



UNIVERSITI PUTRA MALAYSIA

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

MD. MUNIR HAYET KHAN

FK 2007 42

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

MD. MUNIR HAYET KHAN

**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-substrate 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

Pengerusi: Salmiaton Ali, PhD

Fakulti: Kejuruteraan

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.

ACKNOWLEDGEMENTS

In the name of ALLAH, The Most Gracious and The Most Merciful, all my appreciations, gratitude and gratefulness for HIS endless favors to complete this research. My heartiest thanks to Dr. Salmiaton Ali, chairman of the supervisory committee, and Associate Professor Dr. Fakhru'l-Razi Ahmadun, member of supervisory committee, for their precious guidance, invaluable advice, untiring assistance, encouragement, motivation and social support that enabled me to accomplish the Master program smoothly and efficiently.

I am especially extremely indebted to Associate Professor Dr. Md. Zahangir Alam, IIUM, Malaysia, member of supervisory committee, for arranging this whole research work in the Environmental Biotechnology Laboratory there, for his valuable guidance, constructive suggestions, encouragement, motivation and generous help throughout my study period. I am deeply obliged for his suggestions and inspiration in the preparation of the research proposal on early stage of the study and preparation of this thesis. I am also sincerely grateful to him and his family for openhearted social support in Malaysia.

My appreciation also goes to all of my teachers especially to Prof. Dr. Azni Idris and Dr. Luqman Chuah Abdullah. I am also grateful to the staff members of all laboratories of Biotechnology Engineering in IIUM, especially to Sister Suharti and the staff members in UPM (Biochemical, Environmental, Process and Computer lab), Mr. Termizi, Mr. Joha and Mr. Ismail for their gentle assistance throughout



the course of the study. My special thanks to Dr. Abdullah Al Mamun (IIUM) for generous supports and to Dr. Luqman Chuah Abdullah for helping to buy some valuable chemicals of this research work. I like to thank Ms. Christina for her help to translate the abstract of my thesis from English into Bahasa Melayu. Grateful acknowledges are extended to all the staff members of the Department of Chemical and Environmental Engineering for their sincere support, help and cooperation.

I would like to convey special thanks to my friends and Bangladeshi fellows Dr. Rowson Kamal, Dr. Noman, Dr. Niaz Pasa, Dr. Ataur Rahman, Dr. Hamid, Mrs. Mahafuza, Mrs Tabassum, Mrs Shampa, Mr. Enayet Karim, Dr. Sabira Khatun, Dr. Abul Hossain Molla, Dr. Salim Khan (Malaysia) for their cooperation and encouragement during my study period in Malaysia. Special thanks also go to Mr. Parvez Iqbal, Mr. Jakir Hossen, Mr Sarwar Jahan, Mr. Shofiquzzaman, Mr. Altab hossain, Mr. Obaydullah , Mr. A. Latif, Mr. Mohammad (Iran) and Ms Norhayati for their company, help and encouragement to finish the study smoothly.

My warmest special thanks go to Md. Nazmus Saadat (KKK) and friend Md. Abdul Mannan Sarkar (KKA) for their constant encouragement, moral support, ideas, inspirations, full computer using support and time to time discussions for continuing research. I would like to give special thanks to Lab mate Mr. Ajie, Ms. Maria, Mr. Fadhlán of IIUM and Mrs. Hind, Mrs. Roshanida, Ms. Sukaina, Ms. Fateme, Mr. Ahmed, and Mr. Omar of UPM for their help and cooperation.



I am very much grateful to the authority of International Islamic University Malaysia (IIUM) for providing support by allowing to perform this research work during the study period.

My heartfelt and warmest appreciation goes to my mother, brothers, sisters, other relatives, Mr. Charles Wilson and Mr. Ying Kei Thomas Ha who always encouraged and supported me during the study period at abroad. Their sacrifices and affections are motivated me to complete this study a great success in my carrier.



