



UNIVERSITI PUTRA MALAYSIA

***BIOLOGICAL PRE-TREATMENT OF BANANA PSEUDO STEM
WITH WHITE ROT FUNGI AND ITS EFFECTS ON
PULP AND PAPER PROPERTIES***

NORHASLIDA BINTI RAZALI

FH 2015 13



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By

NORHASLIDA BINTI RAZALI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of
Philosophy**

November 2015

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Doctor of Philosophy

BIOLOGICAL PRE-TREATMENT OF BANANA PSEUDO STEM WITH WHITE ROT FUNGI AND ITS EFFECT ON PULP AND PAPER PROPERTIES

By

NORHASLIDA BINTI RAZALI

November 2015

Chairman : Assoc Prof. Rasmina Halis, PhD
Faculty : Forestry

Wood fibre is known to be a major raw material for most paper industries all over the world. However, a great concern in forest resources conservation calls for an overall plan for utilization of non-woody plants. This study was carried out using banana pseudo stem (*Musa acuminata* var. *truncata*) as potential raw material for pulp and paper. In paper production, chemicals disposal process requires high cost and causes negative effects to the environment. Biopulping has showed potential in providing environmentally-friendly technology for pulp and paper production. This research was aimed at investigating the effect of fungi pre-treatment in lignin removal prior to the pulping process and their effect to the pulp and paper properties. Research studies have been geared toward properties of banana pseudo stem, fungi pre-treatment on banana pseudo stem, optimisation of soda pulping process and biosoda pulping process.

Properties of banana pseudo stem include botanical aspects, anatomical structures, fibre morphology and chemical compositions. From the anatomical observation, it showed that banana pseudo stem consists of fibre bundle, vascular bundle and lots of parenchymatous cells. Banana pseudo stem fibres were discovered to have long fibre which range from 3.34 mm to 4.19 mm and had lower runkel ratio, high coefficient of suppleness and higher felting power. Chemical composition of banana pseudo stem consisted of more holocellulose (71.68%), alpha-cellulose (68.30%) and remarkable lower lignin content (14.34%).

Fungal pre-treatment study was carried out to determine the best fungus species and a suitable pre-treatment period to be used in biosoda pulping. The pre-treatment was done using banana chips that were inoculated with *Pycnoporus sanguineus* and *Oxyporus latemarginatus* in 5, 10 and 15 days incubations. *P. sanguineus* appeared to grow much faster compared to *O. latemarginatus* on banana chips and gave the highest weight loss (29.36%). *P. sanguineus* secreted sole ligninolytic enzyme Laccase with the highest level of activity which was 6.07 U/ml in 15 days and showed preference to degrade

mainly on lignin (24.85% reduction) with minimal loss of holocellulose and alpha-cellulose. *O. latemarginatus* was found to secrete lignin peroxidase and manganese peroxidase and appeared to degrade more on lignin (18.92%) and cellulose (45.93%).

Soda pulping process was carried out at different cooking time (30 and 60 mins) and different alkali percentage (10, 12, 14 and 16%). It was found that a combination of 30 mins cooking time and 14% alkali produced higher pulp yield (40.91%) and also gave better result in pulp and paper properties.

Biosoda pulping was carried out using *P. sanguineus* with the incubation period of 15 days. Pulp and paper properties of soda pulping and biosoda pulping were compared. Biosoda pulping appeared to give the highest pulp yield with 47.57% and also better in tensile index, burst index, and tear index and also brightness. Scanning Electron Microscope (SEM) observation of treated handsheets indicated that there were less voids between fibres, and the fibres collapsed onto each other which contributed to good bonding. From the research, the fungal pre-treatment prior to pulping process helped to improve pulp and paper properties.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan Ijazah Doktor Falsafah

PRA-RAWATAN BIOLOGI KE ATAS BATANG PISANG DENGAN MENGUNAKAN KULAT PUTIH DAN PENGARUHNYA TERHADAP SIFAT PULPA DAN KERTAS

Oleh

NORHASLIDA BINTI RAZALI

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Gentian kayu merupakan bahan utama dalam industri kertas di dunia ini. Walaubagaimanapun, kesedaran yang timbul terhadap pemuliharaan sumber hutan telah memperkenalkan penggunaan tumbuhan bukan kayu. Kajian ini dijalankan dengan menggunakan batang pisang (pisang hutan) sebagai bahan mentah yang dilihat berpotensi untuk industri pulpa dan kertas. Dalam penghasilan kertas, proses pelupusan bahan kimia ini memerlukan kos yang tinggi dan mendatangkan kesan buruk terhadap alam sekitar. Bio pemulpaan dilihat berpotensi dalam menghasilkan teknologi pembuatan kertas yang mesra alam. Kajian ini adalah bertujuan untuk mengenal pasti kesan pra-rawatan kulat sebelum proses pemulpaan dalam menceraikan lignin dan kesannya terhadap sifat pulpa dan kertas yang dihasilkan. Kajian ini meliputi pencirian terhadap sifat-sifat batang pisang, pra-rawatan kulat ke atas batang pisang, serta penghasilan mempulpa soda dan biosoda yang optimum.

