CELLULASE PRODUCTION BY A LOCALLY ISOLATED FUNGAL STRAIN GROWN ON OIL PALM EMPTY FRUIT BUNCH

By

AZHARI BIN SAMSU BAHARUDDIN

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

December 2006

Dedicated to:

My Loving and Caring Wife:

Nor Asma Ab. Razak

My Sweet Child:

Muhammad Akmal Azhari

My Loving and Supporting Parents:

In memory of my dad, Samsu Baharuddin Daud, who nurtured and gave me strong spirit

and

my mom, Jamaliah Adnan, who cares and understand

My Beloved Sister and Brother:

Suzana Samsu Baharuddin

Muhammad Hafiz Samsu Baharuddin

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

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Chairman : Professor Mohd Ali Hassan, PhD

Faculty : Engineering

The bioconversion of a local lignocellulosic material, i.e, oil palm empty fruit bunch (OPEFB) was studied in 250 ml Erlenmeyer flasks and locally designed rotary drum and tray cabinet bioreactors. The aim of this study is to utilize the OPEFB for the production of cellulolytic enzymes and sugars, as it offers an enormous economic potential for the bioconversion of agro-industrial residues generally regarded as waste. Bioconversion profiles suggest that the cellulolytic enzymes production from OPEFB in solid substrate fermentation (SSF) was better when using mono culture than co-culture condition with locally isolated fungal strains (*Aspergillus niger* EB4 and *Trichoderma sp* EB5). *A. niger* EB4 produced the highest cellulolytic enzymes activity (FPase 4.3 U/g, CMCase 8.2 U/g, β-glucosidase 19.1 U/g) at day 7 fermentation with carboxymethylcellulose (CMC) as pre-culture cellulase inducer. Soluble protein and reducing sugars were determined to evaluate fungal growth and substrate uptake in the SSF by the fungal strains. Scanning electron microscopy (SEM) showed the capability of these local fungal strains in mono and co-culture

conditions for OPEFB degradation. The bioconversion of pre-treated OPEFB for cellulolytic enzymes production by *A. niger* EB4 was successfully achieved in tray cabinet bioreactor (static condition, without forced aeration) which mimicked SSF conditions in flasks experiment. It is possible to obtain 3.2 ± 0.26, 6.3 ± 0.38, 19.0 ± 0.85 U/g enzyme activity of FPase, CMCase, and β-glucosidase respectively after 6 days fermentation. The extracted crude enzyme from tray cabinet bioreactor experiment was partially purified using ammonium sulphate precipitation. The results showed that protein fraction at 80% ammonium sulphate saturation had managed to precipitate the cellulolytic enzymes with recoveries of 8.1% (2.1 fold), 7.7% (2.0 fold) and 5.8% (1.5 fold) for β-glucosidase, CMCase and FPase respectively. The molecular weights of precipitated cellulolytic enzymes were estimated to be 67 and 120 kDa using SDS-PAGE analysis. The results of saccharification for 5% (w/v) pre-treated OPEFB using enzymatic hydrolysis suggest that the reducing sugars production was significantly affected by the concentration of enzymes and its purity. The use of precipitated cellullases at 3% (v/v) enzyme concentration (6 ml/g substrate) of *A.niger* EB4 successfully hydrolyze pre-treated OPEFB to produce 7.7 g/l of reducing sugars which corresponds to 27.8% conversion (55.3% cellulose fraction). Due to its rich organic nature, OPEFB can serve as a potential and feasible substrate for microbial process in the production of value added products such as cellulolytic enzymes and sugars.

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

PENGHASILAN SELULASE OLEH KULAT PENCILAN TEMPATAN DARI TANDAN KOSONG KELAPA SAWIT

Oleh

AZHARI BIN SAMSU BAHARUDDIN

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Biopenukaran bahan lignoselulosa tempatan seperti tandan kosong kelapa sawit (TKKS) dikaji di dalam 250 ml kelalang Erlenmayer dan bioreaktor yang direkabentuk tempatan seperti drum berputar dan kabinet dulang. Matlamat kajian ini ialah untuk memanfaatkan penggunaan TKKS bagi penghasilan enzim selulase dan gula, dimana ia menawarkan potensi ekonomi yang besar dalam biopenukaran hasil sampingan agro-industri yang sering dikenalpasti sebagai sisa bahan buangan. Profil biopenukaran mencadangkan bahawa penghasilan enzim selulolitik daripada TKKS di dalam fermentasi substrat pepejal adalah lebih baik menggunakan kultur tunggal berbanding dalam keadaan kultur bersama oleh kulat tempatan terpencil (*Aspergillus niger* EB4 dan *Trichoderma sp* EB5). Kulat *A.niger* EB4 didapati menghasilkan aktiviti enzim selulase yang tertinggi (FPase 4.3 U/g, CMCase 8.2 U/g, β-glukosidase 19.1 U/g) pada hari ke 7 fermentasi dengan CMC sebagai penggalak penghasilan selulase semasa pra-kultur. Protein boleh larut dan gula penurun diukur untuk menilai pertumbuhan kulat dan pengambilan substrat oleh kulat di dalam

