SOLID-STATE FERMENTATION OF PALM KERNEL CAKE AND COMPOSTING OF OIL PALM EMPTY-FRUIT BUNCHES USING MICROBES

SHARON LAU YU LING

BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2009

SOLID-STATE FERMENTATION OF PALM KERNEL CAKE AND COMPOSTING OF OIL PALM EMPTY-FRUIT BUNCHES USING MICROBES

SHARON LAU YU LING

THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2009

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN TESIS

JUDUL : ____

____

____

IJAZAH : ____

____

SAYA : ____________________________________ **SESI PENGAJIAN** : ______________________________

(HURUF BESAR)

Mengaku membenarkan tesis *(LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

[ ] SULIT (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di AKTA RAHSIA RASMI 1972)

[ ] TERHAD (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

[ ] TIDAK TERHAD

Disahkan oleh:

____________________ (TANDATANGAN PENULIS)

Alamat Tetap: ________________

____

____

____

TARIKH: ______________

________________________ (TANDATANGAN PUSTAKAWAN)

____

(NAMA PENYELIA)

TARIKH: ________________

Catatan:
*Potong yang tidak berkenaan.
*Jika tesis ini SULIT dan TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
*Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana Secara Penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

25 May 2009

Sharon Lau Yu Ling
PB20068033

CERTIFICATION

NAME : **SHARON LAU YU LING**

MATRIC NO. : **PB20068033**

TITLE : **SOLID-STATE FERMENTATION OF PALM KERNEL CAKE AND COMPOSTING OF OIL PALM EMPTY-FRUIT BUNCHES USING MICROBES.**

DEGREE : **MASTER OF SCIENCE (BIOTECHNOLOGY)**

VIVA DATE : **19 MAY 2009**

DECLARED BY

SUPERVISOR
Assoc. Prof. Dr. Clemente Michael Wong Vui Ling ____________________

ACKNOWLEDGEMENT

I would like to take this opportunity to thank my esteemed supervisor, Associate Professor Dr. Clemente Michael Wong Vui Ling who guided and helped me in so many different ways that it is truly an honor to have known such great mentor. His assistance in various ways has helped me to accomplish this work successfully.

Special recognition goes to the Ministry of Science, Technology and Innovation of Malaysia for granting me as one of their recipient of the National Science Fellowship for session 2006/2007. Similarly, Biotechnology Research Institute (BRI) and Postgraduate Centre of Universiti Malaysia Sabah and Korean Research Institute of Bioscience and Biotechnology (KRIBB) deserve the acknowledgement for providing me the chance to gain invaluable experiences by attending seminars, symposiums and research attachment. Special appreciation also goes to Malaysian Agricultural Research and Development Institute (MARDI) for their kind assistance in this study.

I am very much indebted to many individuals who helped in many ways throughout my study; Prof. Datin Dr. Ann Anton for her dynamism and invaluable suggestions, Dr. Kim Chul Ho for his exuberance and generosity, Dr. Choi Min Ho for his patience and substantial guidance and Dr. Sahilah Abdul Mutalib for her optimism and continuous support.

My appreciation also goes to friends and colleagues from BRI Lab, Chemical Engineering Department of School of Engineering and Information Technology and Microbiology Lab of MARDI; Awang Muhammad Sagaf, Kenneth Francis Rodriques, Adrian Ng, Chelven Lim Ai Chen, Grace Joy Chin Wei Lie, Gordon John Thomas, Elaine Remi Douglas Telajan, Shuhadah Mustapha, Tam Heng Keat, Chin Hooi Chuan, Saw Horng Yuan, Wong Yoke Phui, Tang Sui Yan and Jeffrey Lim for their advice, technical help, suggestion and remarkable discussion of ideas. My appreciation is also extended to the lab assistants Vidarita Maikin, Richard Dailis, Azian Awg Besar and Rudi Boliku for their helpfulness and cheerfulness in the lab.

My MSc. thesis would not have been possible without the support and prayer from family and friends. My deepest gratitude goes to both my parents for having faith in me, my sisters Valerie, Supang and Rachel, who stood by me, friends that never-endingly praying for me, Prof. Son, Sagau and Sarah Lee, Ps. Nam, Ps. Kusam, Eleanor Muyou and family, Mary Marlyna Markus, Angeline Tan Huay Lin, Lily Kok Heng Feng and friends from SIB Alamesra and EP group.

Above all, I thank God for embarking me on a journey that I know where it'll lead to, for nurturing me in faith and helping me to stand strong through hard times, comforting me through difficult moments and forever faithful throughout the ages.

The Lord upholds all who fall, and raises up all who are bowed down....Psalms 145:14

ABSTRACT

SOLID-STATE FERMENTATION OF PALM KERNEL CAKE AND COMPOSTING OF OIL PALM EMPTY-FRUIT BUNCHES USING MICROBES.

