LIGNIN DEGRADATION OF BANANA STEM WASTE USING *Streptomyces badius*

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I declare that this thesis entitled “Lignin Degradation of Banana Stem Waste Using *Streptomyces badius*” is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.”

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Special Dedication to my family members,
my friends, my fellow colleague
and all faculty members

For all your care, support and believe in me.

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ABSTRACT

In this project, the ability of *Streptomyces badius* (*S. badius*) to degrade lignin in banana stem waste was studied. In order for the lignin degradation to occur fermentation process which involved interaction between *S. badius* and the banana stem waste was conducted. Based on the growth analysis, fermentation process by *S. badius* takes only one day and 6 hours in 250 ml medium. As for the lignin analysis content, Klason (72% H_2SO_4) method was employed. There are 5 major step involved in Klason method which are drying, addition of sulfuric acid, addition of distilled water, filtration, and last but not least is drying and moisture removal step. Lignin content was obtained by weighing the final mass of the banana stem waste sample after the final step in Klason method. Following formula were used to determine the percentage of lignin being degraded in the fermentation process.

$$\text{Lignin degradation} = \frac{[\text{\% lignin in control}] - [\text{\% lignin in sample}]}{[\text{\% lignin in control}]} \times 100\%$$

Based on the result of the experiment, *Streptomyces badius* was able to degrade lignin contain in banana stem waste as much as 23%.

ABSTRAK

Projek ini bertujuan untuk mengkaji kebolehan *Streptomyces badius* untuk menguraikan lignin. Bagi mewujudkan penguraian lignin berlaku, proses penapaian yang melibatkan interaksi di antara *S. badius* dan sisa batang pisang dijalankan. Berdasarkan analisis pertumbuhan bakteria, didapati *S. badius* boleh hidup selama satu hari dan 6 jam di dalam 250 ml media. Bagi analisis kandungan lignin pula, kaedah Klason(72% H_2SO_4) diguna pakai. Terdapat 5 langkah utama di dalam kaedah ini iaitu, pengeringan, penambahan asid sulfurik, penambahan air suling, penapisan dan juga pengeringan dan penyingkiran kelembapan. Kandungan lignin boleh didapati dengan menimbang berat akhir sampel sisa batang pisang pada langkah terakhir dalam kaedah Klason. Formula berikut digunakan bagi menentukan peratusan lignin yang diurai.

$$\%\text{Lignin diurai} = \frac{[\%\text{ lignin di dalam kawalan}] - [\%\text{ lignin di dalam sampel}]}{[\%\text{ lignin di dalam kawalan}]} \times 100\%$$

Berdasarkan keputusan eksperimen, peratusan terbanyak lignin yang diuraikan oleh *Streptomyces badius* mampu mencapai sehingga 23%.

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LIST OF SYMBOLS/ABBREVIATIONS

DNA	-	Deoxyribonucleic acid
g	-	gram
H_2O	-	water
H_2O_2	-	Hydrogen peroxide
ha	-	hectar
HCS	-	Hazard Communication Standard
hr	-	hour
ISO	-	International Organisation for Standardization
LiP	-	Lignin peroxidase
mg/L	-	milligram per liter
MAO	-	Malaysia Agriculture Organization
Min	-	minutes
mL	-	mililiter
MnP	-	Manganese Peroxidase
PHA	-	polyhydroxyalkanoates
PHB		poly-b-hydroxy butyrate
UN	-	United Nation
v/v	-	volume per volume
v/w	-	volume per weight
w/v	-	weight per volume
w/w	-	weight per weight
WWII		world war II
%	-	percentage
°C	-	degree Celsius

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CHAPTER 1

INTRODUCTON

1.1 Background of Study

Our environment nowadays is polluted mostly because of the chemical based materials that cannot be degraded and containing toxic chemical substance like plastic. Thus, these contribute to the solid waste management problem. Generally, plastic is one kind of synthetic polymers that cannot be degraded in natural environment. The characteristic of these synthetic polymers raised a global ecological problem. Besides, by burning it at low temperature and at open area the toxicity gaseous will be released to the air.

To solve the problem of solid waste management problem, a production of the polymers capable of degrading into components harmless for both animate and inanimate nature is the most radical approach (Hong Chua et al.,1999). Therefore the production of bioplastic is an alternative of conventional plastic produce as it is so called environmental friendly product.

Polymers of fatty acid hydroxyl derivatives or so-called polyhydroxyalkanoates (PHAs) are the most widely used and intensely developed biodegradable polymers (Hong Chua et al.,1999). Bioplastic differ with the traditional plastic which made from polyethylene polymer. Bioplastic are actually made of renewable biomass sources such as vegetable oil, corn starch or microbiota. By comparing with conventional plastic which derived from petroleum, bioplastic seems to be sustainable products since plant and microbiota are living things that can be grew. These differ with traditional plastic which based on the fossil fuel which may finish one day.

