Faculty of Resource Science and Technology

Banana Stem as an Alternative Raw Materials for Bioethanol Production

Anis Sofia Binti Azmi (72118)

Bachelor of Science with Honours
(Resource Biotechonology)
2022

Banana Stem as an Alternative Raw Material for Bioethanol Production

Anis Sofia Binti Azmi

A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of Science with Honours

(Resource Biotechnology)

SUPERVISOR: DR. DAYANG SALWANI AWANG ADENI

Programme of Resource Biotechnology

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2022

UNIVERSITI MALAYSIA SARAWAK

Grade: ____________

Please tick (√)

Final Year Project Report	√
Masters	
PhD	

DECLARATION OF ORIGINAL WORK

This declaration is made on the 06 day of July 2022.

Student's Declaration:

I, **Anis Sofia Binti Azmi, 72118,** from **Faculty Resource and Science Technology** hereby declare that the work entitled, **Banana Stem as an Alternative Raw Material for Bioethanol Production** is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

15 July 2022
Date submitted

Anis Sofia Binti Azmi (72118)

Supervisor's Declaration:

I**, Associate Professor Dr. Dayang Salwani bt Awang Adeni,** hereby certify that the work entitled **Banana Stem As An Alternative Raw Material For Bioethanol Production** was prepared by the above named student, and was submitted to the "FACULTY" as a * partial/full fulfillment for the conferment of **Bachelor of Science with Honours (Resource Biotechonology),** and the aforementioned work, to the best of my knowledge, is the said student's work

Received for examination by:____________ Date: 06 July 2022
(Dayang Salwani Bt Awang Adeni)

I declare this Project/Thesis is classified as (Please tick (√)):

☐ **CONFIDENTIAL** (Contains confidential information under the Official Secret Act 1972)*
☐ **RESTRICTED** (Contains restricted information as specified by the organisation where research was done)*
☑ **OPEN ACCESS**

Validation of Project/Thesis

I therefore duly affirmed with free consent and willingness declared that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abide interest and rights as follows:

- This Project/Thesis is the sole legal property of Universiti Malaysia Sarawak (UNIMAS).
- The Centre for Academic Information Services has the lawful right to make copies for the purpose of academic and research only and not for other purpose.
- The Centre for Academic Information Services has the lawful right to digitise the content to for the Local Content Database.
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis for academic exchange between Higher Learning Institute.
- No dispute or any claim shall arise from the student himself / herself neither third party on this Project/Thesis once it becomes sole property of UNIMAS.
- This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student except with UNIMAS permission.

Student's signature________________ (06 July 2022)

Supervisor's signature: ________________ (06 July 2022)

Current Address:
1133A, Kampung Bukit Besar, 21100, Kuala Terengganu, Terengganu

Notes: * If the Project/Thesis is **CONFIDENTIAL** or **RESTRICTED**, please attach together as annexure a letter from the organisation with the period and reasons of confidentiality and restriction.

[The instrument was duly prepared by The Centre for Academic Information Services]

ACKNOWLEDGEMENT

Alhamdulillah praised to Allah S.W.T for His blessings and guidance, whereby I would not be able to complete my Final Year Project research. First and foremost, I would like to offer my utmost gratitude appreciation to my supervisor, Dr. Dayang Salwani Awang Adeni for giving me a chance to conduct this research as well as improving my scientific skills. I would also like to thank her for the advice, constant support, encouragement and guidance throughout my difficulties in accomplishment of this research project.

I sincerely appreciate and thank all the postgraduate students in the for their time in sharing their knowledge and offering their assistance to me during this research especially Mr. Mohammad Helmi. I would like to express my special thanks to my fellow colleagues, Anis Jasmin Azimin, Norfahirah Lamdani, Muhammad Hilmi Mohd Idris, Isaac Una Albert, Muhammad Alfishah Muhammad Riza and Abdul Azim Bin Zainol Abidin for their time, support and sincere assistant while I was conducting the research.

