BIOCONVERSION OF AGRICULTURAL SOLID WASTE (BANANA TREE STEM) BY MIXED CULTURE FOR USE AS CARBON SOURCE FOR FERMENTATION MEDIUM

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BIOCONVERSION OF AGRICULTURAL SOLID WASTE (BANANA TREE STEM) BY MIXED CULTURE FOR USE AS CARBON SOURCE FOR FERMENTATION MEDIUM

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A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
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April 2009

I declare that this thesis entitled “Bioconversion of Agricultural Solid Waste (Banana Tree Stem) By Mixed Culture for Use as Carbon Source for Fermentation Medium” 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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“Specially dedicated to my beloved father, mother and sisters”

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ABSTRACT

Lignocellulosic waste can be useful if the cellulose can be freed from the lignin so that the cellulose can be converted into sugar and be used as the carbon source for fermentation medium. The glucose can be produced by anaerobic digestion which involve the process of breaking up the lignin components or delignification and later on followed by cellulose degradation. This thesis reports on the research in the delignification of lignocellulose in banana stem waste and the degradation of the cellulose into glucose. The mixed culture was collected from a banana plantation field. The process is carried out at ambient temperature in two anaerobic digesters with different organic loading rates and also with and without acclimatization respectively. From the result, it is found that glucose is recovered at the end of the digestion process but having different conversion with the anaerobic digester having the higher loading rates and acclimatized culture giving higher conversion. The percentage of lignin loss due to the delignification that occurred is determined by analyzing the sample using Klason (72% sulphuric acid) Method. The recovered glucose from the anaerobic digestion is tested as fermentation medium for *Saccharomyces cerevisiae* (yeast) and the results had shown that the yeast is capable of utilizing the glucose as carbon source. Hence, it can be concluded that the banana stem waste can be bioconverted into carbon source for fermentation medium.

ABSTRAK

Bahan buangan berlignoselulosa boleh mendatangkan manfaat apabila selulosa dapat dipisahkan daripada lignin dan seterusnya dijadikan sebagai sumber karbon bagi medium untuk fermentasi. Glukos dapat dihasilkan sebagai sumber karbon dalam proses fermentasi melalui pencerna anaerobik yang melibatkan proses pemecahan lignin dan seterusnya diikuti oleh proses penguraian selulosa. Tesis ini membincangkan proses penguraian lignoselulosa kepada glukos daripada bahan buangan batang pisang menggunakan kultur campuran yang dikumpulkan dari kebun pisang. Proses ini dijalankan pada suhu persekitaran di dalam dua pencerna anaerobik berlainan yang mempunyai kadar muatan bahan organik yang berbeza serta dilakukan proses penyesuaian dan tidak disertai oleh proses penyesuaian. Daripada keputusan kajian, didapati bahawa glukos terhasil pada akhir proses pencernaan anaerobik namun pada kadar pertukaran yang berbeza di mana kadar pemerolehan glukos adalah lebih tinggi di dalam pencerna anaerobik yang mempunyai kadar muatan bahan organik yang lebih tinggi dan disertai penyesuaian terlebih dahulu. Kemudian, kadar peratusan kehilangan lignin turut ditentukan menerusi kaedah Klason (72% asid sulfurik). Akhir sekali, glukos yang telah terhasil diuji sebagai medium fermentasi bagi *Saccharomyces cerevisiae* (yis) dan melalui pemerhatian yang dibuat, yis dapat menggunakan glukos tersebut sebagai sumber karbon. Justeru, dapat disimpulkan bahawa bahan buangan batang pisang dapat ditukarkan secara biologi kepada sumber karbon bagi proses fermentasi secara pencernaan anaerobik mengunaakan kultur campuran.

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LIST OF SYMBOLS/ABBREVIATIONS

DNS	-	Di-Nitro Salicylic Acid
Glu.	-	Glucose
g	-	gram
h	-	hour
mg/L	-	milligram per liter
Min	-	minutes
mL	-	mililiter
v/v	-	volume per volume
v/w	-	volume per weight
w/v	-	weight per volume
w/w	-	weight per weight
g/L	-	gram per liter
%	-	percentage
°C	-	degree Celsius

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The bio-based chemical industry is growing rapidly day by day. Over the long term, the requirement for the fermentable sugars as the feedstock to support the bio-based chemical industry will be very high to meet the demand of the industry. The existing commercial microbial fermentation is utilizing the glucose to produce the ethanol, acetic acid, amino acids, antibiotics and also other chemicals. The future of fermentation technology will be greatly enhanced by lignocelluloses conversion (Dale, 1987). The fermentable sugars can be found in agricultural waste and also in crops and the sugars can be produced either directly or is derived from the polysaccharides such as the cellulose and hemicelluloses. Cellulose and hemicelluloses are carbohydrates that can be broken down by enzymes, acids, or other compounds to simple sugars, and then fermented to produce other product such as the renewable electricity, fuels, and biomass based products (Puri, 1984; Wyman and Goodman, 1993; van Wyk, 2001)

The banana stem waste has a high organic content (83%); with 15±20% (w/w) lignin and cellulose which gives it a sheath-like texture (Kalia *et al.*, 1999). In tropical and subtropical areas, the banana is produced in large quantities. The total planted area of banana in Malaysia in 2001 was estimated to be 33,704.2 hectares (MAO, 2006). Normally, after the harvesting of the banana fruits, the whole plant which are the stem,

leaves and also the rhizome is left at the plantation field for natural degradation and this process will take several months. However, these banana wastes can be utilized effectively for the releasing of sugars for carbon source which can later on be used as fermentation medium (Khalil *et al*,. 2006).

Before the desired carbon source which is glucose can be obtained from the banana stem waste, a sequence of procedures should be done first. The process is the degradation of the lignin (delignification) and also the degradation of polysaccharide (cellulose degradation) which are the components of the lignocelluloses.

1.2 Objective

The aim of this study/research is to biologically convert the agricultural solid waste (banana tree stem) to carbon source for fermentation medium. Also, this study is done to investigate the delignification of banana stem waste by mixed culture. Lastly, it is also the aim of this study to produce the carbon source from the cheap source (banana stem waste).

1.3 Scope of Study

The scope of doing this study is to investigate the anaerobic digestion of the lignin and polysaccharide components of the banana stem waste which will further be converted into the carbon source for the fermentation medium. In converting the agricultural solid waste as the carbon source for the fermentation medium, several processes will be conducted. First of all is the process of acclimatizing the anaerobic microflora from the soil which is taken from the banana plantations at ambient temperature which is the best temperature for mixed culture from garden soil (Benner *et al*,. 1984) and also the

optimum temperature for lignin degradation (Bumpus and Aus, 1985;Janshekar and Fiechter, 1988). After several months of acclimatization, the sample is transferred into the anaerobic digester to be degraded. The products of the anaerobic decomposition are then being analyzed using the DNS method for glucose and also using the Klason method for lignin constituents.

