UNIVERSITI PUTRA MALAYSIA

MATHEMATICAL MODELLING FOR GROWTH OF ASPERGILLUS NIGER FTCC 5003 DURING SOLID-STATE FERMENTATION ON PALM KERNEL CAKE

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

June 2005

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

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Chairperson: **Associate Professor Suraini Abd-Aziz, PhD**

Faculty: **Faculty of Biotechnology and Biomolecular Sciences**

An attempt to obtain the first model for growth of *Aspergillus niger* FTCC 5003 in solid-state fermentation (SSF) using palm kernel cake (PKC) as substrate was investigated. Initially, it was found that pre-germination of fungal spores occurred after 10 hours fermentation. Hence, 10 hours after fermentation process is used to denote 0 day in the present work. Both protein and glucosamine contents were well correlated to the fungal dry weight in SSF on support material as described by the equations, whereby fungal dry weight = (protein concentration – 0.10) / 3.03 and fungal dry weight = (glucosamine concentration - 6.57) / 222.89, respectively. These equations were then used as calibration standard for indirect biomass estimation for growth of *Aspergillus niger* FTCC 5003 in SSF on PKC system. Estimated fungal dry weight based on protein and glucosamine concentration, PKC dry weight and β-mannanase activity were determined in the study on growth of *Aspergillus niger* FTCC 5003 in SSF using PKC as substrate. It was found that the relationship between PKC dry weight and estimated fungal dry weight based on protein concentration and between PKC dry weight and β-mannanase well described the growth pattern of *Aspergillus niger* FTCC 5003 in this system with correlation

coefficient values of -0.97 and 0.97, respectively. However, the relationship between PKC and estimated fungal dry weight based on glucosamine concentration was poor as the correlation coefficient value between these two variables was – 0.30. An initial kinetic study showed that the experimental data for growth of *Aspergillus niger* FTCC 5003 in SSF on PKC was less significant based on the Monod growth model. The study on logistic growth model found that the simulation curve fitted well to the experimental data for growth of *Aspergillus niger* FTCC 5003 during SSF on PKC which estimated fungal dry weight was based on protein concentration. The deviation between simulated and experimental data was not significant at significance probability of 5%. However, the simulation curve did not fit well to the experimental data for growth of *Aspergillus niger* FTCC 5003 in SSF on PKC which estimated fungal dry weight was based on glucosamine concentration. In the study of growth model based on β-mannanase activity, the growth model for estimated fungal dry weight based on glucosamine was not successfully determined and developed. Nevertheless, a new model was successfully obtained and proposed for the growth model based on β-mannanase activity for estimated fungal dry weight based on protein concentration. The proposed model was able to simulate the growth and reflect the actual growth profile of *Aspergillus niger* FTCC 5003 in SSF on PKC. Besides, the value of prob> $|T|$ in β-mannanase activity based growth model for estimated fungal dry weight based on protein concentration (0.95) found to be closer to 1 indicating that it was able to simulate the *Aspergillus niger* FTCC 5003 growth profile more precisely in this system compared to logistic growth model for estimated fungal dry weight based on protein concentration which the value of prob> $|T|$ was 0.76.

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

MODELLING MATEMATIK UNTUK PERTUMBUHAN *ASPERGILLUS NIGER* FTCC 5003 SEMASA FERMENTASI FASA PEPEJAL PADA HAMPAS ISIRONG KELAPA SAWIT

By

GAN SIEW HUNG

Jun 2005

Pengerusi: **Profesor Madya Suraini Abd-Aziz, PhD**

Fakulti: **Fakulti Bioteknologi dan Sains Biomolekul**

Percubaan untuk mendapatkan model pertama bagi pertumbuhan *Aspergillus niger* FTCC 5003 semasa fermentasi fasa pepejal (FFP) pada hampas isirong kelapa sawit (PKC) telah dijalankan. Pada peringkat permulaan pre-germinasi fungi diperolehi selepas 10 jam proses fermentasi. Oleh yang demikian, masa sifar pada kajian yang seterusnya mewakili 10 jam selepas proses fermentasi. Korelasi kandungan protein dan glukosamin dengan berat kering fungi dalam FFP yang dijalankan ke atas bahan sokongan adalah baik. Kedua-duanya diwakili oleh persamaan-persamaan berikut: berat kering fungi = (kepekatan protein – 0.10) / 3.03 dan berat kering fungi = (kepekatan glukosamin – 6.57) / 222.89. Persamaan-persamaan yang diperolehi digunakan sebagai piawaian untuk penentu ukuran anggaran biomas secara tidak langsung untuk pertumbuhan *Aspergillus niger* FTCC 5003 dalam FFP pada PKC. Berat kering fungi dianggarkan melalui kepekatan protein dan glukosamin, berat kering PKC dan aktiviti enzim β-mannanase telah ditentukan di dalam kajian mengenai pertumbuhan *Aspergillus niger* FTCC 5003 di dalam FFP pada PKC. Kajian menunjukkan bahawa hubungan di antara berat kering PKC dan berat kering fungi yang dianggar melalui kepekatan protein dan hubugan di antara berat kering