The main concern of the oil palm industry is the growing amount of by-product and wastes produced from the processing of oil palm each year. On average, for every ton of Fresh Fruit Bunches (FFB) processed, 200kg of Empty-Fruit Bunches (EFB), 670kg of Palm Oil Mill Effluent (POME) and 30 kg of Palm Kernel Cake (PKC) are produced. Thus, zero waste strategy has to be adopted to maintain the competitive edge of the booming industry. The experimental work of this study aims to effectively utilize wastes by adopting solid-state fermentation of PKC to convert it to poultry feed and composting of EFB into biofertilizer. PKC is an excellent source of protein and energy and is used extensively in the animal feed industry. However, the main problems faced in the application of PKC as animal feed especially in the non-ruminants are the high fiber content and limitation in amino acid contents of untreated PKC. Thus, solid-state fermentation (SSF) has become a method of preference for the purpose of upgrading the quality of PKC involving the use of microbes as it stimulates the natural environment of most microorganisms. Two species of fungi, *Emericella nidulans* var. *nidulans* (Eidam) Vuillermin, *Cladosporium herbarum* (Pers.) Link BGB and three strains of bacteria *Bacillus subtilis*, which are good beta-mannanase producing strains were used in combinations to improve the nutritive value of PKC as animal feed through lab-scale solid-state fermentation. With the fermentation operating conditions set to 20% of inoculum, 110% moisture content, pH 7.0 and PKC particle size 0.8 mm, five types of mixed culture treatments were tested. Treatment of PKC with the combination of *Emericella nidulans* (4DP5) and *Bacillus subtilis* (7DY7) showed advantages over other treatments. This treatment produced the highest increase in reducing sugar content in PKC to 418.3%. In addition to that, this treatment also gave the lowest increase in aflatoxin level of only 26.8% from 9.99 ppb (aflatoxin content in untreated PKC) to 12.67 ppb, which is below 20 ppb, an acceptable level in animal feed. Simultaneously, EFB and POME have been used extensively as mulch and organic fertilizer in the oil palm plantations. Thus, composting has been singled out as a useful method in converting EFB, which is essentially organic in nature, into humus that is suitable for crop production. One hundred and thirteen potential isolates from the four main groups of microbes namely bacteria, actinomycetes, yeasts and fungi were selected to form a concoction of inoculum, which was applied during composting of EFB in a model backyard-scale system. Mechanically-shredded EFB and raw POME, subjected to an open system composting employing the 4 days turn-over method help to accelerate the rate of EFB composting. The rate of EFB composting was sped up to within 28 days. With the aide of inocula, EFB composting was further improved in terms of maturity and nitrogen and phosphorus content in the compost. EFB added with compost, nitrogen-fixing and phosphate-solubilizing inoculum (Pile A) yielded the carbon/nitrogen value of 18.7 on day 28 from the initial value of 71 compared to 21.2 (Pile B) and 26.4 (control). Pile A also showed an increase of 2.19% in nitrogen content and 0.18% in phosphorus content.

