Optimization of Ethanol Production from Waste Bananas

by

MUHAMMAD FARID BIN MAZLAN (12730)

Dissertation submitted in partial fulfilment of the requirements for the

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Universiti Teknologi PETRONAS

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CERTIFICATION OF APPROVAL

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A project dissertation submitted to the
Chemical Engineering Programme
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Approved by :

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(Dr Oh Pei Ching)

UNIERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK
May 2013

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

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MUHAMMAD FARID BIN MAZLAN

ABSTRACT

Biofuel has been gaining momentum in terms of research and development. Since there are various factors such as recent rise in oil prices, support from government subsidies, and growing concern about global warming make biofuel the focal point of the public and researchers. The recent studies has come out with a new method for the production of ethanol using banana which is preferable compared to the old method of using other types of food-based feedstock such as corn, sugar cane, and potato. Various operating parameters available for the reaction also helps in providing significant effect on the products. Thus, different researches have to be done to further enhance and improve the production of ethanol from banana.

Hence, this research study focuses on optimizing the ethanol production from banana using different parameters such as temperature and yeast concentration. The findings of this study can be used as a basis for comparison with other literature readings on the banana ethanol production having different operating conditions and parameters. Then, the effects of these different parameters are studied and optimized using Response Surface Methodology method. For the first part of the experiment, the study will focus on the different characteristics of banana especially on the growth rate effect on the weight of banana. While for the second part of the experiment, optimization of ethanol production from banana will be the main focus.

This project dissertation serves to provide an introduction to the project, its background study, literature review, and also the detailed planning and methodology to carry out this research project to meet its objectives. It is expected that different parameters such as temperature and yeast concentration will affect the production of ethanol from banana which can further be optimized using the Response Surface Methodology method. The optimum yeast concentration and temperature for production of bioethanol from banana pulp is at 30°C and 0.10% w/v yeast concentration while for peel is at 40°C and 0.10% w/v yeast concentration, respectively.

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First and foremost, all praises goes to Allah the Almighty for the strengths and His blessing in completing my Final Year Project. I believe everything has been planned carefully by Him and I would not make it this far without Him.

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Above all, I would like to thank my beloved family and friends for constantly giving support, advices, and guidance from the start of my final year project until the end. There were the one who always there beside me and help me out in every way they could in the times of trouble.

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CHAPTER 1

PROJECT BACKGROUND

1.1 Background of Project

Being the highlight for this project, ethyl alcohol or easily known as ethanol is a volatile, flammable, colourless liquid. It is commonly used as a feedstock in chemical industry to make a wide variety of products. It is also used in the automotive fuel industry. With the current technology, there are cars that run on 100% ethanol or fuel mixtures containing part gasoline and part ethanol. According to factmonster.com (February 17, 2013), most of the automobiles manufactured in U.S. since 1998 have been manufactured with equipment that allow them to run on E85. a mixture of 85% ethanol and 15% gasoline . The amount of harmful gases emission can also be reduced with the uses of ethanol as fuel for motor vehicles.

Biofuel is a type of fuel with energy derived from biological carbon fixation. It has been around for a long time but the discoveries of huge petroleum deposits made it largely forgotten. However, with the recent rise in oil prices, support from government subsidies, and growing concern about global warming, biofuels have been regaining popularity. Banana is one of the main feedstock for biofuels. With the easy maintenance and high sugar content, they have a great potential in this industry and act as a much cheaper alternative fuel sources.

This project studies the different stages of banana's ripeness and conditions which further require multiple test and analysis. In order to increase the accuracy and precision of the results, Response Surface Methodology is used. The main idea is to use a sequence of experimental design to obtain an optimal response by exploring the relationships between several explanatory or response variables. This method also helps in optimizing the selected parameters.

1.2 Problem Statement

Based on 2011 statistics, India led the world in banana production with 145,000,000 metric tonnes which covers around 20% of the worldwide crop. This is followed by Uganda which covers 8% of the total worldwide crop. Banana or scientifically known as Musa Spp are also among the popular fruit crop in Malaysia. There are around 29,270 hectares of banana planted which produce more than 294,530 metric tonnes of fresh banana in year 2012. The worst part is, approximately a tonne of waste made up of skins, leaves, and stems are produced for every ten tonnes of bananas. To avoid wastage, this banana wastes can be turned into a new energy source.

The existing production method such as the corn ethanol industry that is rapidly growing will cause significant environmental damage without significantly reducing the dependence on fossil fuels. The production of this corn energy produce a heavy carbon footprint which causes high concentration of greenhouse gases released. The features available on banana is proven in producing less amount of harmful gases which will help in controlling the environmental damage.

Besides that, the increased soil degradation in Malaysia have been negatively affecting the soil fertility. The soil properties affected such as nutrient content, water holding capacity, and acidity reduce the chances of the plants to live. Banana can be easily maintained and grown on less fertile land, especially on land that has been degraded by farming compared to the other bioethanol producing plants. It can also produce much higher amount of energy compared to corn.

1.3 Objectives

1. To study on the characteristics of banana on different stages of ripeness.
2. To study the effect of temperature and yeast concentration for ethanol production from banana.
3. To optimize the ethanol production by using Response Surface Methodology method.

