EFFECT OF DIFFERENT ORGANIC LOADING RATE ON BIOLOGICAL PRETREATMENT TO CHEMICAL PULPING PROCESS

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DECLARATION

I declare that this thesis entitled “Effect of different organic loading rate on biological pretreatment to chemical pulping process” is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ....

Name : Ku Marsilla Binti Ku Ishak
Date : November 2006

DEDICATION

Special Dedication to my…

Beloved parents,

KU ISHAK KU ISMAIL
NORMAH IBRAHIM

Sisters,

KU ESYRA HANI KU ISHAK
KU IZZAIRA KU ISHAK

For Their Love, Support, Advices, Help and Best Wishes,

and also to my lovely friends. Thank you, for your supporting.

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As preparing this thesis, I have used the experiences and knowledge of a large number of specialists. It is therefore appropriate and necessary to acknowledge their contributions.

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ABSTRACT

Pulping is the process by which the fibres in the wood or non wood are separated and treated to produce pulp. Commercial non wood pulp production has been estimated to be 6.5% of the global pulp production and is expected to increase. One of the materials found to be promising was banana stem waste, which is very good source of cellulose. These approaches offer several advantages, since these raw materials can be produced annually and have generally lower lignin contents and lower than wood-based materials. A lignin content in banana stem waste is about 12.25%. The effect of different organic loading rate on biological pretreatment to chemical pulping process was initiated in this studies using mix culture that was obtained in banana plantation. The samples that have been treated appeared to give lower lignin contents than untreated. The cellulose composition for treated samples could be say as maintained although there are a very slight depreciation because of the degradation of cellulose give higher content of glucose. The organic loading rate used in this study are 2,5 and 8g/liter day.Organic loading rate of 5g/liter day are the most efficient and have the maximum production rate by the mix culture while the others are not so encouraging. This may have been caused by the limitation of one of the parameter that is the substrate. Theoretically, biological pretreatment is effective, more beneficial and have high interest in the industry due its potential to save energy, reduce chemical needs and have less impact on environment.

ABSTRAK

Pempulpaan adalah proses di mana gentian di dalam bahan kayu dan juga bahan bukan kayu dipisahkan dan diberi rawatan untuk menghasilkan pulpa. Pengeluaran hasil bahan bukan kayu telah dikaji dan didapati bahawa 6.5% daripada pengeluaran antarabangsa dijangka meningkat. Salah satu bahan yang menjanjikan pulangan yang baik ialah sisa batang pisang kerana ia adalah sumber selulosa yg baik. Batang pisang ini mempunyai banyak kelebihan, tambahan pula pengeluarannya yang tetap dan mempunyai kandungan lignin yang rendah. Kandungan lignin di dalam batang pisang adalah 12.25%. Kesan pelbagai kadar beban organik dalam rawatan biologi yang diaplikasikan terhadap pempulpaan kimia adalah kajian utama menggunakan campuran mikroorganisma. Sampel yang telah diberi rawatan menunjukkan kadar lignin yang paling rendah. Komposisi selulosa yang menjalani rawatan biologi boleh dikatakan sekata kerana terlalu sedikit kadar penurunan dan pemakan lignin telah menukarkan glukosa kepada selulosa. Kadar beban organik yang digunakan ialah 2,5 dan 8 gram/liter hari. Kadar beban organik 5g/liter hari menunjukkan kadar paling effisien dan mempunyai kadar pengeluaran paling maksima berbanding yang lain. Ini adalah kerana had terhadap parameter yang digunakan iaitu beban organik. Eksperimen ini telah membuktikan bahawa rawatan biologi terhadap pulpa adalah effisien, lebih bermanfaat dan akan mendapat pulangan yang baik di dalam industri. Ini adalah kerana potensinya yang mampu menjimatkan penggunaan tenaga, mengurangkan penggunaan bahan kimia dan tidak mencemarkan alam sekitar.

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OLR - Organic Loading Rate

HRT - Hydraulic Retention Time

TAPPI - Technical

mL - Mililiter

^{o}C - Degree Celcius

L - Liter

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CHAPTER 1

INTRODUCTION

U.S. pulp and paper mills are a world leader in the production of pulp and paper. These mills produce 9 million tons of pulp annually and 26 billion newspapers, books, and magazines. They account for 35% of pulp produced in the world and make up 16% of the pulp mills in the world. Using wood to make paper is a fairly recent innovation. In the 1800s, fiber crops such as linen fibres were the primary material source, but a shortage led to experimentation with other materials. Around 1850, a German named Friedrich Gottlob Keller crushed wood with a wet grindstone to obtain wood pulp. Further experimentation by American chemist C.B. Tilghman and Swedish inventor C.F. Dahl enabled the manufacture of wood pulp using chemicals to break down the fibres.

