



UNIVERSITI PUTRA MALAYSIA

**PRODUCTION OF CELLULASES BY INDIGENOUS FUNGI
(*ASPERGILLUS* SP. AND *TRICHODERMA* SP.) FOR SUBSEQUENT USE
IN BIOCONVERSION OF PALM OIL MILL EFFLUENT SOLID TO SUGAR**

WONG KOK MUN

FBSB 2005 37

**PRODUCTION OF CELLULASES BY INDIGENOUS FUNGI
(*ASPERGILLUS* SP. AND *TRICHODERMA* SP.) FOR SUBSEQUENT
USE IN BIOCONVERSION OF PALM OIL MILL EFFLUENT SOLID
TO SUGAR**



By

WONG KOK MUN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science**

October 2005

Specially dedicated to,

My beloved Family



© COPYRIGHT UPM

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

**PRODUCTION OF CELLULASES BY INDIGENOUS FUNGI
(*ASPERGILLUS* SP. AND *TRICHODERMA* SP.) FOR SUBSEQUENT
USE IN BIOCONVERSION OF PALM OIL MILL EFFLUENT SOLID
TO SUGAR**

By

WONG KOK MUN

October 2005

Chairman: Professor Mohd Ali Hassan, PhD

Faculty: Biotechnology and Biomolecular Sciences

The isolation of fungi was done from sources such as palm oil plantation, rotten fruits and et cetera, where out of fifty colonies isolated, six strains showed positive result on cellulose degradation and only two cellulolytic fungi were selected to undergo optimization. They were identified as *Aspergillus* sp and *Trichoderma* sp respectively. In the optimization process, different parameters were studied in order to increase the cellulases activity for both the fungi. Different types of substrates were tested such as POME solid, carboxymethylcellulose (CMC) and sugarcane bagasse. From the results obtained, sugarcane bagasse was the best substrate to induce the production of cellulases from the fungi. Therefore, in the optimization process sugarcane bagasse was used to examine the effect of different parameters conditions such

as temperature, nitrogen source, inoculum size and inoculum age. The fermentation that was carried out at 37°C, mixture of nitrogen sources consisted of (NH₄)₂SO₄, KNO₃, peptone and urea, 10% (v/v) inoculum size and at 48 hours of inoculum age proved to be the best conditions for cellulases production. For *Aspergillus sp*, 0.85 U/mL FPase, 2.03 U/mL CMCase and 3.00 U/mL β-glucosidase were obtained whereas for *Trichoderma sp* 0.90 U/mL FPase, 3.00 U/mL CMCase and 0.11 U/mL β-glucosidase were recorded respectively. The crude cellulase obtained was precipitated with ammonium sulphate to be further utilized in the saccharification process. The temperature stability and pH profile of the crude enzyme were also examined. The saccharification process for sugar production was carried out in controlled parameters such as different chemical pretreated POME solids, different ratio of enzyme concentration and different pH. The highest reducing sugars and glucose produced by using the crude enzyme were 22.8 g/L and 14.8 g/L respectively. Comparison on sugar production from POME solid by using diluted commercial cellulase enzyme for sugar production from POME solid was also done. About 0.23 g of reducing sugars and 0.15 g of glucose per gram of substrate was yielded by using the crude enzyme in the saccharification process. In conclusion, the objectives of the study were achieved with the isolation of local cellulase-producing fungi.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

**PENGHASILAN ENZIM SELULASE OLEH KULAT TEMPATAN
(*ASPERGILLUS SP.* DAN *TRICHODERMA SP.*) UNTUK DIGUNAKAN BAGI
BIOPENUKARAN PEPEJAL POME KEPADA GULA**

