Effect of Ammonium Sulphate on Bioethanol Production from Banana Hydrolysate

Sandi Lau Chien Chien (71477)

Bachelor of Science with Honours
(Resource Biotechonology)
2022

Effect of Ammonium Sulphate on Bioethanol Production from Banana Hydrolysate

Sandi Lau Chien Chien

A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of Science with Honours
(Resource Biotechnology)

SUPERVISOR: DR. NURASHIKIN BINTI SUHAILI

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 13 day of July 2022.

Student's Declaration:

I SANDI LAU CHIEN CHIEN, 71477, FACULTY RESOURCE SCIENCE AND TECHNOLOGY (PLEASE INDICATE STUDENT'S NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled, Effect of Ammonium Sulphate on Bioethanol Production from Banana Hydrolysate 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.

13 JULY 2022	SANDI LAU (71477)
Date submitted	Name of the student (Matric No.)

Supervisor's Declaration:

I, **NURASHIKIN BINTI SUHAILI** (SUPERVISOR'S NAME), hereby certify that the work entitled, Effect of Ammonium Sulphate on Bioethanol Production from Banana Hydrolysate (TITLE) was prepared by the above named student, and was submitted to the "FACULTY" as a * partial/full full fulfillment for the conferment of Bachelor of Resource Bioetchnology (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work

Received for examination by: NURASHIKIN BINTI SUHAILI Date: 13 JULY 2022
(Name of the supervisor)

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 Chien (13 JULY 2022)

Supervisor's signature: Ashikin (13 JULY 2022)

Current Address:

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

First and foremost, thank God for his blessings and guidance, which lead me to complete this Final Year Project research. Besides, I would like to express my gratitude to my supervisor, Dr. Nurashikin binti Suhaili, for allowing me to perform this research and improve my scientific skills. I would like to thank her for guiding and assisting me throughout this work.

I would also like to thank all the postgraduates in the Biochemistry Lab for their time in sharing their knowledge and helping me during this period, especially Miss Faezzah.

I am grateful to all my friends as well, especially my labmates, Christie Ling and Sharon Khiong for assisting me in this work. Not forget to mention my roommate, Karen Hon Jing Yi and also Yit Hui Ying, who support and help me while I was completing this research.

My heartfelt gratitude to my parents for their unwavering support and encouragement as this research progressed. I would like to thank my brothers as well for soothing and motivating me during the research.

Effect of Ammonium Sulphate on Bioethanol Production from Banana Hydrolysate

Sandi Lau Chien Chien

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

ABSTRACT

Banana is an important and one of the earliest crops produced in the agriculture history. However, its harvest causes a huge number of agricultural wastes. In order to avoid environmental issues, banana agro wastes was utilised to produce value-added products such as bioethanol. The information on the influence of nitrogen sources on bioethanol production from banana hydrolysate is still limited. Therefore, this study was carried out to study the effect of ammonium sulphate $(NH_4)_2SO_4$, as a nitrogen source on the bioethanol production from banana hydrolysate. The banana stems were hydrolysed using liquozyme and spirizyme enzymes producing banana hydrolysate. The resulting banana hydrolysate was used as a substrate for bioethanol production by *Saccharomyces cerevisiae*. Different concentrations of ammonium sulphate were applied, namely 0, 2, 4, and 6 g/L. The fermentation performance was analysed in terms of cell growth, substrate consumption, and bioethanol production. The results showed that the addition of 2 g/L of ammonium sulphate to the fermentation resulted in the highest bioethanol concentration, 6.65 g/L, which was 4-fold over that in the control experiment. Additionally, the glucose consumption was the highest, 98.12%. The results indicated that the introduction of ammonium sulphate in the banana hydrolysate fermentation caused a positive effect on the bioethanol production.

Key words: Ammonium sulphate, banana hydrolysate, bioethanol, fermentation, nitrogen.