I would also express my deepest appreciation to my beloved parents, Mr. Azmi Mat Ali and Mdm. Azlina Shamsuddin for helping me to complete my research as well as the encouragement and love throughout the progression of this research. Their sincere prays and advice helped me to keep moving and faced all the difficulties during completion of this project. Their hopes give me a greater strength and motivation to give out my best to accomplished the project.

Banana Stem as an Alternative Raw Material for Bioethanol Production

Anis Sofia Binti Azmi

Resource Biotechnology Programme
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

Bioethanol production from agricultural waste is known to be a promising alternative in replacing the use of fossil fuels in order to prevent sudden depletion of non-renewable source and reduce environmental pollution. Banana stem waste is an abundant source which can be easily found in Malaysia since banana plantation conduct at large scale. Banana stem has been proven to contain glucose, sucrose, starch and lignocellulosic compound which can potentially be utilize as carbon source for bioethanol production at industry scale. Therefore, this is conducted to determine the potential of raw starch and free sugar of banana stem waste in producing an efficient amount of bioethanol. Research had been performed and it is found that starch and sugar available in banana stem do have the potential in producing ethanol. Based on the results obtained, banana stem dry matter was identified to consist of higher amount of starch compared to banana stem sap with 0.615 g/L and 0.016 g/L respectively. Thus, banana stem dry matter was further analyses through conduction of enzymatic hydrolysis to breakdown starch into glucose. Enzymatic hydrolysis was done in three different concentrations which are 5%, 10% and 15% of samples to identify which concentration have the most efficient rate of conversion to be used in fermentation step. Samples of 5% concentration showed the highest rate conversion among all three which is 61.87%. The 10% concentration showed low conversion rate that is 39.17% while enzymatic hydrolysis process of concentration 15% are not able to be completed. The high conversion rate of 5% concentration indicates a potential in efficient bioethanol production after completed fermentation process. By the end of the research, the result obtained on ethanol production is 8.5 g/L which also proved the high rate conversion of 88%. The results achieved indicates that banana stem does have the potential in bioethanol production.

Key words: Banana stem, bioethanol, enzymatic hydrolysis, fermentation.

ABSTRAK

Penghasilan bioetanol daripada sisa pertanian diketahui sebagai alternatif yang memberangsangkan dalam menggantikan penggunaan bahan api fosil untuk mengelakkan kekurangan sumber yang tidak boleh diperbaharui secara tiba-tiba dan mengurangkan pencemaran alam sekitar. Sisa batang pisang adalah sumber yang banyak yang boleh didapati dengan mudah di Malaysia kerana tanaman pisang dilakukan secara besar-besaran. Batang pisang telah terbukti mengandungi glukosa, sukrosa, kanji dan sebatian lignoselulosa yang berpotensi digunakan sebagai sumber karbon untuk penghasilan bioetanol pada skala industry. Oleh itu, ini dijalankan untuk menentukan potensi kanji mentah dan gula bebas dalam sisa batang pisang dalam menghasilkan jumlah bioetanol yang cekap. Penyelidikan telah dilakukan dan didapati bahawa kanji dan gula yang terdapat dalam batang pisang mempunyai potensi dalam menghasilkan etanol. Berdasarkan keputusan yang diperolehi, bahan kering batang pisang dikenal pasti mempunyai daripada jumlah kanji yang lebih tinggi berbanding sap batang pisang dengan masing-masing 0.615 g/L dan 0.016 g/L. Oleh itu, bahan kering batang pisang telah dianalisis lebih lanjut melalui pengaliran hidrolisis enzim untuk memecahkan kanji ke dalam glukosa. Hidrolisis enzim dilakukan dalam tiga kepekatan yang berbeza iaitu 5%, 10% dan 15% sampel untuk mengenal pasti kepekatan mana yang mempunyai kadar penukaran yang paling cekap untuk digunakan dalam langkah penapaian. Sampel kepekatan 5% menunjukkan penukaran kadar tertinggi di antara ketiga-tiganya iaitu 61.87%. Kepekatan 10% menunjukkan kadar penukaran yang rendah iaitu 39.17% manakala proses hidrolisis enzim kepekatan 15% tidak dapat diselesaikan. Kadar penukaran tinggi kepekatan 5% menunjukkan potensi dalam penghasilan bioetanol yang cekap selepas proses penapaian selesai. Diakhir penyelidikan, keputusan yang diperolehi pada penghasilan etanol adalah 8.5 g / L yang juga membuktikan penukaran kadar tinggi sebanyak 88%. Keputusan yang dicapai menunjukkan bahawa batang pisang mempunyai potensi dalam penghasilan bioetanol.