1.4 Scope of Study

Banana is known to be in three different main stages of ripeness which are green, normal ripe and over ripe. Based on the first objective, the characteristics of banana on these three different stages are studied using dry ash analysis and dry matter analysis. While for the second objective, studies are done in order to identify the parameters such as temperature and yeast concentration involve in optimizing the ethanol production. Besides that, the design expert software by Stat-Ease is used in order to optimize the selected parameters and achieve the third objective in optimizing the ethanol production.

1.5 Feasibility of the Project within Scope and Time Frame

The optimization of yeast concentration and temperature will be conducted using RSM method by Design Expert Software by StatEase. This study will be divided into two main stages. The first stage will focus on the characteristic of the bananas while for the second stage, the study will focus on extracting the ethanol from banana. As shown in Table 2, this project will be carried out in the given time frame and will cover the scope of study.

CHAPTER 2
LITERATURE REVIEW

2.1 Biofuel as Alternative Fuel

Non-conventional or advanced fuels are any materials or substances that can be used as fuels. Normally known as alternative fuels, they have the same purpose which is to store energy. More than a dozen alternative fuels are now under production or development for use in alternative fuel vehicles and advanced technology vehicles. Biofuels are one of the alternative fuel developed and it is a renewable energy. Other examples of renewable energies are photovoltaic, wind, hydropower, and many more. As mentioned by Hossain *et al.* (2008), different forms of bioenergy can be produced from a wide range of biomass sources. Biofuel can be either solid, liquid or gas fuel made from relatively recently dead biological material but the most common sources of biofuels are photosynthetic plants.

Sustainable biofuels are essential to ensure a constant, secure supply of energy for individuals and industry. Increase in demand of fossil fuels, combined with depletion of mineral oil reserves has led to the development of eco-friendly concepts (Demirbas, 2007). It is also stated by Demirbas (2008), demand of the energy increases with the increase of the world population and urbanization . Thus, development of alternative energy might help in reducing the problems related to the high demand of fossil fuels and depletion of the mineral oil reserves.

As mentioned by Himmel *et al.* (2007), the negative impacts of fossil fuel on the environment and the unstable oil market are the factors that lead to the constant search for alternative fuels. The phenomenon of pollution of the environment has increased in many countries of the world. Biofuels emits less green house gas while producing less harmful emission during combustion (Dhabekar and Chandak, 2010).

2.2 Banana as Biofuel

According to scientists, approximately one tons of wastes are produced for every ten tons of bananas, made up of skins, leaves, and stems. In Columbia for example, banana fruit surplus production amounts to 850,000 tonnes per year with generation of more than 1,150,000 tonnes per year of associated residual biomass (Bohorquezz and Herrera, 2005). The banana fruit and its associated residual biomass can be converted into glucose which can be used as feedstock to produce ethanol by fermentation and distillation.

Depletion of the mineral oil reserves combined with the increase in demand of fossil fuels has led to the development of eco-friendly concepts (Demirbas and Demirbas, 2007). Implementation of ethanol as fuel decreases fossil consumption and further reduce the contribution to the global environmental crisis. It is considered biodegradable and sulphur free. As mentioned by Wang *et al.* (1999), the carbon released during combustion process has a vegetable origin and as a consequence, it does not contribute to the increase of carbon dioxide in the atmosphere. This is much helpful in reducing the global warming.

With the increasing demand on biofuels production, many new technologies are developed and improvement on the available technologies are done in order to ensure that all opportunities and challenges related to biofuels are covered. As stated by Akin-Osanaiye *et al.* (2005), ethanol production by fermentation faces competition with ethanol production from petroleum-based products. However, as the values of the petrochemical were increased, fermentation of ethanol received more attention (Ahmeh *et al.*, 1988).

2.3 Fermentation Process

Fermentation comes from the Latin verb, *fevere*, to boil. It is a process of chemical change caused by organisms or their products and it is the oldest of all biotechnological processes. This anaerobic process is able to release energy from glucose even without the present of oxygen. As mentioned by Pierre-Yves Bouthyette (2008), the definition of fermentation can be very broad and encompasses various phenomena but there is one thing in common for each case, introduction of microorganisms, voluntarily or not.

Normally fermentation is referred to the process used to make wine or beer. However, it actually plays a much greater role in our lives. Based on Rolle and Satin (2002), this process are believed to have been developed over the years by women, in order to preserve food for times of scarcity, to impart desirable flavour to foods, and to reduce toxicity. It is also known that in modern-day life, the wide spectrum of foods marketed both in developing and industrialized countries, not only for the benefit of preservation and safety but also for their highly appreciated sensory attributes.

The microorganisms can be bacteria, yeast or even molds. Each of the microorganisms work in their own ways, with different end products. This also means that there are different types of fermentation process available. Based on Ensymm.com (February 22, 2013), the growth of micro organisms or other cells results in a wide range of products and each culture operation has one or few set objectives.

2.4 Distillation Process

In the realm of chemical practice, the physical separation of mixtures is an extremely important consideration. Distillation is a very scalable technique and energy-intensive. It is much cheaper compared to other comparable chromatographic approaches. According to Rosemary Hoegger (1998), distillation is one of the oldest techniques of separating liquid or molten substances.

Rosemary Hoegger (1998) stated that this method is fast, simple, and effective for cleaning and separation. It separates solvents and volatile substances from non-volatile liquid and solids. Hinkel and Junghans (2004) stated that distillation is the separation of the constituents of a liquid mixture by partial vaporization and subsequent condensation, taking advantage of differences in volatility.

