

# IMMOBILISATION OF XYLANASE FOR XYLOOLIGOSACCHARIDES PRODUCTION FROM MERANTI WOOD SAWDUST

SITI SABRINA BINTI MOHD SUKRI

Doctor of Philosophy

UNIVERSITI MALAYSIA PAHANG



## **SUPERVISOR'S DECLARATION**

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy.

---

(Supervisor's Signature)

Full Name : PROF. DATIN DR. MIMI SAKINAH BINTI ABDUL MUNAIM

Position : PROFESSOR

Date :

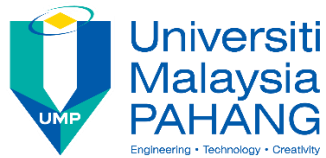
---

(Co-supervisor's Signature)

Full Name : DR. NOORMAZLINAH BINTI AHMAD

Position : SENIOR LECTURER

Date :



## **STUDENT'S DECLARATION**

I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

---

(Student's Signature)

Full Name : SITI SABRINA BINTI MOHD SUKRI

ID Number : PKB 14007

Date :

IMMOBILISATION OF XYLANASE FOR XYLOOLIGOSACCHARIDES  
PRODUCTION FROM MERANTI WOOD SAWDUST

SITI SABRINA BINTI MOHD SUKRI

Thesis submitted in fulfillment of the requirements  
for the award of the degree of  
Doctor of Philosophy

Faculty of Engineering Technology  
UNIVERSITI MALAYSIA PAHANG

MAY 2018

## ACKNOWLEDGEMENTS

First of all, I wish to express my sincere appreciation and thanks to my supervisor, Prof. Datin Dr. Mimi Sakinah Abdul Munaim, whose expert guidance permitted me to achieve this final result. Her willingness to mentor, discuss and critique is highly appreciated.

I gratefully acknowledge the assistance and cooperation of all laboratory staffs of Faculty of Chemical and Natural Resources and Faculty of Engineering Technology, UMP who helped me in many ways and made my stay in UMP pleasant and unforgettable. I am also deeply indebted to Uitm and Ministry of Higher Education for funding and scholarship provided during my study.

My sincere appreciation also extends to all my colleagues and friends who have provided assistance at various occasions. Their views and tips are useful indeed. Unfortunately, it is not possible to list all of them in this limited space.

Thanks to my family, especially my parents, who has endured my many absences during my research period. My parents always supported and encouraged me to do my best in all matters of life. Yours prayer for me was what sustained me thus far. I cannot find the appropriate words that could properly describe my appreciation for their devotion, support, and faith in my ability to attain my goals. To them I dedicate this thesis.

## TABLE OF CONTENT

<b>DECLARATION</b>	
<b>TITLE PAGE</b>	
<b>ACKNOWLEDGEMENTS</b>	<b>ii</b>
<b>ABSTRAK</b>	<b>iii</b>
<b>ABSTRACT</b>	<b>iv</b>
<b>TABLE OF CONTENT</b>	<b>v</b>
<b>LIST OF TABLES</b>	<b>xii</b>
<b>LIST OF FIGURES</b>	<b>xiv</b>
<b>LIST OF SYMBOLS</b>	<b>xviii</b>
<b>LIST OF ABBREVIATIONS</b>	<b>xix</b>
<b>CHAPTER 1 INTRODUCTION</b>	<b>1</b>
1.1 Background of Study	1
1.2 Problem Statement	3
1.3 Objectives	5
1.4 Scopes of the Study	6
1.5 Significance of the Study	8
1.6 Novelty of the Study	9
1.7 Thesis Outline	11
<b>CHAPTER 2 LITERATURE REVIEW</b>	<b>12</b>
2.1 Introduction	12
2.2 Functional Food Ingredients	12