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:

Professor Azni Idris, PhD
Department of Chemical and Environmental Engineering
Universiti Putra Malaysia
43400 UPM Serdang
Selangor
(Chairman)

Professor Ali Hasan, PhD
Faculty of Biotechnology and Molecular Science
Universiti Putra Malaysia
43400 UPM Serdang
Selangor
(Internal Examiner)

Associate Professor Tey Beng Ti, PhD
Department of Chemical and Environmental Engineering
Universiti Putra Malaysia
43400 UPM Serdang
Selangor
(Internal examiner)

Associate Professor Azlina Harun, PhD
Center for Chemical Engineering Studies
Engineering Campus, Universiti Sains Malaysia
14300 Nibong Tebal, Seberang Perai Selatan
Pulau Pinang
(External Examiner)

HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



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:

Salmiaton Ali, PhD

Lecturer
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

Fakhru'l-Razi Ahmadun, PhD

Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

Md. Zahangir Alam, PhD

Associate Professor
Faculty of Engineering
Universiti Islam Antarabangsa, Malaysia
(Member)

AINI IDERIS, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xxi
CHAPTER	
1 INTRODUCTION	1
1.1 Problem Statement	1
1.2 Background of the Study	3
1.3 Justification of Research	5
1.4 Objectives of Study	7
1.5 Thesis arrangement	7
2 LITERATURE REVIEW	9
2.1 Lignocellulosic materials	9
2.1.1 Cellulose	9
2.1.2 Hemicelluloses	11
2.1.3 Lignin	12
2.1.4 Other cell wall components	12
2.2 Degradation of lignocellulosic material	13
2.2.1 Degradation of cellulose and wood products	13
2.2.2 Degradation of hemicelluloses	15
2.2.3 Degradation of lignin	15
2.2.4 Pretreatment of substrate	16
2.3 Applications of lignocellulosic materials	18
2.3.1 Chemicals	19
2.3.2 Bio-fuel	20
2.3.3 Other high-value byproducts	20
2.3.4 Various Enzymes	22
2.4 Production cost of enzyme	24
2.5 Screening for microorganisms with enzymes	26
2.6 Potential microorganisms	27
2.6.1 Fungi	27
2.6.2 Bacteria	28
2.6.3 <i>Trichoderma species</i>	28
2.6.4 <i>Phanerochaete chrysosporium</i>	31
2.7 Cellulase enzyme	33
2.8 Solid State Fermentation	38
2.8.1 Introduction, comparison and review of SSF process factors	38



	2.8.2 Physical and Chemical factors of SSF	44
	2.8.3 Microbial factor of SSF	46
2.9	Optimization	51
3	MATERIALS AND METHODS	54
3.1	Experimental Materials	55
3.1.1	Substrate and Co-Substrate	55
3.1.2	Fungal strain	55
3.1.3	Mineral solution	56
3.1.4	Equipments	57
3.1.5	Consumable items	57
3.1.6	Chemicals and reagents	57
3.2	Experimental Methods	57
3.2.1	Sample preparation	57
3.2.2	Preparation of Inoculum	58
3.2.3	Solid state fermentation	58
3.2.4	Extraction method	59
3.2.5	Total Cellulase Activity Assay Using Filter Paper	60
3.2.6	Measurement of Endo-1,4- β -glucanase using Carboxymethyl-cellulose (CMCase)	63
3.2.8	Glucosamine determination	65
3.2.9	Reducing sugar estimation	66
3.2.10	pH Analysis	68
3.3	Optimization of Solid state fermentation process factors for the treatment of Rice straw by screened fungi <i>Phanerochaete chrysosporium</i> in shake flask	68
3.4	Evaluation of Production performance for the Solid state fermentation of Rice straw under optimized process factors to produce cellulose enzyme	72
4	RESULTS AND DISCUSSION	74
4.1	Selection of potential fungi for the production of cellulase using rice straw as substrate in shake flask by solid state fer- -mentation (SSF) process	74
4.1.1	Total Cellulase Activity using Filter paper	74
4.1.2	Endo- 1,4- β -glucanase by carboxymethylcellulose (CMC)	78
4.1.3	Glucosamine Estimation	80
4.1.4	Reducing sugar estimation	81
4.1.5	pH Anylysis	82
4.2	Statistical Optimization of Solid State Fermentation Process factors for the production of cellulases by potential fungi <i>Phanerochaete Chrysosporium</i>	83
4.2.1	Regression model of the Plackett-Burman technique	84
4.2.2	Interaction between moisture and mineral content	92
4.2.3	Interaction between moisture content & co-substrate	93
4.2.4	Interaction between moisture content and inoculum	98
4.2.5	Interaction between mineral content and wheat flour	98
4.3	Regression model by Plackett-Burman design methodology	