Bioplastic is biodegradable which means that microbes are able to degrade them at suitable conditions, where as the conventional plastics mostly are non-degradable since the materials used are excessive in molecular size. Polyhydroxyalkanoates (PHAs) have been find out as better choices for biodegradable plastics since it has similar characteristic to conventional plastics such as polypropylene and their complete biodegradability.

Common material used in producing bioplastic that are a widespread storage material in many micro-organisms is poly b-hydroxy butyrate(PHB) which is one type of polyhydroxyalkanoates (PHAs) that are relatively high concentration with high productivity. In many micro-organisms like Azotobacter, Bacillus, and Nocardia, PHB accumulates as energy reserve material. They are also known to be non toxic. Although, PHAs have advantages over the conventional plastics, high production cost seems to be the problem before the widespread application of bioplastic is possible. Current cost of PHAs is ~10 times higher than that of conventional plastics (Hong Chua et al., 1999).

Recently, much effort has been devoted to isolating bacterial strains that can produce PHAs from cheap carbon sources (Hong Chua et al., 1999). For instance, tapioca which is a food had been used as the raw material of the carbon sources for the bacteria producing bioplastic to grow. Therefore, a consideration to use other sources which is not food and more cheap was thought.

One of the materials found to be promising was banana stem waste, which is a very good source of cellulose. Banana stem is proposed to replace the tapioca usage since they are not food and can be obtain at lower price as they are considered as waste because after harvesting fruit, the stem which is known as pseudostem is traditionally wasted and used as organic materials for the soil. In Malaysia alone, the total banana plantation areas are almost 34 thousands hectares (MAO, 2006). Therefore, economic utilization of these banana palm waste will be advantageous.

Banana stem (banana fiber) consist of lignocelluloses. Since lignocelluloses is where the cellulose located, delignification or lignin degradation process need to be conducted towards the banana fiber before the cellulose itself need to be degrade into its monomer to be provided as media for bacteria producing biopolymer could be grow. Lignin is a heterogeneous and irregular arrangement of phenylpropanoid polymer that resists chemical or enzymatic degradation to protect cellulose. Lignin degradation was done by the action of the enzyme in bacteria or fungi that break down the lignin into carbon dioxide and water.

1.2 Objectives

The aim of this research is to employ the sugar (cellulose) consist in banana stem waste as carbon sources for the bacteria producing biopolymer. Therefore our objective is as below;

- To study the submerged fermentation of banana stem waste using *Streptomyces badius*
- To study the amount of lignin degraded in banana stem waste using *Streptomyces badius.*

1.3 Scope of Study

There are 2 scopes being studied in this research. They are as follows:

- Submerged fermentation is used as a fermentation condition for lignin degradation of banana stem using *Streptomyces badius* .
- Lignin content of banana stem waste being degrade by *Streptomyces badius* analyzed using Klason(72% H_2SO_4) method.

1.4 Problem Statement

This research is proposed to determine whether the *Streptomyces badius* could degrade the lignin in banana stem before it can be use as a medium for bacteria producing biopolymer to grow in order to produce bioplastic. This research is a further research on finding the suitable carbon sources as a nutrient to grow bacteria.

This is due to the cost required to produce the bioplastic that used food as nutrient supplier at high cost such as tapioca. Tapioca had been used as the raw material for the sources of sugar. Since food is likely global crisis where the supply is insufficient besides consideration of cost, tapioca seems not a suitable candidates. Meanwhile, banana stem is considered as waste since they are mostly can only grow its fruits once. They also degraded by themselves if not being cut down after fruit harvesting. Rather than let them disposed by themselves, banana stem is suggested as a better choices to overcome this problem.

However, the characteristic of banana which consist of lignin is a barricade for this problem to be overcome. Lignin in banana stem need to be lignified before the cellulose could be degraded into its monomer which is glucose residue by breaking the glycosidic bond in order to get carbon sources that can be provided into the medium. Therefore, in order to degrade the lignocelluloses consist in banana stem, bacteria strain, *Streptomyces badius* is used.

Streptomycetes are Gram-positive aerobic members of the order Actinomycetales within the classis Actino-bacteria (Stackebrandt et al., 1997). Previous study had proved that the *Streptomyces badius* able to degrade lignin. Therefore the ability of this bacteria strain is applied in degrading lignin in banana stem waste.

1.5 Rationale and Significance

The significance of this research is that we can eliminate the used of tapioca as carbon sources of the medium used for bacteria growth since it is food and costly, thus using banana stem is its alternative way to reduce the cost of producing bioplastic. Moreover, banana stem can be reused as something useful instead of letting it degraded by itself to provide organic material for the soil. Besides, banana plantation is one of the biggest plantations in Malaysia and they are easy to cultivate since it is suitable with our country condition.