2.2.1	Xylooligosaccharides (XOS)	15
2.2.2	Properties of XOS	16
2.2.3	Applications of XOS	17
2.2.3.1	XOS in Food Products	18
2.2.3.2	XOS for Pharmaceutical Applications	19
2.2.3.3	XOS for Agricultural and Feed Applications	19
2.3	Lignocellulose Biomass	20
2.3.1	Lignocelluloses Composition	21
2.3.1.1	Lignin	22
2.3.1.2	Cellulose	23
2.3.1.3	Hemicellulose	24
2.3.2	Production of XOS from Lignocellulosic Materials	27
2.3.2.1	Lignocellulose Biomass from Wood Sawdust	30
2.4	Method for XOS Production	30
2.4.1	Chemical Method	31
2.4.2	Autohydrolysis	32
2.4.3	Enzymatic Hydrolysis Methods	33
2.4.3.1	Effect of Reaction Conditions on Enzymatic Hydrolysis	36
2.5	Enzymatic Hydrolysis for XOS Production	36
2.5.1	Free Enzyme	38
2.5.2	Immobilised Enzyme	38
2.5.3	Enzyme Immobilisation Techniques	42
2.5.3.1	Entrapment	43
2.5.3.2	Covalent Binding	44
2.5.3.3	Adsorption	46
2.5.3.4	Crosslinking	47
2.5.3.5	Comparison between Different Immobilisation Techniques	47
2.5.3.6	Immobilisation Technique Applied for Xylanase	50
2.5.4	Parameters Affecting the Performance of Immobilised Xylanase	54

2.5.4.1	Effect of Immobilisation Techniques	54
2.5.4.2	Effect of Immobilisation Carrier Materials	55
2.5.4.3	Effect of Enzyme Loading	56
2.5.5	Characterisation of Xylanase Immobilisation	58
2.5.5.1	pH, Thermal, and Storage Stability	58
2.5.5.2	Thermodynamics Study	59
2.5.5.3	Kinetics Study	60
2.5.5.4	Reusability	63
2.6	Optimisation Strategies	64
2.6.1	One-Factor-at-a-Time (OFAT)	65
2.6.2	Fractional Factorial Design (FFD)	65
2.6.3	Response Surface Methodology (RSM)	66
2.7	Summary	67
<b>CHAPTER 3 METHODOLOGY</b>		<b>68</b>
3.1	Introduction	68
3.1.1	Stage 1: Characterisation and Recovery of Xylan from MWS	68
3.1.2	Stage 2: Immobilisation of Xylanase	69
3.1.3	Stage 3: Enzymatic Hydrolysis for XOS Production	70
3.2	Materials	73
3.2.1	Chemicals and Reagents	73
3.2.2	Meranti Wood Sawdust (MWS)	73
3.2.3	Enzyme	74
3.3	Preparation of MWS	74
3.4	Characterisation of MWS	74
3.4.1	Determination of Total Solids and Moisture Contents	75
3.4.2	Determination of Extractives	75
3.4.3	Determination of Holocellulose	76



3.4.4	Determination of $\alpha$ -Cellulose	76
3.4.5	Determination of Hemicellulose	77
3.4.6	Determination of Xylan Content	77
3.4.7	Determination of Lignin	77
3.4.8	Determination of Ash Content	78
3.5	Recovery of Hemicellulosic-xylan from MWS	78
3.6	FTIR Analysis of Commercial and MWS Xylan	79
3.7	Immobilisation of Xylanase	79
3.7.1	Preparation of Enzyme Dilution	79
3.7.2	Enzyme Assay	80
3.7.3	Immobilisation of Xylanase using Different Techniques	81
3.7.3.1	Xylanase Immobilisation using a Single Entrapment Technique	82
3.7.3.2	Xylanase Immobilisation using a Single Covalent Binding Technique	82
3.7.3.3	Xylanase Immobilisation using a Combination Technique of Entrapment and Covalent Binding	82
3.7.3.4	Scanning Electron Microscopy (SEM)	83
3.7.4	Optimising Process Conditions for Xylanase Immobilisation	83
3.7.4.1	Parameter Design for Xylanase Immobilisation using OFAT	84
3.7.4.2	Identifying the Significant Variables for Xylanase Immobilisation using FFD	84
3.7.4.3	Optimisation of Critical Variables for Xylanase Immobilisation	85
3.7.4.4	Optimisation and Validation of the Results	86
3.7.5	Characterisation of Immobilised Xylanase	86
3.7.5.1	pH Stability	86
3.7.5.2	Thermal Stability	87
3.7.5.3	Determination of Thermodynamic Parameters	87
3.7.5.4	Storage Stability of Free and Immobilised Xylanase	89