1.4 Problems Statement

The banana stem is one of the agricultural solid wastes which comprises of the lignocellulosic agricultural waste. The waste can be useful if the cellulose can be freed from the lignin so that the cellulose can be converted into sugar and be used as the compound for fermentation medium. The cellulose from the lignocellulosic waste can be hydrolyzed by acid to glucose, but much of the glucose will be destroyed during the process. Also, enzymatic hydrolysis using mixtures of enzymes, such as cellulase and hemicellulaces can be used to avoid the destruction of sugars associated with acid treatments (hydrolysis) of lignocellulosic material. These enzymes will provide high yields of glucose and also other fermentable sugars with minimal sugar losses when combined with effective pretreatment of lignocellulosics. However, these enzymes are currently too costly to be used in large scale conversion of lignocellulosic materials to fermentation substrates. The cost of carbohydrate raw material influences the economy of many fermentation processes, hence the cost play a decisive role in future and scope of industries employing fermentation processes (Dale, 1987; Castellanos *et al*., 1995). Therefore, the use of mix culture from the soil can be a new alternative to produce the glucose for fermentation medium via the anaerobic digestion of the lignocellulosic substances which is not only cheap but in turn will also utilize the waste to be converted into useful products.

CHAPTER 2

LITERATURE REVIEW

2.1 Bioconversion

The bioconversion is the biological processes for the conversion of wastes to fuels include ethanol fermentation by yeast or bacteria, and methane production by microbial consortia under anaerobic conditions. Bioconversion is referred to as the enzyme-mediated conversion of organic substrates, such as cellulose, to other more valuable substances, such as protein, and also sugars by other organisms. The conversion of biomass to useable energy, as by burning solid fuel for heat, by fermenting plant matter to produce fuel, as ethanol, or by bacterial decomposition of organic waste to produce methanol is also referred to as bioconversion (Okonko *et al*., 2006).

2.2 Banana

Banana (*Musacea sp*.) which is a herbaceous monocots grows in large quantity in both the tropical and also the subtropical area. In 2001, there was approximately 33,704.2 hectares of the total planted area of banana in Malaysia (MAO, 2006). Banana plants range in height from 0.8m to more than 15m. Each contains the flattened and modified stem which is called the 'pseudostem'. This 'pseudostem' consists of concentric layers of leaf sheath and column of large leaves (Ennos *et al.,* 2000). The leaves, which are among

the largest of all the banana plants, can become up to 9 ft long and 2 ft wide. After the banana fruit is harvested, the 'pseudostem' is usually being left and wasted. According to N. Saha and G.P. Nagori, banana stem contains 22% lignin and 35% cellulose on dry weight basis.

2.3 Lignocellulose

Lignocellulose is mainly composed of cellulose, hemicellulose, and lignin (Sjöström, 1993). Fengel and Wegener , (1989) and Argyropoulos and Menachem , (1997) estimate that there is 2.5−4×1011 tons of cellulose and 2−3×1011 tons of lignin in the earth, representing 40% and 30% of organic matter carbon, respectively, with other polysaccharides comprising 26%. A variety of fungi and bacteria can break down the lignocellulose using hydrolytic and oxidative enzymes (Erikson *et al*., 1999) Photosynthesis and degradation of lignocellulose are essential for the global carbon cycle (Brown 1985, Colberg 1988). The degradation rate is governed by temperature, moisture content, and type of lignocellulose (Rayner and Boddy , 1988, Kuhad *et al*., 1997). A warm, wet environment in contrast to a cold, dry one enhances degradation (Rayner and Boddy , 1988), and herbaceous litter degrades considerably faster than wood (Kuhad *et al*.,1997).

2.4 Lignin

Lignin is a natural composite material in all vascular plants, providing the plant with strength and ridigity (Brown, 1985).It is the third most abundant natural polymer present in nature after cellulose and hemicelluloses. The estimated amount of lignin on earth is 300 billion metric tonnes with an annual biosynthetic production rate of 20 billion metric tones (Argyropoulos and Menachem, 1998).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. Nonetheless, some organisms, particularly fungi, have developed the necessary enzymes to break lignin apart. The initial reactions are mediated by extracellular lignin and manganese peroxidases, primarily produced by white-rot fungi (Kirk and Farrel, 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). The enzymatic equipment for depolymerizing lignin can be found in fungi and bacteria. Several types of enzymes involved in degradation have been described (Kirk & Farrell, 1987). These include monooxygenases (phenoloxidases, laccases), dioxygenases and peroxidases.