***Kata kunci**: Batang pisang, bioethanol, hidrolisis enzim, penapaian.*

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
α	Alpha
BS	Banana Stem
Bioenergy	Biological Energy
Bioethanol	Biological Ethanol
Biofuel	Biological Fuel
Biomass	Biological Mass
CO_2	Carbon dioxide
C_2H_5OH	Ethyl alcohol
D	Dried weigh
DNS	Dinitrosalicylic acid
g	Gram
g/g	Gram per gram
g/L	Gram per liter
H_2SO_4	Sulphuric acid
HPLC	High Performance Liquid Chromatography
KI	Potassium Iodide
L	Liter
m	Meter
mL	Milliliter
ml/min	Milliliter per minute
M	Molarity
NADH.	Nicotinamide adenine dinucleotide hydrogen

nm	Nanometer
starch/L	Starch over liter
UV	Ultraviolet
w/v	Weigh per volume
W	Wet weigh

CHAPTER 1

INTRODUCTION

The growing demand for energy in the twenty-first century is expected to be met by the increase of energy-intensive production, as the consumer populations continue to grow. According to Faizal *et al.* (2019), Malaysia had undergone rapid development which led to surging energy demand. The energy sector in Malaysia is heavily dependent on conventional fossil fuels, including oil and natural gas, which mainly contributes towards the negative impacts on the environment due to high emission of carbon dioxide (CO_2). Renewable energy is critically required to replace conventional fossil fuel in effort of preventing fuel depletion, which is predicted to be completely exhaust in 2052 (Faizal *et al.*, 2019). Apparently, Malaysia is known to have abundant natural resources and is therefore well-positioned to further explore the use of bioenergy as an alternative energy.

Malaysia has a tropical climate and high humidity that provide a favourable environment for agriculture and forestry (Ho, 2018). Kayat *et al.* (2016) stated that Malaysia is the world's second-largest producer of bananas and fourth-largest exporter and harvests approximately 29,000 hectares of land and produces 294,000 metric tons of bananas each year. Banana stem wastes produce 200 kilograms of discarded waste for every 60 kilograms of bananas harvested. These banana stems will normally be disposed as waste despite the fact that it may consist useful properties which had not been discovered yet. The amassing of banana stem waste will eventually lead towards environmental pollution and accumulation of greenhouse gas released, mainly due to incineration process from the landfills (Aili Hamzah *et al.*, 2021). Thus, the increasing number of waste production will worsen the environmental condition.

According to Chin and H’ng (2013), the Malaysian government launched a new strategy in 2011, National Biomass Strategy 2020, that proposes a 10 percent mandate for bioethanol blending in gasoline by 2020 to cut down greenhouse gas emissions. Currently, bioethanol production in Malaysia is derived from raw materials such as sugar cane, beet or starch from cereals (Chin and H’ng, 2013). However, sugarcane is known to be a crucial source of sugar production in this country with approximation of 1.57 million metric tons in 2021 (Hirschmann, 2022). Production of bioethanol from sugarcane, beet and cereal may induce decline in sugar production’s profit in the future prospect. In order to solve the problems faced, an alternative option is to utilize banana stem wastes into renewable source for bioethanol production. Thus, research and study on composition and properties of banana stem is prominent to ensure that it achieves the standard requirement as renewable source of energy. As part of biomass resources, banana stem appears to be a promising alternative which can be utilize as feedstock for bioethanol production. This is because the residue of banana stem consists of sap that are rich in sugary substances, which is present in liquid form.