ABSTRAK

Pisang adalah penting dan merupakan salah satu tanaman terawal yang dihasilkan dalam sejarah pertanian. Walau bagaimanapun, penuaiannya menyebabkan sejumlah besar sisa pertanian. Untuk mengelakkan isu-isu alam sekitar, buangan agro pisang digunakan untuk menghasilkan produk nilai tambah seperti bioethanol. Maklumat mengenai pengaruh sumber nitrogen terhadap pengeluaran bioethanol dari pisang hidrolisis adalah terhad. Oleh itu, kajian ini dijalankan untuk mengkaji kesan ammonium sulfat $(NH_4)_2SO_4$ sebagai sumber nitrogen pada pengeluaran bioethanol dari pisang hidrolisis. Batang pisang dihidrolisis menggunakan enzim liquozyme dan spirizyme untuk menghasilkan hidrolisis pisang. Hydrolyzate pisang yang dihasilkan digunakan sebagai substrat untuk pengeluaran bioethanol oleh Saccharomyces cerevisiae*. Kepekatan ammonium sulfat yang berbeza digunakan, iaitu 0, 2, 4, dan 6 g/L. Prestasi penapaian dianalisis dari segi pertumbuhan sel, penggunaan substrat, dan pengeluaran bioethanol. Keputusan menunjukkan bahawa penambahan 2 g/L ammonium sulfat kepada penapaian menghasilkan kepekatan bioethanol tertinggi, 6.65 g/L, yang 4 kali ganda di atasnya dalam eksperimen kawalan. Di samping itu, penggunaan glukosa adalah yang tertinggi, 98.12%. Hasilnya menunjukkan bahawa pengenalan ammonium sulfat dalam penapaian hidrolisis pisang menyebabkan kesan positif terhadap pengeluaran bioethanol.*

Kata kunci: *Ammonium sulfat, hydrolysate pisang, bioethanol, penapaian, nitrogen*

TABLE OF CONTENT

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	percentage
°C	degree celcius
L	litre
mL	millilitre
µL	microlitre
g	gram
g/L	gram per litre
rpm	revolution per minute
ANOVA	analysis of variance
HPLC	High Performance Liquid Chromatography
$(NH_4)_2SO_4$	ammonium sulphate
NaOH	sodium hydroxide
OH-group	hydroxide group
S. cerevisiae	*Saccharomyces cerevisiae*
YM broth	yeast malt broth

CHAPTER 1

INTRODUCTION

1.1 Study Background

Bananas are commonly farmed in big plantations in tropical and subtropical nations, and approximately 106.54 million tonnes of banana fruits are produced each year, accounting for about 16% of global food output (Mohapatra and Sutar, 2010). According to Baig et al. (2004), banana is a prominent cash crop in Malaysia, but its harvest results in a large amount of agricultural wastes. The banana agro wastes, including dried leaves, stem and rachis are employed as substrates that release sugars. The wastes are lignocellulosic residues that can be used in the manufacturing of second-generation bioethanol (Guerrero et al., 2018).

Bioethanol is a non-petroleum-based alternative energy source that can help to increase oil supply security while also reducing the negative environmental effects of fossil fuels (Tan et al., 2019). It is the most widely used and commercially manufactured biofuel. As stated by Kusmiyati and Sukmaningtyas (2018), bioethanol is manufactured from basic sources that contain reduced sugar and can be fermented, such as fibres, molasses, fruits, and other components. The second-generation bioethanol is made from lignocellulosic feedstocks and the lignocellulosic materials are derived from forestry, agro-industries as well as food wastes, which are renewable, abundant, and cheap (Maitan-Alfenas et al., 2015). Consequently, global demand for bioethanol production is steadily expanding (Awasthi et al., 2015).

Gupta et al. (2019) claimed that the abandoned banana leaves and stem will emit a lot of gases including carbon dioxide, methane, and hydrogen sulphite to the air. They may also cause an outbreak of *Fusarium oxysporum*, a well-known banana fungus, which

affects the banana production (Pei et al., 2014). Therefore, the banana wastes are utilised appreciably in producing the bioethanol to reduce the environmental issues.