Oleh

WONG KOK MUN

October 2005

Pengerusi: Professor Mohd Ali Hassan, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Pengasingan telah dilakukan daripada sumber seperti ladang kelapa sawit, buah yang reput dan sebagainya. Daripada lima puluh koloni kulat, enam jenis kulat telah menunjukkan keputusan yang positif dalam selulosa degradasi dan dua selulolitik fungus telah dipilih untuk melalui proses optimisasi iaitu *Aspergillus sp.* dan *Trichoderma sp.* Dalam proses optimisasi, parameter yang berbeza digunakan untuk meningkatkan aktiviti selulase daripada kedua-dua kulat tersebut. Kesesuaian substrak yang berbeza seperti pepejal POME, hampas tebu dan 'carboxymethylcellulose' (CMC) turut diuji. Secara keseluruhannya, hampas tebu dipilih bagi proses optimisasi yang seterusnya kerana ia dapat mencetus peningkatan dalam penghasilan enzim selulase. Selain itu, perubahan dalam parameter yang berbeza seperti suhu, sumber nitrogen, saiz inokulasi dan umur inokulasi turut dikaji. Keputusan didapati keadaan yang paling sesuai bagi penghasilan selulase adalah fermentasi pada suhu 37°C, campuran sumber nitrogen, 10% saiz inokulasi dan penggunaan inokulasi yang berusia 48 jam. Bagi *Aspergillus sp.*, 2.85 U/ml FPase, 2.03 U/ml CMCcase dan 3.0 U/ml β -glucosidase

berjaya dihasilkan. Bagi *Trichoderma sp.* pula, 0.90 U/ml FPase, 3.0 U/ml CMCase dan 0.11 U/ml β -glucosidase telah dikesan. Enzim selulase mentah yang terhasil kemudian dimendakan dengan garam ammonium sulfat untuk digunakan dalam proses sakarifikasi. Profil kestabilan suhu dan pH bagi enzim mentah ini turut dikaji. Proses sakarifikasi dalam penghasilan gula dijalankan dalam parameter yang terkawal seperti pepejal POME pra-rawat secara kimia, nisbah enzim dan keadaan pH yang berbeza. Kandungan gula penurun dan glukosa yang tertinggi berjaya dihasilkan dengan menggunakan enzim selulase mentah adalah masing-masing sebanyak 22.8 g/L dan 14.8 g/L. Perbandingan dengan menggunakan komersial enzim selulase dalam penghasilan gula daripada pepejal POME juga dikaji. Lebih kurang 0.23g gula penurun / g substrak dan 0.15 g glukosa / g substrak terhasil daripada enzim mentah dalam proses sakarifikasi. Sebagai kesimpulan, objektif kajian telah dicapai dengan pengasingan kulat tempatan yang berupaya menghasilkan enzim selulase.

ACKNOWLEDGEMENTS

I wish to express my deepest appreciation and gratitude to my supervisor Prof. Dr. Mohd Ali Hassan and members of the supervisory committee Assoc. Prof. Dr. Suraini Abd Aziz, Prof. Dr. Mohamed Ismail Abdul Karim and Prof. Dr. Vikineswary, S for their advice and guidance throughout the project.

To my fellow colleagues and seniors; Sim Kean Hong, Cheong Weng Chung, Phang Lai Yee, Nor Aini, Voon Phooi Tee, Ooi Kim Yng, Azhari, Rafein, Munir and Syahman thank you for your help

A special thanks to my parents, sister and relatives for their support and love. To Sook Fong, thank you for your support, advice and patient throughout my study and in my life.

I certify that an Examination Committee has met on 12 October 2005 to conduct the final examination of Wong Kok Mun on his Master of Science thesis entitled “Production of Cellulases by Indigenous Fungi (*Aspergillus* sp. and *Trichoderma* sp.) for Subsequent Use in Bioconversion of Palm Oil Mill Effluent Solid to Sugar” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

LING TAU CHUAN, PhD

Lecturer
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

ROSFARIZAN MOHAMAD, PhD

Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

ARBAKARIYA ARIFF, PhD

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

KOPLI BUJANG, PhD

Associate Professor
Centre for Technology Transfer and Consultancy
Universiti Malaysia Sarawak
(External Examiner)