In this project, sap from banana stem was analysed to observe the valuable properties which can be obtained from crop residue. It is stated that the common composition found in sugary liquid extracted from the banana stem sap includes glucose, sucrose, xylose, mannose, arabinose and rhamnose (Arpit & Sudhir, 2017; Celignis Analytical, 2021; Ma, 2015). Each type of sugar content has its own specific functional used in various industry. The sugar content found in the sap will issue a great opportunity for crop residues to be fully utilized as renewable source mainly in biofuel production. However, the amount and concentration of free sugar, starch as well as sugar classification are yet to be known. Therefore, this study concerns the amount of free sugar and starch content, sugar classification as well as the potential of ethanol production from sugar found in banana stem sap.

1.1 Objectives

The purpose of this study is:

1. To determine the approximate free sugar and starch content of banana stem.

2. To analyse total sugar concentration of banana stem used in this study upon completing enzymatic hydrolysis process.

3. To determine the potential of bioethanol production from banana stem.

CHAPTER 2

LITERATURE REVIEW

2.1 Banana Plantation in Malaysia

In Malaysia, banana is the second most widely cultivated fruit with a total production of 530,000 metric tonnes and total value estimation of RM294.5 million per year (Rushdan *et al.*, 2017). Malaysia has a tropical humid climate which is highly suitable for banana plantation. This is because banana crop prefers to grow in a temperature ranging from 15˚ to 35˚C with approximately 75% to 85% of relative humidity (Important banana varieties cultivated in different states of India are given below: BANANA, n.d.). There are several common species of banana planted in Malaysia known as *Musa sapientum*, *Musa cavendishii*, *Musa acuminate*, *Musa balbisiana* and *Musa nana* (Knoema, 2019; Rushdan *et al.*, 2017; Sharifah, 2014).

2.1.1 The Utilization of Banana Stem

Banana stem include the middle and inner layers that tightly wrapped the soft central core called pseudo-stem as shown in **Figure 1**. The stem provides support to other parts of the tree such as leaves, flower and banana fruits. There are approximately 2.12 million tonnes of banana trunk discard openly every year in Malaysia (Rushdan *et al.*, 2017). Despite the fact that banana stem considered as crop waste, it is actually rich in various type of nutrients include protein, fat, free sugar, starch and fibre. Rushdan *et al.* (2017) found that banana stem contains 33% of cellulose, 32% of hemicellulose and 18% of lignin. On the other hand, according to the article written by Ma (2015) stated that banana stem has the amount of 3.4% free sugar, 27.3% starch and 56.9% of total carbohydrate.

Figure 1 The cross section of banana stem.

Nowadays, most industrial sectors seek for natural fibre to replace the used of synthetic fiber due to its non-degradable properties which lead to serious environmental pollution problems (Preethi *et al*., 2013). The fibre in banana stem possess an excellent quality to produce garments and furniture, thus it is preferred more compared to synthetic fibre (Ma, 2015). Banana stem also commonly utilized as feed material for animals, paper industry and clothing (Ma, 2015). Based on study made by Akpabio *et al.* (2012), they discovered that banana stem waste contains abundant amount of carbohydrate approximately 50% (Dayod & Abat, 2018). Two methods can be performed on the carbohydrate content which is saccharification to convert carbohydrate into sugar (glucose) and fermentation process to produce alcohol such as ethanol (biofuel) through mechanism action of yeast, *Saccharomyces cerevisiea* (Akpabio *et al.*, 2012).

2.2 Polymeric Compound of Crop Waste and its Utilization

2.2.1 Crop Sap

Sap is a liquid substance that is commonly found in plant. There are two type of sap presence in a plant known as xylem sap and phloem sap. Xylem sap contains water, minerals and low level nutrient that flows from roots to the upper parts of plant such as leaves and stem. On the other hand, phloem sap is rich with nutrients such as sugar and hormones which distributed from leaves to stem and roots. During photosynthesis, carbohydrate is produced and stored in tree as starch. The starch will then be converted into sucrose which is dissolved in the sap. Sap is not only essential to provide nutrients and energy for the plant, but also edible as food source to humankind.