3.7.5.5	Reusability of Immobilised Xylanase	89
3.8	Enzymatic Hydrolysis for XOS Production	89
3.8.1	Preparation of Enzymatic Hydrolysis using Free and Immobilised Xylanase for XOS Production from Commercial and MWS Xylan	90
3.8.2	Effect of Enzymatic Hydrolysis Conditions on XOS Production	90
3.8.2.1	Enzymatic Hydrolysis using Free Xylanase (FX) and Substrate from Commercial Xylan (CX) and MWS Xylan (MX)	91
3.8.2.2	Enzymatic Hydrolysis using Immobilised Xylanase (IX) and Substrate from Commercial Xylan (CX) and MWS Xylan (MX)	91
3.8.3	Determination of Kinetic Parameters	92
3.9	Reusability of Immobilised Xylanase for XOS Production	94
3.10	Analytical Methods	94
3.10.1	Estimation of Total Reducing Sugar	94
3.10.2	XOS Quantification	94
3.11	Summary	95
<b>CHAPTER 4 RESULTS AND DISCUSSION</b>		<b>96</b>
4.1	Introduction	96
4.2	Raw Material from Meranti Wood Sawdust (MWS)	97
4.2.1	Characterisation of MWS	98
4.2.1.1	Lignocellulosic Composition of MWS	98
4.2.2	Xylan Recovery from MWS	99
4.2.2.1	Identification of the Functional Groups in Xylan using Fourier Transform Infrared (FTIR)	101
4.2.3	Summary	105
4.3	Immobilisation of Xylanase	106
4.3.1	Determination of the Best Immobilisation Technique	106
4.3.1.1	Different Immobilisation Technique for Xylanase	106

4.3.1.2	Mechanism of Xylanase Immobilisation by Different Techniques	109
4.3.1.3	Scanning Electron Microscope (SEM) Analysis of Alginate Beads	115
4.3.2	Design of Parameters for Xylanase Immobilisation by Combination Technique of Entrapment and Covalent Binding using One-Factor-at-a-Time Approach	117
4.3.2.1	Effect of Sodium Alginate Concentration	117
4.3.2.2	Effect of CaCl <sub>2</sub> Concentration	119
4.3.2.3	Effect of Agitation Rate	120
4.3.2.4	Effect of Enzyme Loading	121
4.3.2.5	Effect of Glutaraldehyde Concentration	123
4.3.3	Screening of Parameters for Xylanase Immobilisation by Fractional Factorial Design (FFD)	125
4.3.3.1	Main Effects Analysis for Screening	126
4.3.3.2	Interactions between Parameters	130
4.3.4	Optimisation of Xylanase Immobilisation Using Response Surface Methodology (RSM)	133
4.3.4.1	Interaction between Variables	135
4.3.4.2	Verification Experiment and Adequacy of the Model for Xylanase Immobilisation	140
4.3.4.3	Optimisation Point Prediction	141
4.3.5	Characterisation Studies on Immobilised Xylanase	142
4.3.5.1	pH Stability	142
4.3.5.2	Thermal Stability	144
4.3.5.3	Thermodynamics Study	146
4.3.5.4	Storage Stability	155
4.3.5.5	Enzyme Kinetics of Free and Immobilised Xylanase	156
4.3.5.6	Reusability of Immobilised Xylanase	159
4.3.6	Summary of Xylanase Immobilisation	161
4.4	Enzymatic Hydrolysis for XOS Production	163

