymatic hydrolysis of oil palm frond as model substrate using the developed co-immobilized enzymes was also done.

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APPENDIX A

SOLUTION PREPARATION

A1. Preparation of Sodium Citrate Buffer Solution

210 g of citric acid monohydrate added with 750 mL of distilled water. NaOH then was added around 50 to 60 g slowly until the pH reach 4.3. The solution was diluted to 1 L until reach pH 4.5 by adding NaOH. Prepared 1 M citrate buffer was diluted to 0.05 M concentration with pH adjusted to pH 4.8 and stored for further use.

A2. Preparation of DNS Reagent

In preparation of 1 L of DNS reagent, about 600 mL of distilled water stir in hot plate at 100 °C. 10 g of DNS was added and allowed it to dissolve. After that, 16 g of NaOH were gradually added and also allowed it to dissolve. Then, 300 g of Rochelle salt (sodium potassium tartrate) were added and stirred it for 20-30 min and warm at temperature 45 ℃ The mixture was cooled down and dilute to 1000 mL before stored in Schott bottle

APPENDIX B

STANDARD CURVE

B.1 DNS Glucose Standard Curve

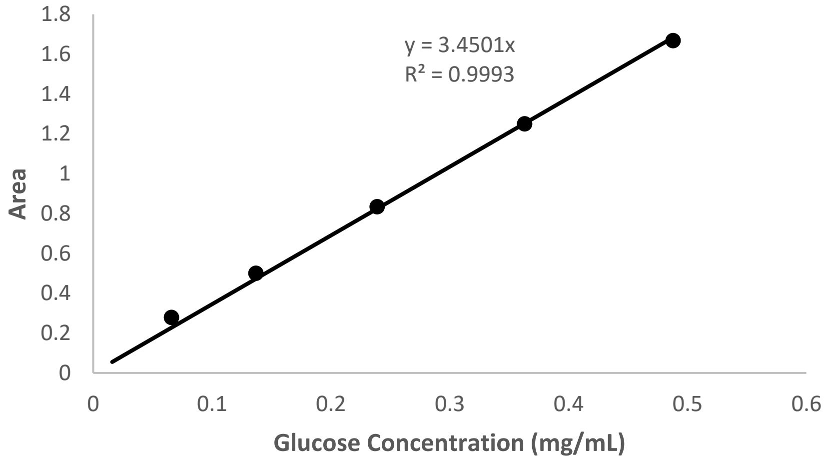


Figure B1 DNS standard curve for glucose

B.2 HPLC Glucose Standard Curve

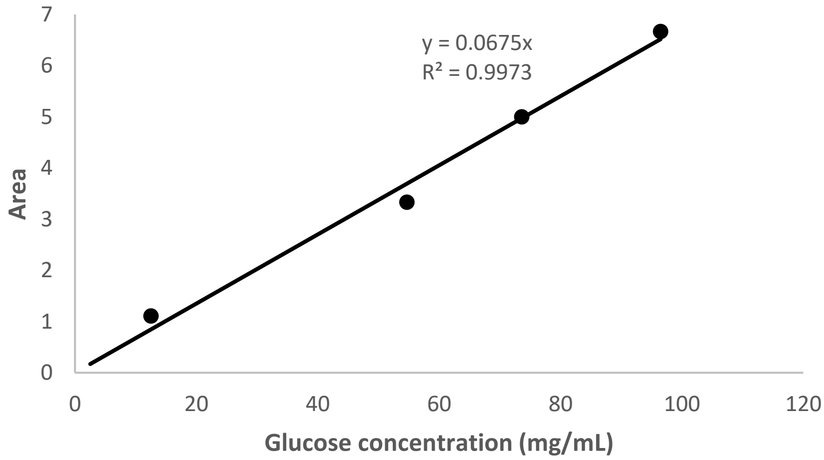


Figure B2 HPLC standard curve for glucose

APPENDIX C

MATHEMATICAL EQUATIONS

C1. Activity Retention

$$\%\ Activity\ retention = \frac{Total\ enzyme\ activity\ in\ M-HMMS\ (Unit\ activity)}{Total\ enzyme\ activity\ used\ for\ preparing\ immobilized\ enzyme\ (Unit\ activity)} \times 100\ \% \quad \text{(C1)}$$

C2. Protein Concentration Calculation (Bradford Assay)