Pulping is the process of converting wood to separated pulp fibers for papermaking.Wood consists of two primary components: cellulose and lignin. Cellulose, which is the fibrous component of wood, is used to make pulp and paper. Lignin is the “glue” that holds wood fibers together. Pulping is the process which reduces wood to a fibrous mat by separating the cellulose from the lignin. Pulping processes are generally classified as chemical, mechanical, or semi-chemical. The three chemical pulping methods are known as kraft, sulfite, and soda. Of these, the kraft and sulfite processes are most common (Scott et al, 1995).

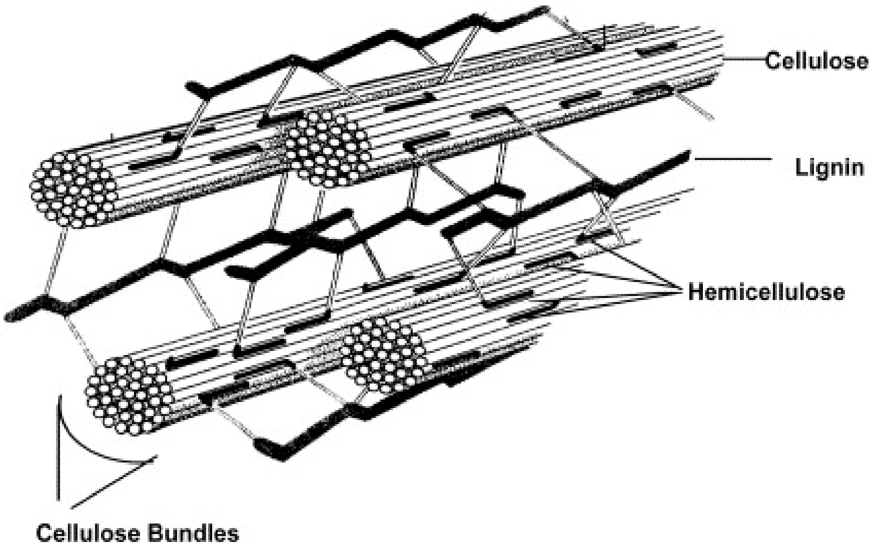


Figure 1.1 Fibre with three components: Lignin, Cellulose and Hemicellulose

Over the years, an increasing preoccupation regarding forest preservation and rational use of forest and agricultural residues has occurred. This fact was mainly motivated by the increasing consumption of wood fibre-based products. This demand is currently solved by using increasing fibres. Thus in some paper grades, more than 50% of raw materials are secondary fibres. In many parts of the world, local supplies of wood cannot support the demand for pulp. Alternative sources of fibre are needed. Annual plants could also be a new sources of lignocellulosic fibres for papermaking.

Non-wood fibre such as straw, kenaf, bamboo and banana waste are becoming increasingly important to the pulp and paper industry. This is understandable because their use has been confined almost exclusively to countries where resources for wood are scant, or indeed, are not available. A non-wood fibre pulp can be very cheaper for local use than imported wood pulp and locally produced non wood fibres can save foreign currency as compared with wood pulp imported at the same or even at a lower price.

Research is under way to develop biological pulping, similar to chemical pulping but using certain species of fungi that are able to break down the unwanted lignin, but not the cellulose fibres. Biopulping is defined as the pretreatment of material pulped with a lignin-degrading fungus prior to pulping. Research shows that the fungal treatment is very effective for chemical pulping process. This research has a high interest in the industry due to its potential to save energy, reduce chemical needs (both pulping and bleaching), and have less impact on the environment. An environmentally sound approach for pulp production would be to use non-wood fibres together with biological pretreatment (Wikipedia, 2006).

Organic loading rate is a parameter about amount of organic material put into a reaction medium per unit time. Bacteria have a maximum production rate depending on the type of reactor and substrate. Organic loading is one of parameters used to describe this production rate. Bacteria and microorganism has their specific growth rate that will achieve a maximum production rate when they degrade substrate. Different organic rate give a different impact to the reaction rate.

1.2 Objectives

i. To study the effect of different organic loading rate(OLR) on biological pretreatment to chemical pulping process.

1.3 Scope of study

i. To study the effects of biological pretreatment on the chemical pulping process
ii. To study the lignin biodegradation of banana stem waste
iii. To determine the for lignin, cellulose and glucose composition.
iv. To determine the effective organic loading rate on biological preteratment to chemical pulping process.