Sifat-sifat batang pisang meliputi aspek botani, anatomi, morfologi gentian dan kandungan kimianya. Struktur anatomi menunjukkan batang pisang terdiri daripada kelompok-kelompok gentian, kelompok-kelompok vaskular dan taburan tisu parenkima yg banyak. Batang pisang didapati mempunyai gentian yang panjang dengan purata gentian sepanjang 3.34mm hingga 4.19mm dan mempunyai nisbah runkel yang lebih rendah dan tinggi pekali kelembutan dan kuasa lipatan. Gentian mempunyai kandungan kimia holoselulosa (71.68%), alfa-selulosa (68.30%) dan lignin (14.34%).

Kajian pra-rawatan kulat dijalankan untuk menentukan spesies kulat terbaik dan tempoh masa penderaman yang sesuai. Cip batang pisang dirawat dengan menggunakan *Pycnoporus sanguineus* dan *Oxyporus latemarginatus* selama 5, 10 dan 15 hari. *P. sanguineus* dilihat membiak dengan cepat berbanding *O. latemarginatus* dan memberikan pengurangan berat yang paling tinggi (29.36%) ke atas cip pisang. *P. sanguineus* hanya merembeskan enzim laccase dengan kadar tertinggi sebanyak 6.07 U/ml yang dilihat pada hari ke-15 dan menunjukkan keutamaan mengdegradasi lignin (24.85% pengurangan)

berbanding holoselulosa dan alfa selulosa. *O. latemarginatus* merembeskan enzim Lignin peroxidase dan manganese peroxidase dan mengdegradasi lebih kepada lignin (18.92%) dan selulosa (45.93%).

Mempulpa secara soda dijalankan dengan masa masakan yang berbeza (30 dan 60 minit) dengan kepekatan alkali yang berbeza (10, 12, 14 dan 16%). Mempulpa secara soda ke atas cip pisang yang dimasak pada pembolehubah yang berbeza menunjukkan kombinasi 30 minit masa masakan dan 14% kepekatan alkali menghasilkan pulpa yang banyak (40.91%) dan memberi sifat kertas yang paling baik.

Pemulpaan biosoda telah dijalankan menggunakan *P. sanguineus* dengan penggeraman selama 15 hari. Sifat pulpa dan kertas daripada pemulpaan soda dan biosoda dibandingkan. Keputusan menunjukkan bahawa mempulpa secara biosoda menghasilkan pulpa sebanyak 47.57% dengan indeks regangan, indeks pecahan, indeks koyakan dan kecerahan yang lebih baik. Pemerhatian daripada SEM menunjukkan kertas terawat kurang lompong antara gentian dan gentian jatuh di antara satu sama lain menghasilkan prekatan yang baik. Daripada kajian ini, didapati pra-rawatan kulat sebelum proses mempulpa membantu meningkatkan sifat-sifat pulpa dan kertas.

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I certify that a Thesis Examination Committee has met on 26 November 2015 to conduct the final examination of Norhaslida binti Razali on her thesis entitled "Biological Pre-treatment of Banana Pseudo Stem with White Rot Fungi and its Effects on Pulp and Paper Properties" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS

AA	Alcohol acetone
AD	Air dried
AC	Alpha cellulose
ANOVA	Analysis of variance
APMP	Alkaline Peroxide Mechanical pulping
CED	cupriethylenediamine
CO ₂	Carbon dioxide
COD	Chemical oxygen demand
C ₂ H ₄ O ₂	Glacial acetic acid
CSF	Canadian standard of freeness
CSL	Corn steep liquor
<i>C. versicolor</i>	<i>Coriolus versicolor</i>
CW	Cold water
DP	Degree of polymerization
ECF	Elemental chlorine free
FRIM	Forest Research Institute Malaysia
HW	Hot water
HC	Holocellulose
KMnO ₄	Kalium permanganate
Lac	Laccase
LiP	Lignin peroxidase
MC	Moisture content
MnP	Manganese peroxidase
Mt	Million tonne