Protein concentration was calculated by using the following equation:

$$Protein\ concentration, \frac{mg}{mL} = \frac{A_{595}}{M_{std}} \times DF \quad \text{(C2)}$$

where:

A_{595}: The absorbance of Bradford assay read at 595 nm

M_{std}: Slope from the standard curve equation of BSA

DF: Dilution factor of the protein

C3. Xylanase Activity Calculation (DNS Assay)

One unit (U) of enzyme activity is defined as the amount of enzyme required to produce 1 μmol xylose per min under optimum conditions. Unit activity per volume enzyme (U/mL) was calculated using the following equation:

$$Activity, \frac{U}{mL} = \frac{A_{575,enzyme} - (A_{575,EC} + A_{575,SC})}{M_{std} \times MW\ xylose \times 5\ \text{min} \times 0.1\ mL} \times 1000 \times DF_{enzyme} \qquad (C3)$$

where:

$A_{575,enzyme}$: The absorbance of assay with the enzyme read at 575 nm

$A_{575,EC}$: The absorbance of assay with the enzyme in buffer without substrate, read at 575 nm (EC; Enzyme control)

$A_{575,SC}$: The absorbance of assay with the substrate in buffer without enzyme, read at 575 nm (SC; Substrate control)

M_{std}: Slope from the standard curve equation of xylose

MW: Molecular weight of xylose; 150.13 g/mol

5 min: Incubation time

0.1 mL: Volume of enzyme used in the assay

DF_{enzyme}: Dilution factor of the enzyme used for the assay

C4. Kinetic Parameters Calculation

The calculation of kinetic parameters of free enzymes, EAC and EAPC are shown below. Given the data in table C1'

Table C1 Data for calculation of kinetic parameters using xylan

Substrate concentration, S (g/L)	Velocity, V (mM/min)			1/S (g/L)	1/V (min/mM)		
	Free	EAC	EAPC		Free	EAC	EAPC
2	0.218	0.115	0.196	0.5	4.586	8.705	5.114
2.5	0.245	0.172	0.213	0.4	4.078	5.845	4.702
10	0.362	0.423	0.499	0.1	2.765	2.365	2.006

1/v versus 1/c was plotted for the given data to ensure that substrate saturation was achieved. The plot is shown in Figure C1.

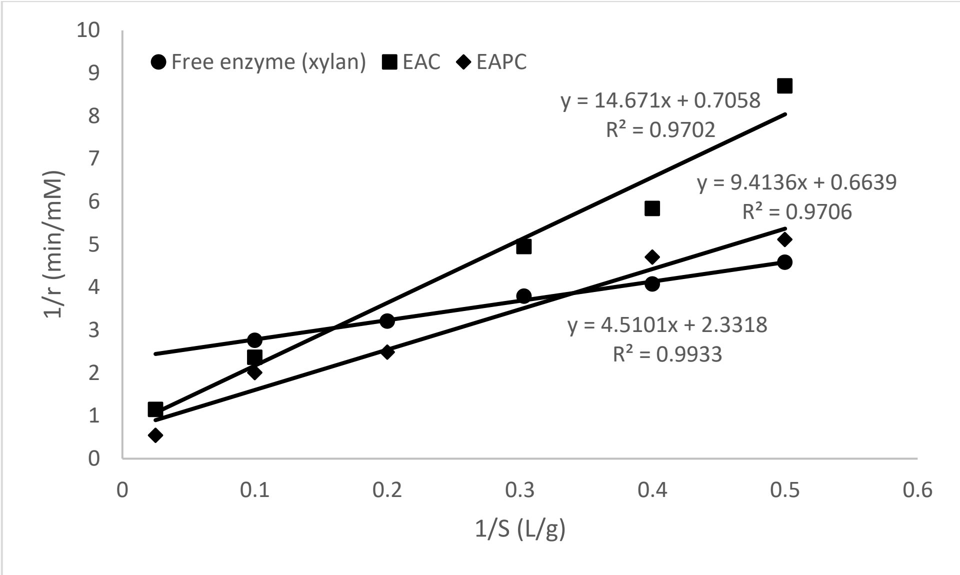


Figure C1 The plot of 1/v versus 1/c for kinetic parameters determination of free enzymes, EAC and EAPC towards birchwood xylan.