1.4 Problem statement

Due to the wood pulp production about 35% in the world today, it is expected this demand will increase in the future as raw material become more difficult to obtain. Wood pulp also are more expensive compared to noon wood pulp. Commercial of non wood pulp has been estimated to be 6.5% of the global pulp production and it is expected to increase (Paavilainen et al, 1996). A intensive study about non wood pulp is not enough explore yet by our industries. According to recent research and studies, chemical pulping use a lot of chemical, energy and have environmental impact when it use chemicals to break the lignin contents in a fiber (Atchison, 1987) . The chemical uses increases energy where it require a high temperature and a long time for cooking condition. Futhermore, the waste produce by chemical pulping will affect our environment if it is not manage properly.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

U.S pulp and paper mills are a world leader in the production of pulp and paper. These mills produce 9 millions tons of pulp annually and 26 billion newspaper, books, and magazines. They account for 35% of pulp produced in the world and make up 16% of the pulp mills in the world. The fact is that the world paper and paperboard demand which was 313.3 million metric tonnes in the year 2000, is expected to exceed 320 million metric tonnes by the end of 2002 (Environmental Protection Agency, 1997). The demand growth, however, has shifted to the emerging market in Asia, which is anticipated to account for a third of the world's paper consumption within the next 15 years. It is predicted that the demand for the world's paper in the region will grow at an annual average of 6% to 10% over the long term (Malaysia's Paper And Paper Products Industry, 2004).

At present, Malaysia has 19 paper manufacturing companies including one integrated pulp and paper mill located in Sipitang, Sabah. Since coming into operation in the early '70s, Sabah Forest Industries Sdn. Bhd. continues to produce pulp only for its own consumption. The mill turns pulp made from mixed tropical hardwood species into fine quality printing and writing paper. Others, including Kimberly Clark (M) Sdn. Bhd., continue to use virgin pulp as their main raw material despite having introduced some recycled tissue paper (Malaysian Timber Council, 2004).

Pulping is the process by which the fibres in the wood are separated and treated to produce pulp. Different pulping processes are used depending on the raw material and end product (Nilsson et al, 1995). Pulping processes are generally classified as chemical, mechanical, or semi-chemical. In chemical pulping, wood is cooked in a “digester” at elevated pressure with a appropriate chemicals which dissolve the lignin and leave behind the cellulose. In mechanical pulping, wood is pressed against a grinder which physically separates the fibers. Mechanical pulping, which is energy intensive, produces an opaque product which is weak and discolors easily when exposed to light. Semi-chemical pulping uses a combination of chemical and mechanical methods. The wood chips are partially cooked with chemicals, and the remainder of the pulping is accomplished mechanically (Environmental Protection Agency, 1997).

On an annual basis, large quantities of agricultural plants and their residues are available in the United States and throughout the world. Most of these resources would make an excellent alternative source of fiber for paper manufacturing, particularly in light of the increasing pressure being placed on the nation's forests, both from diminishing return and environmental legislation. The fungal treatment of wood chips can reduce pollution, reduce pulping chemicals, and improve paper properties. An environmentally sound approach for pulp production would be to use non wood fibers together with biological treatments (Gary M. Scott, 2001).

A new example of industrial biotechnology for fiber is biopulping -- using a fungus to convert wood chips to paper pulp while reducing energy use and pollutants. Other fibers from plants and animals include cotton, wool, silk, linen, leather, umber and paper. Biopulping is defined as the pretreatment of wood chips with a lignin-degrading fungus prior to pulping. Previous work has established the efficacy of biopulping for mechanical pulping, resulting in energy savings of over 30%. This research transfers that technology to kraft pulping (National Health Museum, 1999).

2.2 Materials for Pulping

2.2.1 Wood

Wood pulp is the most common material used to make paper. The timber resources used to make wood pulp are referred to as pulpwood. Wood pulp generally comes from softwood trees such as spruce, pine, fir, larch and hemlock, but also some hardwoods such as eucalyptus and birch. The major environmental impacts of wood pulping come from its impact on forest resources and from its waste by-products. The number of trees consumed depends on the type of paper, whether made by using the groundwood process or the kraft process. It has been estimated that based on a mixture of softwoods and hardwoods 40 feet tall and 6-8 inches in diameter, it would take a rough average of 24 trees to produce a ton of printing and writing paper, using the kraft chemical pulping process (Thompson et al, 1992).

Table 2.1 Lignin Content in Wood Plant

Wood Plant Material	Lignin Content %
Aspen	25
Pine	26
Spruce	30

Table 2.1 shows lignin content in three example of wood plant material. In many countries, wood is not available in sufficient quantities to meet the rising demand for pulp and paper (Atchison et al 1987). In recent years, active research has been undertaken in Europe and North America to find a new, non woody raw material for paper production.

2.2.2 Non Wood

The earliest information on the use of non woody plant species as surfaces for writing dates back to 3000 BC in Egypt, where the pressed pith tissue of papyrus sedge (*Cyperas papyrus L.)* was the most widely used writing material. Actual papermaking was discovered by a Chinese, Ts’ai Lun when he found a way of making sheets using fibres from hemp rags and mulberry *(Morus alba L.)*. Straw was used for the first time as a raw material for paper in 1800, and in 1827 the first commercial pulp mill began operations in the USA using straw (Atchison and McGovern 1987).

Commercial non wood pulp production has been estimated to be 6.5% of the global pulp production and is expected to increase (Paavilainen, 1998). China produces 77% of the world’s non wood pulp (Paavilainen et. al. 1996). In China and India over 70% of raw material used by the pulp industry comes from non-woody plants. The main sources of non-wood raw materials are agricultural residues from monocotyledons, including cereal straw and bagasse, a fibrous residues from processed sugar cane, bamboo, reeds and some grass plants are also grown or collected for the pulp industry (Paavilainen et al 1996b).