Based on Seader (1998), the separation process requires three things. A second phase must be formed in order for both liquid and vapor phases to present and able to contact each other on each stage within a separation column. Other than that, the components must have different volatilities so that partition will be created between the two phases to different extent. This is the phase where it differs from absorption and stripping with the phase created by thermal means. Lastly, the two phases can be separated by gravity or other mechanical means.

2.5 Response Surface Methodology

Response Surface Methodology or also known as RSM is a collection of mathematical and statistical techniques that are very beneficial for modelling and analysis of problems (Montgomery, 2005). With the main idea of using a sequence of designed experiments to obtain an optimal response, RSM explores the relationships between several explanatory variables and one or more response variables. The first goal for RSM is to find the optimum response and as stated by Oehlert (2000) it is important to find the compromise optimum that does not optimize only one response when there are many responses.

Started with the aim to model experimental responses, it was then migrated into the modelling of numerical experiments (Box and Draper, 1987). The only differences is, the type of error generated by the response. With the purpose in optimizing defined response of interest, RSM proved to be a useful tool for the analysis of problems during which a certain response of concern is usually influenced by different reaction variables. It is also known for being successfully employed for the optimization of parameters for the production of enzymes and ethanol (Liu et al, 2007).

Based on Cornel (1990) RSM can be viewed from three major standpoints. The techniques are used to find the optimum value of the response if the system response is well discovered or the techniques are used to at least gain a better understanding of the overall response system if discovering the best value is beyond the available resources of the experiment. Besides that, a simplified equivalent response surface may be obtained by a few numbers of runs to replace a complicated analysis.

CHAPTER 3
METODOLOGY

3.1 Overall Process Flowchart

Figure 1 shows the overall process flowchart for the whole experiment. The experimental works for this project will fully done in laboratory.