		103
4.3.1	Interaction between moisture content and inoculum	111
4.3.2	Interaction between moisture and co-substrate	114
4.3.3	Interaction between inoculum and mineral content	114
4.3.4	Interaction between moisture content and mineral	120
4.3.5	Plot of test variables	123
4.4	Evaluation of production performance for the Solid State Fermentation (SSF) of rice straw under optimum process factors to produce cellulase enzymes	127
5	CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES	132
5.1	Conclusions	132
5.2	Recommendations for future studies	134
	REFERENCES	136
	APPENDICES	A.1
	BIODATA OF THE AUTHOR	B.1
	LIST OF PUBLICATIONS	B.2



LIST OF TABLES

Table		Page
2.1	Types of lignocellulosic materials and their current uses	18
2.2	Annual production of chemicals which could potentially be made from fermentation	19
2.3	Lignocellulose contents of common agricultural residues and wastes	23
2.4	Comparison between liquid and solid substrate fermentations	40
2.5	Main groups of microorganisms involved in Solid State Fermentation	47
3.1	The levels of parameter for process conditions in first phases for 3-level Plackett-Burman design of experiments	69
3.2	The factorial design of experiments by Plackett-Burman method of MINITAB software.	70
3.3	The ranges of parameter of process conditions in second phase of optimization	70
3.4	Data Matrix (randomized) for the experimental design by Plackett-Burman technique for the process parameter of moisture, inoculum, co-substrate and mineral under Minitab software.	72
3.5	Optimized factors of SSF process for treatment of rice straw	73
4.1	Comparison of cellulase production by different fungal species and substrates	77
4.2	The coefficient of determination (R^2) of regression model for Yield	85
4.3	ANOVA for the selected quadratic model, Y(FPA)	86
4.4	ANOVA for the selected quadratic model, Y(CMC)	87
4.5	Statistical analysis showing coefficient of t-value and p-value, Y(FPA)	88



4.6	Statistical analysis showing coefficient of t-value and p-value, Y(CMC)	89
4.7	Actual values of coded data of x_1 , x_2 , x_3 and x_4	91
4.8	Plackett-Burman design matrix along with experimental and predicted values of yield	92
4.9*	The coefficient of determination (R^2) of regression model for yield	105
4.10	ANOVA for the selected quadratic model, Y(FPA)	106
4.11	ANOVA for the selected quadratic model, Y(CMC)	106
4.12	Statistical analysis showing coefficient of t-value and p-value, Y(FPA)	107
4.13	Statistical analysis showing coefficient of t-value and p-value, (CMC)	108
4.14	Actual values of coded data of x_1 , x_2 , x_3 and x_4	109
4.15	Plackett-Burman design matrix along with the experimental and predicted values	110
4.16	Predicted production of cellulase at different values of test variables using regression model	124

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



LIST OF FIGURES

Figure	Page	
2.1	Structure of cellulose microfibrils	11
3.1	Flow chart of experimental design	54
3.2	Rice Straw	55
3.3	Growth culture of four fungi (a) <i>Trichoderma harzianum</i> , (b) <i>Trichoderma spp.(1)</i> , (c) <i>Trichoderma spp.(3)</i> and (d) <i>Phanerochaete chrysosporium</i> on PDA plate	56
3.4	Extracted samples of Enzyme	60
3.5	Glucose Standard curve (FPase)	62
3.6	Glucose standard curve for CMCCase	64
3.7	Glucosamine standard curve	65
3.8	Standard curve for reducing sugar estimation	68
4.1	Enzyme activity of cellulase production	76
4.2	Enzyme activity in cellulase production (CMCCase)	79
4.3	Glucosamine estimation in cellulase production	80
4.4	Reducing sugar released in cellulase production	81
4.5	pH analysis in shake flask	82
4.6	The interaction between Moisture (x_1) and Mineral (x_2) for Y(FPA) (a) 3D wireframe plot and (b) Contour plot curves, Inoculum and co-substrate at center point values	94
4.7	The interaction between Moisture and Mineral content for Y (CMC)-yield (a) 3D wireframe plot and (b) Contour plot curves, Inoculum and co-substrate at center point values	95
4.8	The interaction between Moisture and Wheat flour for Y (FPA)-yield (a) 3D wireframe plot and (b) Contour plot curves, Inoculum and mineral at center point values.	96
4.9	The interaction between Moisture and Wheat flour for Y(CMC) :	