Table 2.2 Chemical Composition of some natural fibres

Fibre	Cellulose	Hemi-cellulose %	Lignin %
Banana	60-65	6-8	5-10
Coir	43	<1	45
Cotton Lint	90	6	-
Flax	70-72	14	4-5
Jute	61-63	13	5-13
Ramic	80-85	3-4	0.5
Sisal	60-67	10-15	8-12
Straw	40	28	18
Sun Hemp	70-78	18-19	4-5

Table 2.2 shows the chemical composition of some natural fibre in non wood plant. From the table, banana, flax, ramic and sun hemp could be a good source of cellulose. One of the materials found to be promising was banana stem waste, which is very good source of cellulose. In Madeira Island (Portugal) the production of banana *(Musa acuminata Coll)* has a capital economica; importance. This agricultural activity generates a large amount of residues, because each plant produces only one bunch of bananas, after its harvesting the bare pseudo-stems are cut and usually left in the soil plantation to be used as organic material. Thus, it could be estimated that few tons per hectare are produced annually. In this context, we have recently started a research program aiming to deep the knowledge on chemical and structural constitution of banana plants (Oliveira et al., 2002) and the use of its cellulosic fibres in papermaking composition and as reinforcing fibres in composite materials (Faria et al., 2002).

Table 2.3 Chemical and Elemental Composition of Banana Stem (TAPPI test method)

Name	Banana Stem
Chemical Composition (wt% dry)	
Ash	10.7
SiO2	1.59
Extractive	4.76
Holocellulose	30.5
Lignin	12.25
Cellulose	40.2
Elemental composition (wt% dry)	
Carbon	38.2
Hydrogen	5.3
Nitrogen	0.3
Oxygen	43.4
Calorific value, kJ/g-dry	15.7

Table 2.3 shows chemical and elemental composition of banana stem. Lignin content in banana stem is about 12.25%. These approaches offer several advantages, since these raw materials can be produced annually and have generally lower lignin contents and lower than wood-based materials (Atchison, 1993). They are more easily delignified and require milder and faster cooking conditions comparing with wood fibre sources. The resulting pulp can be used mainly in paper and board, fibre-board and composite materials.

2.2.3 Comparison of wood and non wood materials

The difference in lignin content is probably the most striking overall feature of the differences. As is well known, pulping processes for wood are designed principally to eliminate this particular plant constituent with the minimum of damage to the paper-making properties of the cellulose fibres present. The fact that there is so much less in non wood fibres precludes many of the results of fundamental research on this aspect of paper-making’s being applied directly to non wood. In a sense this limitation has some indirect advantages. The most important of these is that the relatively complicated chemical reactions involved in removal of lignin from the plant arise to a reduced extent with non wood fibres (Grant et al, 2000).

There are three factors that affect the usage of non wood materials. In the first place, the non wood fibres required a simple process than wood fibres. Secondly the mills that use this fibres are generally smaller production units than those concerned with wood pulp manufacture. Thirdly, the use of a straightforward alkaline cook involves a minimum of chemicals and those required are the simplest nature. It follows the from the three factors, a non wood fibre pulp can be very cheaper for local use than imported wood pulp and locally-produced non wood fibres can save foreign currency as compared with wood pulp imported at the same or even at a lower price (Grant et al, 2000).

2.3 Biodegradation of Lignin

The recent thrust in bioconversion of agricultural and industrial wastes to chemical feedstock has led to extensive studies on cellulolytic enzymes produced by fungi and bacteria. Cellulose is a potentially valuable resource for fibre, fuel and feed. Lignocellulose is the major structural component of woody plants and non-woody plants such as grass and represents a major source of renewable organic matter. Lignocellulose consists of lignin, hemicellulose and cellulose. The chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value (Malherbe and Cloete, 2003).

Table 2.4 The composition of lignocellulose (Paavilainen, 1998)

LIGNOCELLULOSE	
1) Lignin	Lignin is further linked to both hemicelluloses and cellulose forming a physical seal around the latter two components that is an impenetrable barrier preventing penetration of solutions and enzymes. lignin is the most recalcitrant to degradation whereas cellulose, because of its highly ordered crystalline structure, is more resistant to hydrolysis than hemicellulose.
2) Hemicellulose	Hemicellulose macromolecules are often polymers of pentoses (xylose and arabinose), hexoses (mostly mannose) and a number of sugar acids.
3) Cellulose	cellulose is a homogenous polymer of glucose.