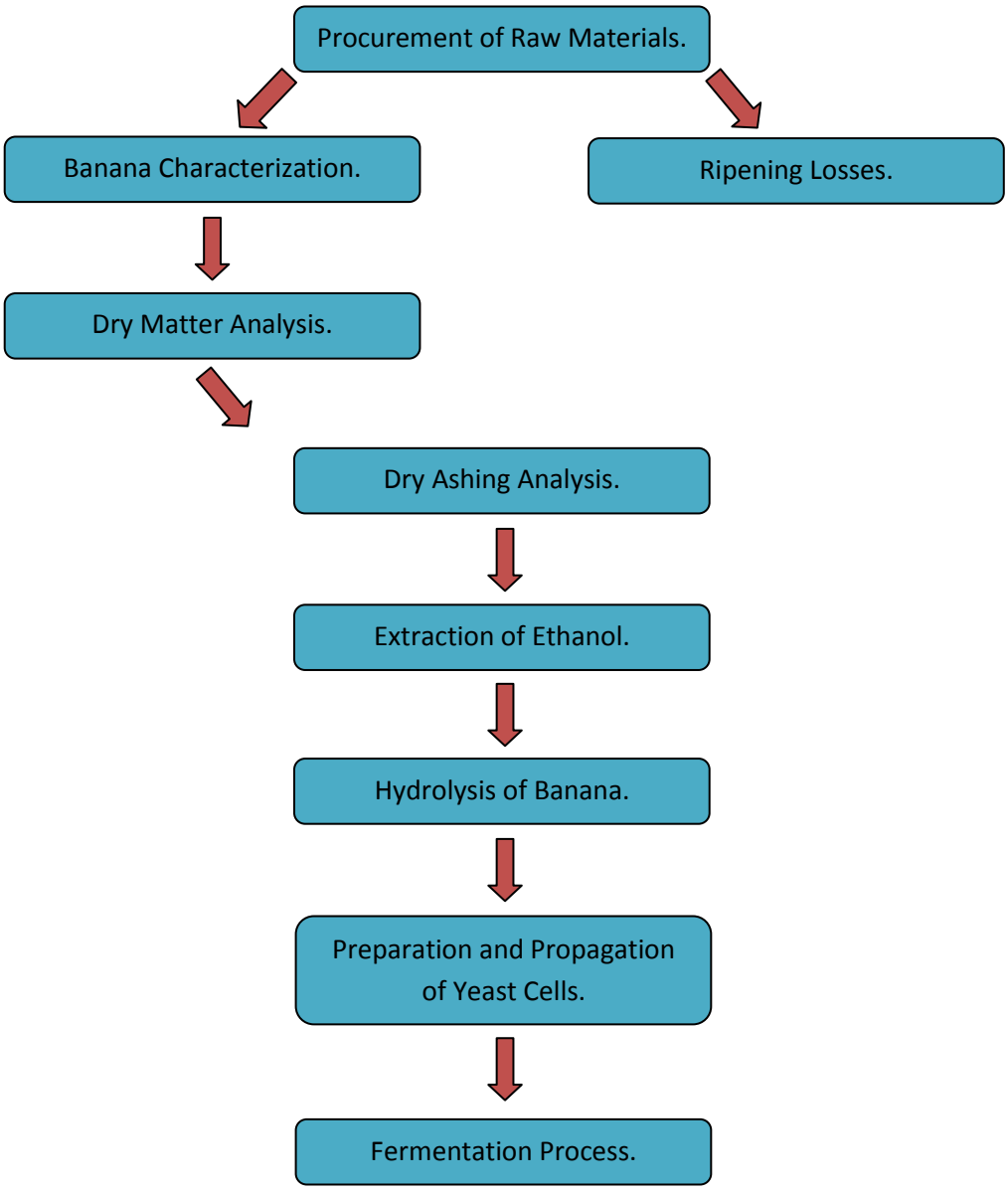

Figure 1 : Overall Process Flowchart

3.2 Banana Characterization

1. 18 bananas are selected at random and weighed.
2. The whole banana is peeled before the pulp and peel are weighed separately.
3. The samples are hand chopped, sampled, and immediately frozen until analyzed.
4. The components are tested for (3.2.1) dry matter analysis and (3.2.2) dry ashing analysis using procedures in Standard Methods (APHA,1992).
5. Characteristics of the whole fruit are calculated as a weighted average of the component parts based on their fraction of the whole fruit.
6. Steps 1 to 5 are repeated six times for each stage of ripeness and analyzed.

3.2.1. Dry Matter Analysis

1. The empty container is weighed and recorded.
2. The feed is placed in the container.
3. The total weight of container with feed are measured and recorded.
4. To determine the weight of feed before drying, the weight of the container is subtracted from the total weight.
5. The feed is dried thoroughly.
6. The container and feed are weighed immediately after drying.
7. To determine the weight of feed after drying, the weight of the container is subtracted from the total weight.
8. The weight of dry feed is divided by the weight of the wet feed.
9. The results are multiplied by 100 to get a percentage.

3.2.2. Dry Ashing Analysis

1. The empty porcelain crucible is weighed and recorded.
2. The feed is placed in the porcelain crucible.
3. The total weight of feed and porcelain crucible are measured and recorded.
4. To determine the weight of feed before ashing, the weight of the porcelain crucible is subtracted from the total weight.
5. The samples are transferred into the microwave and held at 500 °C to 600 °C for 24 hours.
6. The porcelain crucible and feed are weighed immediately after drying.
7. To determine the weight of feed after ashing, the weight of the porcelain crucible is subtracted from the total weight.
8. The weight of ashes is divided by the weight of the feed before ashing.
9. The results are multiplied by 100 to get a percentage.

3.3 Ripening Losses

1. Fourteen fresh green bananas are weighed separately.
2. Five bananas are peeled and components being sampled.
3. On day six, the remaining bananas are weighed again and five are peeled and sampled.
4. On day ten, the remaining bananas are weighed again, peeled, and sampled.

3.4 Extraction of Ethanol

1. Eight plastic cups are filled with 60 g mashed up banana peel.
2. 150 ml of water is measured and poured into each of the cup.
3. 6.25 g of Active-Dry Yeast is added to each cup and sealed with saran wrap.
4. Four cups of mashed up banana are left out to be fermented for 24 hours.
5. While the other four cups of mashed up banana are left out to be fermented for 48 hours.
6. Four of the cups are distilled after 24 hours while the other four cups are distilled after 48 hours.
7. The obtained ethanol are then distilled again for 15 minutes.
8. The samples obtained are tested for ethanol.
5. Steps 1 to 8 are repeated using mashed up banana pulp.

3.5 Hydrolysis of Banana

1. 13g of banana feedstock is hand chopped and mashed with a pastry cutter.
2. The hand chopped banana are then transferred into 20 separate polycarbonated baffle flasks.
3. 87 g of deionized water is added into the polycarbonated flask containing the hand chopped banana.
4. Sulphuric acid is added at 0 M, 0.25 M, 0.5 M, and 0.75 M.
5. The solution are transferred to an autoclave and held at 121 °C for 15 minutes.
6. The solution is filterred using vacuum filtration using coarse filter paper.
7. The hydrolysate is collected in receiver flask.
8. The sugars are analyzed using refractometer.

3.6 Preparation and Propagation of Yeast Cells

1. Dried yeast powder is added into a 150 ml Erlenmeyer Flask containing 50ml glucose yeast extract.
2. The flask is held on 100 rpm incubator at 30 °C for 48 hours.
3. The mixture are transferred into 250 ml Erlenmeyer Flask containing 100ml glucose yeast extract broth.
4. 50 ml of prepared culture is transferred into 1000 ml Erlenmeyer Flask containing 500 ml of glucose yeast extract.
5. The flask is held on 100 rpm incubator at 30 °C for 24 hours.
6. The cells are transferred to a 50 ml centrifuge tube.
7. The cells are centrifuged at 4 °C for 10 minutes.

3.7 Fermentation Process

1. 1.2L of hydrolysate is put into a 2 L batch fermenter.
2. The hydrolysate is neutralized and supplemented with a concentrated nutrient solution in order to have a final concentration of 0.3 % weight per volume of yeast extract and 0.2 % weight per volume peptone.
3. The residual pre-treated biomass is stored in a sterile bag and frozened.
4. The fermenter containing hydrolysate is heated to a temperature of 80 °C for 30 minutes and agitated at 250 rpm.
5. The fermentation is performed at temperature, pH and time according to the runs obtained from Design Expert software.
6. The fermenter is inoculated with 120ml of yeast inoculums at concentration of $1x10^9$ cells/ml.
7. The agitation speed is reduced and maintained at 200 rpm.
8. The pH is maintained using 5 N HCL and 10 N NaOH.
9. The samples are drawn at 3 hours intervals and analyzed for sugar and ethanol concentration.