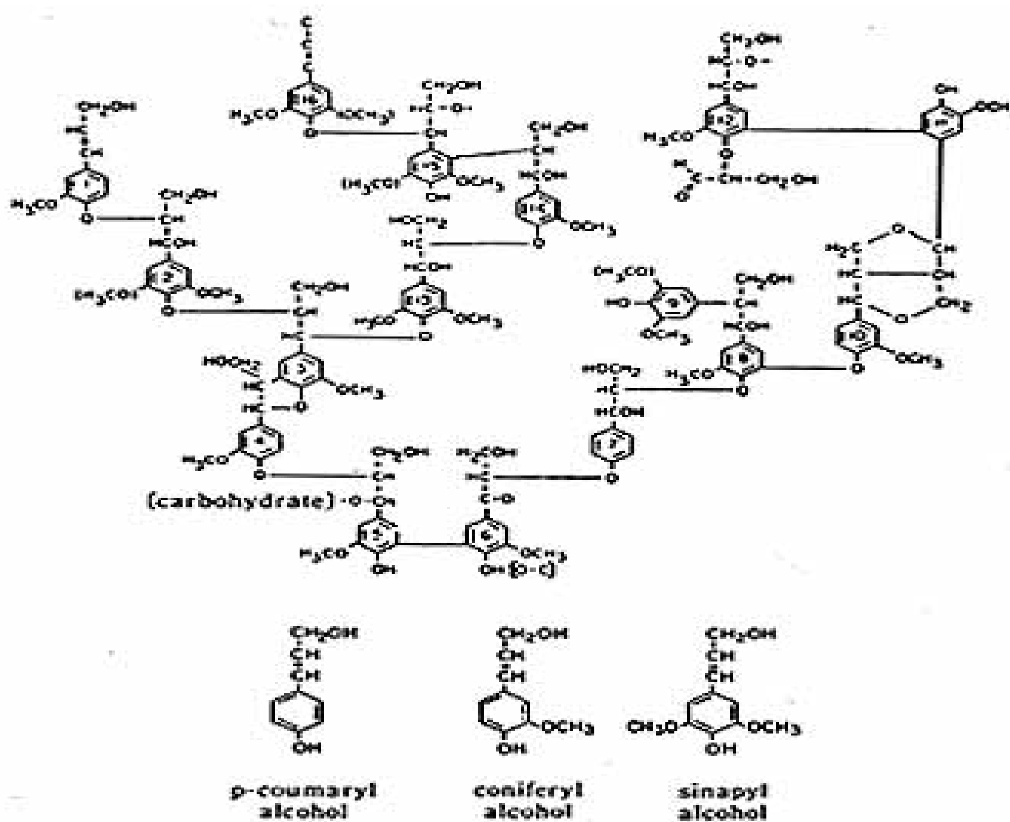

Figure 2.1: Lignin Structure

2.5 Cellulose

Cellulose is the main constituents of plant cell walls comprising about 50% of wood. Cellulose is a long chain of glucose molecules, linked to one another primarily with β,1-4 glycosidic bonds. The simplicity of the cellulosic structure, using repeated identical bonds, means that only a small number of enzymes are required to degrade this material. Cows and other ruminants create an environment in their rumen which encourages the microbial degradation, converting cellulose to volatile fatty acids and microbial biomass which the ruminant can then digest and use.

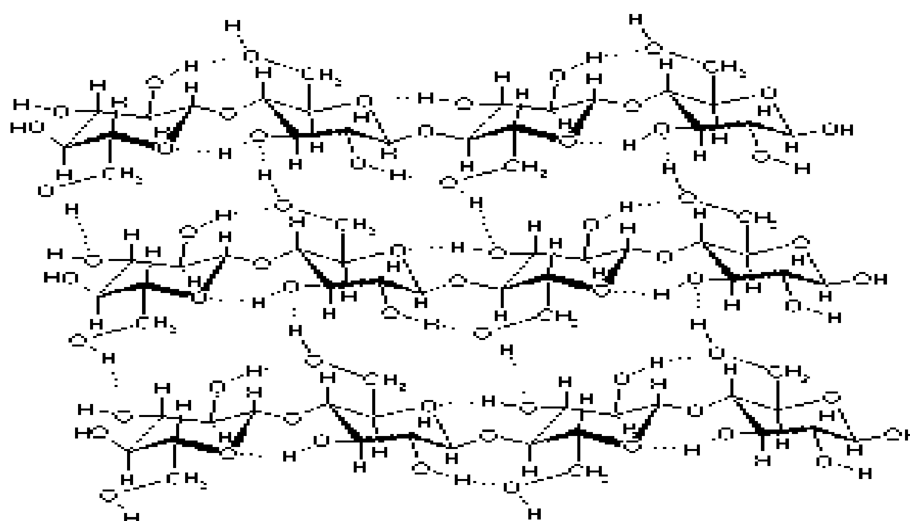

Figure 2.2: Cellulose Structure

2.6 Hemicelluloses

Hemicelluloses are branched polymers of xylose, arabinose, galactose, mannose, and glucose. Hemicelluloses bind bundles of cellulose fibrils to form microfibrils, which enhance the stability of the cell wall. They also cross-link with lignin, creating a complex web of bonds which provide structural strength, but also challenge microbial degradation (Ladisch *et al*, 1983; Lynch, 1992)

2.7 Lignin-degrading microorganisms

2.7.1. Aerobic bacteria

Pure bacterial cultures are unable to perform efficient lignin degradation (Blanchette, 1995; Daniel and Nilsson, 1998). The lignin degradation mechanism of bacteria is more specific than that of fungi in which one bacterial species is able to cleave only one type of bond in the lignin polymer (Vicuña *et al.,* 1993). Thus, bacteria degrade lignocellulose in mixed cultures, either in mixed bacterial cultures, or more commonly, in bacterial and fungal cultures together (Vicuña *et al*., 1993; Daniel and Nilsson, 1998). Actinomycetes live in environments rich in lignocellulose, such as soil, compost, heaps of hay, straw, or wood chips (Lacey 1988). Actinomycetes frequently degrade, modify, or solubilize lignin polymer, especially lignin of gramineous plants, to acid precipitable polymeric lignin (Crawford et al. 1983, Adhi *et al*., 1989, Ball *et al*., 1989, Pasti *et al*., 1991, Spiker *et al*. 1992). While degrading the lignocellulosic complex, actinomycetes may also polymerize lignin fragments (Crawford, 1988) or mineralize lignin to some extent (Haider and Trojanowski ,1980). Although lignin mineralization by actinomycetes is not as efficient as by fungi, it is still more efficient than by unicellular bacteria.

2.7.2 Anaerobic conditions

In nature, most lignocellulose is degraded by aerobic microorganisms, but a substantial amount is also degraded under anaerobic conditions, such as in soil and compost microenvironments (Atkinson *et al*. 1996, Durrant, 1996). Hackett *et al*., (1977) and Odier and Monties (1983) observed no degradation of lignin in anaerobic conditions. However, in the study of Benner and Hodson (1985), a mixed population isolated from compost mineralized 2-4% of lignin and 14-22% of Kraft lignin under anaerobic conditions at 55°C. According to Colberg and Young (1985), a mixed population isolated

from activated sludge was able to cleave the β-O-4 linkage of low molecular mass lignin in anaerobic conditions, producing monoaromatic compounds. Mineralization of lignin was 6% (Colberg and Young, 1985). In rumen, up to 50% of lignin is either solubilized or transformed into a soluble lignincarbohydrate- complex and a variable amount is digested, although the biochemical pathways are unknown (Susmel and Stefanon ,1993).

2.7.3 Mixed populations

The lignin degradation studies of pure microbial cultures have a limited value in understanding the process of mixed populations in soil and in compost because the complex populations involve several interactions that may either stimulate or inhibit the lignin-degrading organisms (Rayner and Boddy ,1988, Carlile and Watkinson , 1994). In forest soil, lignocellulose and lignin are mainly degraded by basidiomycetous litter decomposing fungi, whereas in arable soil and in compost, microfungi are mostly responsible for the lignin degradation since Basidiomycotina are not able to compete with other organisms in these environments (von Klopotek, 1962; Brown 1985). In wood chip or sawdust piles, thermophilic microfungi and bacteria usually dominate, but white rot fungi, such as *Phanerochaete chrysosporium* and *Pleurotus ostreatus*, have been found as well (Rayner and Boddy , 1988; Eaton and Hale ,1993). In mixed populations of soils and composts, actinomycetes are important for lignin degradation, especially at temperatures too high for fungi, but also because they stimulate lignin degradation of some fungi (Waksman and Cordon 1939, Waksman *et al.* 1939a, 1939b, Crawford ,1988, Rüttimann *et al.*, 1991).