HASANAH MOHD. GHAZALI, PhD

Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

MOHD ALI HASSAN, PhD

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

SURAINI ABD AZIZ, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

MOHAMED ISMAIL ABDUL KARIM, PhD

Professor
Kulliyah of Engineering
International Islamic University, Malaysia
(Member)

VIKINESWARY, S, PhD

Professor
Faculty of Science
Universiti Malaya
(Member)

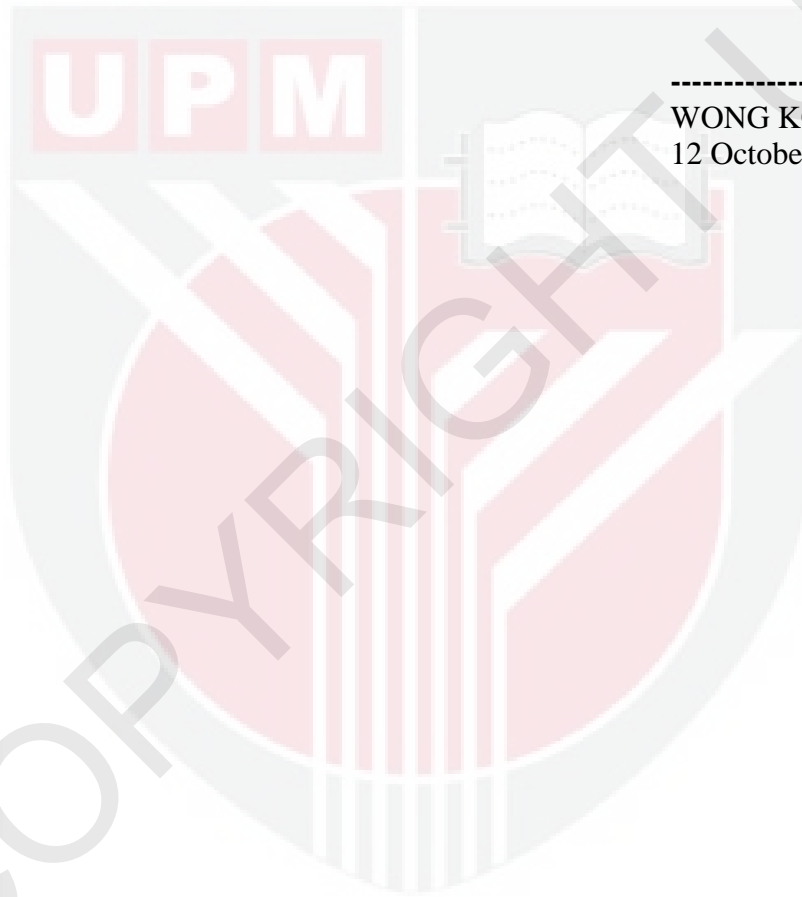
AINI IDERIS, PhD

Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for the 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 UPM of other institutions.



WONG KOK MUN
12 October 2005

TABLE OF CONTENTS

DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xv
LIST OF FIGURES	xvi
LIST OF PLATES	xxi
LIST OF ABBREVIATIONS	xxii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	4
2.1 Palm Oil Industry	4
2.1.1 Palm Oil Mill Effluent (POME)	6
2.1.2 Characteristics of Palm Oil Mill Effluent	7
2.2 Sugarcane Industry	8
2.3 Features of Lignocellulose	9
2.3.1 Lignocellulosics	9
2.3.2 Sources of Lignocellulosic Materials	9
2.3.3 Cellulose	10
2.3.4 Hemicellulose	12
2.3.5 Lignin	13
2.3.6 Degradation of Lignocellulose	14
2.4 Fungi	15
2.5 Cellulolytic Enzyme	17
2.5.1 Cellulases	17
2.5.2 Endoglucanase (EC 3.2.1.4)	18
2.5.3 Exoglucanase (EC 3.2.1.91)	18
2.5.4 β -Glucosidase (EC 3.2.1.21)	19
2.5.5 Synergistic Action Between Cellulases	19
2.5.6 Products from Lignocellulose Hydrolysis	20
2.5.7 Applications of Cellulases	21
3 GENERAL MATERIALS AND METHODS	24
3.1 General Plan of the Experimental Work	24
3.2 Enzymes	25
3.3 Substrate for Fermentation Processes	25
3.3.1 Sugarcane Bagasse	28
3.3.2 Carboxymethylcellulose	29
3.3.3 Palm Oil Mill Effluent Solid	29
3.4 Microorganism and Maintenance	29
3.5 Medium Composition	30

