UNIVERSITI PUTRA MALAYSIA

SOLID STATE FERMENTATION OF RICE STRAW FOR PRODUCTION OF CELLULASES BY SELECTED FUNGI

MD. MUNIR HAYET KHAN

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

June 2007

DEDICATION

TO

MY FATHER, MOTHER AND
MY YOUNGEST UNCLE
(A. TAHER KHAN)

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

SOLID STATE FERMENTATION OF RICE STRAW FOR PRODUCTION OF CELLULASES BY SELECTED FUNGI

By

MD. MUNIR HAYET KHAN

June 2007

Chairman: Salmiaton Ali, PhD

Faculty: Engineering

The production of cellulases from rice straw (RS) by four fungi: *Trichoderma harzianum (SCahmT105)*, *Trichoderma spp.(1) (STP101)*, *Trichoderma spp.(3) (STP103)* and *Phanerochaete chrysosporium* was investigated. The microbial treatment using solid state fermentation was conducted in 250 ml Erlenmeyer flasks considering rice straw as a major solid substrate. The highest cellulase activities such as 25.53 U/g of rice straw due to filter paper activity and 42.86 U/g of rice straw due to carboxymethyl cellulose activity were obtained at day 4 of cultivation using *Phanerochaete chrysosporium* for the purpose of selecting the best fungus among these four strains. Glucosamine for growth and reducing sugar as substrate utilization indicator were observed to evaluate the fermentation of rice straw in the experiment and pH values were recorded as well.

Four process parameters of the solid state fermentation namely moisture content, mineral content, co-substratre and inoculum size with three levels of each parameter were used to optimize the production of cellulases by Plackett-Burman technique under factorial design. The results for first phase of optimization showed that the production of cellulases were higher i.e. 26.43 U/g of rice straw due to filter paper of activity and 46.25 U/g of rice straw due to carboxymethyl cellulose activity compared to the production obtained during the earlier study of selecting best strain among four fungi but the optimum regions of the surfaces was not found. Second phase of optimization was conducted to determine the actual optimum conditions within the ranges of variables tested. The experimental data were used to develop second order polynomial models considering linear, quadratic and interaction effects of the variables (factors). The optimum values obtained at second phase of optimization for moisture content, inoculum size, co-substrate and mineral content were 50% (v/w), 10% (v/w), 1% (w/w) and 5% (v/w) respectively.

Using the final model equations the process factors/variables were tested by increasing or decreasing the values within the ranges of parameters tested and optimum production of cellulases were obtained to be 30.18 U/g of rice straw (FPU) and 53.93 U/g of rice straw (CMCase) for *Phanerochaete chrysosporium* with the optimum process conditions. A final experiment with these optimum process parameters of SSF was conducted to evaluate the production of cellulases as well as the validation of the models which indicated the production of 29.46 U/g of rice straw due to filter paper activity and 54.83 U/g of rice straw due to carboxymethyl cellulose activity in the laboratory which approved the optimum production obtained with 2.4% and 1.6% error, respectively.

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

PENAPAIAN JERAMI PADI DALAM BENTUK PEPEJAL BAGI PENGHASILAN SELULOSA DENGAN MENGGUNAKAN KULAT TERPILIH

Oleh

MD. MUNIR HAYET KHAN

Jun 2007

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Penghasilan selulosa dari jerami padi (RS) oleh empat kulat: *Trichoderma harzianum* (SCahmT105*), Trichoderma spp.(1)* (STP101), *Trichoderma spp.(3)* (STP103) dan *Phanerochaete chrysosporium* telah dikaji. Rawatan mikrob ini menggunakan penapaian dalam bentuk pepejal telah dijalankan dalam termos Erlenmeyer 250 ml dengan mengambil kira jerami padi sebagai substrat pepejal utama. Aktiviti selulosa tertinggi seperti 25.53 U/g jerami padi dari aktiviti kertas turas dan 42.86 U/g jerami padi dari aktiviti selulosa karboximetil telah diperolehi pada hari ke-4 penanaman menggunakan *Phanerochaete chrysosporium.* Glucosamine sebagai petanda pertumbuhan dan penurunan gula sebagai utilisasi substrat telah diperhatikan untuk menilai penapaian jerami padi di dalam eksperimen nilai pH juga direkodkan.

Empat parameter proses iaitu kandungan lembapan, kandungan bahan mineral, substrat bersama dan saiz inokulum dengan tiga aras bagi setiap parameter

digunakan untuk mengoptimasi penghasilan selulosa menggunakan teknik Plackett-Burman dibawah penggunaan rekabentuk faktorial. Fasa optimasi pertama menunjukkan penghasilan selulosa yang lebih tinggi iaitu 26.43 U/g jerami padi dari aktiviti kertas turas dan 46.25 U/g jerami padi dari aktiviti selulosa karbosimetil berbanding penghasilan yang diperolehi semasa kajian awal pemilihan strain yang terbaik tetapi kawasan optimum permukaan tidak dapat ditemui. Fasa kedua optimasi telah dijalankan untuk menentukan keadaan optimum yang sebenar di dalam julat yang diuji. Data eksperimen telah digunakan untuk membentuk model regresi polinomial kelas kedua dengan mempertimbangkan kesan-kesan linear, kuadratik dan interaksi. Nilai-nilai optimum yang diperolehi di fasa kedua optimasi bagi kandungan lembapan, saiz inokulum, substrat bersama dan kandungan bahan mineral adalah 50% (v/w), 10% (v/w) 1% (w/w) dan 5% (v/w) masing-masing.