3.8 Tools/ Equipments Required

Table 1 : Chemicals and Equipments Required

Type	No.	Name	Amount
CHEMICAL	1	Active-Dry Yeast	200g
	2	Deionized Water	
	3	Sulphuric Acid (0.25M, 0.5M, 0.75M)	
	4	Dried Yeast Powder	
	5	Glucolin	
	6	Hydrochloric Acid (5N)	
	7	Sodium Hydroxide (10N)	
GLASSWARES & EQUIPMENTS	8	Stopwatch	2
	9	Autoclave	1
	10	Refrigerator	1
	11	High Performance Liquid Chromatography	1
	12	Distillation Apparatus	1
	13	Spatula	1
	14	Beaker	9
	15	Weighing Scale	1
	16	Knife	1
	17	Oven	1
	18	Blender	1
	19	Porcelain	9
	20	Plastic Cup	16
	21	Saran Wrap	1
	22	Polycarbonated Baffle Flasks	20
	23	Autoclave	1
	24	Coarse Filter Paper	
	25	Refractometer	1
	26	Erlenmeyer Flask (50ml, 100ml, 150ml, 250ml, 1000ml)	4
	27	Incubator	1
	28	Centrifuge Tube (50ml)	12
	29	Sterile Bag	12
SOFTWARE	30	Design Expert Software	1

3.9 Project Activities & Key Milestones

Table 2 : Project Activities and Duration

No.	Activity	Duration
1	Banana Characterization	4 hours
2	Dry Matter Analysis	2 hours
3	Dry Ashing Analysis	26 hours
4	Ripening Losses	11 days
5	Extraction of Ethanol	53 hours
6	Hydrolysis of Banana	3 hours
7	Preparation and Propagation of Yeast Cells	53 hours
8	Fermentation Process	5 hours
Total		410 hours

The total time estimated for this project to be completed is within 410 hours or equivalent to 17 days. Most of the steps involve simple methods but need a long waiting time in order to be completed. Due to that reason, some of the steps such as banana characterization, ripening losses, and preparation and propagation of yeast cells can be done simultaneously to reduce the duration short.

3.9.1 Gantt Chart for FYP I

Table 3 : Gantt Chart for FYP I

No.	Detail	Week														
		1	2	3	4	5	6	7		8	9	10	11	12	13	14
1	Selection of Project Topic															
2	Preliminary Research Work															
3	Preparation of Extended Proposal															
4	Submission of Extended Proposal															
5	Proposal Defence															
6	Meeting with Supervisor															
7	Order for Equipments and Chemicals															
8	Booking of Laboratory															
9	Procurement of raw materials															
10	Banana Characterization															
11	Dry Matter Analysis															
12	Dry Ashing Analysis															
13	Ripening Losses															
14	Submission of Interim Draft Report															
15	Submission of Interim Report															

3.9.2 Gantt Chart for FYP II

Table 4 : Gantt Chart for FYP II

No.	Detail	Week														
		1	2	3	4	5	6	7		8	9	10	11	12	13	14
1	Meeting with Supervisor															
2	Procurement of raw materials															
3	Extraction of Ethanol															
4	Hydrolysis of Banana															
5	Preparation and Propagation of Yeast Cells															
6	Fermentation Process															
7	Submission of Progress Report															
8	Pre-SEDEX															
9	Submission of Draft Report															
10	Submission of Dissertation															
11	Submission of Technical Paper															
12	Oral Presentation															
13	Submission of Project Dissertation															

CHAPTER 4

RESULT AND DISCUSSION

4.1 Banana Characteristics

For the banana characterization, fourteen bananas were purchased green from the local grocery store. The initial weight of each bananas were measured on the first day. One banana was weighed, peeled and sampled for each day for fourteen days. The characteristic such as the colour and weight were also recorded.