In recent years, numerous studies and reports on bioethanol production from agricultural banana wastes have been published. There are some reports on the production from banana peels, banana stem and banana leaves. As derived from the previous work by Kusmiyati and Sukmaningtyas (2018) that studied on the effect of different alkaline concentrations in pretreatment of the banana stem, higher concentration of NaOH produced the greatest level of bioethanol. In addition, Kusmiyati et al. (2018) had proposed their work on the simultaneous saccharification and fermentation (SSF) of the acid pretreated banana stems using varied ratios of mixed cultures; *Aspergillus niger*, *Trichoderma reesei*, and *Zymomonas mobilis*. As the outcomes, the maximum ethanol content was obtained when the cultivation ratios of *A. niger: T. reesei: Z. mobilis* were 1:1:2 at pH 5 in comparison to pH 4 and pH 6.

1.2 Objectives

The objectives of this study are:

1. To examine the effects of ammonium sulphate on bioethanol production from banana hydrolysate
2. To investigate the optimal concentration of ammonium sulphate for the production of bioethanol from banana hydrolysate

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction to bioethanol

Bioethanol is known as a biofuel produced from biological feedstocks such as sugar-based raw material, starchy material, and lignocellulosic biomass via microbial fermentation (Balat et al., 2008). It is environmental-friendly, safe, and renewable, which leads to its capability in minimizing greenhouse gases emissions. According to Mohd Azhar et al. (2017), bioethanol is ethyl alcohol, a second-generation bioethanol with chemical formula of C_2H_5OH. Feedstocks high in sucrose such as sugar cane, sugar beet, and fruits as well as high in starch like corn, wheat, rice, and potato are used in the first-generation bioethanol process (Mohd Azhar et al., 2017). Lignocellulosic biomass, such as wood, straw, and agricultural wastes, is applied in the manufacturing of second-generation bioethanol whereas third-generation bioethanol is made from algal biomass, including microalgae and macroalgae (Mohd Azhar et al., 2017).

Bioethanol has a greater octane number than gasoline, as well as a wider range of flammability limitations, faster flame speeds, and higher heat vaporisation (Balat et al., 2008). These qualities enable a higher compression ratio, a shorter burn period, and a leaner burn engine in an internal combustion engine, resulting in theoretical efficiency improvements over gasoline. Besides, bioethanol is soluble in all proportions with water, as well as ether, acetone, benzene, and some other organic solvents (Ritslaid et al., 2010). Its chemical role is dominated by the OH-group, which can readily aid reactions in the chemical industry such as dehydration, halogenation, ester formation, and oxidation (Ritslaid et al., 2010).

Balat et al. (2008) stated that bioethanol is the most frequently utilised biofuel in the transportation industry, with a lengthy history as an alternative fuel. Bioethanol has

established itself as the most demanding engine fuel in the world because it functions as an octane enhancer in unleaded gasoline, resulting in cleaner combustion and less pollution (Balat and Balat, 2009).

2.2 Process involved in bioethanol production from agricultural waste

The process of producing bioethanol is determined by the feedstocks used. In general, bioethanol production consists of three basic steps: pretreatment, hydrolysis, and fermentation procedures. As mentioned by Mohd Azhar et al. (2017), pretreatment of substrates is common in order to minimise their size and make the following procedures easier. The hemicellulose and cellulose of the substrates are hydrolysed into sugars that can be fermented, whereas the fermentation of these carbohydrates into bioethanol is delegated to *Saccharomyces cerevisiae*. Bioethanol is recovered through purification techniques before it can be utilised as a fuel.