ABSTRAK

Masalah utama dalam industri pemprosesan kelapa sawit adalah jumlah produk sampingan serta bahan buangan yang dijanakan setiap tahun. Secara purata, pemprosesan satu tan metrik buah kelapa sawit bersama tangkainya (FFB) akan menghasilkan 200 kg tangkai kelapa sawit tanpa buah (EFB), 670 kg efluen daripada kilang pemprosesan kelapa sawit (POME) dan 30 kg dedak isirong kelapa sawit (PKC). Oleh itu, strategi untuk mengkosongkan bahan buangan perlu diiktiarkan berikutan perkembangan pesat industri ini. Kajian ini berusaha untuk mencari kaedah penggunaan bahan buangan secara efektif dengan mengaplikasikan kaedah penapaian keadaan pejal (SSF) PKC untuk dijadikan makanan ayam serta penghasilan baja kompos daripada EFB. PKC mengandungi sumber protein dan tenaga yang tinggi dan adalah digunakan secara meluas dalam industri penghasilan makanan ternakan. Walau bagaimanapun, masalah yang dihadapi dalam aplikasi PKC sebagai makanan ternakan terutamanya ternakan bukan ruminan adalah masalah kandungan seratnya yang tinggi serta kandungan beberapa asid amino yang rendah dalam PKC yang belum dirawat. Oleh itu, kaedah SSF menjadi pilihan untuk meningkatkan kualiti PKC dengan melibatkan penggunaan mikrob kerana SSF menyediakan keadaan yang sesuai untuk kebanyakan mikroorganisma. Dua spesies fungi iaitu Emericella nidulans var. *nidulans* (Eidam) Vuillermin *serta Cladosporium herbarum* (Pers.) Link BGB *dan tiga jenis bakteria Bacillus subtilis yang dikenalpasti sebagai stren yang menghasilkan enzim beta-mannanase telah digunakan dalam beberapa bentuk kombinasi untuk meningkatkan kualiti PKC melalui SSF berskala makmal. Dengan keadaan penapaian ditetapkan kepada 20% inokulum, 110% kelembapan, pH 7.0 dan saiz partikel PKC pada 0.8 mm, lima jenis kombinasi mikrob telah diuji. Rawatan dengan kombinasi Emericella nidulans (4DP5) dan Bacillus subtilis (7DY7) menunjukkan kelebihannya berbanding jenis rawatan yang lain. Rawatan ini menghasilkan peningkatan gula ringkas dalam PKC sebanyak 418.3%. Selain itu, rawatan ini juga menghasilkan peningkatan kandungan aflatoksin yang paling rendah iaitu 26.8% daripada 9.99 ppb (kandungan aflatoksin dalam PKC tanpa rawatan) kepada 12.67 ppb, iaitu lebih rendah daripada tahap maksima 20 ppb yang dibenarkan dalam makanan ternakan. Pada masa yang sama, EFB dan POME digunakan secara meluas sebagai sungkupan dan baja organik di ladang-ladang kelapa sawit. Oleh itu, penghasilan kompos adalah kaedah yang berguna untuk menukarkan EFB kepada humus yang sesuai untuk pertanian. Sebanyak 113 mikrob yang berpotensi daripada empat kumpulan utama mikrob iaitu bakteria, actinomycetes, yis dan fungi dipilih untuk menghasilkan inokulum yang akan digunakan semasa penghasilan kompos daripada EFB. EFB yang yang telah dihancurkan mesin ditambah dengan POME dipercepatkan tahap pengkomposannya dengan bantuan kaedah pengaulan setiap empat hari. Tahap pengkomposan EFB dipercepatkan sehingga 28 hari. Dengan bantuan inokulum, kualiti kompos EFB turut meningkat dari segi kematangan kompos, kandungan nitrogen serta fosforus. EFB yang ditambah inokulum dalam Pile A menghasilkan nilai karbon/nitrogen 18.7 pada hari ke-28 daripada nilai 71 pada hari ke-0 berbanding 21.2 (Pile B) dan 26.4 (kontrol). Pile A juga menunjukkan peningkatan sebanyak 2.19% dalam kandungan nitrogen dan 0.18% dalam kandungan fosforus.*

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

Abs	Absorbance
ADF	Acid detergent fiber
BLAST	Basic Local Alignment Search Tool
Bp	Base pair
BSA	Bovine serum albumin
C	Carbon
C:N	Carbon nitrogen ratio
Ca	Calcium
$CaCl_2$	Calcium chloride
CF	Crude fiber
CFU	Colony forming unit
CP	Crude protein
CPO	Crude palm oil
Cu	Copper
CYC	Czapek-dox yeast extract casein agar
DNA	Deoxynucleic acid
DNS	Dinitrosalicylic acid
dNTP	Any deoxynucleoside
EDTA	Ethylenediaminetetra-acetate
EFB	Empty-fruit bunch
FFB	Fresh-fruit bunch
g	Gram
gds	Gram dry sample
HCl	Hydrochloric acid
K	Potassium
Kb	Kilobase pairs
Kg	Kilogram
LBG	Locust bean gum
M	Molar
MARDI	Malaysia Agricultural Research and Development Institute
Mg	Magnesium
$MgCl_2$	Magnesium chloride
ml	Mililitre
mm	Milimeter
mM	Milimolar
mm^3	Milimeter cube
N	Nitrogen
NA	Nutrient agar
NaOH	Sodium hydroxide
NB	Nutrient broth
NCBI	National Center for Biotechnology Information
NDF	Neutral detergent fiber
nm	Nanometer
OD	Optical density
OPF	Oil palm frond

OPT	Oil palm trunk
P	Phosphorus
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PKC	Palm kernel cake
pmol	Picomole
POME	Palm oil mill effluent
Ppb	Parts per billion
Rpm	Revolution per minute
SCA	Starch Casein agar
SDS	Sodium dodecyl sulphate
SmF	Submerged fermentation
sp.	Species
SSF	Solid-state fermentation
TBE	Tris borate EDTA
U	Unit
Uv	Ultraviolet
V	Voltage
w/v	Weight per volume
YEPD	Yeast extract peptone dextrose agar
%	Percentage
/	Per
°C	Degree Celsius
µg	Microgram
µl	Microlitre
µmol	Micromole

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The main concern of the oil palm industry is the growing amount of by-products and wastes produced from the processing of oil palm each year as the country is the world's leading producer and exporter of palm oil products. Palm kernel cake (PKC) is an important oil palm by-product as more than two million tons of PKC are produced annually. The industry also produces about 8 million tons of empty-fruit bunches (EFB) and 20 million tons of palm oil mill effluent (POME); a massive amount of biomass waste produced throughout Malaysia each year (Chew, 2002).