Day 1 : Green


Day 2 : Green


Day 3 : Green


Day 4 : Green


Day 5 : Green


Day 6 : Green


Day 7 : Green

Figure 2 : Green Bananas

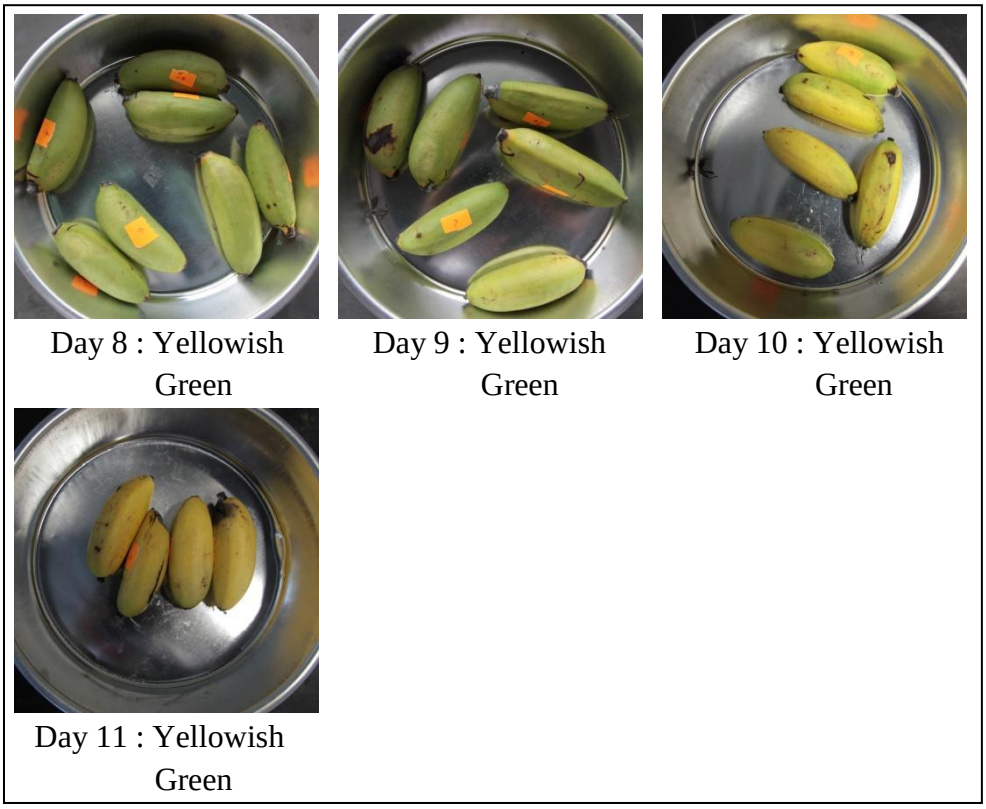

Figure 3 : Yellowish Green Bananas

Figure 4 : Yellow Banana

For the first seven days, the colour of bananas remained green as shown in Figure 2. The colour started to turn yellowish-green as shown in Figure 3 only on day eight and reached its normal ripeness on day twelve where it turned into yellow as in Figure 4.

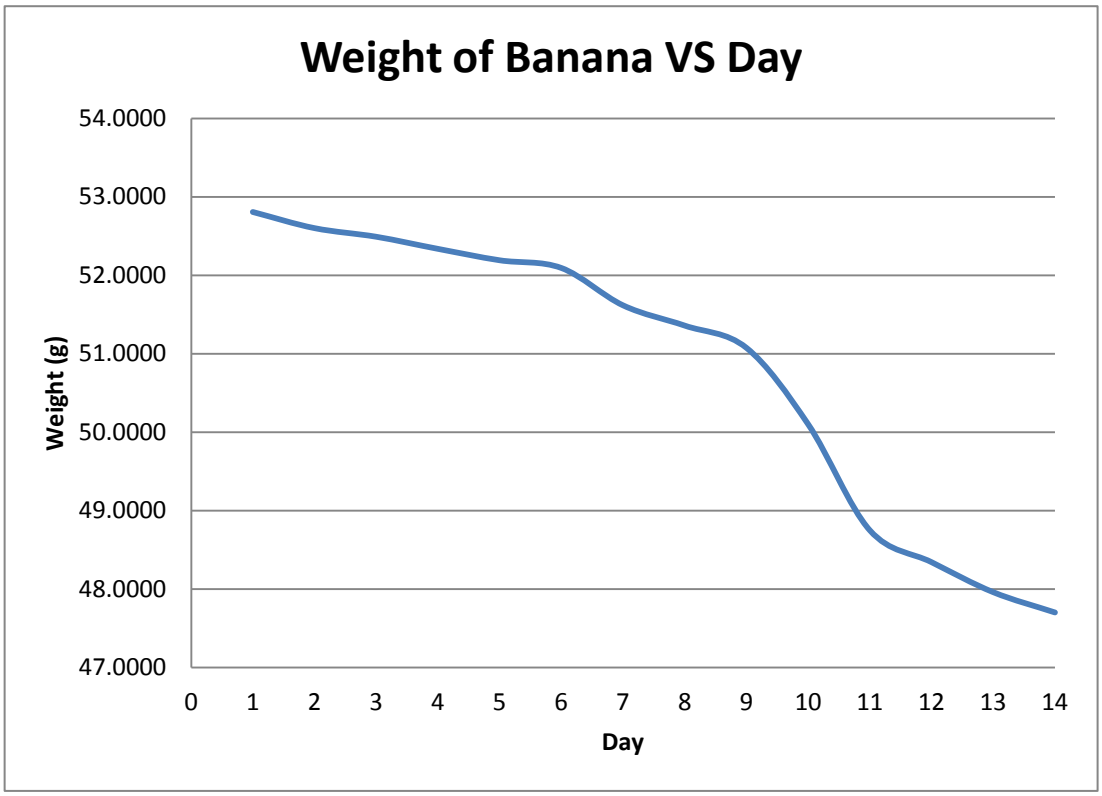

Figure 5 : Changes of Weight of Banana for period of Fourteen Days

One banana was randomly selected to remain unpeeled and weighed daily for the period of fourteen days. The resulted changes of the weight of banana were tabulated in a graph shown in Figure 5. It is clearly shown the decreased of weight of banana during the growth period of fourteen days. There were also a drastic dropped of weight of banana during day nine to day eleven where the banana having a changes from green to yellow banana or known as the normal ripe banana. We can conclude that the reduction in weight of bananas were due to losses associated with both the drying of the banana and the internal metabolic activities.

4.2 Dry Matter Analysis

Eight randomly selected bananas were weighed and peeled. The pulp and the peel were then heated separately at 60°C with 30 minutes time interval. By using the formula, the percentage of dry weight for both pulp and peel of the bananas were determined.

$$Percentage\ of\ Dry\ Weight\ = \frac{Weight\ of\ Dry\ Pulp}{Weight\ of\ Wet\ Pulp}\ X\ 100\%$$

$$Percentage\ of\ Dry\ Weight\ = \frac{Weight\ of\ Dry\ Peel}{Weight\ of\ Wet\ Peel}\ X\ 100\%$$

The resulted percentage of dry weight for the pulp and peel of the randomly selected bananas are tabulated in the graphs shown in Figure 6 and Figure 7.