The basic and most significant stage in the bioconversion of lignocellulosic to bioethanol is the pretreatment process. Physical, chemical, biological, and physiochemical methods are commonly used for pretreatments. All these pretreatment approaches aim to promote enzymatic saccharification by increasing the porosity of lignocellulosic materials, which increases fermentable sugar yields (Mosier et al., 2005). Mechanical milling is used to grind the substrate during physical pretreatment. Some of the most frequent chemical pretreatments are acid pretreatment and alkaline pretreatment. In addition, biological pretreatment involves a variety of fungal species, whereas physicochemical pretreatment comprises ammonia fiber explosion and steam.

Acid pretreatment removes hemicellulose from lignocellulosic materials, exposing cellulose for enzymatic response, while alkaline pretreatment removes lignin and

numerous uronic acid substitutions on hemicellulose, lowering the enzyme activity (Silverstein et al., 2007). According to Haq et al. (2016), acid pretreatment is usually done by using nitric acid, sulphuric acid, or hydrochloric acid, with dilute acid pretreatment being the most effective and extensively used approach. On the flip side, sodium hydroxide and calcium hydroxide are utilised as alkaline pretreatment. In comparison to other pretreatment procedures, this requires less heat and pressure to produce high saccharification yield (Haq et al., 2016).

Usman et al. (2016) stated that the hydrolysis stage aids in the breakdown of polysaccharides into monomers, allowing for efficient fermentation and distillation. Acid hydrolysis and enzymatic hydrolysis are the two procedures used. Enzymatic hydrolysis is substrate specific, with enzymes like cellulase, endo-gluconase, exo-gluconase, or glucosidase interacting directly with cellulose (Ko et al., 2009). Cellulase enzyme, which can be generated from bacteria or fungi, is the most common form of enzyme used for hydrolysis. As stated by Banerjee et al. (2010), dilute acid hydrolysis (0.7% - 3%) necessitates a high temperature (200-240 ºC), whereas concentrated acid hydrolysis necessitates a huge amount of acid, making it uncommercial and costly.

The hydrolysate results from the acid pretreatment of lignocellulosic material is utilised for bioethanol fermentation. In view of the fact that the lignocellulose hydrolysate contains not only glucose but also variety of monosaccharides, such as xylose, mannose, galactose, arabinose, and oligosaccharides, microorganisms such as *S. cerevisiae* should be necessary to successfully ferment these sugars to produce bioethanol in the industrial scale (Katahira et al., 2006). *S. cerevisiae* is one of the most successful bioethanol producing yeasts as it contributes high bioethanol synthesis from hexoses, and high tolerance to bioethanol and other inhibitory chemicals in lignocellulosic biomass acid hydrolysates (Haq et al., 2016).

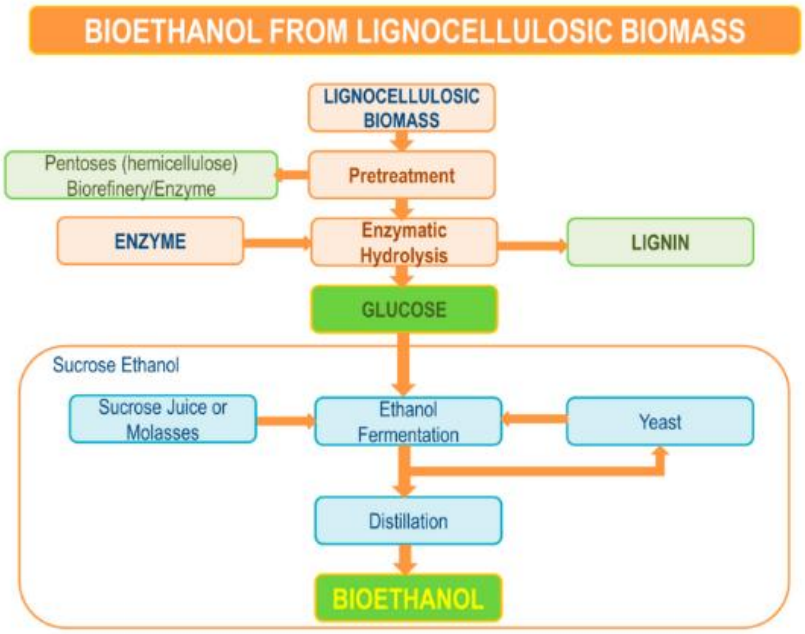

Figure 1: Pathway of bioethanol production from lignocellulosic biomass (Vasić et al., 2021).