	(a) 3D wireframe plot and (b) Contour plot curves, Inoculum and mineral at center point values	97
4.10	The interaction between Moisture and Inoculum size for Y(FPA) : (a) 3D wireframe plot and (b) Contour plot curves, co-substrate and mineral at center point values	99
4.11	The interaction between Moisture and Inoculum for Y(CMC) : (a) 3D wireframe plot and (b) Contour plot curves, co-substrate and mineral at center point values	100
4.12	The interaction between Mineral and Wheat flour for Y(FPA) : (a) 3D wireframe plot and (b) Contour plot curves, Inoculum and moisture at center point values	101
4.13	The interaction between Mineral and Wheat flour for Y(CMC) : (a) 3D wireframe plot and (b) Contour plot curves, Inoculum and moisture at center point values	102
4.14	The interaction between Moisture and Inoculum for Y(FPA) (a) 3D wireframe plot and (b) Contour plot curve, mineral and co-substrate at center point values	112
4.15	The interaction between Moisture and Inoculum for Y(CMC) (a) 3D wireframe plot and (b) Contour plot curve, mineral and co-substrate at center point values	113
4.16	The interaction between Moisture and Co-substrate for Y(FPA): (a) 3D wireframe plot and (b) Contour plot curves, mineral and inoculum at center point values	116
4.17	The interaction between Moisture and Co-substrate for Y(CMC) (a) 3D wireframe plot and (b) Contour plot curve, mineral and inoculum at center point values	117
4.18	The interaction between Inoculum and Mineral for Y(FPA): (a) 3D wireframe plot and (b) Contour plot curves , moisture and co-substrate at center point values	118
4.19	The interaction between Inoculum and Mineral for Y(CMC): (a) 3D wireframe plot and (b) Contour plot curves, moisture and co-substrate at center point values	119
4.20	The interaction between Moisture and Mineral for Y(FPA): (a) 3D wireframe plot and (b) Contour plot curves, inoculum and co-substrate at center point values	121
4.21	The interaction between Moisture and Mineral for Y(CMC): (a)	



	3D wireframe plot and (b) Contour plot curves, inoculum and co-substrate at center point values	122
4.22	Plot of test variables for Y(FPA) (a) linear effect (b) quadratic effect (c) interaction effect.	125
4.23	Plot of test variables for Y(CMC) (a) linear effect (b) quadratic effect (c) interaction effect	126
4.24	Solid state fermentation (SSF) of rice straw under optimum process factors using <i>Phanerochaete chrysosporium</i> at day 4 of fermentation period, (a) With inoculum, (b) Control (without inoculum)	127
4.25	Cellulase enzyme production by <i>Phanerochaete chrsosporium</i>	128
4.26	Glucosamine concentration for 5 days fermentation period	129
4.27	Reducing sugar released during 5 days of fermentation	130
4.28	pH values in shake flask	131

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	Dinitrosalicylic 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	<i>Phanerochaete chrysosporium</i>
RS	Rice straw
RSM	Response Surface Methodology
rpm	Rotation per minute
R ²	Coefficient of Determination
<i>spp.</i>	Species
SSB	Solid state bioconversion
SSF	Solid state fermentation
SmF	Submerged fermentation
STP	Sewage treatment plant
T	<i>Trichoderma harzianum</i>
T-1	<i>Trichoderma spp.(1)(STP101)</i>
T-3	<i>Trichoderma spp.(3)(STP103)</i>
UV	Ultra Violet
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 tonnes (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 sequester 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