3.5.1	LCA (Miura Agar)	30
3.5.2	Basal Medium	31
3.5.3	POME Solids Agar	31
3.5.4	Potato Dextrose Agar (PDA)	32
3.6	Inoculum Preparation	32
3.7	Fermentation	32
3.8	Analytical Methods	33
3.8.1	Determination of Total Reducing Sugars	33
3.8.2	Determination of Glucose	33
3.8.3	Determination of Cellulose, Hemicellulose and Lignin Content	35
3.8.4	Measurement of Cellulase Activities	39
3.8.5	Soluble Protein Analysis	42
4	SCREENING AND ISOLATION OF LOCAL FUNGI STRAINS	44
4.1	Introduction	44
4.2	Material and Methods	45
4.2.1	Source of Screening	45
4.2.2	Screening and Isolation of Local Fungi	46
4.2.3	Analytical Procedures	46
4.3	Results	47
4.3.1	Screening and Isolation of Fungi from Various Sources	47
4.3.2	Production of Cellulase Enzyme and Reducing Sugar	49
4.3.3	Growth of Fungi on Cellulolytic Agar	52
4.3.4	Photomicrograph and Morphological Characteristics of Isolate A and Isolate T	55
4.4	Discussion	57
4.5	Conclusion	60

5	OPTIMIZATION OF FERMENTATION CONDITIONS FOR CELLULASE PRODUCTION BY LOCAL ISOLATES	61
5.1	Introduction	61
5.2	Materials and Methods	63
	5.2.1 Substrates	63
	5.2.2 Inoculum Preparation	63
	5.2.3 Analytical Procedures	63
5.3	Results	64
	5.3.1 Effects of Different Types of Substrate on Cellulase Production from <i>Aspergillus</i> sp	64
	5.3.2 Effects of Different Types of Substrate on Cellulase Production from <i>Trichoderma</i> sp	68
	5.3.3 Optimization Conditions for Cellulase Production	72
5.4	Discussion	106
5.5	Conclusion	109
6	SACCHARIFICATION OF PALM OIL MILL EFFLUENT (POME) SOLID TO SUGAR USING CELLULASE OF LOCAL ISOLATES	110
6.1	Introduction	110
6.2	Materials and Methods	111
	6.2.1 Crude Enzyme Preparation	111
	6.2.2 POME Solid Preparation	111
	6.2.3 Analytical Procedures	111
6.3	Results	112
	6.3.1 The Enzyme Activities of Crude Cellulase and Commercial Cellulase and Chemical Composition of POME Solid	112
	6.3.2 The Reaction Temperature Profile of Crude Cellulase	114
	6.3.3 The Reaction pH Profile of Crude Cellulase from <i>Aspergillus</i> sp and <i>Trichoderma</i> sp	116
	6.3.4 Residual Cellulases Activity of <i>Aspergillus</i> sp	118
	6.3.5 Residual Cellulases Activity of <i>Trichoderma</i> sp	120
	6.3.6 The Effects of Different Chemicals and Concentrations on Pretreatment of POME Solid for Sugar Production	122
	6.3.7 The Effects of Enzyme Ratio on POME Solid for Sugar Production	126
	6.3.8 The Effects of pH on POME Solid for Sugar Production	128
	6.3.9 Production of sugar from POME solid using Crude Cellulase and Commercial Cellulase	129
6.4	Discussion	132
6.5	Conclusion	

7	GENERAL DISCUSSION, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK	136
	REFERENCES	140
	APPENDICES	148
	BIODATA OF THE AUTHOR	153