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CHAPTER 2

LITERATURE REVIEW

2.1 Bioplastic

Bioplastics are made of renewable biomass sources, such as vegetable oil, corn starch, pea starch or microbiota, rather than traditional plastics which are derived from petroleum. They are used either as a direct replacement for traditional plastics or as blends with traditional plastics.

Most industry understands that bioplastic term must refer to plastic that is made from biological sources. Wood cellulose can also be used to produce cellulose film which is one of the oldest plastic ever made. Technically either bio or petroleum based plastic are able to be biodegrade where they are able to be degraded under suitable condition using microbes. They are considered as non-biodegradable if its degradation occurs at slow rate.

There are some conventional plastic that are considered bio-degradable and can be added to improve the performance of commercial bioplastic. There are some bioplastic that are non-degradable and it is called as durable.

The degree of biodegradation varies with temperature, polymer stability, and available oxygen content. Consequently, most bioplastics will only degrade in the tightly controlled conditions of commercial composting units. An internationally agreed standard, EN13432, defines how quickly and to what extent a plastic must be degraded under commercial composting conditions for it to be called biodegradable. This is published by the International Organisation for Standardization ISO and is recognised in many countries, including all of Europe, Japan and the US. However, it is designed only for the aggressive conditions of commercial composting units. There is no standard applicable to home composting conditions.

Ultraviolet light and oxygen can be used to degrade the conventional plastic such as polyethylene. Specially modified petrochemical-based plastics producers often used the term biodegradable plastic for their product that are appear to be biodegrade. Therefore stabilizing chemical is added to prevent their product from being 'biodegrade'. However there is more another precise terminology to define this type of degradation such as 'degradable plastic' or 'photodegradable plastic' as there are no microbial used to for the plastic to degrade. (Wikipedia 2008)

2.1.1 Poly b-hydroxy Butyrate

A wide variety of industrial and consumer plastic products have been increasingly regarded as a source of solid waste-management problems because of their nondegradable properties. In Hong Kong, 14 weight percents of the 10,000 tons of municipal solid wastes produced each day are plastic packaging materials and disposable products. Although plastics usage and plastics-waste generation is forecast to increase at 15%/year over the next decade (Chua et al. 1995), there has been considerable interest in developing environmentally-friendly materials to substitute for conventional plastics (Chang et al 1994;Chua et al. 1995).

Polyhydroxyalkanoates (PHAs) have been recognized as better candidates for biodegradable plastics owing to their similar material properties to conventional plastics and their complete biodegradability. Approximately 300 different isolated microorganisms synthesize and accumulate PHAs as carbon and energy storage materials under the condition of limiting nutrients in the presence of excess carbon source. Among the various members of PHAs, only three kinds of PHAs, poly(3-

hydroxybutyrate), [P(3HB)],poly(3-hydroxybutyrate-co-3 hydroxyvalerate) ,[P(3HB-Co-3HV)], and poly(3-hydroxyhexanoate-co-3-hydroxyoctanoate) [P(3HHx-co-3HO)], have been produced to a relatively high concentration with high productivity (Sang 1996). Although, PHAs have advantages over the conventional plastics, widespread application of PHAs is hampered by high production cost. The current cost of PHAs is ~10 times higher than that of conventional plastics (Chua et al. 1995). Substantial reduction in production cost is necessary before widespread applications in packaging and disposable products are possible. In the last several years, fermentation strategies for the production of PHAs with high productivity have been relatively well developed to bring down the overall cost. Recently, much effort has been devoted to isolating bacterial strains that can produce PHAs from cheap carbon sources.

PHB material was first described in 1926 by Lemoigne. PHB is a very common and widespread storage material in many micro-organisms. PHB has been found to be a very basic polymer of variety of chemically similar polymers, the polyhydroxyalkonates.

Poly b - hydroxy butyrate (PHB) accumulates as energy reserve material in many micro-organisms like Alcaligenes, Azotobacter, Bacillus, Nocardia, Pseudomonas, Rhizobium etc. Polypropylene (PP) and Poly b - hydroxy butyrate (PHB) had physical properties which are almost identical that make them comparable. Repeating unit that making the PHB chain is $CH(CH_3)$-CH_2-CO-O. What makes PP and PHB differ with each other is that PHB degrade completely, where as PP is vice versa. Since degradation simply occur at sediment condition , PHB is describe to be more easier to be degrade compare to PP since it sinks while PP floats.

Alcaligenes eutrophus and Azotobacter beijerinckii can accumulate up to 70% of their dry weight of PHB. These micro-organisms can produce the polymer in environment of Nitrogen and Phosphorus limitation. Minimum 40-50% of the dry weight of this polymer is required for making the process commercially viable.