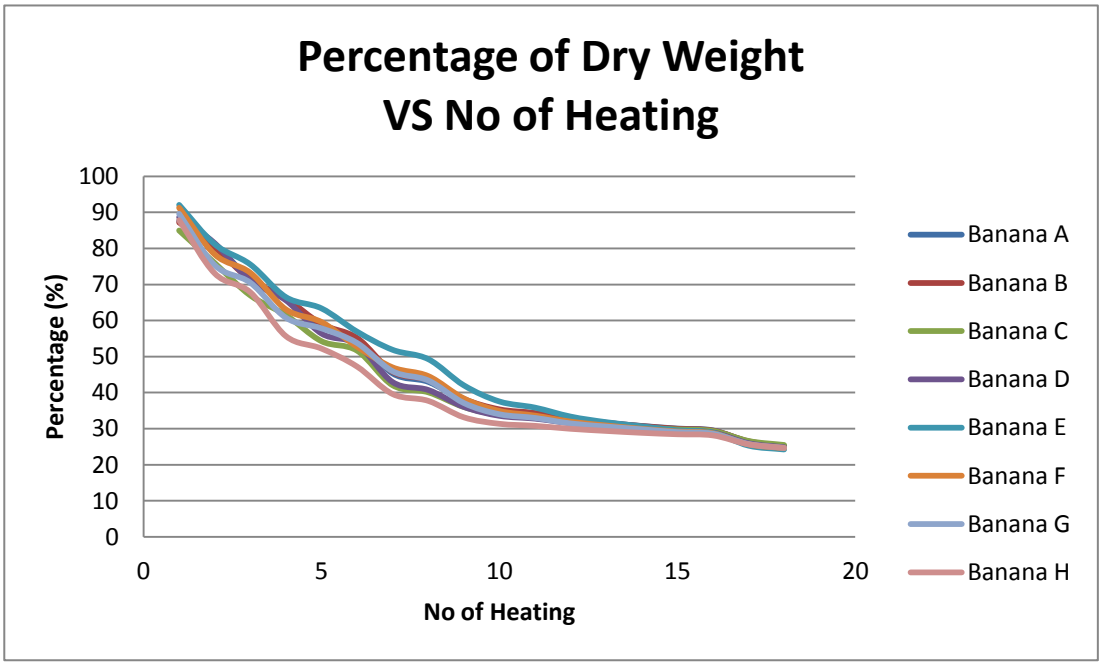

Figure 6 : Effect of Heating on Percentage of Dry Weight for Pulp of Banana

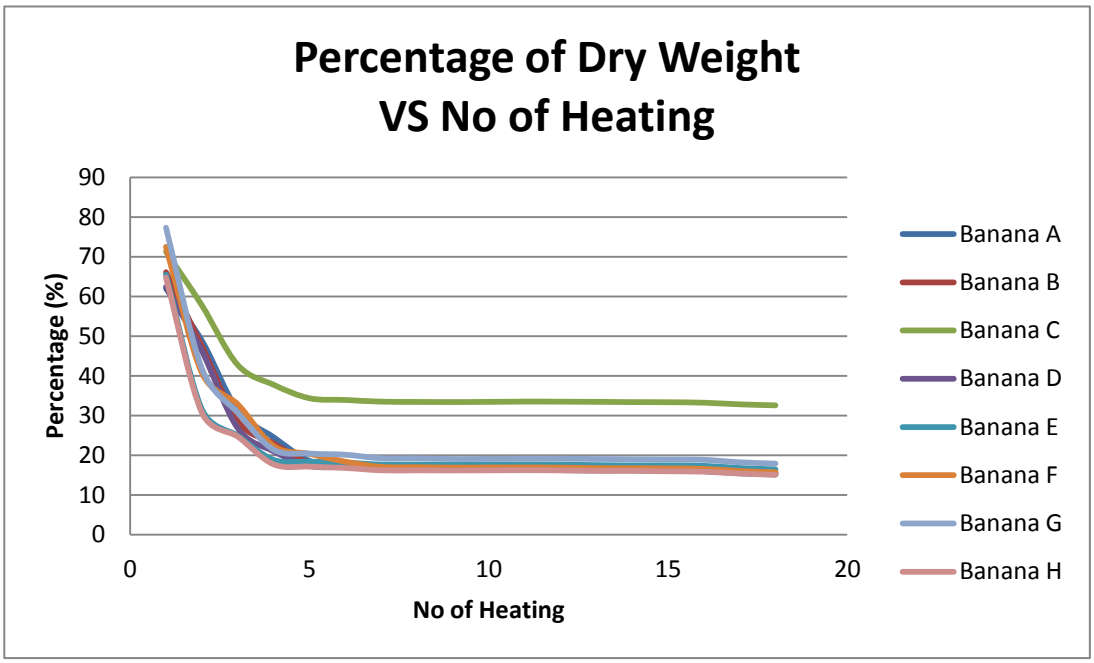

Figure 7 : Effect of Heating on Percentage of Dry Weight for Peel of Banana

This dry matter analysis is a method used to determine the total major and trace element content in the bananas. It measures the mass of the bananas when it is completely dried. The dry matter of the bananas would be solids consist of all its constituents except the water content. When the percentage of dry weight reached a steady point, it shows that there is no water left inside the banana and it was completely dried.

