IMMOBILISATION OF XYLANASE FOR XYLOOLIGOSACCHARIDES PRODUCTION FROM MERANTI WOOD SAWDUST

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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.

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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.

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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 collegues 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.

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LIST OF SYMBOLS

%	Percentage
% (w/v)	Percentage weight per volume
% (w/w)	Percentage weight per weight
µL	Microliter
µm	Micrometer
µmol	Micromole
Å	Angstrom (a unit of length)
cm^{-1}	Reciprocal centimeters
D-values	Decimal reduction time
E_a	Activation enery
E_d	Activation energy for denaturation
g/g	Gram per gram
g/L	Gram per liter
g/mol	Gram per mole
h	Planck constant (11.04 x 10^{-36} J min)
$J \cdot mol^{-1}\ K^{-1}$	Joule per mole times Kelvin
K	Kelvin
k_B	Boltzman constant (1.38 x 10^{-23} $J \cdot K^{-1}$)
k_d	Thermal inactivation rate constant
k_d	Deactivation rate constant (min^{-1})
kDa	Kilodalton
$kJ \cdot mol^{-1}$	Kilojoule per mole
K_m	Michaelis-Menten constant
M	Molar concentration
mg/g	Miligram per gram
mg/mL	Milligram per mililiter
mg/mL·min	Milligram per mililiter times minutes
min^{-1}	Reciprocal minutes
mL	Mililiter
mL/min	Mililiter per minutes
°C	Degree celcius
R	Gas constant (8.314 $J \cdot mol^{-1}\ K^{-1}$)
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_{max}	Maximum reaction rate
V_o	Initial reaction rate
V	Reaction rate
z-values	The temperature increase needed for 90% of decrease in the *D*-values
ΔG°	Gibbs free energy
ΔH°	Enthalpy
ΔS°	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	Dinitrosalycyclic 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

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ABSTRAK

Keterbatasan enzim bebas atau larut seperti tidak boleh digunakan semula, kestabilan yang lemah, dan sensitiviti kepada denaturasi boleh di atasi dengan pengunaan enzim pegun. Penghasilan produk nilai tambah, xilooligosakarida (XOS) daripada bahan baru yang boleh diperbaharui, iaitu habuk kayu Meranti (MWS) dengan menggunakan xilanase pegun mempunyai banyak kelebihan berbanding enzim larut. Tujuan kajian ini adalah untuk menyediakan xilanase pegun untuk penghasilan XOS daripada MWS melalui proses hidrolisis berenzim. Xilan daripada MWS diekstrak menggunakan kaedah piawaian holoselulosa klorit. Sebelum proses hidrolisis berenzim, xilanase pegun disediakan menggunakan teknik pemegunan yang berbeza terdiri daripada pemerangkapan tunggal, pengikatan kovalen tunggal dan gabungan pemerangkapan dan pengikatan kovalen. Keadaan optimum pemegunan xilanase ditentukan dengan menggunakan reka bentuk uji kaji sistematik yang merangkumi satu-faktor-satu-masa (OFAT) untuk mengkaji kesan setiap parameter, diikuti oleh reka bentuk eksperimen separa faktor (FFD), digunakan untuk proses penentuan parameter penting dan seterusnya penentuan keadaan optimum oleh kaedah gerak balas permukaan (RSM) untuk memperolehi hasil pemegunan xilanase yang maksimum. Keputusan mendapati kandungan xilan dalam MWS adalah sebanyak 21.89% dan hasil pemulihan xilan selepas proses ekstraksi adalah sebanyak 39.45% daripada jumlah asal xilan yang terdapat dalam komposisi MWS. Untuk proses pemegunan xilanase, teknik pengabungan pemerangkapan dan pengikatan kovalen menunjukkan hasil pemegunan tertinggi (65.83%) berbanding dengan teknik tunggal, masing-masing hanya menyumbang sebanyak 31.98% dan 48.46%. Keadaan pemegunan xilanase yang optimum oleh RSM diperolehi pada 16.76% (w/w) kepekatan gluraldehid, 3.13% (w/v) kepekatan natrium alginat, dan 178 U pemuatan enzim dengan hasil maximum pemegunan sebanyak 82.61%. Proses pemegunan enzim telah meningkatkan kestabilan pH dari 7.0 kepada 8.0 dan kestabilan suhu dengan mengalihkan suhu optimum dari 50 kepada 60 °C. Kajian termodinamik menunjukkan bahawa xilanase pegun telah menurunkan sedikit E_a dari 15.24 kepada 14.80 kJ·mol^{-1}, dimana ianya menunjukkan peningkatan kecekapan pemangkinan xilanase. Xilanase pegun juga menunjukkan kestabilan operasi yang baik dengan mengekalkan kira-kira 81% dan 60% daripada aktiviti awalnya semasa kitaran kedua dan ketiga. Xilanase pegun pada keadaan optimum kemudiannya digunakan dalam hidrolisis berenzim untuk proses degradasi xilan, dan penghasilan jumlah XOS dan derivatifnya dibandingkan dengan tindak balas enzim bebas dengan xilan komersial. Jumlah hasil XOS tertinggi daripada xilan MWS dengan tindak balas xilanase pegun adalah sebanyak 53.61 mg/g pada keadaan hidrolisis terbaik pada 2% (w/v) kepekatan substrat, 48 jam hidrolisis dan pada suhu 55 °C. Semasa hidrolisis, xilanase pegun menghasilkan XOS dengan tahap polimerisasi (DP) yang rendah, terdiri daripada xilobios (X2), xilotrios (X3), dan xilotetraos (X4) daripada pemecahan xilan MWS, yang mana merupakan jenis oligomer yang lebih baik dan sesuai terutamanya untuk aplikasi dalam industri makanan. Kajian kebolehgunaan semula xilanase pegun dalam penghasilan XOS dapat mengekalkan 70% daripada pengeluaran XOS awal semasa kitaran kedua dengan kebolehgunaan semula sebanyak lima kitaran berturut-turut. Mengambil kira kebolehlaksanaan ekonomi dan aplikasi industri, MWS menunjukkan potensi sebagai sumber substrat baru untuk penghasilan XOS. Xilanase pegun melalui teknik penggabungan juga menunjukkan hasil yang baik dari segi kestabilan dan kebolehgunaan semula untuk proses hidrolisis berterusan.