Dengan menggunakan persamaan model terakhir, faktor proses tersebut diuji dengan menaikkan atau menurunkan nilai tersebut dalam julat parameter yang diuji dan penghasilan selulosa optimum yang didapati adalah 30.18 U/g jerami padi dari aktiviti kertas turas dan 53.93 U/g jerami padi dari aktiviti selulosa karbosimetil untuk *Phanerochaete chrysosporium* menggunakan keadaan proses optimum. Satu eksperimen terakhir dengan parameter proses optimum penapaian keadaan pepejal telah dijalankan untuk menilai penghasilan selulosa dan juga untuk persetujuan model tersebut, dan didapati penghasilan sebanyak 29.46 U/g jerami padi dari aktiviti kertas turas dan 54.83 U/g jerami padi dari aktiviti selulosa karbosimetil di dalam makmal telah diperolehi yang mana telah mengesahkan penentuan penghasilan optimum dengan kesilapan 2.4% dan 1.6% masing-masing.

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I certify that an examination committee has met on 26th June 2007 to conduct the final examination of Md. Munir Hayet Khan on his Master of Science thesis entitled "Solid State Fermentation of Rice straw for Production of Cellulases by selected Fungi" 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 was 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 Supervisory Committee were as follows:

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Date: 15 November 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.

MD. MUNIR HAYET KHAN

Date: 5th October 2007

TABLE OF CONTENTS

LIST OF TABLES

* Table 4.9 – 4.15 (Refer to Second phase of optimization)

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
A_w	Activity of Water
CMC	Carboxymethylcellulose
CMCase	Carboxymethylcellulose assay
CER	CO_2 Evolution rate
C/N	Carbon/Nitrogen ratio
DP	Degree of polymerization
DNS	Dinitrosalicyclic acid
DOE	Design of experiment
FPA	Filter paper activity
FPU	Filter paper unit
FPase	Filter paper assay
GLOX	Glyoxal oxidase
IU	International Unit
IIUM	International Islamic University Malaysia
LiP	Lignin Peroxidases
LSB	Liquid State Bioconversion
LSF	Liquid state fermentation
MnP	Manganese peroxidases
MARDI	Malaysian Agricultural Research Development Institute
NS	Nutrient Salts
OCR	O_2 Consumption rate

PC	*Phanerochaete chrysosporium*
RS	Rice straw
RSM	Response Surface Methodology
rpm	Rotation per minute
R^2	Coefficient of Determination
spp.	Species
SSB	Solid state bioconversion
SSF	Solid state fermentation
SmF	Submerged fermentation
STP	Sewage treatment plant
T	*Trichoderma harzianum*
T-1	*Trichoderma spp.(1)(STP101)*
T-3	*Trichoderma spp.(3)(STP103)*
UV	Ultra Violate
v/v	Volume/volume
v/w	volume/weight
WF	Wheat flour
w/w	Weight/Weight
Y(FPA)	Yield for filter paper activity
Y(CMC)	Yield for carboxymethylcellulose activity

CHAPTER 1

INTRODUCTION

1.1 Problem Statement

There were 113 rice-producing countries in 2000, where 10 countries produced more than 10 million tones (Mt) annually, 20 produced between 1 and 9.99 Mt, 35 produced between 100 000 and 999 999 tonnes, and 48 less than 100 000 tonnes (Nguyen, 2002). The total paddy planted areas for Malaysia in the year 2000 was about 600,287 hectares producing 2,050,306 tonnes of paddy. The regions which are devoted to rice production are Kedah (31.05%), Sarawak (19.41%), Perak (11.81%) and Kelantan (11.38%) reported by Ludin et. al (2004). Malaysia is about 65% self sufficient in rice supply and another 35% is imported from Thailand and Vietnam. Paddy straw and rice husk are generated as biomass residue during the harvesting and milling processes. The paddy straw is left in the paddy field and the rice husk is generated in the rice mill. Both of the biomass is disposed to landfill or by open burning. Only a small quantity of rice husk is used for energy generation and other application such as silica production and composting. The amount of rice husk and paddy straw generated in the future are dependent on the planted area, paddy yield and government policies on agriculture. Ludin et. al (2004) reported that the Malaysian government plans to increase the yield from the existing rate to 10 metric tonne per hectare in the future. According to the United Nations estimation, by 2020 the world population will have swollen to around 8 billion people where 5 billion of whom will be rice consumers. It is estimated that the world's rice harvest should increase from 560 million tonnes to 840 million tonnes per year to meet the demand. As a result more rice straw will be produced creating environmental problems.

Rice straw is produced throughout the world as a byproduct of rice cultivation. The options for the disposition of rice straw are limited by the great bulk of material, slow degradation in the soil, harboring of rice stem diseases, and high mineral content. The paddy fields should be cleaned from straw to make ways for the next crops. Soil incorporation and field burning have been the major practices for removing the rice straw. Field burning is fast, economical and removes disease organisms, but is now tightly regulated. Rice straw burning and soil incorporation have global environmental risk implications. The carbon content from rice straw is about 40%, and the burning of 500,000 tonnes of rice straw may return 200,000 tonnes of carbon into the atmosphere (Bainbridge, 1997). This carbon is fixed during the growing season by photosynthesis and there is little net gain. If the straw is incorporated in the soil it increases methane emissions which are more damaging than the byproducts of burning. Methane is a special concern for global warming; each molecule of methane has 20-25 times the heat capturing potential of a carbon dioxide molecule. Even allowing for the lower level of emissions, the net impact on global warming would be 10-15 times worse than the effects of carbon dioxide from field burning (Bainbridge, 1997). The use of rice straw for other purposes that would store or quester carbon would decrease emissions and reduce global warming risks. Incorporation is slower, more expensive and may foster rice diseases. Since neither of these traditional methods is ideal, additional alternatives have been sought and developed. One of the major alternative uses is as a