PKC dan activiti enzim β-mannanase telah menghuraikan bentuk pertumbuhan *Aspergillus niger* FTCC 5003 di dalam sistem ini dengan baik; dimana, nilai pekali korelasi masing-masing adalah -0.97 dan 0.97. Walaubagaimanapun, hubungan antara berat kering PKC dan berat kering fungi yang dianggar melalui kepekatan glukosamin adalah kurang baik dengan pekali korelasi, -0.30. Kajian awal mengenai kinetik pertumbuhan menunjukkan bahawa data eksperimen untuk pertumbuhan *Aspergillus niger* FTCC 5003 di dalam FFP pada PKC didapati kurang signifikan daripada model pertumbuhan Monod. Pada kajian model pertumbuhan logistik, didapati bahawa keluk simulasi adalah sepadan dengan data daripada eksperimen untuk pertumbuhan *Aspergillus niger* FTCC 5003 di dalam FFP pada PKC yang mengganggarkan berat kering fungi melalui kepekatan protein. Sisihan antara data simulasi dan data eksperimen adalah tidak bermakna pada kebarangkalian 5%. Walaubagaimanapun, keluk simulasi pertumbuhan *Aspergillus niger* FTCC 5003 di dalam FFP pada PKC yang mengganggarkan berat kering fungi melalui kepekatan glukosamin adalah didapati tidak sepadan dengan data daripada eksperimen. Kajian seterusnya mengenai model pertumbuhan berdasarkan aktiviti enzim β-mannanase gagal memperolehi model dapat diperolehi untuk anggaran berat kering fungi melalui kepekatan glukosamin. Sebaliknya, satu model baru telah dicadangkan untuk pertumbuhan berdasarkan aktiviti enzim β-mannanase dengan menggunakan anggaran berat kering fungi melalui kepekatan protein. Model yang dicadangkan ini didapati berupaya untuk mensimulasikan pertumbuhan dan menggambarkan pertumbuhan sebenar *Aspergillus niger* FTCC 5003 di dalam SSF pada PKC. Selain itu, nilai kebarangkalian > $|T|$ pada model pertumbuhan berdasarkan aktiviti enzim β-mannanase untuk berat kering fungi yang dianggarkan melalui kepekatn protein (0.95) yang lebih mendekati nilai 1 menunjukkan bahawa ianya berupaya

mensimulasikan pertumbuhan *Aspergillus niger* FTCC 5003 dengan lebih tepat berbanding dengan model pertumbuhan logistik untuk berat kering fungi yang dianggar melalui kepekatan protein dimana nilai kebarangkalian > $|T|$ hanya 0.76.

AKNOWLEDGEMENTS

Thanks to God for His blessing and His faithfulness upon completion of this research project and thesis. The author would like to express her sincere appreciation and respect to Assoc. Prof. Dr. Suraini Abd-Aziz, the chairperson of the supervisory committee for her understanding, supervision, advice and suggestions during the research as well as critical reviewing of the manuscripts. Deepest gratitude and sincere appreciation is also extending to Prof. Dr. Mohamed Ismail Abdul Karim and Prof. Dr. Mohd Ali Hassan, members of the supervisory committee for their constructive criticisms, guidance and time along the research and preparation of this thesis.

The heartfelt thanks and appreciation is also extending to family members, especially Dad in memory and Mom for their love, sacrifices and care; family members for their care and encouragement throughout these years. The author would also like to express her deep sense of thanks to Mr. Teoh Gim Hooi for his understanding, encouragement, wonderful patience and care. The author is also grateful to Mrs. Noraini Samat for her encouragement, generous help and suggestions from the very beginning and during unexpected time throughout this project.

Finally, special thanks to all members of the Bioprocessing Laboratory in Strategic Livestock Research Centre, MARDI Serdang, Enzyme Engineering and Bioprocess Engineering Laboratory, FSMB, UPM and fellow friends for their endless assistance and cooperation. Acknowledgements are also extended to the Ministry of Science, Technology and Environment (MOSTE), Malaysia for their financial support on RMK8 Top-down NBD project (01-03-03-003 BTK/ER/008).