2.3 Potential of banana wastes for bioethanol fermentation

Banana is a popular and important fruit crop that originates in Southeast Asia and spreads throughout the Pacific region (Padam et al., 2014). As stated by Tan et al. (2019), following oil palm, rubber, and paddy, banana production falls in fourth place in Malaysia, and it is the second most extensively farmed fruit crop. Banana is the prominent term for herbaceous plants in the Musa genus that are grown primarily for their fruit. In the year 2001, Malaysia's total banana plantation area was 33,704.2 hectares (Khalil et al., 2006).

Majority of the edible bananas are developed essentially for their fruits, therefore when the consumable banana fruit is harvested, the entire plant becomes underutilised by-products and agro wastes (Padam et al., 2014). Nevertheless, the sugar is greatly concentrated throughout the banana agro wastes such as stem, dried leaves, and rachis (Padam et al., 2014). These banana wastes can then be used as sustainable substrates in bioethanol production to avoid environmental problems like groundwater pollution.

Banana stem is among the lignocellulosic material that can be fermented to produce bioethanol. According to Kusmiyati et al. (2018), the banana stem composes of 44.6% cellulose, 36.0% hemicellulose and 19.4% lignin. Due to its relevant cellulose level and low lignin level, the banana stem has the capability to be bioethanol feedstock (Kusmiyati et al., 2018).

There are copious works on different parts of banana wastes for bioethanol production. As an example, banana stems, banana peels, rachis, and leaves have been studied as feedstock materials for production of bioethanol. Regarding the studies done by Usman et al. (2016), banana peels are used as substrates of lignocellulosic biomass to investigate the viability of producing bioethanol. Another illustration is about the utilisation of banana stem juice as the source for bioethanol production (Gupta et al., 2019). In this study, banana sap was used to produce bioethanol. It was a colourless appearance of water after collected from the outer sheath of banana stem.

2.4 Roles of ammonium sulphate in fermentation

Ammonium sulphate is an inorganic salt, having the chemical formula $(NH_4)_2SO_4$. Its IUPAC name is ammonium tetraoxosulphate (VI). As stated by National Center for Biotechnology Information [NCBI] (2022), ammonium sulphate exists as a fine white and odourless crystalline solid that dissolves in water but insoluble in acetone, alcohol, and ether. It has a density of 1.77 g/cm3 and melting point at 235 to 280 ^oC (NCBI, 2022). A central nitrogen atom bonds with four nitrogen atoms to form the tetrahedral structure of ammonium sulphate. Along with it, a central sulphur atom is linked to four oxygen atoms in the sulphate anion. This sulphate anion then holds the two ammonium molecules together.

Ammonia reacts with sulphuric acid to produce ammonium sulphate. Besides, calcium sulphate is plentiful in mineral form found in numerous places of the world. As a result, calcium sulphate is combined with ammonia and water to form ammonium sulphate. When ammonium sulphate reacts with alkaline substances, it releases ammonia gas.

Ammonium sulphate is used in many different industries. The most typical of its application is as a soil fertiliser, especially for alkaline soil. It consists of 21% nitrogen and 24% sulphur. An ammonium ion is released when it is placed into the damp soil. This produces a little amount of acid, which maintains the soil's pH equilibrium. It also provides nitrogen that helps plants thrive. Furthermore, ammonium sulphate is employed as an herbicide as well because it burns the plants' leaves, kills them, or weakens them enough to be easily removed.