2.8 Mixed culture

A group or colony of microorganisms present in a specific, localized location.

2.9 Anaerobic digestion

Anaerobic digestion is the process of decomposting of organic matter by a microbial consortium in an oxygen-free environment (Pain and Hepherd, 1985). It is designed to encourage the growth of anaerobic bacteria particularly the methane producing bacteria. This bacteria decrease the organic solids by reducing them to soluble substances and gases such as the methane and also the carbon dioxide.

The anaerobic digestion process is accomplished by four steps which are (1) hydrolysis of insoluble polymers by enzymes, (2) acidogenic fermentation with acetate is the main end product plus production of volatile fatty acids along with carbon dioxide and hydrogen, (3) acetogenesis which is the breakdown of volatile fatty acids to acetate and hydrogen and lastly, (4) methanognesis which is the conversion of acetate, formaldehyde, hydrogen and also carbon dioxide into methane and water.

CHAPTER 3

METHODOLOGY

This chapter will discuss about the process and procedures for the conversion of banana stem waste into the carbon source for the fermentation medium. In this study, the process is done anaerobically in a specialized anaerobic digester which is equipped with the gassing ports and also the sampling points. The anaerobic digestion process will be run by using anaerobic tank with the capacity of 10 liters for both of the experimental setup namely the Set A and Set B. Finally the glucose and also the lignin determination will be shown as analysis procedures in this experiment.

3.1 Banana Stem Waste

The banana stem waste was collected from the nearest banana plantation located at Batu 10, Jalan Gambang, Pahang Darul Makmur.

3.2 Microorganisms

The microorganisms used in this study are the mixed culture.

3.2.1 Source

The mixed culture was collected from the soil of banana plantation at the depth of 5-10 cm from the earth surface.

3.2.2 Culture Conditions

The mixed culture is acclimatized with banana stem waste anaerobically at ambient temperature.

3.2.2.1 Anaerobic Acclimatization Procedures and Experimental Setup (Set A)

Sections of sediment cores at a depth of 5-10 cm were removed to collect the anaerobic microflora from the soil. Immediately, the soil were sealed with butyl rubber stoppers and transported to the laboratory for processing within 4 hours. Sediments were placed in Schott bottle (specially equipped with gassing ports) so that continuous flow of N_2 is maintained in the bottle.20 mL of distilled water and 20 mg of banana stem is placed in the bottle. Sediments were homogenized for 1 minute.20 mL aliquouts were removed from sampling port and being dispersed into 35 mL serum bottles. Serum bottle were stoppered with butyl rubber stoppers. Soil sample in the serum bottle were flushed with N_2 for 5 minutes. Samples were incubated for 1 month. After 1 month, the sample is placed in an anaerobic tank with 1 Liter capacity containing 10 g of banana stem waste. The anaerobic tank was stoppered with the butyl rubber. Then, it was flushed with N_2 for 5 minutes and is incubated for another 1 month. Later, the samples from the 1 Liter anaerobic digesters are transferred into another anaerobic digester with the capacity of 10 liters with the additional of total solid. Anaerobic digestion will be run for 5 weeks. The

anaerobic digestion is monitored and the sample is taken out from the digester at every week for the analysis of the anaerobic digestion products.

3.2.2.2 Experimental Setup (Set B)

Sections of sediment cores at a depth of 5-10 cm were removed to collect the anaerobic microflora from the soil. Immediately, the soil were sealed with butyl rubber stoppers and transported to the laboratory for processing within 4 hours. Sediments were placed in anaerobic digester so that continuous flow of N_2 can be maintained.6 L of distilled water and 1015 g of banana stem is placed in the anaerobic digester with the capacity of 10 L.The sample is flushed with N_2 for 5 minutes. Anaerobic digestion will be run for 5 weeks and is incubated for another 1 month. The anaerobic digestion is monitored and the sample is taken out from the digester at every week for the analysis of the anaerobic digestion products.

3.3 Analysis method

3.3.1 Preparation of Di-Nitro Salicylic Acid (DNS) Reagent

To determine the glucose in each of the sample, the DNS method (Miller, 1959) was performed by measuring the optical density of the samples at 560 nm. The DNS reagent was firstly prepared by dissolving 10 g of NaOH, 2 g of phenol, 10 g of 3,5-dinitrosalicylic acid and 182 g of sodium-potassium tartarate in 600 mL of distilled water in the amber bottle to prevent the phenol oxidation to phenolic compound. Then, the content is topped up with distilled water to 1 L. The mixture was heated and stirred on a hot plate stirrer for overnight. Later, the reagent was then cooled down to ambient temperature and is kept at 4°C.

3.3.2 Determination of Glucose by DNS Method

A 1 mL of sample is taken out and put in a lightly capped test tube. Then 2 mL of DNS reagent is added to each of test tube. The solution was vigorously shaken and later was heated at 90 ^{o}C for 10 min to develop red-brown colour. After that, the sample is cooled at room temperature in a cold water bath for another 10 min. The solution was later diluted with additional 10 mL distilled water and analyzed for glucose concentration. The absorbance was recorded using UV-visible spectrophotometer at 560 nm.

3.3.3 Klason's Method

In determining the lignin content, Klason (72% H2SO4) Method is used. The banana stem waste is dried in oven at 105 °C for 1 hour. After 1 hour, 1 gram of sample is taken out and placed in 100 mL beaker. 20 mL of 72 % sulphuric acid is added to the sample and the sample is left at room temperature (25 °C) for two hours. 560 mL of distilled water was added to the sample which had been heated at 100 °C in shaking water bath for 2 hours. Sample is filtered and been washed with distilled water to make sure that the sample is free from acid. Sample was then dried in the oven at 105 °C for 1 hour. The sample was placed in the desicator to ensure that the sample is free from moisture. The sample is left in the desiccator for 2 days. The sample is weighed and measured and later being labeled with w1.