Table 2.4 shows the composition of lignocellulose in plant. Because of the difficulty in dissolving lignin without destroying it and some of its subunits, its exact chemical structure is difficult to ascertain. In general lignin contains three aromatic alcohols (coniferyl alcohol, sinapyl and pcoumaryl). Lignin is further linked to both hemicelluloses and cellulose forming a physical seal around the latter two components

that is an impenetrable barrier preventing penetration of solutions and enzymes. Hemicellulose macromolecules are often polymers of pentoses (xylose and arabinose), hexoses (mostly mannose) and a number of sugar acids while cellulose is a homogenous polymer of glucose.

Of the three components, lignin is the most recalcitrant to degradation whereas cellulose, because of its highly ordered crystalline structure, is more resistant to hydrolysis than hemicellulose. Alkaline (Chahal, 1992) and acid (Nguyen et al, 1993) hydrolysis methods have been used to degrade lignocellulose. Weak acids tend to remove lignin but result in poor hydrolysis of cellulose whereas strong acid treatment occurs under relatively extreme corrosive conditions of high temperature and pH which necessitate the use of expensive equipment. Also, unspecific side reactions occur which yield non-specific by-products other than glucose, promote glucose degradation and therefore reduce its yield.

2.3.1 Microorganisms

A diverse spectrum of lignocellulolytic microorganisms, mainly fungi (Baldrian et al, 2003) and bacteria (McCarthy et al, 1987) have been isolated and identified over the years and this list still continues to grow rapidly. Already by 1976 an impressive collection of more than 14 000 fungi which were active against cellulose and other insoluble fibres were collected (Mandels and Sternberg, 1976). Despite the impressive collection of lignocellulolytic microorganisms only a few have been studied extensively and mostly *Trichoderma reesei* and its mutants are widely employed for the commercial production of hemicellulases and cellulases (Esterbauer et al, 1991). This is so, partly because *T. reesei* was one of the first cellulolytic organisms isolated in the 1950’s and because extensive strain improvement and screening programs, and cellulase industrial production processes, which are extremely costly, have been developed over the years in several countries. *T. reesei* might be a good producer of hemi-and cellulolytic enzymes but is unable to degrade lignin. The white-rot fungi belonging to the basidiomycetes are the most efficient and extensive lignin degraders (Akin et al, 1995)with *P.*

chrysosporium being the best-studied lignin-degrading fungus producing copious amounts of a unique set of lignocellulytic enzymes. *P. chrysosporium* has drawn considerable attention as an appropriate host for the production of lignin-degrading enzymes or direct application in lignocellulose bioconversion processes (Bosco et al, 1999).

Table 2.5 Macromolecular Composition and General Properties of Micro-organisms (Malherbe S, 2003)

	Bacteria	Yeast	Fungi	Algae
Doubling time(hours)	1-3	2-6	5-12	6-24
Crude protein (% dry cell weight)	40-80	40-60	30-45	40-50
Nucleic acids (%)	8-20	5-15	6-13	45-51
Carbohydrates and fats (%)	10-30	10-40	10-45	34.6-45
Ash contents (%)	4-10	4-10	4-10	5-8
Temperature range (^oC)	22-55	25-40	25-50	25-32
pH range	5-7	3-5	6-8	6.9-9.6

Protein of microbial origin, called single-cell protein, or microbial protein, can be derived from a variety of micro-organisms, both unicellular and multicellular namely, bacteris, yeasts, fungi, or microscopic algae. The macromolecular composition and general properties of these organisms are shown in Table 2.5.

2.3.1.1 Bacteria

Bacteria likely to be found in a compost heap are aerobic bacteria that specialize in breaking down organic compounds and thrive in temperatures ranging up to 170°F (77°C). Bacterial populations differ from pile to pile, depending upon the raw materials of the compost, degree of heat, amount of air present, moisture level, geographical location of the pile, and other considerations. Bacteria are single-celled and can be shaped like a sphere, rod, or a spiral twist. They are so small that it would take 25,000 bacteria laid end to end to take up one inch on a ruler, and an amount of garden soil the size of a pea may contain up to a billion bacteria. Most bacteria are colorless and cannot make carbohydrates from sunshine, water, and carbon dioxide the way more complex green plants can. Some bacteria produce colonies; others are free-living. All reproduce by means of binary fission. In binary fission, the nucleus splits in two and a new cell wall grows crosswise over the middle of the cell. Each half contains one of the two nuclei, so that a new individual is produced from a single bacterial cell. Under the best conditions, a colony of bacteria can multiply into billions in a very short time. The life span of one generation of bacteria is about 20 to 30 minutes, so that one cell may yield a progeny of billions of individuals in half a day. Bacteria are the most nutritionally diverse of all organisms, which is to say, as a group, they can eat nearly anything. Most compost bacteria are heterotrophic, meaning that they can use living or dead organic materials. Some are so adaptable that they can use more than a hundred different organic compounds as their source of carbon because of their ability to produce a variety of enzymes. Usually, they can produce the appropriate enzyme to digest whatever material they find themselves on. In addition, respiratory enzymes in the cell membrane make aerobic respiration possible as an energy source for compost bacteria (Wikipedia, 2006).