NaClO ₂	Sodium Chlorite
NaOH	Sodium hydroxide
OD	Oven dried
<i>O. latemarginatus</i>	<i>Oxyporous latemarginatus</i>
<i>P. chrysosporium</i>	<i>Phanerochatae chrysosporium</i>
<i>P. sanguineus</i>	<i>Pycnopus sanguineus</i>
PDA	Potato dextrose agar
PEG	Polyethylene glycol
RMP	Refiner mechanical pulp
SEM	Scanning Electron Microscope
SSF	Solid state fermentation
TAPPI	Technical Association of the Pulp and Paper Industry
TCF	Totally chlorine free
TEM	Transmission Electron Microscope
TMP	Thermomechanical pulp

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background move

The pulp and paper industry is one of the largest industrial sector in the world, and an important source of employment in many countries. A sustainably managed pulp and paper industry can bring many benefits to the local economy and people, particularly in rural areas. The demand for paper and the unacceptable large ecological footprint of current paper production requires the need for alternatives in terms of new raw materials and improved processing methods for paper production. Roughly, more than 1 Mt of paper are used every day (Anon, 2013).

In many parts of the world, local supplies of wood cannot support the demand for pulp. As a result, the search for non-wood raw materials in paper making industry has been given more attention due to the rising consumption of wood resources for the paper production. From 1970s to present time, the non-wood plant fibre pulping capacity has increased on a global basis two to three times as fast as the wood pulping capacity. It was also forecasted that during the next decade, the non-wood pulp production will annually grow at an average of 6%, which is three times as fast as the production of pulp on wood basis. Initially, non-wood fibre pulping occurred in regions where wood supply had been reduced to levels insufficient to sustain paper making, and an alternative source of fibre feedstock was mandatory (Laftah and Abdul Rahaman, 2015). Non-wood lignocellulosic fibre resources have the potential to complement conventional wood supplies because it does not compete with agricultural crops for fertile land, relies on larger biomass yields, have short cycles and rapid regeneration, and are of comparatively low price (Cherubini, 2010).

Banana pseudo stem is one example of non-wood fibres, which can be described as lignocellulosic that comprises primarily of cellulose, hemicellulose and lignin (Khalil *et al.*, 2006; Norhaslida *et al.*, 2014; Low *et al.*, 2015). In Malaysia, as the second most widely cultivated fruit crop, banana cultivation covers almost 10% or 33,584 ha of the total fruit area (297,860 ha), with a total production of 535,000 tonnes (Mohamad Roff *et al.*, 2012; Low *et al.*, 2015). Banana pseudo stem produces a large amount of biomass residues (approximately 88% of the banana plant), and in Malaysia it is usually discarded as an agricultural waste from banana plantation (Low *et al.*, 2015). Banana stem has long been known as one of the potential cellulose sources and recommended as a suitable source of fibre for pulp and paper industry (Faria *et al.*, 2006). Cellulose is a linear and high molecular weight polymer of natural and biodegradable material that can be found in the banana pseudo stem.

The pulp and paper industry is traditionally known to be the large contributor to environmental world pollution due to its large consumption of energy and

chemicals. Biotechnological methods offer potential opportunities for changing the pulp and paper industry towards more environmentally friendly and efficient operations compared to the conventional method (Ferraz *et al.*, 2008). Biotechnology in pulp and paper industry has found its way in the commercialized applications in using pre-treatment with microorganisms (bacteria, fungi, enzymes etc.) in biopulping, biobleaching, biodeinking, pulp and paper mill effluents (Bajpai, 2006; Fatriasari *et al.*, 2010; Fillat *et al.*, 2012; Singh *et al.*, 2013; Sharma *et al.*, 2015;).

Biopulping is the fungal pre-treatment with wood decay fungi to biodegrade wood chips and other lignocellulosic materials prior to mechanical or chemical pulping. This pre-treatment intensively alters and removes lignin and other chemical compound in the substrate (wood chips) especially extractives, hemicellulose and ash content. Biopulping provides several benefits where the pre-treatment process helps to soften and loose the fibres, improves chemical penetration and reduces electrical energy during chemical and mechanical pulping (Van beek *et al.*, 2007; Islam *et al.*, 2008; Rosli *et al.*, 2008). In some cases, it helps to increase pulp yield, improves fibre properties and enhances paper quality. One of the promising microorganisms for biological pre-treatment is white rot fungi that belong to class basidiomycete.