3.4 Preparation of Glucose Standard Curve

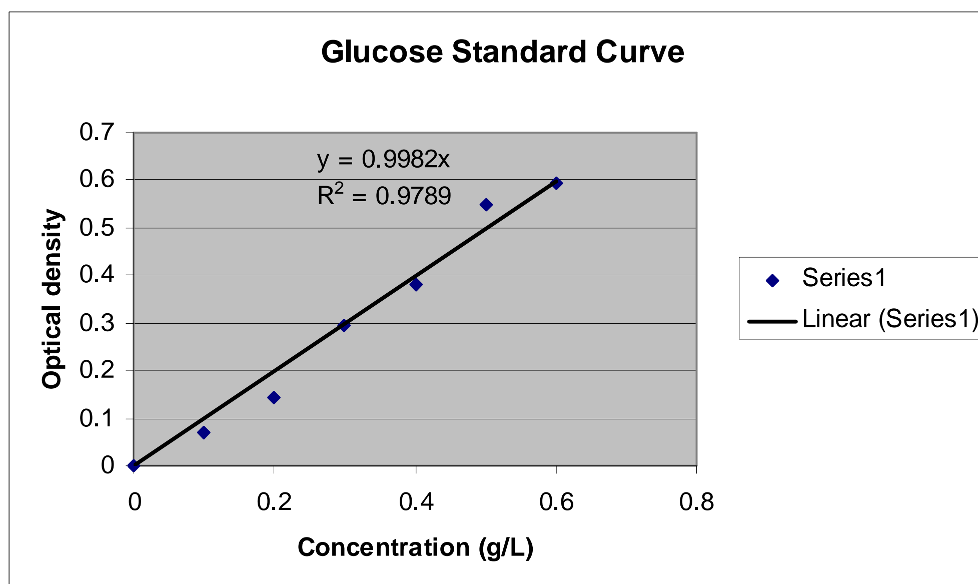

Figure 3.1: Glucose Standard Curve

The above graph was constructed based on the data obtained after the standard glucose was tested by using DNS method. This graph was important in order to find the concentration of glucose in every sample being tested. The data for the standard curve can be found at **Appendix B.1**. This preparation is needed in order to find the concentration of tested sample using the equation obtained from the graph. A 1g/L glucose stocksolution is prepared by adding 0.1g of D (+)-glucose anhydrous into 100 mL of distilled water. The solution is stirred until all glucose was dissolved in the water. Next, 6 different concentrations of glucose solution is prepared from 1g/L of glucose stock solution. The first concentration is 0.1 g/L is prepared by adding 0.1mL of glucose solution into 0.9 mL of distilled water. The procedure repeated for the next 5 concentrations. After all of the glucose solution is ready, 1 mL of each glucose solution from different concentration is put into in a lightly capped test tube with the addition of 2 mL DNS reagent at each and every test tubes. Next, all the solution is being test with DNS method at the wavelength of 560 nm.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

In this study of the bioconversion of agricultural solid waste which is the banana stem waste, the delignification and also the cellulose degradation was studied in two different system of anaerobic digesters which involve the degradation of banana stem waste with acclimatization for 2 months in the anaerobic digester labeled as Set A and another system of anaerobic digester containing banana stem waste without acclimatization and was being labeled as Set B by the soil mixed culture taken from banana plantation . The anaerobic digestion is done at ambient temperature.

In this experiment, the experimental setup for Set A and Set B had the different organic loading rate. Therefore, in order to determine the exact concentration for glucose in each set, a basis which is 1 g/mL had been selected as the equivalence for each set. Then, both set were compared according to the ratio.

Organic loading rate for Set A: 0.5722 g substrate/mL distilled water
Organic loading rate for Set B: 0.1692 g substrate/mL distilled water

Set A : Set B

0.5722/0.1692: 0.1692/0.1692 ≡ 3.3817: 1

4.1.1 Determination of Glucose Concentration

Using the equation of y = 0.9982 x, the concentration of glucose in Set A and B can be determined; in which y denotes the optical density and x denotes the concentration. However, for every calculation in Set A, all the x values need to be multiplied with the ratio number of 3.3817 so that the comparison of the concentrations for both Set A and Set B were done on the same basis.

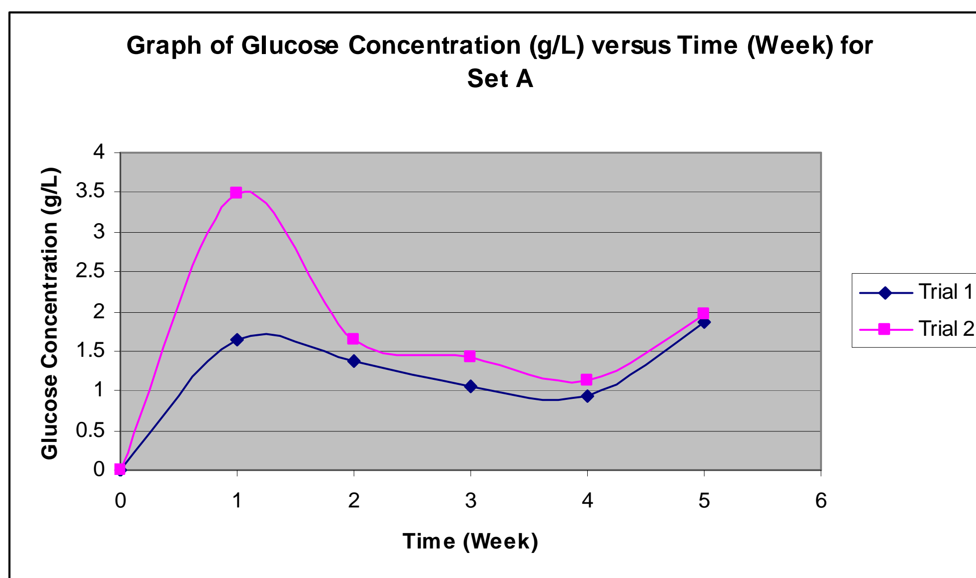

Figure 4.1: The Variation of Glucose Concentration over Time for Set A

Based from the observation from Figure 4.1, the glucose is produced due to the cellulose degradation that had occurred in the anaerobic digester of Set A. At first, the concentration of glucose rise exponentially. However, after 1 week, the concentration is

slowly decreasing. This is because the other species of the mixed culture had consumed the glucose which had firstly being degraded by the species present in the mixed culture.