2.3.1.2 Actinomycetes

The characteristically earthy smell of newly plowed soil in the spring is caused by actinomycetes, a higher form of bacteria similar to fungi and molds. Actinomycetes are especially important in the formation of humus. While most bacteria are found in the top foot or so of topsoil, actinomycetes may work many feet below the surface. Deep under the roots they convert dead plant matter to a peat-like substance. While they are decomposing animal and vegetable matter, actinomycetes liberate carbon, nitrogen and ammonia, making nutrients available for higher plants. They are found on every natural substrate, and the majority are aerobic and mesophilic. Five percent or more of the soil's bacterial population is comprised of actinomycetes. The reason bacteria tend to die rapidly as actinomycete populations grow in the compost pile is that actinomycetes have the ability to produce antibiotics, chemical substances that inhibit bacterial growth (Wikipedia, 2006).

2.3.1.3 Fungi

Fungi are many-celled, filamentous or single-celled primitive plants. Unlike more complex green plants, they lack chlorophyll, and, therefore, lack the ability to make their own carbohydrates. Most of them are classified as saprophytes because they live on dead or dying material and obtain energy by breaking down organic matter in dead plants and animals. Like the actinomycetes, fungi take over during the final stages of the pile when the compost has been changed to a more easily digested form. The best temperature for active fungi in the compost heap is around 70° to 75°F though some thermophilic forms prefer much greater heat and survive to 120°F (Wikipedia, 2006).

2.3.1.4 Aerobic Process

Aerobic process is one that requires oxygen. Aerobic composting is characterised by the generation of heat and the rapid multiplication of specific oxygen loving species of microorganisms, bacteria and fungi. It is natural, but usually requires intervention to ensure that air (oxygen) and water do not become exhausted within the heap. Conversely, an anaerobic process is one devoid of oxygen. (Totally anaerobic decomposition processes are used on an industrial scale in highly controlled conditions for rapidly breaking down almost any organic materials e.g. car tyres.) Typically, an ‘open’ mix of compostable materials with sufficient bulky fibrous matter (e.g. ‘long’manure) will start off with a strong aerobic action. Oxygen loving organisms multiply rapidly if sufficient nitrogen is available, producing carbon dioxide, water and heat. At about 50^oC these go to sleep or die off, and bacteria called aerobic thermophiles take over - but even these have their limits (65^oC – 70^oC). Too high a temperature and a restricted oxygen supply can lead to rapid cooling as these organisms die, run for cover or mutate - leading to anaerobic conditions, or cessation if the constituents dry out. Degrees of both processes may co-exist in typical garden heaps but predominantly anaerobic conditions are cooler, slower, odiferous and are best avoided for horticultural composting. Rapid and safe composting is predominantly a high temperature aerobic process. This tends to kill most weeds and a seed, speeds decomposition, avoid dangerous by-products and sets the scene for nitrogen fixation in later stages (Wikipedia, 2006).

2.3.2 Parameters affecting lignin biodegradation

Biological is a process that carried out by bacteria, which have to be kept in a healthy condition and in good living conditions. The bacteria have to be grown and nurtured in the process to get a good production of lignin degradation. The aerobic degradation process can be carried out quite different conditions. All of these conditions have specific influences on the fibre production. Additionally, from a technological viewpoint, the biological process can also be carried out in more than one reactor, which has some, mainly economical, implications (BTG Biomass Technology Group, 2003).

Bacteria have a temperature range in which they are most productive in terms of production rates, growth rates and substrate degradation performance. The several groups of bacteria involved in aerobic digestion all have (slightly) different temperature optimums. Temperature is very important to the biological activity taking place. Low outside temperatures slow the activity down, while warmer temperature speed up lignin degradation. This results in two main temperature ranges in which degradation process usually can be performed optimally and most economically. These ranges are: 25-38°C called the mesophilic range, and 50-70°C called the thermophilic range. These ranges have different characteristics, advantages and disadvantages of which the most important ones are: compared to the mesophilic process, the thermophilic process usually results in a higher degradation of the substrate at a faster rate at the expense of a less stable process. It is less attractive from an energetic point of view since more heat is needed for the process (BTG Biomass Technology Group, 2003).

The groups of bacteria needed for degradation is not only have an optimum temperature but also an optimum acidity at which they are most productive. Unfortunately, for the different groups of bacteria the optimum pH-value (measure for acidity) is not the same. The complexity of the entire system is increased by the fact that the intermediate products of the digestion have a tendency to lower the pH, making the later steps in the process more difficult. This makes balancing the pH in the reactor an important design and operation issue (BTG Biomass Technology Group, 2003).