It was demonstrated that the plot is a linear graph with Lineweaver-Burk plot:

$$\frac{1}{V_0} = \left[\frac{K_m}{V_{max}}\right] \cdot \left[\frac{1}{[c]}\right] + \frac{1}{V_{max}} \quad \text{(C4)}$$

Table C2 Parameters obtained from Lineweaver-Burk plot

	Free	EAC	EAPC
Linear equation	y=4.5101x+2.3318	y=14.671x+0.7058	y=9.4136x+0.6639
$V_{max} = \frac{1}{c}$	$\frac{1}{2.3318} = 0.43\ mM/min$	$\frac{1}{0.7058} = 1.42\ mM/min$	$\frac{1}{0.6639} = 1.51\ mM/min$
K_m=m.V_{max}	4.5101x0.43=1.93 g/L	14.671x1.42=20.97 g/L	9.4136x1.51=14.18 g/L
$\frac{V_{max}}{K_m} \times MW_{xylose} \times 1000$	$\frac{0.43}{1.93} \times 150.13 \times 1000 = 33.45 \times 10^3 min^{-1}$	$\frac{1.42}{20.97} \times 150.13 \times 1000 = 10.25 \times 10^3 min^{-1}$	$\frac{1.51}{14.18} \times 150.13 \times 1000 = 15.99 \times 10^3 min^{-1}$

APPENDIX D

STANDARD PROCEDURE FOR INSTRUMENTS

D1. HPLC Analysis

Name of Instrument: Agilent 1260 Infinity II LC
Name of Software: Agilent 1200 Chemstation
Mobile phase: Deionised water (Filtered via 0.2 μm and degassed)
Apparatus: Degasser, pump, detector, oven, column, guard column, computer, HPLC syringe and needle

High Performance Liquid Chromatography (Agilent)

1. Column and guard column were attached to the appropriate lines.
2. Deionized water was degassed using vacuum filter and the probe was inserted into the water.
3. The degasser, pump and detector were switched on.
4. The computer was switched on and the Breeze software was opened.
5. The line for the flow of the mobile phase was set to 100%.
6. Pump prime was carried out using a syringe.
7. The pump valve was pushed to the right and pump purge was started.
8. Method was set by opening a new method in the Breeze software.
9. The system was equilibrated for a few seconds to recognize the new set method.
10. The flow rate was increased to 0.05 ml/min and pressure was observed. After the pressure was stable, the flow rate was increased gradually. Connecting lines were checked for any leakage.
11. When the flow rate reached half of the desired flow rate, the oven was switched on.
12. The flow rate was increased gradually until the desired flow rate was reached.

13. After flow rate and temperature reached the set up conditions, detector was purged until the reading was stable (until +0000 reading was shown on the detector's screen).
14. The detector was unpurged.
15. The system was equilibrated until a stable baseline was achieved.
16. After finished equilibration, standards and samples can be injected into the column.