Sap can be extracted and process into value added product since it contains abundant amount of free sugar and starch. According to an article written by Sjogren (2013), the average sugar concentration of sap is approximately between 20 to 25 percent. Some crop waste has sap that is rich in free sugar content such as banana trunk. Sap extracted from banana trunk consist of free sugar such as glucose and sucrose (Ma, 2015). The total free sugar in sap of banana trunk has a great potential to be utilized as value added products such as food product like sugar and ethanol for bioethanol production.

2.2.2 Starch

Starch is a granular, organic compound that is white in colour, produced in plant through photosynthesis. Starch is a complex carbohydrate that binds glucose molecules in α 1,4 linkage, which comprise of linear polymer amylose as its simplest form and amylopectin in branched form as shown in **Figure 2** (Britannica, 2021, Willfahrt *et al.*, 2019). There are many types of starchy plants, which are mainly from vegetables group that include sweet potatoes, yam, corn, wheat, rice and legumes. Starch is kept in granules form in the chloroplasts and storage organs of plant located in roots or stem, such as tuber of potatoes, pith of sago, roots of cassava plant and seeds of rice, corn and wheat (Britannica, 2021).

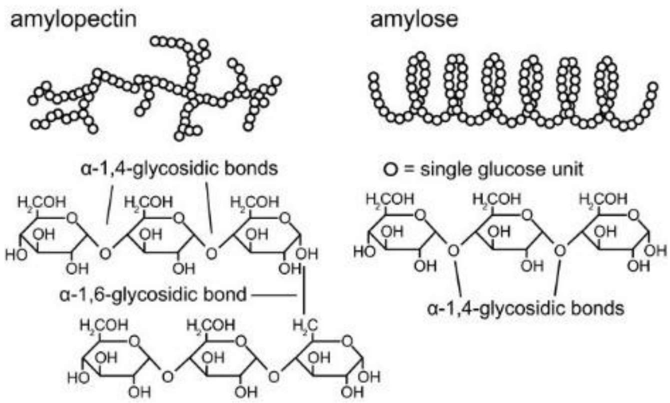

Figure 2 The chemical structure of starch (Willfahrt *et al.*, 2019).

According to Padi and Chimphango (2021), corn is composed of approximately 60% to 70% of starch and known to be the most reliable source of starch feedstock compared to other conventional source which are potatoes, cassava and wheat. Starch that is obtained from plants can be used in paper manufacturing and textile industry or processed into food product as well as feedstock for biofuels production. Starch compound also exist in crop waste that consist of undesirable parts of plant which is non marketable such as leaves, peels, bagasse, stubble and stem. For example, 27.3% of starch content in can be extracted from banana stem (Ma, 2015). Starch obtain from crop must undergo starch hydrolysis process prior to conversion to glucose, maltose, fructose and maltodectrins, where the final product obtain is highly depends on the mode of action of the enzyme such as amylase or amylolytic enzymes (Bednarska, 2015; De Souza *et al.*, 2019).

2.3 Classification of Sugar

Sugar, which can also be known as saccharides, is a simple form of carbohydrate that is divided into four categories, which are monosaccharides, disaccharides, polysaccharide and sugar alcohol. These sugar are categorized based on its number of monomers chain which they are composed of. According to Dotson (2018), Monosaccharide is the simplest form of carbohydrate and it is composed of a single monomer. On the other hand, disaccharides consist of two sugar monomers while polysaccharides build out of multiple units of sugar. The general type of sugar that can be found in the sap of industrial crops include glucose, sucrose, xylose, arabinose, mannose, and starch. The concentration of the sugar depends on the light intensity and the rate of photosynthesis.