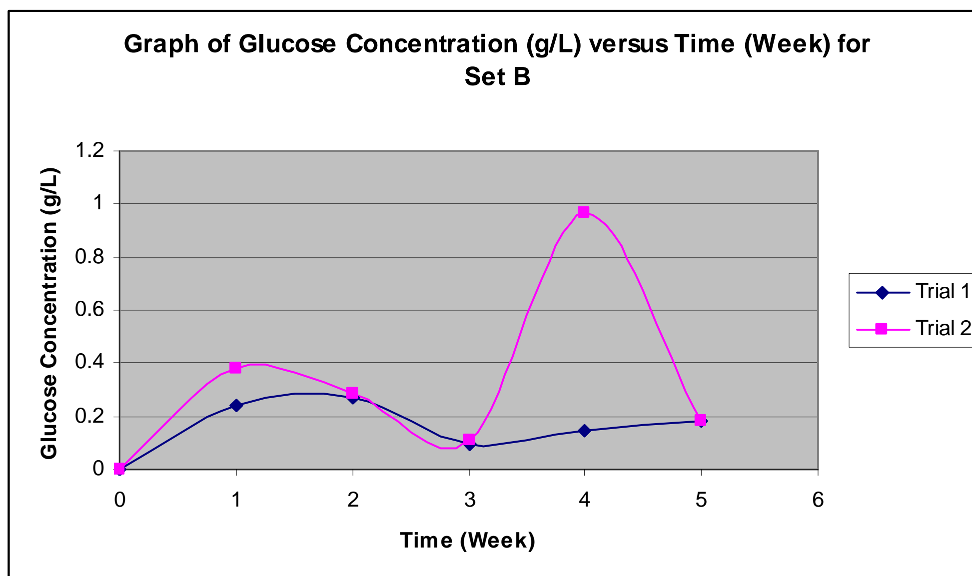

Figure 4.2: The Variation of Glucose Concentration over Time for Set B

From Figure 4.2, the glucose is also being produced in Set B showing that the cellulose degradation had also occurred. The pattern is also the same as it was in Set A in which the glucose concentration was firstly increase exponentially and was started to decrease after 1 week. This is also due to the variations in the activities of the mixed culture in which a different species of mixed culture was degrading the glucose while another species present in the mixed culture consume the glucose for their metabolic activities. However in Set B, there was slightly an error occurred at week 4 for Trial 2 where the glucose concentration is suddenly rise. The error must had occurred during the analysis of glucose by the Dinitrosalicylic Acid Method (DNS Method) where the test tube used in the analysis may had been not clean enough and there was a leftover of glucose. Also, the error may had been contributed by the usage of recycled cuvette used itself which may have been contaminated with other chemicals and had not been cleaned

thoroughly. This may interfere the transmittance of the UV light causing the error in the reading of the optical density.

Supposedly, at the early stage of the digestion process which is the first 2 weeks, glucose analysis should be done more frequent which is at least at every 2 days interval so that the pattern of the reaction that occurred can be observed better. This is because; the digestion process is almost to complete after 2 weeks causing the pattern is seen to be flat and thus the changes at which the conversion is optimum and the point when it began to deplete can not be clearly and accurately determined.

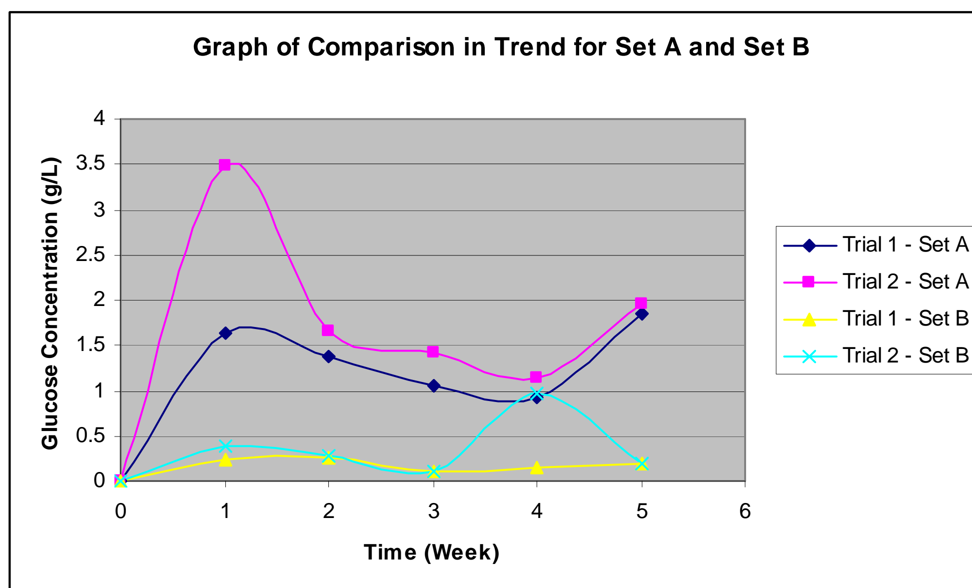

Figure 4.3: Comparison in Trend for Glucose Concentration in Set A and Set B

From Figure 4.3, the trends for glucose being produced and later consumed can be observed and compared. From the graph, the concentration for glucose in Set A is higher compared to Set B in both of the experimental trials, showing that the higher conversion of glucose occurred in Set A. This can be explained by the higher organic loading rate in Set A which is 0.5722 g TS/mL of distilled water but only 0.1692 g TS/mL of distilled water in Set B. Also, the acclimatization of the banana stem waste with the soil had been done for 2 months only in Set A and is neglected in Set B.

4.3 Determination of Degraded Lignin

From both the Figure of 4.4 and 4.5, the percentage of degraded lignin over time in Set A and Set B respectively can be observed. The analyses were done at week 3, week 4 and week 5. Using Klason Method, the amount of lignin being removed in the digestion process can be determined. In Klason Method, the 72% sulphuric acid which had been used will dissolve the polysaccharide and also the protein present in the sample leaving the lignin as the residue. The amount of lignin removed is determine by comparing the controlled experiment which is the banana stem waste which was not being digested in the anaerobic digester with the amount of lignin leftover after the analysis using Klason Method.

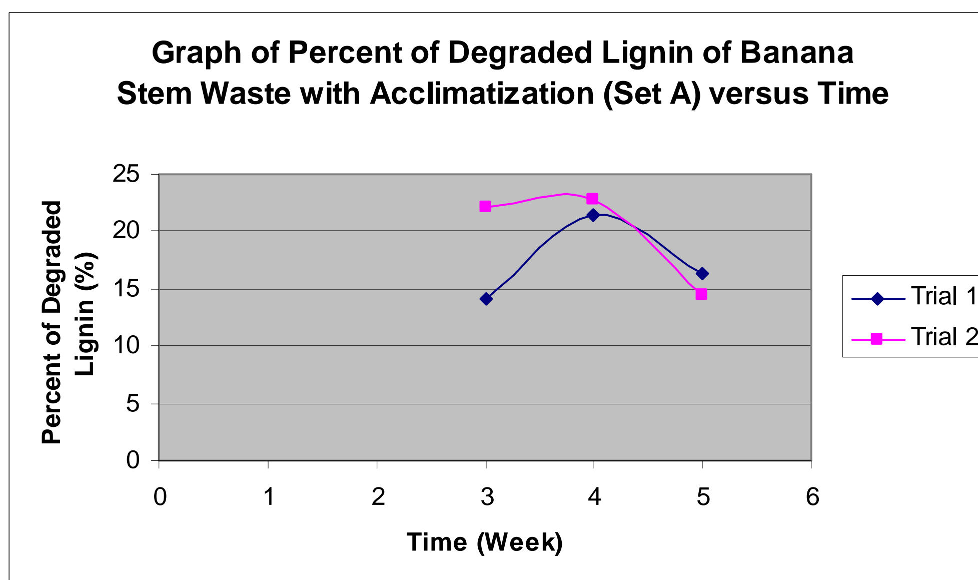

Figure 4.4: Percentage of Degraded Lignin over Time in Set A

Based from Figure 4.4, it can be seen that the degraded lignin was increasing with the increasing of time except for the first trial in week 5. There is actually and error which had occurred in which the surrounding area of the filter paper is slightly burned.