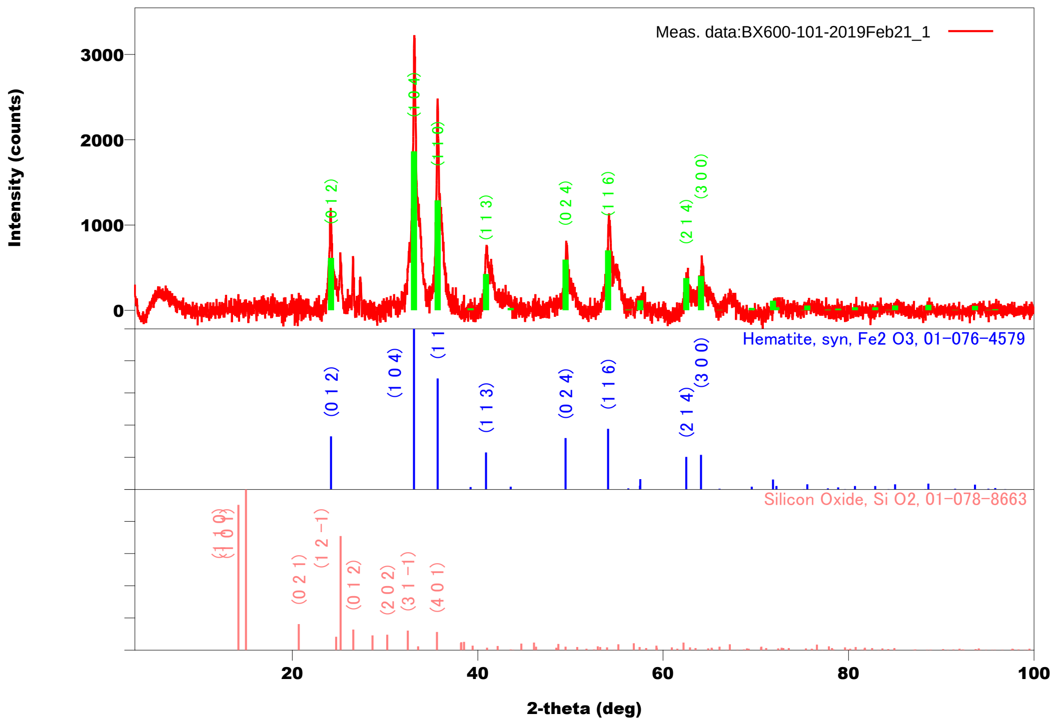
APPENDIX E

XRD ANALYSIS

Qualitative analysis results

Phase name	Formula	Figure of merit	Phase reg. detail	DB card number
Hematite, syn	Fe2 O3	0.438	ICDD (PDF-2 Release 2015 RDB)	01-076-4579
Silicon Oxide	Si O2	1.127	ICDD (PDF-2 Release 2015 RDB)	01-078-8663

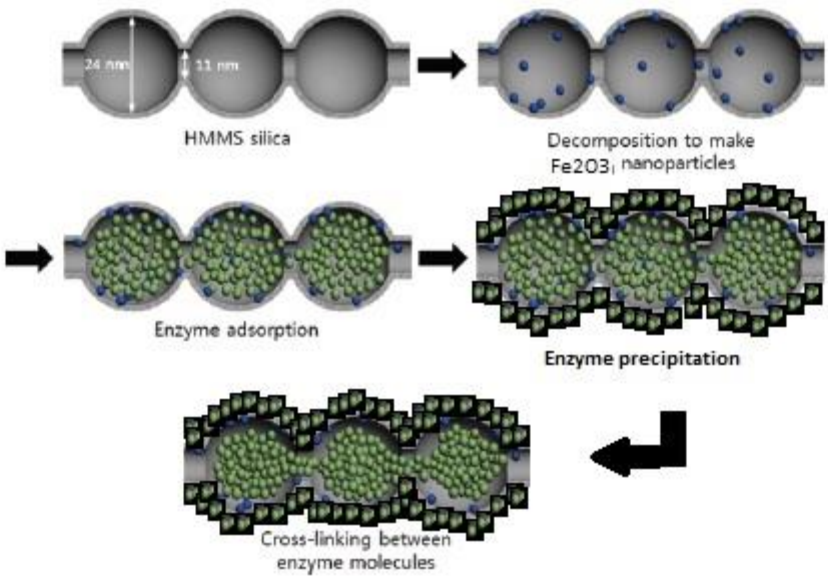
Phase name	Formula	Space group	Phase reg. detail	DB card number
Hematite, syn	Fe2 O3	167 : R-3c,hexagonal	ICDD (PDF-2 Release 2015 RDB)	01-076-4579
Silicon Oxide	Si O2	148 : R-3,hexagonal	ICDD (PDF-2 Release 2015 RDB)	01-078-8663



Peak list

No.	2-theta(deg)	d(ang.)	Height(counts)	FWHM(deg)	Int. I(counts deg)	Int. W(deg)
1	5.63(16)	15.7(4)	117(11)	1.97(16)	245(30)	2.1(4)
2	13.8(11)	6.4(5)	20(4)	6.1(12)	126(30)	6(3)
3	24.102(8)	3.6894(12)	779(28)	0.306(18)	492(12)	0.63(4)
4	25.209(10)	3.5299(13)	451(21)	0.13(3)	122(6)	0.27(3)
5	26.576(5)	3.3514(7)	490(22)	0.10(3)	85(7)	0.17(2)
6	33.149(9)	2.7003(7)	2108(46)	0.416(16)	1777(21)	0.84(3)
7	35.661(12)	2.5156(8)	1582(40)	0.366(17)	1046(21)	0.66(3)
8	40.89(4)	2.2050(18)	414(20)	0.83(6)	535(19)	1.29(11)
9	49.55(3)	1.8382(11)	503(22)	0.44(5)	429(14)	0.85(7)
10	54.030(13)	1.6958(4)	557(24)	1.08(5)	802(29)	1.44(11)
11	64.20(15)	1.450(3)	182(13)	2.21(14)	428(36)	2.4(4)
12	67.3(2)	1.391(4)	84(9)	1.0(2)	89(14)	1.1(3)