4.4.1	Effect of Reaction Conditions on Enzymatic Hydrolysis for Total XOS and its Derivatives Production	163
4.4.1.1	Effect of Substrate Concentration	164
4.4.1.2	Effect of Hydrolysis Time	168
4.4.1.3	Effect of Temperature	171
4.4.2	Kinetic Studies on Enzymatic Hydrolysis for XOS Production	174
4.4.3	Reusability of Immobilised Xylanase for XOS Production from MWS and Commercial Xylan	178
4.4.4	Summary of Enzymatic Hydrolysis for XOS Production	180
<b>CHAPTER 5 CONCLUSION AND RECOMMENDATIONS</b>		<b>182</b>
5.1	Conclusion	182
5.2	Recommendations	184
<b>REFERENCES</b>		<b>186</b>
<b>APPENDIX A BUFFER AND REAGENT PREPARATION</b>		<b>215</b>
<b>APPENDIX B STANDARD CURVE</b>		<b>217</b>
<b>APPENDIX C EXPERIMENTAL DATA</b>		<b>220</b>
<b>APPENDIX D LIST OF PUBLICATIONS</b>		<b>232</b>

## LIST OF TABLES

Table 2.1	Dietary oligosaccharides or functional foods with their natural sources and industrial production processes	13
Table 2.2	Main composition of several lignocellulosic biomass	21
Table 2.3	Sources of lignocellulose materials (LCM) residues, pretreatments for xylan extraction, and different methods for XOS production	29
Table 2.4	Summary of XOS production using different methods, advantages, and disadvantages	35
Table 2.5	Comparison of immobilisation techniques and their advantages and disadvantages	49
Table 2.6	Immobilisation techniques by various carriers for different enzymes	51
Table 2.7	Kinetic parameters obtained upon xylanase immobilisation by different technique and xylanase sources	62
Table 3.1	List of chemicals used in this study	73
Table 3.2	Variation of process parameters for xylanase immobilisation using OFAT	84
Table 3.3	Experimental range and levels of independent process variables	85
Table 3.4	Variation in process parameters for XOS production from commercial xylan using OFAT	91
Table 4.1	Lignocellulosic composition of MWS	99
Table 4.2	Physical appearance of xylan	101
Table 4.3	Wavenumber and its assignment for commercial and MWS xylan	104
Table 4.4	Effect of single and combination techniques for xylanase immobilisation on xylanase activity	107
Table 4.5	Physical properties of calcium alginate beads	109
Table 4.6	Experimental design of $2^{5-1}$ FFD with xylanase immobilisation yield	126
Table 4.7	Analysis of variance (ANOVA) of the first order model for xylanase immobilisation yield	127
Table 4.8	The percentage of contribution of each main factors and their interaction	129
Table 4.9	Experimental design and results of the CCD for xylanase immobilisation	133
Table 4.10	Analysis of variance (ANOVA) for response surface quadratic model for the immobilisation of xylanase	134

Table 4.11	Results of model validation for xylanase immobilisation yield	140
Table 4.12	Verification experiment at optimum process conditions	141
Table 4.13	Comparison of optimisation results of xylanase immobilisation using OFAT and CCD of RSM	142
Table 4.14	Inactivation kinetic parameters of free and immobilised xylanase toward thermal processes	152
Table 4.15	Thermodynamic parameters for thermal inactivation of free and immobilised xylanase	155
Table 4.16	Apparent kinetic parameters of free and immobilised xylanase	158
Table 4.17	Maximum XOS yield from OFAT study	174
Table 4.18	Kinetic parameters of free and immobilised xylanase	175