This is due to the sulphuric acid which may still present and was not being filtered and washed properly.

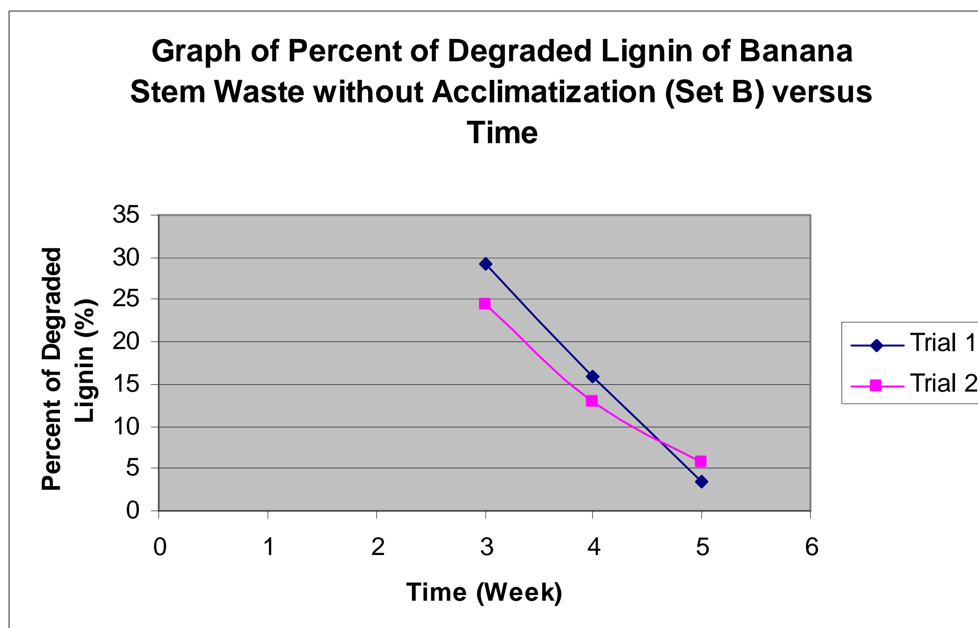

Figure 4.5: Percentage of Degraded Lignin over Time in Set B

In Figure 4.5, the amount of degraded lignin was decreasing with the increasing of time. However, there was also an error which had occurred here in which the surrounding area of the filter paper for both trial 1 and trial 2 in this Set B were also burned. As a result, the measurement of the leftover amount was inaccurate causing the value to be lower than the real value. Therefore, when the comparison is done with the controlled data, the calculated values showed that the percentage of degraded lignin was higher than the values it should be. The data should show that percent of lignin degraded should increase with time or stay constant.

Figure 4.6: Dried Sample at 105°C in Week 3

For the first run on week 3, the error may had occurred due to excessive sulphuric acid which may still present and was not being filtered thoroughly causing to the burning of the filter paper. However, there is another reason which had contributed to this phenomenon. At this first run of analysis, the filter paper which had been used were the non- ash less and it is not resistant to the high temperature as high as 105°C which was required for the drying process. The filter paper turned to be yellowish after some time it was being put in the oven. Therefore, another type of filter paper which was the ash less had been used as the replacement for the next runs on week 4 and week 5.

Figure 4.7: Dried sample at 105°C in Week 4

Figure 4.8: Dried Sample at 105°C in Week 5

4.3 Observation of Mixed Culture

Just after the digestion process was complete after 5 weeks, some aliquots had been taken out from the anaerobic digester. Then, streaking method had been done to observe the colony that may present in the mixed culture. From the morphological of the colony being formed, it was found that the mixed culture comprised of bacteria.

Figure 4.9: Colony of Mixed Culture Taken from Anaerobic Digester

4.4 Utilization of Glucose as Fermentation Medium

In order to determine whether the degraded cellulose which had formed the glucose can be used as the fermentation medium, the *Saccharomyces cerevisiae* (yeast) had been activated using the forming glucose from the digestion process as the sole carbon source. However, some other nutrients which were needed for the growth of *Saccharomyces cerevisiae* had also been prepared which were the yeast extract and also the peptone. However, the yeast is activated in 3 different medium which (1) containing only yeast extract and peptone, (2) containing only glucose and (3) containing glucose, yeast extract and also peptone.

Figure 4.10: *Saccharomyces cerevisiae* in Different Liquid Mediums at Early Stage of Activation

Figure 4.11: *Saccharomyces cerevisiae* in Different Liquid Mediums After 18 Hours

After 18 hours of activation, the physical changes of the liquid medium were observed. The colour of the medium had slightly changed. However, there was no proof to show that the yeast was able to live in the prepared medium, therefore streaking plate is done. From the observation, it was found that the yeast can live in the liquid medium containing the glucose being produced via the anaerobic digestion as it live on the medium containing only yeast extract and peptone. However, the growth was optimum and the colony was more distributed in the medium containing the combination of yeast extract, peptone and glucose. So, these observations shows that the glucose formed can be used as the fermentation medium.

Figure 4.12: Colony of *Saccharomyces cerevisiae* Inoculated from Medium Containing Yeast Extract and Peptone Only

Figure 4.13: Colony of *Saccharomyces cerevisiae* Inoculated from Medium Containing Glucose as Produced in the Degradation of Delignified Cellulose

Figure 4.14: Colony of *Saccharomyces cerevisiae* Inoculated from Medium Containing Glucose, Yeast Extract and Peptone

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

The soil mixed-culture is capable of delignifying the banana stem waste anaerobically. After delignification, cellulose can be recovered and being degraded to form the glucose. The glucose can be used for as the fermentation medium.