Hematite, syn	Scale factor	s	162(5)
	FWHM	U	2.4700
		V	0.0000
		W	0.0001
	Asym. factor	A0	0.4123
		A1	3.1276
	Decay rate factor	etaL0/mL0	0.9954
		etaL1/mL1	0.0000
		etaL2/mL2	0.0000
		etaH0/mH0	1.3350
		etaH1/mH1	0.0000
		etaH2/mH2	0.0000
	Preferred orientationMarch-Dollase	h	0
		k	0
		l	0
		March coefficient	1.000000
Silicon Oxide	Scale factor	s	14.4(4)
	FWHM	U	0.0000
		V	0.0000
		W	0.2724
	Asym. factor	A0	0.1219
		A1	2.5061
	Decay rate factor	etaL0/mL0	1.1538
		etaL1/mL1	-0.5627
		etaL2/mL2	0.0000
		etaH0/mH0	1.5398
		etaH1/mH1	-0.5627
		etaH2/mH2	0.0000
	Preferred orientationMarch-Dollase	h	0
		k	0
		l	0
		March coefficient	1.000000


24 nm
11 nm
HMMS silica
Decomposition to make
Fe2O3 nanoparticles
Enzyme adsorption
Enzyme precipitation
Cross-linking between
enzyme molecules

APPENDIX F

THERMODYNAMIC CALCULATIONS

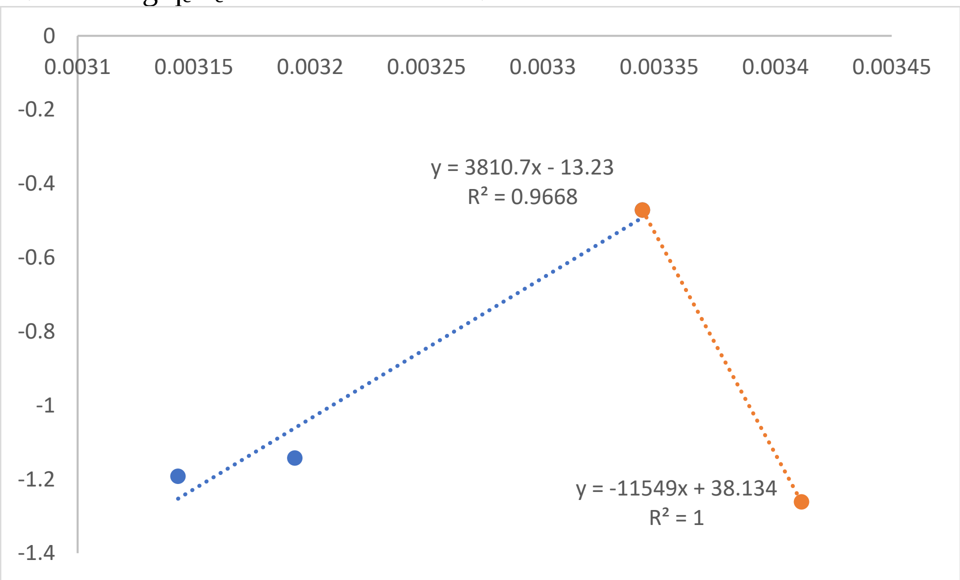
1. Using Equation (3.7) to plot log q_e/c_e vs 1/T:

$$\log \frac{q_e}{C_e} = \frac{\Delta S°}{2.303R} - \frac{\Delta H°}{2.303RT} \qquad (3.7)$$

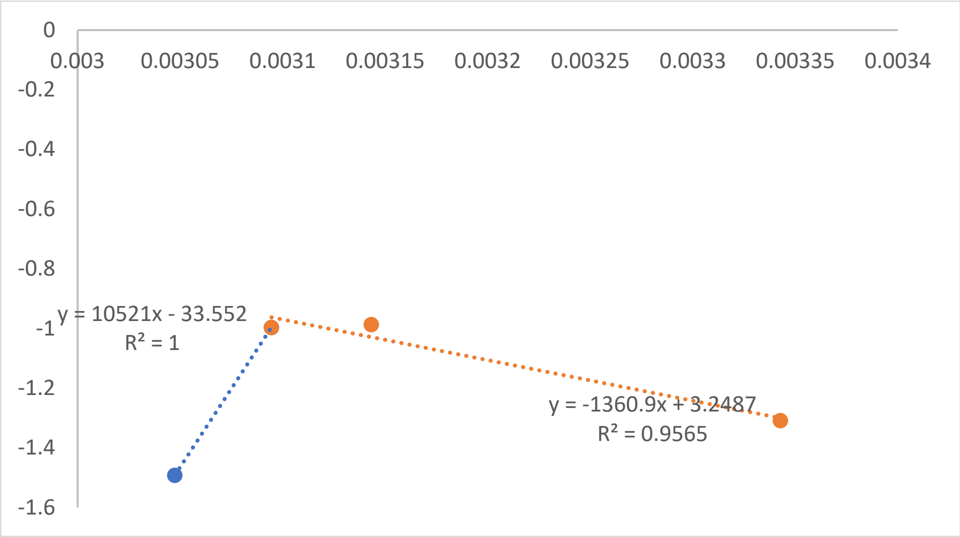
2. Data:

Enzymes	Temperature (K)	Log q_e/c_e	1/T
Cellulase	293.15	-1.261547597	0.003411223
	299.15	-0.471397776	0.003342805
	313.15	-1.142811201	0.003193358
	318.15	-1.191639358	0.003143171
Xylanase	293.15	-1.30872281	0.003411223
	299.15	-0.987263159	0.003342805
	313.15	-0.996169436	0.003193358
	318.15	-1.492226199	0.003143171

3. Plot log q_e/c_e vs 1/T for cellulase:



4. Plot log q_e/c_e vs 1/T for xylanase:



5. Calculate ΔS° and ΔH°

$$Slope = -\frac{\Delta H°}{2.303R}$$

$$\Delta H° = -Slope * 2.303R$$

$$\Delta H° = -slope * 2.303 * 8.3145$$

$$Intercept = \frac{\Delta S°}{2.303R}$$

$$\Delta S° = intercept * 2.303 * 8.3145$$

6. Calculate ΔG°

$$\Delta G° = \Delta H° - T\Delta S°$$

APPENDIX G

LIST OF PUBLICATIONS

Journal with Impact Factor

1. **Nurul Jannah Sulaiman**, Azmi Fadziyana Mansor, Roshanida A. Rahman, Rosli Md. Illias & Shalyda Md. Shaarani. (2019). Adsorption kinetics of cellulase and xylanase immobilized on magnetic mesoporous silica. *Chemical Engineering & Technology, 42*(9), 1825-1833. https://doi.org/10.1002/ceat.201800657. **(Q2, IF: 2.418)**

Indexed Journal

1. **Nurul Jannah Sulaiman**, Roshanida A. Rahman & Rosli Md. Illias (2019). Effect of cross-linked enzyme aggregates in hierarchically mesocellular mesoporous magnetic silica preparation conditions towards enzyme activity retention. *Malaysian Journal of Fundamental and Applied Sciences, 15*(1), 6-12. https://doi.org/10.11113/mjfas.v15n2019.1205 **(Indexed by WOS)**

2. **Nurul Jannah Sulaiman**, Roshanida A. Rahman & Norzita Ngadi (2014). Precipitation of cellulase and xylanase for cross-linked enzyme aggregates. *Jurnal Teknologi (Sciences and Engineering), 68*(5), 17-20. http://dx.doi.org/10.11113/jt.v68.3024. **(Indexed by SCOPUS)**

Non-indexed Journal

1. **Nurul Jannah Sulaiman** & Roshanida A. Rahman (2016). New advancement on cross-linked enzyme aggregates within magnetically-separable mesoporous silica. *Applied Mechanics and Materials, 818*, 276-280. https://doi.org/10.4028/www.scientific.net/AMM.818.276.

Non-indexed Conference Proceedings

1. **Nurul Jannah Sulaiman,** Roshanida A. Rahman & Mazura Jusoh (2013). Preparation of cross-linked enzyme aggregates from cellulase and xylanase. *Proceedings of the 8th Curtin University Technology, Science and Engineering International Conference (CUTSE 2013).* 3-4 December 2013. Miri, Sarawak: Curtin University.

2. **Nurul Jannah Sulaiman** & Roshanida A. Rahman (2015). Effects and interaction of preparation parameters on the fabrication of combine cross-linked enzymes aggregates in magnetic mesoporous silica using two-level factorial design. *Proceedings of Asian Congress on Biotechnology 2015 (ACB2015).* 15-19 November 2015. Kuala Lumpur, Malaysia: Asian Federation of Biotechnology.