Classification	Sub-group	Examples
Sugars (DP 1-2)	• Monosaccharides • Disaccharides • Sugars alcohols/polyols	• Glucose, fructose galactose, mannose, arabinose, xylose, erythrose, and others. • Sucrose, isomaltulose, lactose, maltose, trehalose, and others. • Sorbitol, mannitol, lactitol, xylitol, erythritol
Oligosaccharides (DP 3-9)	• Maltodextrins* (Malto-oligosaccharides) • Non-digestible oligosaccharides • Starch	• *Contain: glucose, maltose gluco-oligosaccharides • Raffinose, stachyose, fructo-oligosaccharides (FOS), arabino-oligosaccharides (AXOS), and others. • Amylose, amylopectin, and modified starches.
Polysaccharides (DP >9)	• Non-starch polysaccharides (NSP) • Resistant starch (RS)	• Pectin, cellulose, hemicellulose, hydrocolloids (Arabic gum, guar gum, others). • RS type 1,2,3, and 4

Figure 3 The classification of carbohydrate (Brouns, 2020).

2.4 Bioethanol

Bioethanol, chemically known as ethyl alcohol (C_2H_5OH), is a colourless liquid that is environmentally friendly and has a low toxicity level (Chin & H'ng, 2013). According to Lee and Lavoie (2013), bioethanol can be classified under three categories; First-generation (biomass as feedstock), second-generation (wide range of feedstock from lignocellulosic to municipal solid waste) and third generation (algal biomass and feedstock from CO_2 utilization). The first-generation bioehanol, such as ethanol and biodiesel, are derived directly from edible biomass. This is a first-generation bioethanol is known to be the simplest form of biofuel. Ethanol is produced from the fermentation of C6 sugars, such as glucose and sucrose, which is obtained from plant sources by using classical or genetically modified yeast known as *Saccharomyces cerevisiae*. The most common feedstocks used in first-generation bioethanol are derived from cop-based raw materials derived from corn, potato, sugarcane and beets (Lee & Lavoie, 2013).

Based on an article written by Chin and H'ng (2013), conversion of sugar-containing biomass into bioethanol is easier compared to starchy materials and lignocellulosic biomass because the disaccharide biodegrading requires less processing prior to conversion by yeasts. However, it is also stated in the article that the yield of ethanol produced from starchy materials is greater than that from sugar-containing materials because of the higher amount of fermentable sugars (glucose) present in the original starchy material. (Chin & H'ng, 2013). Oner *et al*. (2005) explain that the conversion of starch into ethanol involves two distinct steps. The first step in the fermentation process involves saccharification, or the breakdown of starch into sugar using an amylolytic microorganism or enzymes such as glucoamylase and α-amylase. The second step involve the process of sugar conversion ethanol using yeast such as *Saccharomyces cerevisiae.*

2.5 Enzymatic Hydrolysis

Enzymatic hydrolysis is a crucial step to prepare biomass prior to fermentation method for maximum yield production of bioethanol. Enzymatic hydrolysis can then break the polysaccharides into fermentable monosaccharides and disaccharides. The conversion of lignocellulose and starch into simple sugars can be done through either enzymatic hydrolysis or chemical hydrolysis. However, according to Maitan-Alfenas *et al.* (2015), it is stated that enzymatic hydrolysis is more preferred compared to chemical hydrolysis due to the reduced energy requirement, the milder conditions needed during the process and lesser fermentation inhibitor products are produced. Banana stem consist of starch and lignocellulosic compound which can be used as carbon source for the yeast in the fermentation process.

Soccol *et al.* (2011) stated that enzymatic hydrolysis involves a complex multi-step heterogenous process. Enzymatic hydrolysis commonly has 3 stages procedure which include gelatinization, liquefaction and saccharification (Souza & Magalhães, 2010). Based on an article written by Borglum (1980), it is stated that during gelatinization process, a viscous suspension is formed, which then transform into liquid due to the breakdown of starch into dextrins by the addition of α-amylase enzyme that is partially hydrolyses the α -1,4 bonds in starch. Then, in the saccharification process α-1,4 bonds are further hydrolysed along with α-1,6 bonds to convert dextrins into glucose by the aid of enzyme glucoamylase.