## LIST OF FIGURES

Figure 2.1	General process in lignocellulose bioconversion into value-added products	15
Figure 2.2	Basic structures of xylose and XOS	16
Figure 2.3	Main applications of XOS	17
Figure 2.4	Basic structure of lignocellulose	22
Figure 2.5	Lignin monomers (a) <i>p</i> -coumaryl, (b) coniferyl, (c) sinapyl alcohols, the building blocks of lignin	23
Figure 2.6	Schematic diagram of lignin structure of hardwood	23
Figure 2.7	Illustration of cellulose chain	24
Figure 2.8	Structures of main component of hemicelluloses	25
Figure 2.9	General idea of XOS production by chemical method	31
Figure 2.10	General idea of XOS production by steam or autohydrolysis method	32
Figure 2.11	General idea of XOS production by enzymatic hydrolysis method	33
Figure 2.12	Application of immobilised enzymes	41
Figure 2.13	Different immobilisation techniques	43
Figure 2.14	Illustration of immobilisation techniques	48
Figure 3.1	Flowchart of research activities	71
Figure 3.2	Schematic diagram of operational framework of this study	72
Figure 3.3	Appearance of the (a) raw MWS and (b) screened MWS	74
Figure 3.4	Set up for calcium alginate beads preparation	81
Figure 3.5	Determining initial velocity	93
Figure 4.1	Physical appearance of (a) commercial xylan and (b) MWS xylan	101
Figure 4.2	FTIR spectra of (a) commercial xylan and (b) xylan from MWS	103
Figure 4.3	Calcium alginate beads for xylanase immobilisation	108
Figure 4.4	Formation of calcium alginate beads	110
Figure 4.5	Immobilisation technique for xylanase by single entrapment	110
Figure 4.6	Immobilisation technique for xylanase by single covalent binding	112
Figure 4.7	Schematic illustration of the reaction of hydroxyl-support for covalent immobilisation with glutaraldehyde as an activating agent	113

Figure 4.8	Immobilisation technique for xylanase by combination of entrapment and covalent binding	114
Figure 4.9	Enzymatic reaction of immobilised xylanase by combination technique of entrapment and covalent binding with the substrate (xylan) for the formation of products (XOS)	114
Figure 4.10	Surface morphology of calcium alginate beads: (A) without xylanase, (B) entrapment with xylanase, and (C) combination of entrapment and covalent binding with xylanase at 1,000× magnification	116
Figure 4.11	Effect of sodium alginate concentrations on xylanase immobilisation yield at 0.2 M of CaCl <sub>2</sub> , 200 rpm, 100 U of xylanase activity, and 6% (w/w) of glutaraldehyde	118
Figure 4.12	Different shapes of alginate beads at different sodium alginate concentrations	119
Figure 4.13	Effect of CaCl <sub>2</sub> concentration on xylanase immobilisation yield at 3% (w/v) of sodium alginate, 200 rpm, 100 U of xylanase activity, and 6% (w/w) of glutaraldehyde	120
Figure 4.14	Effect of agitation rate on xylanase immobilisation yield at 3% (w/v) of sodium alginate, 0.3 M of CaCl <sub>2</sub> , 100 U of xylanase activity, and 6% (w/w) of glutaraldehyde	121
Figure 4.15	Effect of enzyme loading on xylanase immobilisation yield at 3% (w/v) of sodium alginate, 0.3 M of CaCl <sub>2</sub> , 200 rpm, and 6% (w/w) of glutaraldehyde	122
Figure 4.16	Effect of glutaraldehyde concentration on xylanase immobilisation yield at 3% (w/v) of sodium alginate, 0.3 M of CaCl <sub>2</sub> , 200 rpm, and 200 U of xylanase activity	124
Figure 4.17	Half normal plot of effects of 2 <sup>5-1</sup> fractional factorial design	128
Figure 4.18	Pareto chart of each main factors and their interaction during the experiment of the immobilisation of xylanase	129
Figure 4.19	Effects between sodium alginate concentration and glutaraldehyde concentration towards the immobilisation yield: (a) interaction graph, (b) 3D surface plot	131
Figure 4.20	Effects between enzyme loading and glutaraldehyde concentration towards the immobilisation yield: (a) interaction graph, (b) 3D surface plot	131
Figure 4.21	Interaction and 3D response surface plots for immobilisation yield as a function of glutaraldehyde and sodium alginate concentrations	137
Figure 4.22	Interaction and 3D response surface plots for immobilisation yield as a function of glutaraldehyde concentration and enzyme loading	138
Figure 4.23	Interaction and 3D response surface plots for immobilisation yield as a function of sodium alginate concentration and enzyme loading	139