The properties of PHB are listed as follows:

1. PHB is water insoluble and relatively resistant to hydrolytic degradation. This differentiates PHB from most other currently available biodegradable plastics, which are either water soluble or moisture sensitive.
2. PHB shows good oxygen permeability.
3. PHB has good ultra-violet resistance but has poor resistance to acids and bases.
4. PHB is soluble in chloroform and other chlorinated hydrocarbons.
5. PHB is biocompatible and hence is suitable for medical applications.
6. PHB has melting point 1750C., and glass transition temperature 150C.
7. PHB has tensile strength 40 MPa which is close to that of polypropylene.
8. PHB sinks in water while polypropylene floats. But sinking of PHB facilitates its anaerobic biodegradation in sediments.
9. PHB is nontoxic.

2.2 Banana Stem

Banana plant stem is called pesudostem that grows to 6 to 7.6 meters (20-25 feet) tall. It grow from the corm which is the roots of for banana plant terminology .The pseudostem that cultivate will grow its leaves that are 2.7 meters long and 60 cm wide .The stem also responsible in producing the banana heart that are single, sterile and is a male banana flower. As for the female flowers, they are produced further up the stem produce actual fruit. Fertilization is not required for the fruit production. Banana stem contain fiber that is described as lignocelluloses. The term lignocellulose is usually used to describe the plant waste fibers. These lignocelluloses are comprised of cellulose, hemicelluloses and lignin. Lignocellulosics include wood, agricultural residues, water plants, grasses, and other plant substances (Rowell et al. 2000). Plant fibers are used to produce fuel, chemicals, enzymes, and food. The composition, properties and structure of plant waste fiber are suitable to be use as composite, textile, and pulp and paper manufacture.

Biomass, including agricultural crops and residues, forest resources, and residues, animal and municipal wastes, is the largest source for cellulose in the world. Approximately 2×10^{11} tons of lignocelluloses are produced every year, compared with 1.5×10^{8} tons of synthetic polymers. Organic plant wastes such as oil palm, pineapple, banana, and coconut fiber are annually renewable, available in abundance and of limited value at present. These lignocellulosic byproducts could be a principal source for fibers, chemicals, and other industrial products (Reddy and Yang 2005). In Malaysia alone, there are a large area of plantation of oil palm (3.87 million ha.), coir (147 thousands ha.), banana (34 thousands ha.), and pineapple (15 thousands ha).

Natural fibers are described to have many advantages such as low density, appropriate stiffness and mechanical properties and high disposability and renewability. Since they are waste and living things they are recyclable and biodegradable. Lignocellulose fibers in banana are obtained from the pseudostem of banana plant (Musa sepientum). These fibers have a good mechanical property. The ‘‘pseudo-stem’’ is a clustered, cylindrical aggregation of leaf stalk bases. Waste banana stem has a high organic content (83%); with 15-20% (w/w) lignin and cellulose which gives it a sheath-like texture.

2.3 Lignin and Lignin Degradation

Lignin can be found in all vascular plants, mostly between the cells and in the cell walls. It gives the shape of the plants, makes plants become firm and that gives "fiber" in our food. It functions to regulate the transport of liquid in the living plant and it enables trees to grow taller and compete for sunshine. and researchers see it as a disposal mechanism for metabolic wastes. Whereas, lignin degradation is a process to break down lignin.

2.3.1 Lignin

Lignin is a complex polymer of phenylpropane units, which are cross-linked to each other with a variety of different chemical bonds. This complexity has thus far proven as resistant to detailed biochemical characterization as it is to microbial degradation, which greatly impedes our understanding of its effects. Some organism such as bacteria and fungi excrete enzymes that are used to degrade lignin. The initial reactions are mediated by extracellular lignin and manganese peroxidases, primarily produced by white-rot fungi (Kirk and Farrell, 1987). Actinomycetes can also decompose lignin, but typically degrade less than 20 percent of the total lignin presents (Crawford, 1986; Basaglia et al., 1992). Lignin degradation is primarily an aerobic process, and in an anaerobic environment lignin can persist for very long periods (Van Soest, 1994).

Since lignin is the most recalcitrant component of the plant cell wall, the higher the proportion of lignin in the fibers, thus the lower the bioavailability of the substrate. The effect of lignin on the bioavailability of other cell wall components is thought to be largely a physical restriction, with lignin molecules reducing the surface area available to enzymatic penetration and activity (Haug, 1993).

2.3.2 Lignin Degradation

Lignin degradation is a process where lignin was break down to produce carbon dioxide and water. Lignin is a heterogenous and irregular arrangement of phenylpropanoid polymer that resists chemical or enzymatic degradation to protect cellulose (Ohkuma et al 2001). Previously, white rot fungi is the only organism known that can degrade lignin into water and carbon dioxide.