fermentasi substrat pepejal. Imbasan mikroskopi elektron menunjukkan kebolehan kulat-kulat tempatan ini bagi degradasi TKKS. Biopenukaran pra-rawatan TKKS untuk penghasilan enzim selulase oleh *A.niger* EB4 telah berjaya dihasilkan di dalam bioreaktor kabinet dulang (keadaan statik, tanpa pengudaraan paksa) yang menyerupai keadaan fermentasi di dalam ujikaji kelalang. Penghasilan sebanyak 3.2 ± 0.26, 6.3 ± 0.38, 19.0 ± 0.85 U/g aktiviti enzim bagi FPase, CMCase, dan β-glukosidase masing-masing boleh diperolehi selepas 6 hari fermentasi. Enzim kasar yang diekstrak daripada bioreaktor kabinet dulang telah ditulenkan sebahagiannya menggunakan pemendakan ammonium sulphate. Pecahan protein pada 80% ketepuan ammonium suphate dapat menulenkan enzim selulotik dengan perolehan sebanyak 8.1% (2.1 fold), 7.7% (2.0 fold) dan 5.8% (1.5 fold) bagi β-glukosidase, CMCase dan FPase. Berat molekul selulase termendak dianggarkan pada 67 dan 120 kDa menggunakan analisis SDS-PAGE. Keputusan ujikaji sakarifikasi bagi 5% (w/v) pra-rawatan TKKS menggunakan hidrolisis enzim mencadangkan bahawa proses ini dipengaruhi secara signifikan oleh kepekatan enzim dan ketulenannya. Penggunaan selulase termendak pada 3% (v/v) kepekatan enzim (6 ml/gram substrat) dari *A.niger* EB4 berjaya menghidrolisis pra-rawatan TKKS untuk menghasilkan 7.7 g/l gula penurun bersamaan dengan 27.8% penukaran (55.3% pecahan selulosa). Berdasarkan kekayaan organik semulajadi, TKKS boleh digunakan sebagai substrat yang berpotensi dan boleh dimanfaatkan bagi proses mikrobial di dalam penghasilan produk bernilai tambahan seperti selulase dan gula.

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I certify that an Examination Committee met on 8th December 2006 conduct the final examination of Azhari bin Samsu Baharuddin on his Master of Science thesis entitled "Cellulases from oil palm empty fruit bunch by local *Aspergillus* and *Trichoderma* strains in solid substrate fermentation" 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:

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

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Date: 8 FEBRUARY 2007

DECLARATION

I hereby declare that the 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 UPM or other institutions.

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AZHARI BIN SAMSU BAHARUDDIN

DATE: 8 JANUARY 2007

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ADF	acid detergent fiber
A. niger	*Aspergillus niger*
a_w	water activity
BSA	bovine serum albumin
Ca^{2+}	calcium ion
dH_2O	distilled water
DNS	Dinitrosalicyclic acid
EtOH	ethanol
FPLC	Fast Performance Liquid Chromatography
LSF	Liquid-state fermentation
MARDI	Malaysia Agriculture Research and Development Institute
ME	metabolism energy
Mg^{2+}	magnesium ion
MW	molecular weight
NaOH	sodium hydroxide
NDF	neutral detergent fiber
OPB	Oil palm biomass
OPEFB	Oil palm empty fruit bunch
PAGE	Polyacrylamide Gel Electrophoresis
PDA	potato dextrose agar
PFF	press fruit fiber
PK	palm kernel
PKC	palm kernel cake
PKO	palm kernel oil
POME	Palm Oil Mill Effluent
rpm	rotations per minute
SEM	scanning electron microscopy
SSF	solid substrate fermentation
SDS	Sodium dodecyl sulfate
TEMED	N,N,N',N'– Tetramethyl Ethylenediamine

Tris-Cl	Tris chloride
U/ml	Unit per ml
UKM	Universiti Kebangsaan Malaysia
UPM	Universiti Putra Malaysia
v/v	volume per volume
w/v	weight per volume
μmole	micromole