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LIST OF ABBREVIATIONS

CRD	Completely Randomised Design
DIAS	Dynex Immunoassay System
DW	Dry weight
ESEM	Environmental Scanning Electron Microscope
FTCC	Food Technology Culture Collection
HCl	Hydrochloric Acid
MARDI	Malaysia Agricultural Research and Development Institute
MF	Membrane filter
MPOB	Malaysia Palm Oil Board
PKC	Palm kernel cake
Prob > |T|	Value of probability of greater |T|
SSF	Solid-state fermentation
TMS	Time Management Software
μ	Specific growth rate
$Y_{x/s}$	Yield coefficient of fungal dry weight obtained per amount of PKC consumed
X_a	Actual fungal dry weight
X_s	Simulated fungal dry weight
X_t	Fungal dry weight at time t

CHAPTER 1

INTRODUCTION

1.0 Introduction

Globalisation and liberalisation are issues that the livestock industry in Malaysia as well as other economic activities have to manage properly for continued well being. Livestock production in Malaysia are range from highly commercial intensive and technology driven production units as in the poultry industry to subsistence animal rearing activities such as ruminant and village chicken production. The livestock industry in Malaysia is actually having the opportunities to expand its export market that has already been initiated.

For continued well being, the industry needs to strive to be more competitive. Unfortunately, the poultry industry that contributes to about 80% of the total ex-farm value in livestock industry is still highly dependent on imported raw feed ingredients such as soybean meal, maize and fishmeal (Hawari, 2001). The high price paid to import feed ingredients has resulted in the high production cost. Malaysian government has expressed concern over the great lost of foreign exchange due to the importation of feed supplies and high cost in poultry production.

Therefore, research and development initiatives particularly in search of cost-effective feed substitute need to be carried out especially in searching for locally available feed resource to be included into poultry diet as alternative ingredients.

Lately, there has been an increase trend towards more efficient utilisation of agricultural by-product such as palm kernel cake (PKC), palm oil mill effluent (POME), sago meal and sugar cane baggase in bioprocesses production. Among the plantation crops in Malaysia, oil palm as a bulk commodity in this country covers a total area of 3,670,243 hectares in year 2002 (MPOB, 2002). Most of the plantation was in Peninsular Malaysia which account for about 60% of the total hectares, while others were in Sabah and Sarawak with 29% and 11%, respectively (MPOB, 2002).

Palm kernel cake is an important by-product of palm kernel oil extraction. Malaysia currently produces an annual quantity of 1,714,522 tonnes of PKC as by-product in the milling of palm kernel oil (MPOB, 2002). Most of the PKC produced are mainly exported to European Union, South Korea, New Zealand, Vietnam, Japan and others for use as animal feed ingredient. About RM 285.5 million has been generated from the exportation of PKC product.

Comparing the value of export of palm oil, PKC constitute only a small percentage of palm oil products and could be considered as not significant in the palm oil industry. However, in the livestock industry the utilisation of PKC as source of animal feed can be significant and important as with a targeted 50% replacement of improved PKC for maize and soybean in imported ingredients, a saving of RM 800 million annually is envisaged.

Many processes have been developed to utilise the agricultural by-product as alternative substrate for the production of value-added products. Biological conversion of agricultural by-product into various value-added products through

solid-state fermentation (SSF) is gaining research interest lately. Over the last 20 years there has been a growing interest in upgrading the solid wastes from agriculture and food processing for use as fermented animal feeds (Pandey, 1992). The conversion of PKC through SSF into value-added product such as poultry feed now becomes popular and is a practical approach in term of full utilisation of locally available biological resource.

Solid-state fermentation is a process whereby microbes of interest will grow and utilise the moist substrate materials in the absence of free water. Many bacteria, yeast and fungi are able to grow on solid substrate and find application in SSF processes. However, filamentous fungi are the most important group of microorganism for SSF processes and dominate in research work owing to their physiological capabilities and hyphal mode of growth. During microbial growth, secretion of hydrolytic enzymes and production of other useful metabolites will upgrade the quality of low nutrient value materials such as PKC. Utilisation and optimisation of fungi *spp* in SSF for PKC digestibility improvement have been extensively studied (Noraini *et al.* 2000; Jaafar *et al.* 2001). Previous study showed that *Aspergillus niger* FTCC 5003 was a suitable microbe for further investigation due to its ability in the depolymerisation of PKC fibre (Noraini *et al.* 2001).