According to Yue et al. (2012), ammonium sulphate is considered as one of the nitrogen sources essential for yeast fermentation. Nonetheless, proficient production of alcohol is not only dependent on carbon sources (Mezule and Dalecka, 2017). Various important elements such as nitrogen, phosphorus, and vitamins must be given to hydrolysate as chemicals introduced throughout or prior to hydrolysis (Chang and Webb, 2017), or included in the fermentation environment for ideal yields.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample preparation

Banana stems were harvested in Kuching, Sarawak. The banana stems were peeled and cut into little pieces. They were dried in an oven at 50 °C until they reached a consistent weight. After that, the dried banana stems were ground with the use of grinder and then sieved to get the banana stem powder.

Figure 2: Banana stems were cut into pieces and dried in an oven.

Figure 3: Dried banana stem was ground and sieved into powder.

3.2 Media preparation

3.2.1 Yeast Malt Broth (YMB)

About 100 ml of distilled water was added to 2.14 g of YMB powder and mixed by using a magnetic stirrer. The mixture was then transferred into a conical flask and sealed with aluminum foil. After the mixture was autoclaved at 121 °C for 90 minutes, half spatula of *Saccharomyces cerevisiae* was added. The mixture was left for overnight incubation at 150 rpm on an incubator shaker.

3.2.2 Nutrient agar

About 300 ml of distilled water was added to 8.4 g of nutrient agar powder and mixed thoroughly with the magnetic stirrer. The mixture was transferred to a conical flask and sealed with aluminum foil before autoclaving at 121 °C for 90 minutes. Then, the nutrient agar was poured onto the petri dish and left to cool in the laminar flow hood. The petri dish was sealed securely with parafilm and put in the refrigerator overnight in an upside-down situation.

S. cerevisiae was extracted from YM broth and streaked on the nutrient agar by using a sterilised inoculating loop. The agar was left overnight to examine the growth of colony.

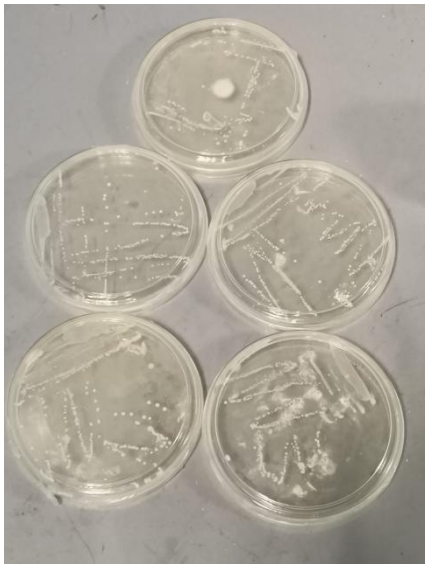

Figure 4: *Saccharomyces cerevisiae* was streaked on the nutrient agar.

3.2.3 Inoculum

For inoculation preparation, 3 g of glucose and 0.75 g of yeast extract were weighed and dissolved in a 100 ml of distilled water. The media was autoclaved for 90 minutes. Then, one colony of *S. cerevisiae* was transferred from the nutrient agar into the media and placed in the incubator shaker for overnight. The media was prepared for bioethanol fermentation.

3.3 Enzymatic hydrolysis

About 10 g of banana stem powder was dissolved in 1 L of water and boiled before enzymatic hydrolysis. In the midst of liquefaction, 20 µL of liquozyme was added and the suspension was heated for 30 minutes. It was left to cool, and water was refilled to 1 L. Afterwards, amid saccharification, 10 µL of spirizyme was added and the mixture was incubated in the oven at 50 °C for 24 hours. The resultant suspension, banana hydrolysate was filtered. Before being utilised as a medium for bioethanol fermentation, it was tested for glucose concentration using High Performance Liquid Chromatography (HPLC).