PKC is obtained from palm kernel after the oil has been extracted by screw pressing method. It is an excellent source of protein and energy for livestock especially ruminants. In Malaysia, PKC is included in the feed for beef cattle up to 80% of the total ration and 50% for dairy cattle (Malaysian Palm Oil Promotion Council, 2002). However, non-ruminants lack the ability to digest fiber in PKC as analysis showed that more than 60% of PKC is cell wall components that consists of 58% mannan, 12% cellulose and 4% xylan (Daud & Jarvis, 1992). Therefore, recommended level of PKC inclusion in the diet of non-ruminants like swine, poultry and fish is only about 20% of the total ration (Malaysian Palm Oil Promotion Council, 2002). Hence, in order to enhance the utilization of PKC as feed in non-ruminants, the fiber content needs to be reduced.

EFB is generated when the fruits from the fresh fruit bunch (FFB) are separated from the bunch stalk through threshing method. EFB are bulk solid residues that require a long time to break down. Palm oil mill effluent (POME) is composed of high polluted effluent (from sterilizer and oil room) and low polluted effluent (steam condensate, cooling water, boiler discharge and sanitary effluent) (Kittikun *et al.*, 2002). EFB and POME have been used extensively as mulch and organic fertilizer in oil palm areas. EFB is either applied as heaps, placed around young palms or in between rows of oil palm trees. EFB is produced at a rate of 25 to 30 tons per hectare and this has given rise to logistic and handling problems which have deterred many planters to take up the method (Chew, 2002). Furthermore, it becomes a good breeding ground for rats and snakes and during rainy season, it provides an ideal condition for fungi to grow which is partly believed to be the cause of ganoderma disease of oil palm.

Upgrading of low quality lignocelluloses into animal feed involving efficient microbes has been a focus of research for a long time. Solid-state fermentation (SSF) has become a method of preference for this purpose as it stimulates the natural environment of most microorganisms, especially fungi (Iluyemi *et al.*, 2006) to reduce fiber content in lignocellulosic materials and at the same time increase the protein level in feed. SSF is generally defined as the growth of microorganisms on moist solid material in absence or near-absence of free water (Pandey *et al.*, 2001).

Composting has been singled out as a useful alternative in managing the massive production amount of EFB as the process converts EFB, which is essentially organic in nature, into humus that is suitable for crop production (Thambirajah & Kuthubutheen, 1989). In general, composting is a process managed by humans involving the cultivation of microorganisms that degrade organic matter in the presence of oxygen.

The experimental work of this study consists of two main parts; (1) solid-state fermentation of palm kernel cake (PKC) and (2) composting of empty-fruit bunches (EFB). In the first part of the study, a selection of microbes isolated from raw EFB and EFB compost were used to produce a concoction of inoculum to be applied during lab-scale solid-state fermentation of PKC. EFB compost is claimed to be a good source of potential microbes as composting involves a dynamic process of organic solids degradation carried out by a complex microbial community. My hypothesis was that the generated mixed-culture will help to improve the nutritive value of PKC as animal feed. As for the second part of the study, another concoction of inoculum was produced and applied during composting of EFB in a model backyard-scale system. The hypothesis was that the generated compost inoculum will help to accelerate composting of EFB.

Significance of Study

The significance or contributions of this study to the field are:

- Increase the quality of palm kernel cake as animal feed to enable higher level of PKC inclusion in the diet of non-ruminants like poultry, swine and fish.
- Starter culture consisting fungal and bacterial inoculum is developed for solid-state fermentation of palm kernel cake.
- Developing composting as a more environmental friendly alternative to dispose off biomass waste of the oil palm industry and at the same time add economical value to EFB by turning EFB into compost, which is not only good for the plantations but also applicable to general agriculture as a whole.
- Developing a better composting method using locally isolated strains.
- Compost inoculum generated from this study can further shorten the time taken in composting of EFB as compared to applying them as mulch.
- Data on the identification of microbes with good potential will be of tremendous help for future study.
- Data on the chemical analysis (nitrogen, phosphorus, potassium and magnesium content) of EFB compost in this study will be useful in determining the quality of EFB compost.
- Data on the distribution of microbes during composting of EFB help us to further understand the complex interaction of microbial community involved.

1.2 Objectives of Study

The objectives of this study were:

- To do lab-scale solid-state fermentation of PKC using mixed-culture.
- To do backyard-scale system composting of EFB using a concoction of potential microbes as inoculants.