White rot fungi have a remarkable ability to produce hydrolytic and oxidative extracellular enzymes i.e. laccase (Lac), manganese peroxidase (MnP), lignin peroxidase (LiP) and cellulase that simultaneously degrade biomass especially lignin and carbohydrate (Dhouib *et al.*, 2005; Dashtban *et al.*, 2009; Ashger *et al.*, 2012; Rozi *et al.*, 2013). Based on the study by Halis *et al.* (2012), *Pycnoporus sanguineus* was found to secrete only Lac and the activity of enzyme can be detected since week 1. While the other fungi, *Oxyporus latemarginatus* showed to produce high rate of MnP and trace of LiP. Rozi *et al.* (2013) who studied on the enzyme activity of four white rot fungi (*O. latemarginatus*, *Rigidoporus vinctus*, *Phanerochaete chrysosporium* and *Coriolus versicolor*) found that all fungi produced all the three ligninolytic enzymes (Lac, LiP and MnP). *O. latemarginatus* secreted highest level of LiP that plays a major role in lignin degradation. For cellulase activity, all four species were found to produce endoglucanase, exoglucanase and β -glucosidase where *O. latemarginatus* showed the highest activity for all the three enzymes. So, *P. sanguineus* and *O. latemarginatus* were found to have the potential to be applied in pre-treatment of banana pseudo stem in this study.

1.2 Problem statement

Forests cover a total of 4 billion hectares worldwide, equivalent to 31% of the total land area (FOA, 2010). Although this figure may seem high, the world's forests are disappearing. Between 1990 and 2000 there was a net loss of 8.3 million hectares per year, and the following decade, up to 2010, there was a net loss of 6.2 million hectares per year (FOA, 2010). Aside from the devastating effects of tropical forest loss has on biodiversity and forest-dependent communities, a major consequence of deforestation and forest degradation is the release of heat-trapping carbon dioxide (CO₂) into the

atmosphere. Forests provide vast carbon sinks that when destroyed emit CO₂ into the atmosphere, either by burning or degradation of organic matter (Moutinho and Schwartzman, 2005). The pulp and paper sector was estimated to generate more than 600 mt of CO₂ emissions per year.

Non-wood plant fibres are another alternative sources of material for the pulp and paper productions and it is also to reduce the forest exploitation and a way of promoting sustainable paper production especially in areas of lacking forest and abundance of annual fibre resources (Ogunsile *et al.*, 2006). Banana pseudo stem is one of the plant fibres that usually discarded as an agricultural waste. This residue can generate great environmental problems when left on the plantation floor. Indirectly, it also can create problems in replanting operations. This residue can turn out to be a source for other value-added products such as pulp and paper. Therefore, exploitation of the banana pseudo stem will be significantly beneficial to the environment and brings additional profits to farmers (Li *et al.*, 2010). In particular previous studies on banana pseudo-stem, it has proven that banana pseudo-stem has approximately higher content of cellulose (64-65%) and has lower content of lignin (11%). It is also has a long and high fibre strength which is suitable for pulp and paper making (Rasmina *et al.*, 2011; Norhaslida *et al.*, 2014).

The pulp and paper industry is looking towards technological improvement in the conventional pulping methods. However, it probably will eventually be necessary to shift to even newer technologies to meet industrial and consumer needs, as well as the environmental requirements. The chemical pulping process is aimed to selectively extract lignin from lignocellulosic material. High cost and energy are needed in order to dispose chemicals in pulp and paper processing. This will cause negative effects to the environment, such as chlorine-based organic compound. Therefore, alternative pulping process such as biopulping is needed in order to reduce environmental pollution and at the same time improve the reduction of pulping time, and improve the properties of pulp (Wolfaardt *et al.*, 2004). Biopulping can be used as a potential solution or eliminating some of the ordinary pulping problems (Rasmina *et al.*, 2010; Singh *et al.*, 2013).

However, not all the fungi are suitable for biopulping. *P. sanguineus* and *O. latemarginatus* were selected in this study based on the previous findings (Rasmina 2010; Tan, 2012; Rozi *et al.*, 2013) which are selective lignin degraders. The enzymatic activity by *P. sanguineus* (Lac, 0.006 U/mL) and *O. latemarginatus* (MnP, 0.0035 U/mL and LiP, 0.0006 U/mL) were the highest detected during fungal pre-treatment (Halis *et al.*, 2012). Apart from that, comprehensive study on biosoda pulping of banana has not yet been established. In order to understand the mechanism of biosoda pulping, several parameters have to be examined and optimized especially on pre-treatment conditions (fungi species, inoculum sizes, chip pre-treatment, incubation time) and right cooking conditions for soda and biosoda pulping (cooking time, active alkali, wood to liquor ratio and cooking temperature).

1.3 Objectives

The general objective was to investigate the effect of fungal pre-treatment in biopulping process on pulp and paper properties of banana pseudo stem.

The specific objectives of the study are:

- 1) To analyze the anatomical structure, fibre morphology and chemical constituents of banana pseudo stem.
- 2) To characterize the fungi morphology and optimize the fungi pre-treatment conditions.
- 3) To determine the best cooking time on soda pulping for banana pseudo stem.
- 4) To evaluate the pulp and paper properties of biosoda pulping.

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