There are different types of techniques available in measuring the growth of microorganism in SSF. Different direct biomass determination such as matrix removal methods by using gelatine matrix, enzyme digestion and membrane filter has been used as an attempt for recovery of fungal biomass in SSF. However, foreign substance & non-digestible residue were found to interfere with the measurement for

methods using gelatine matrix and enzyme digestion. The method of direct peeling off the membrane filter for biomass recovery was reported to be able to prevent the penetration of the fungal hyphae, *Rhizopus oligosporus* into the substrate (Mitchell *et al.* 1989). However, this method obviously cannot be used in actual SSF but could find application in the calibration to indirect method of biomass determination.

Due to the difficulties of measuring the fungal biomass in SSF, many researches for fungal biomass determination have made estimation based on indirect biomass determination or borrowed from other system i.e. submerged liquid fermentation (SmF). It is, however, questionable whether the content in SSF is the same as in SmF. Therefore, biomass estimation which is based on indirect biomass determination in the condition as similar as possible to the actual situation in SSF is clearly superior compared to calibration using SmF and it has been used in several researches.

As biomass is a fundamental parameter in the characterisation of microbial growth and is essential for kinetic studies on SSF, direct biomass determination by peeling off the membrane filter overlaid on top of PKC and SSF on support material have been conducted as to obtain complete recovery of fungal biomass and to find suitable relationships for biomass estimation based on protein and glucosamine determination. The fungal biomass estimation for complete recovery by peeling off membrane filter and SSF on support material based on protein and glucoamine content are important in the investigation of the growth pattern and model development of *Aspergillus niger* FTCC 5003 in SSF on PKC.

Mathematical models represent a convenient, concise and powerful way of describing the phenomena that occurred during SSF and their interactions. It also provides a sound foundation for process development, control, and optimization. Furthermore, they can also guide us in learning where the problems are and how to overcome them. In addition, in carrying out a modelling study, the nature and the parameters of process have to be defined in quantitative terms. Thus, modelling is one that forces a better understanding of the process and promotes methods that accumulate process knowledge.

However, the modelling of phenomena and the performance of SSF have not yet progressed to a stage where it can be fully utilised in industrial application. In this study, the understanding of microbial growth kinetics and model development was important to facilitate the establishment of a good performance SSF bioreactor and the growth of *Aspergillus niger* FTCC 5003 during SSF on PKC before up-scaling and large commercial scale production.

The aim of this study was to obtain models that were able to describe microbial growth and enzyme activity during the growth of *Aspergillus niger* FTCC 5003 in SSF on PKC. The objectives were:

i. To identify the initiation time and complete recovery of fungal biomass by the method of direct peeling off the membrane filter with expectation to determine the initial biomass and find application for fungal biomass estimation from indirect biomass determination for SSF of *Aspergillus niger* FTCC 5003 on PKC.

ii. To obtain suitable relationship for biomass estimation of *Aspergillus niger* FTCC 5003 on support material which have similar condition to actual situation of SSF on PKC based on protein and glucosamine determination.

iii. To obtain experimental profile for PKC dry weight, estimated fungal dry weight based on protein and glucosamine concentration and β-mannanase activity during SSF using PKC as substrate.

iv. To propose simulated models that is able to provide a foundation for prediction of *Aspergillus niger* FTCC 5003 growth and β-mannanase activity during SSF using PKC as substrate.

CHAPTER 2

LITERATURE REVIEW

2.1 Oil palm

Oil palm (*Elaeis guineensis* Jacq.) is one of the important edible oil sources in the tropical region which are mainly grown in countries such as Asia, Central and South America and Africa. It belongs to the family of Palmae, subfamily of Cocoiae and order Spadiciflorae. The genus *Elaeis* is derived from the Greek word "elaion" meaning oil and the species name *guineensis* indicating its origin from the Guines Coast.

It is a monocotyledon plant with chromosome number (2n), 32. It is a monoecious plant whereby the male and female reproductive organs are different but present in the same plant. There are 2 other species of palm of American origin: *E. oleifera* (H.B.K) Cortest and *E. odora* Trail (Gascon *et al.* 1989).

There are no specific varieties of oil palms. They are, however, classified according to the thickness of the shell and the colour of the fruits. Depending on the thickness of the shell, oil palm is distinguished into three forms: thick-shelled, thin-shelled and shell-less oil palms (Godin and Spensley, 1971; Purseglove, 1972).

In Malaysia, the thinner-shelled palms called *tenera*, which have more mesocarp and hence more oil has been cultivated until today. The *tenera* is a cross breed between *dura* (thick-shelled) palms and *pisifera* (shell-less). The oil palm