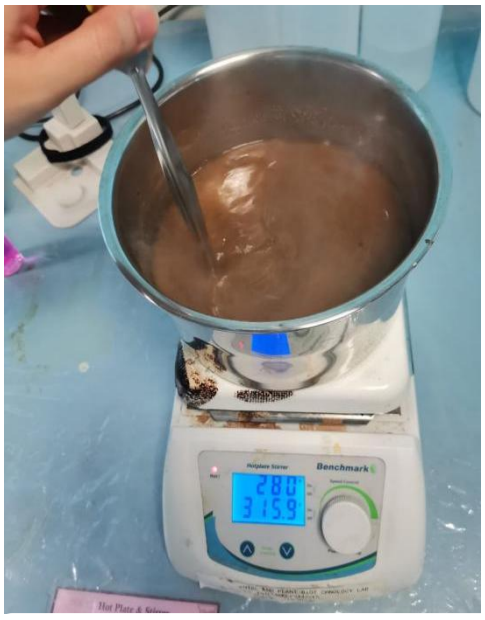

Figure 5: Banana stem powder was boiled and underwent enzymatic hydrolysis.

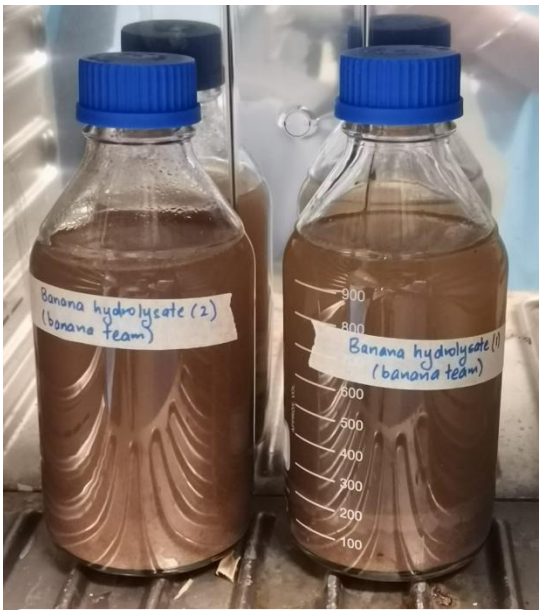

Figure 6: The mixture was incubated in oven for 24 hours.

The resulting hydrolysate from Section 3.3 was used as a substrate for bioethanol fermentation. The fermentations were carried out in batch mode and in 250 mL shake flasks with the working volume of 100 mL. 10 mL of inoculum was involved, and different amount of ammonium sulphate was added to the banana hydrolysate. After the adding of ammonium sulphate, the mixture was autoclaved to avoid contamination. The concentration of ammonium sulphate was adjusted to 0 g/L as a control, 2 g/L, 4 g/L, and 6 g/L. The flasks were secured with aluminum foil to maintain the culture sterility. All the flasks were placed on the shaker at 150 rpm. The samplings were done at 0-hour, 4th hour, 8th hour, and 12th hour.

Figure 7: Fermentations were carried out on the shaker.

3.5 Analytical methods

Cell growth was monitored based on the measurement of dry cell weight, which will be translated to dry cell concentration based on a standard curve. The samples were centrifuged to get supernatant and pellet. The supernatant was filtered for glucose and bioethanol analysis while the pellet was oven-dried to measure dry cell weight of the fermentation residue. In addition, bioethanol was recovered from the fermented solutions via distillation process. The concentration of ethanol and residual glucose were analysed by HPLC technique. All samples were filtered through a filter attached syringe, where 20 μL of each sample was introduced into the HPLC injection inlet. The HPLC run time was set to 10 minutes. The peak area unit was used to convert the HPLC results into a percentage of ethanol concentration, which was done by using a standard curve that made up of many known ethanol concentrations.