In process technology the two main types of process (models) are used, the batch process and the continuous process. In the batch process the substrate is put in the reactor at the beginning of the degradation period after which the reactor is closed for the entire period without adding additional substrate. In the continuous process, the reactor is filled continuously with fresh material and also emptied continuously. In a batch reactor all these reaction steps occur more or less after each other. The degradation process is non-continuous: at the beginning only fresh material is available. Half-way through the degradation period the production rate will be highest and at the end, when only the less material is left, production rate will drop again. In a continuous process, fresh substrate is added continuously, and therefore all reactions will occur at a fairly constant rate resulting in a fairly constant production rate. Several mix forms of these two models are developed in process technology including the so-called plug-flow reactor and the sequencing batch-reactor all of which try to combine the advantages of the two extremes (BTG Biomass Technology Group, 2003).

Biomass can be considered as the mass of organic material from any biological material, and by extension, any large mass of biological matter (Howard et al, 2003). A wide variety of biomass resources are available on our planet for conversion into bioproducts. These may include whole plants, plant parts (e.g. seeds, stalks, and stem), plant constituents (e.g. starch, lipids, protein and fibre), processing byproducts (distiller's grains, corn solubles), materials of marine origin and animal byproducts, municipal and industrial wastes (Smith et al, 1987). These resources can be used to create new biomaterials and this will require an intimate understanding of the composition of the raw material whether it is whole plant or constituents, so that the desired functional elements can be obtained for bioproduct production.

Hydraulic Retention Time (HRT) is one of important parameter that can effect biological pretreatment. HRT is defined as a measure of the average length of time that a soluble compound remains in a constructed reactor (Wikipedia, 2006). Different HRT will give different effect to pretreatment process. The longer a substrate is kept under proper reaction conditions the more complete its degradation will become. But the reaction rate will decrease with increasing HRT. The disadvantage of a longer HRT is the increasing reactor size needed for a given amount of substrate to be treated. A shorter retention time will lead to a higher production rate per reactor volume unit, but a lower overall degradation. These two effects have to be balanced in the design of the full scale reactor (BTG Biomass Technology Group, 2003). Thus, in this study, different HRT will be apply to investigate which HRT will give the best result of lignin biodegradation of banana stem wastes.

Bacteria have a maximum production rate depending on the type of reactor and substrate. Organic loading is one of parameters used to describe this production rate. It is the amount of organic material put into the reaction medium per time unit. The unit is g per liter day (BTG Biomass Technology Group, 2003). For a given system size, higher organic loading rates generally result in lower bioconversion efficiency.

Table 2.6 List of fungi with the highest specific activity (µmol.min-1.mg1) for lignase

Enzyme	Organism	Substrate	Specific Activity	Opt. Temp (°C)	Opt. pH
Manganese peroxidase	Stropharia coronilla	Mn^{2+} + H^+ +H_2O_2	692	25	NA
Laccase	Botrytis cinerea	1,2,4-benzenetriol + O_2/1-naphthol + O_2/3,5-dimethoxy-hydroxy-benzaldazine + O_2/4,5-dimethyl-o-phenylenediamine + O_2/4-amino-N,N'-dimethylaniline + O_2/4-methylcatechol + O_2/ascorbate + O_2/caffeic acid + O_2/catechol + O_2/ ferrocyanide + O_2/gallic acid + O_2/guaiacol + O_2	5778	55	4
Diaryl propane peroxidase (ligninase)	Phanero chaete chrysos porium	1,2-bis(3,4-dimethoxyphenyl)propane-1,3-diol +H_2O_2/1-(3,4-diethoxyphenyl)-1,3-dihydroxy-2-(4-methoxy-phenyl)propane + O_2 + H_2O_2/1-(4-ethoxy-3-methoxyphenyl)-1,2-propene + O_2+H_2O_2/1-(4-ethoxy-3-methoxyphenyl)propane + O_2 + H_2O_2/2-keto-4-thiomethylbutyric acid + H_2O_2/3,4-dimethoxybenzyl	28	23/37	3/4.5

Table 2.7 List of bacteria with the highest specific activity (µmol.min1.mg-1) for cellulase

Enzyme	Organism	Substrate	Specific Activity	Opt. Temp (^{o}C)	Opt. pH
Mannan endo-1,4-ß-mannosidase	Bacillus subtilis	Galactoglucomannan/glucomannans/mannans	514	50-60	5-7
Cellulase	Clostridium thermocellum	Avicel/carboxglucanymethylcellulose/cellulosecellopentaose/cellotetraose/cellotriose	428	75	7
1,3- ß-glucanglucohydrolase	Streptomyces murinus	laminarin	6.7	50	6
1,3-1,4-ß-D-glucanglucanohydrolase	Bacillus macerans	ß-D-glucan/lichenan	5030	60-65	6
1,3- ß-D-glucanglucanohydrolase	Bacillus sp.	3-O-ß-D-Glc-D-Glc-D-Glc-D-Glc/ laminarin	369.6	60	9

Table 2.6 and Table 2.7, compiled from the Brenda Enzyme Data Base (http://www.brenda.uni-koeln) show the microorganism with the highest specific activity under the appropriated conditions.