Figure 4.24	Influence of pH (3.0–9.0) on the relative activity of free and immobilised xylanase at a constant temperature of 50 °C, 60 min of incubation time and 2.0% (w/v) of substrate concentration	143
Figure 4.25	Influence of temperature on the relative activity of free and immobilised xylanase at constant pH (pH 7.0 for free xylanase and pH 8.0 for immobilised xylanase) and 2.0% (w/v) of substrate concentration	145
Figure 4.26	Arrhenius plot to calculate activation energy ( $E_a$ ) of free and immobilised xylanase	147
Figure 4.27	Effect of temperatures on the stability of free and immobilised xylanase at (a) 40 °C, (b) 50 °C, (c) 60 °C, (d) 65 °C, (e) 70 °C, (f) 75 °C, and (g) 80 °C. Reaction conditions: pH 7.0 (free xylanase), pH 8.0 (immobilised xylanase) and 2.0% (w/v) xylan	149
Figure 4.28	First order thermal deactivation of the (a) free xylanase and (b) immobilised xylanase	150
Figure 4.29	Arrhenius plot to calculate activation energy for denaturation ( $E_d$ )	151
Figure 4.30	Temperature dependence of the decimal reduction of free and immobilised xylanase to calculate $z$ -values	153
Figure 4.31	Storage stability of free and immobilised xylanase at 4 °C	156
Figure 4.32	Lineweaver-Burk plot for the estimation of kinetic parameters	157
Figure 4.33	Reusability of immobilised xylanase for total reducing sugar production	160
Figure 4.34	Effect of substrate concentration on total XOS and its derivatives containing xylobiose (X2), xylotriose (X3), xylotetraose (X4), and xylopentaose (X5) production by the reaction of (a) free xylanase with commercial xylan (FXCX), and (b) immobilised xylanase with commercial xylan (IXCX)	165
Figure 4.35	Effect of substrate concentration on total XOS and its derivatives containing xylobiose (X2), xylotriose (X3), xylotetraose (X4), and xylopentaose (X5) production by the reaction of (a) free xylanase with MWS xylan (FXMX), and (b) immobilised xylanase with MWS xylan (IXMX)	166
Figure 4.36	Effect of hydrolysis time on total XOS and its derivatives containing xylobiose (X2), xylotriose (X3), xylotetraose (X4), and xylopentaose (X5) production by the reaction of (a) free xylanase with commercial xylan (FXCX), (b) free xylanase with MWS xylan (FXMX), (c) immobilised xylanase with commercial xylan (IXCX), and (d) immobilised xylanase with MWS xylan (IXMX)	170

Figure 4.37	Effect of temperature on total XOS and its derivatives containing xylobiose (X2), xylotriose (X3), xylotetraose (X4), and xylopentaose (X5) production by the reaction of (a) free xylanase with commercial xylan (FXCX), (b) free xylanase with MWS xylan (FXMX), (c) immobilised xylanase with commercial xylan (IXCX), and (d) immobilised xylanase with MWS xylan (IXMX)	172
Figure 4.38	Lineweaver-Burk plots of free and immobilised xylanase for the estimation of kinetic parameters	175
Figure 4.39	Reusability of immobilised xylanase for total XOS production from MWS and commercial xylan	179