5.2 Recommendations

In order to improve this research, there are several things should be stress out in the future. Firstly, the designs for the anaerobic digester need to be improved and modified. A proper anaerobic digester should at least contain three outlet openings which will enable a better and accurate sampling process and also the sparging of nitrogen gas. Also, the tubes connecting to the system should be tight enough so that the gases produced during the digestion process can be collected by the water displacement and the possibility for the gas to leak out is minimized. Then, a growth profile for the activities and also the metabolisms of the mixed culture being used should firstly being determined. By determining the growth profile, the microbial activities can be observed and this may

gives the right time when the mixed culture should be fed and when the right time for the harvesting should take place in order to get the optimum yield of products. In addition, the mixed culture can also being grown and amplified at the lab via streaking method on the agar plate after the acclimatization had firstly taken place. Then, the colony can be transferred into anaerobic digester to increase the number of the mixed culture. Lastly, the technique of Polymerase Chain Reaction (PCR) can be used to recognize and classify the species that forming the mixed culture.

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APPENDIX A

Appendix A.1: Chopped Banana Stem Waste

Appendix A.2: Banana Plantation Soil Containing Mixed Culture

Appendix A.3: Anaerobic Digester with Acclimatization (Set A)

Appendix A.4: Anaerobic Digester without Acclimatization (Set B)

Appendix A.5: UV-Visible Single Beam Spectrophotometer (Model U-1800)

Appendix A.6: Shaking Water Bath (Model BS-21)

APPENDIX B

Appendix B.1: Data for Standard Curve of Glucose

Concentration (g/L)	Optical Density at 560 nm
0	0.000
0.1	0.071
0.2	0.145
0.3	0.295
0.4	0.381
0.5	0.55
0.6	0.594

Appendix B.2: Optical Density of Banana Stem Waste with Acclimatization (Set A)

Time	Trial 1	Trial 2
Week 1	0.484	1.026
Week 2	0.407	0.486
Week 3	0.313	0.417
Week 4	0.274	0.335
Week 5	0.549	0.576

Appendix B.3: Optical Density of Banana Stem Waste without Acclimatization (Set B)

Time	Trial 1	Trial 2
Week 1	0.242	0.378
Week 2	0.268	0.288
Week 3	0.097	0.110
Week 4	0.149	0.965
Week 5	0.185	0.184

Appendix B.4: Calculation for Glucose Concentration in Set A

Time (Week)	For Set A (Trial 1)	For Set A (Trial 2)
1	When y = 0.484 x =0.484/ 0.9982 X 3.3817 = 1.6397	When y = 1.026 x =0.484/ 0.9982 X 3.3817 = 3.4760
2	When y = 0.407 x =0.407/ 0.9982 X 3.3817 = 1.3787	When y = 0.486 x =0.484/ 0.9982 X 3.3817 = 1.6459
3	When y = 0.313 x =0.313/ 0.9982 X 3.3817 = 0.4849	When y = 0.417 x =0.417/ 0.9982 X 3.3817 = 1.4127
4	When y = 0.274 x =0.484/ 0.9982 X 3.3817 = 0.4849	When y = 0.335 x =0.335/ 0.9982 X 3.3817 = 1.1349
5	When y = 0.549 x =0.484/ 0.9982 X 3.3817 = 0.4849	When y = 0.576 x =0.576/ 0.9982 X 3.3817 = 0.5772

Appendix B.5: Calculation for Glucose Concentration in Set B

Time (Week)	For Set B (Trial 1)	For Set B (Trial 2)
1	When y = 0.242 x = 0.242/ 0.9982 = 0.2424	When y = 0.378 x = 0.378/ 0.9982 = 0.3787
2	When y = 0.268 x = 0.268/ 0.9982 = 0.2685	When y = 0.288 x = 0.288/ 0.9982 = 0.2885
3	When y = 0.097 x = 0.097/ 0.9982 = 0.0972	When y = 0.110 x = 0.110/ 0.9982 = 0.1102
4	When y = 0.149 x = 0.149/ 0.9982 = 0.1493	When y = 0.965 x = 0.965/ 0.9982 = 0.9667
5	When y = 0.185 x = 0.185/ 0.9982 = 0.1853	When y = 0.184 x = 0.184/ 0.9982 = 0.1843

Appendix B.6: Summary of Calculated Data for Glucose Concentration

Time	Set A (With Acclimatization)		Set B (Without Acclimatization)	
	Trial 1	Trial 2	Trial 1	Trial 2
Week 1	1.6397	3.4760	0.2424	0.3787
Week 2	1.3787	1.6459	0.2685	0.2885
Week 3	1.0604	1.4127	0.0972	0.1102
Week 4	0.9283	1.1349	0.1493	0.9667
Week 5	1.8600	1.9514	0.1853	0.1843

Appendix B.7: The Formula for Lignin Analysis Determination

Lignin Leftover	w1/Initial Mass
% of Lignin Removed	[Controlled-Lignin Leftover] X 100%

Appendix B.8: Lignin Analysis: Klason Method (Week 3)

Sample	Initial Mass (g)	w1 (g)	Lignin Leftover (g)	Lignin Removed (%)
Controlled	-	-	0.33260	-
Acclimatized 1	1.0203	0.1957	0.19181	14.079
Acclimatized 2	1.0332	0.1147	0.11112	22.148
Non-Acclimatized 1	1.0745	0.0441	0.04104	29.156
Non-Acclimatized 2	1.0413	0.0913	0.08768	24.492

Appendix B.9: Lignin Analysis: Klason Method (Week 4)

Sample	Initial Mass (g)	Final Mass (g)	Lignin leftover (g)	Lignin Removed (%)
Controlled	-	-	0.3326	-
Acclimatized 1	1.0286	0.1215	0.11812	21.448
Acclimatized 2	1.0346	0.1090	0.10535	22.725
Non-Acclimatized 1	1.0199	0.1776	0.17413	15.847
Non-Acclimatized 2	1.0388	0.2123	0.20437	12.823

Appendix B.10: Lignin Analysis: Klason Method (Week 5)

Sample	Initial Mass (g)	Final Mass (g)	Lignin leftover (g)	Lignin Removed (%)
Controlled	-	-	0.3326	-
Acclimatized 1	1.0120	0.1720	0.1699	16.27
Acclimatized 2	1.0101	0.1900	0.1881	14.45
Non-Acclimatized 1	1.0636	0.3182	0.2992	3.34
Non-Acclimatized 2	1.0105	0.2789	0.2760	5.66

Appendix B.11: Percent of Degraded Lignin of Banana Stem Waste with Acclimatization (Set A)

Time	% of degraded lignin	
	Trial 1	Trial 2
Week 3	14.079	22.148
Week 4	21.448	22.725
Week 5	16.270	14.450

Appendix B.12: Percent of Degraded Lignin of Banana Stem Waste without Acclimatization (Set B)

Time	% of degraded lignin	
	Trial 1	Trial 2
Week 3	29.156	24.492
Week 4	15.847	12.823
Week 5	3.340	5.660