ABSTRACT

The limitations of free or soluble enzyme such as non-reusability, poor stability, and sensitivity to denaturation could be handled by the use of immobilised enzymes. Generating a value-added product, xylooligosaccharides (XOS) from new renewable material, Meranti wood sawdust (MWS) by the used of immobilised xylanase are currently an object of interest due to their benefits over soluble xylanase. The aim of this study is to immobilise xylanase for XOS production from MWS by enzymatic hydrolysis. Xylan from MWS was extracted using a standard chlorite holocellulose method. Prior to enzymatic hydrolysis, immobilised xylanase was prepared using a different technique of immobilisation comprised of a single entrapment, single covalent binding, and a combination of entrapment and covalent binding. The immobilisation conditions were optimised using a systematic experimental design which includes one-factor-at-a-time (OFAT) to study the effects of each parameter, followed by fractional factorial design (FFD) used for screening process to determine the significant parameters, and finally, optimisation by response surface methodology (RSM) to obtain maximum xylanase immobilisation yield. The results showed that xylan content in MWS was 21.89% and the recovery yield of xylan after extraction was 39.45% of original xylan available in MWS. For the immobilisation of xylanase, a combination technique of entrapment and covalent binding showed the highest immobilisation yield (65.83%) compared to single techniques which yielded only 31.98% and 48.46%, respectively. The optimum xylanase immobilisation conditions by RSM were obtained at 16.76% (w/w) of glutaraldehyde concentration, 3.13% (w/v) of sodium alginate concentration, and 178 U of enzyme loading with a maximum immobilisation yield of 82.61%. Immobilisation improved the pH stability from 7.0 to 8.0 and thermal stability by shifting the optimum temperature from 50 to 60 °C. Thermodynamic study indicated that immobilised xylanase slightly lowered the E_a from 15.24 to 14.80 kJ·mol^{-1}, which improves the catalytic efficiency of xylanase. The immobilised xylanase also exhibited a good operational stability, retaining about 81% and 60% of its initial activity during the second and third process cycles. The optimised immobilised xylanase then was applied in enzymatic hydrolysis to degrade the MWS xylan and the production of total XOS and its derivatives were compared to the reaction of free xylanase with commercial xylan. The highest total XOS yield obtained from MWS xylan by the reaction of immobilised xylanase was 53.61 mg/g at their best hydrolysis conditions at 2% (w/v) of substrate concentration, 48 h of hydrolysis, and 55 °C. During hydrolysis, the immobilised xylanase released a lower degree of polymerisation (DP) of XOS, mainly xylobiose (X2), xylotriose (X3), and xylotetraose (X4) from the degradation of MWS xylan, which are the preferable types of oligomers, particularly for food industry applications. From the reusability study, immobilised xylanase in the XOS production was able to retain 70% of its initial XOS production during the second cycle with five consecutive cycles. With respect to economic feasibility and industrial application, the MWS demonstrated the potential as a new source of xylan substrate for XOS production. Immobilised xylanase by a combination technique also showed good results in terms of stability and recycling efficiency for continuous hydrolysis.

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