The formula for calculating dry cell weight was:

$$\text{Dry cell weight} = \frac{(W_{t+d} - W_t)}{0.0015\ \text{L}}$$

where: Wt+d = Weight of tube + dried sample (g)

Wt= Weight of tube (g)

0.0015 L = The volume of microcentrifuge tube in litre

Percentage of glucose consumption (%)

$$= \frac{Initial\ glucose\ concentration\ (g/L) - Final\ glucose\ concentration\ (g/L)}{Initial\ glucose\ concentration\ (g/L)} \times 100\%$$

3.6 Statistical analysis

All studies were carried out in triplicate, and the results were analysed statistically by using one-way analysis of variance (ANOVA) test. The differences were determined as significant when the p value is less than 0.05.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of different ammonium sulphate concentrations on *S. cerevisiae* cell growth during bioethanol fermentation

The effect of different ammonium sulphate concentrations on *S. cerevisiae* cell growth during bioethanol fermentation was investigated. Figure 7 shows the cell growth profile of *S. cerevisiae* during bioethanol fermentation. The control experiment was performed with no ammonium sulphate was added.

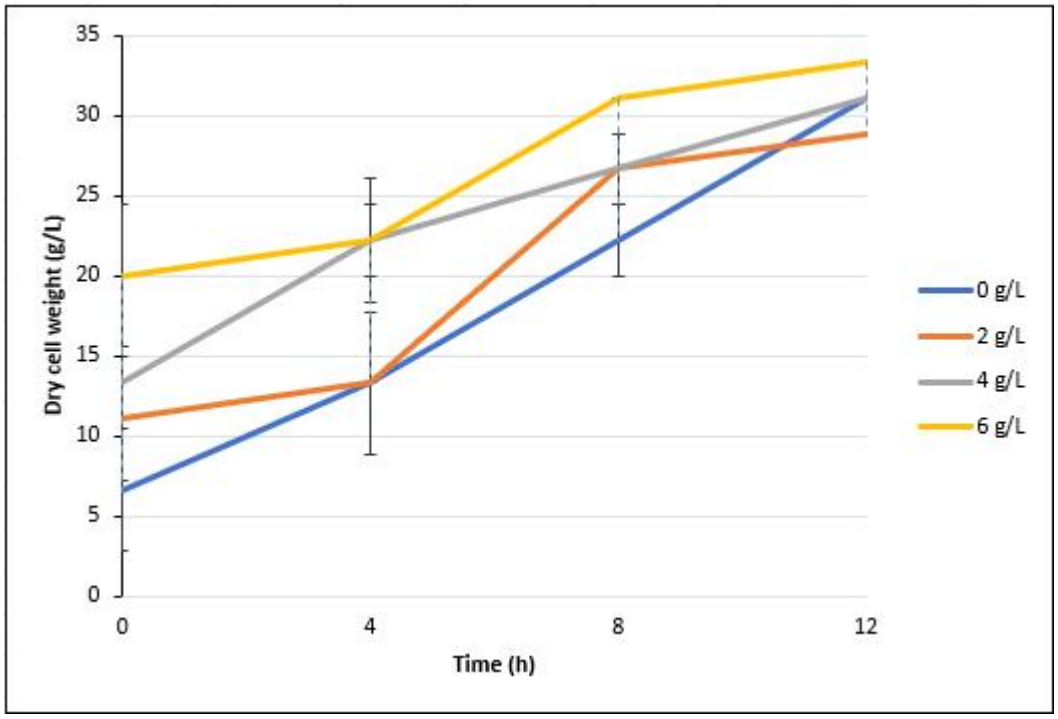

Figure 8: Cell growth profile of *S. cerevisiae* during bioethanol fermentation with different concentrations of ammonium sulphate.

The results showed the *S. cerevisiae* cell growth after the fermentation of banana hydrolysate by varying the amount of ammonium sulphate added. The cell growth increased significantly throughout 12 hours of bioethanol fermentation. *S. cerevisiae* experienced a lag phase at the first four hours of fermentation, then they entered log phase