## LIST OF SYMBOLS

%	Percentage
% (w/v)	Percentage weight per volume
% (w/w)	Percentage weight per weight
$\mu\text{L}$	Microliter
$\mu\text{m}$	Micrometer
$\mu\text{mol}$	Micromole
$\text{\AA}$	Angstrom (a unit of length)
$\text{cm}^{-1}$	Reciprocal centimeters
<i>D</i> -values	Decimal reduction time
$E_a$	Activation energy
$E_d$	Activation energy for denaturation
g/g	Gram per gram
g/L	Gram per liter
g/mol	Gram per mole
<i>h</i>	Planck constant ( $11.04 \times 10^{-36} \text{ J min}$ )
$\text{J}\cdot\text{mol}^{-1} \text{ K}^{-1}$	Joule per mole times Kelvin
K	Kelvin
$k_B$	Boltzman constant ( $1.38 \times 10^{-23} \text{ J}\cdot\text{K}^{-1}$ )
$k_d$	Thermal inactivation rate constant
$k_d$	Deactivation rate constant ( $\text{min}^{-1}$ )
kDa	Kilodalton
$\text{kJ}\cdot\text{mol}^{-1}$	Kilojoule per mole
$K_m$	Michaelis-Menten constant
M	Molar concentration
mg/g	Miligram per gram
mg/mL	Milligram per milliliter
mg/mL $\cdot$ min	Milligram per milliliter times minutes
$\text{min}^{-1}$	Reciprocal minutes
mL	Mililiter
mL/min	Mililiter per minutes
$^{\circ}\text{C}$	Degree celcius
R	Gas constant ( $8.314 \text{ J}\cdot\text{mol}^{-1} \text{ K}^{-1}$ )
$R^2$	Coefficient of determination
Rpm	Rotations per minute
S	Substrate concentration
$t_{1/2}$	Half-life
U	Units of enzyme activity
U/min	Units of enzyme activity per minutes
$V_{\text{max}}$	Maximum reaction rate
$V_o$	Initial reaction rate
V	Reaction rate
<i>z</i> -values	The temperature increase needed for 90% of decrease in the <i>D</i> -values
$\Delta G^{\circ}$	Gibbs free energy
$\Delta H^{\circ}$	Enthalpy
$\Delta S^{\circ}$	Entropy

## LIST OF ABBREVIATIONS

3D	Three Dimensional
ANOVA	Analysis of Variance
CCD	Central Composite Design
CLEAs	Crosslinked Enzyme Aggregates
CLECs	Crosslinked Enzyme Crystals
CLEs	Crosslinked Enzyme
CX	Commercial Xylan
DNS	Dinitrosalicylic acid
DOE	Design of Experiment
DP	Degree of Polymerisation
FFD	Fractional Factorial Design
FOS	Fructooligosaccharides
FOSHU	Foods for Specified Health Uses
FTIR	Fourier Transform Infrared
FX	Free Xylanase
HMF	Hydroxymethylfurfural
HPLC	High Performance Liquid Chromatography
IX	Immobilised Xylanase
IY	Immobilisation Yield
LAB	Lactic Acid Bacteria
LCM	Lignocellulose Materials
LOF	Lack of Fit
MWS	Meranti Wood Sawdust
MX	MWS Xylan
NA	Not Available
NDOS	Nondigestible Oligosaccharides
NREL	National Renewable Energy Laboratory
OD	Optical Density
OFAT	One-Factor-at-a-Time
OPF	Oil Palm Fronds
RID	Refractive Index Detector
RSM	Response Surface Methodology
SD	Standard Deviation
SEM	Scanning Electron Microscope
TAPPI	Technical Association of Pulp and Paper Industry
TS	Tobacco Stalk
X1	Xylose
X2	Xylobiose
X3	Xylotriose
X4	Xylotetraose
X5	Xylopentaose
XOS	Xylooligosaccharides
NM	Not Mentioned