2.4 Chemical Pulping

Pulping is the term used for the process which separates wood fibers. Chemical pulping, dissolving the lignin in the wood to create a pulp, is the most commonly used pulping process. Chemical pulping creates higher sheet strength than mechanical pulping; however, yields 40 to 50 percent pulp, where mechanical pulping yields 95 percent pulp. The two main types of chemical pulping are the more common sulfate pulping (most commonly known as Kraft pulping) and sulfite pulping. Kraft pulping accommodates a variety of tree species, recovers and reuses all pulping chemicals, and creates a paper with a higher sheet strength. Sulfite pulp, however, is easier to bleach, yields more bleached pulp, and is easier to refine for papermaking. The major difference between the two types of chemical pulping is the types of chemicals used to dissolve the lignin (NCASI Technical Bulletin, 1993).

2.4.1 Kraft Pulping

The Kraft process was developed in Germany in 1879 and was first applied to a Swedish mill in 1885. The resulting paper was much stronger than any paper previously made, and therefore the process was named “Kraft”, (German and Swedish for “strength”). Kraft pulping creates dark brown paper which is used for boxes, paper bags, and wrapping paper. Kraft pulp can also be used for writing paper and paperboard when bleached, and for diapers when fluffed. The three main steps involved in Kraft pulping are digestion where wood chips are cooked, washing where black liquor is separated from the pulp and lastly chemical recovery where chemicals are recovered from the black liquor for reuse. Turpentine and tall oil may also be recovered for use or resale.

The first step in pulping wood is to “cook” the wood chips. A digester, heated by steam, “cooks” the wood chips in white liquor (a mix of sodium hydroxide (NaOH) and sodium sulfide (Na_2S) until done. The cooking process dissolves most of the lignin[1] and only some of the hemicellulose[2], leaving mostly cellulose[3] to hold the fibers together.

The digester system may be a batch or a continuous process. Pulp from the blow tank and deknotter is washed with water in a process commonly called brownstock washing. Washing removes weak black liquor from the pulp which is sent to the chemical recovery process. This also prevents contamination during subsequent processing steps. Types of washers used include rotary vacuum washer (most common type of washer), diffusion washers, rotary pressure washers, horizontal belt washers, wash press, and dilution/extraction. The reason Kraft pulping is economically successful is that the used cooking liquor can be recovered and reused in the chemical recovery process. The first step in recovering the chemicals from the black liquor is evaporation. This removes excess water from the black liquor and maximizes the fuel value for the recovery furnace (NCASI Technical Bulletin, 1993).

2.4.2 Sulfite Pulping

Sulfite pulping follows many of the same steps as Kraft pulping. The major difference in sulfite pulping is that the digester "cooks" with a mixture of H_2SO_3 (sulfurous acid) and HSO_{3-} (bisulfite ion in the form of calcium, magnesium, sodium, or ammonium bisulfate). The pulp continues on through the same processes as in the Kraft pulping process. However, the chemicals separated from the pulp in the washers may or may not go into a recovery process. Chemical recovery in sulfite pulping is practiced only if it is economical. If chemical recovery does occur the liquor goes through an evaporator and then to a recovery furnace. Here, smelt is not formed, but ash and SO_2 are formed (NCASI Technical Bulletin, 1993).

CHAPTER 3

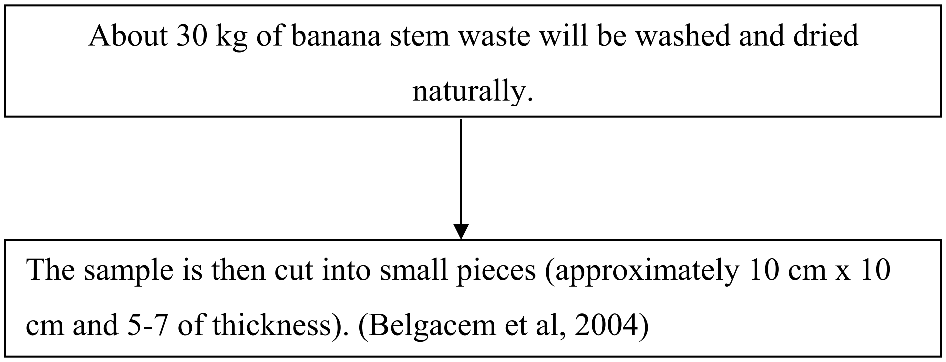
METHODOLOGY

3.1 Introduction

The raw materials that have been used in this experiment is banana stem waste which is obtained from banana plantation near Zaman Restaurant at Jaya Gading. For composition testing, there are three tests that have been used, 72% Lignin Test, Cellulose Testing and Glucose Testing.

3.2 Sample Preparation

Based on Figure 3.1, the sample (banana stem waste) as shown in Figure 3.2, which is procured from banana plantation at Jaya Gading is prepared.



About 30 kg of banana stem waste will be washed and dried naturally.

The sample is then cut into small pieces (approximately 10 cm x 10 cm and 5-7 of thickness). (Belgacem et al, 2004)

Figure 3.1 The banana stem waste preparation