As shown in the graph in Figure 7, the percentage of dry weight for peel of banana has reach a constant value after 5 runs of heating. While, for the pulp of banana, the value is still decreasing even after 18 runs of heating. This shows that the ratio of the water to the banana constituents are higher in the peel of banana compared to the pulp of banana.

4.3 Dry Ashing Analysis

Dry ashing analysis have almost similar objective and steps to the dry matter analysis but for this specific analysis, the pulp and the peel of bananas were heated at extreme temperature of 500°C for 24 hours in a furnace. By using the formula, the percentage of dry weight for both pulp and peel of the bananas after undergo the ashing process were determined.

$$Percentage\ of\ Dry\ Weight\ = \frac{Weight\ of\ Ash\ Pulp}{Weight\ of\ Wet\ Pulp}\ X\ 100\%$$

$$Percentage\ of\ Dry\ Weight\ = \frac{Weight\ of\ Ash\ Peel}{Weight\ of\ Wet\ Peel}\ X\ 100\%$$

The resulted percentage of dry weight for the pulp and peel of the bananas after undergo ashing process are tabulated in the graphs shown in Table 5 and Table 6. Similar to the dry matter analysis, the peel of banana have higher percentage which shows higher ratio between the banana constituent and its water content.

Table 5 : Percentage of Banana Pulp After Ashing

	Banana A	**Banana B**	**Banana C**	**Banana D**
Weight of Wet Pulp (g)	21.8934	20.8957	20.7841	22.0834
Weight of Ash Pulp (g)	0.1562	0.2052	0.1826	0.1723
Percentage (%)	0.71	0.98	0.88	0.78

Table 6 : Percentage of Banana Peel After Ashing

	Banana A	**Banana B**	**Banana C**	**Banana D**
Weight of Wet Peel (g)	9.6644	10.0132	10.0041	8.1597
Weight of Ash Peel (g)	0.3087	0.5120	0.4852	0.3884
Percentage (%)	3.19	5.11	4.85	4.76

4.4 Calibration of Ethanol Concentration

The refractive index of different ethanol concentration are shown. The sample used is in ratio of volume ethanol to distilled water. The significant of ethanol standard calibration is to indicate the present of ethanol component by calculating the percentage of ethanol concentration.

Table 7 : Refractive Index of Ethanol

Volume of Ethanol (ml)	Volume of Distilled Water (ml)	Purity of Ethanol (%)	Refractive Index
0	10	0	1.3326
2	8	19	1.3392
4	6	38	1.3446
6	4	57	1.3493
8	2	76	1.3567
10	0	95	1.3593

As shown in Figure 8, the refractive index is linear to ethanol concentration. The relation can be clearly seen by the equation of :

$$\mathbf{Y = 0.003x + 1.3333}$$

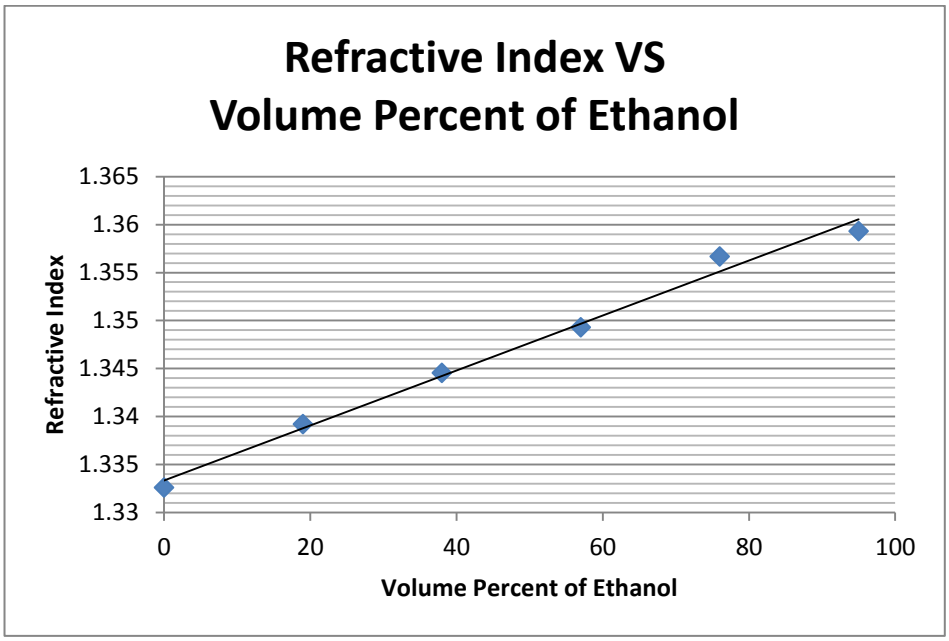

Figure 8 : Refractive Index of Ethanol

4.5 Extraction of Ethanol

During this process of extracting ethanol from bananas, two different fermentation duration were manipulated. The bananas were fermented for 24 hours and 48 hours before being distillate.

Then, the ethanol contain in the bananas were analyzed using the refractometer. Based on the refractive index of the solutions, the concentration of glucose were determined using the formula :

$$\mathbf{n = 0.1363x + 1.2714}$$

where :

n = Refractive Index (RI)

x = concentration of glucose

Table 8 : Concentration of Glucose for Banana Pulp

	Banana	**Refractive Index**	**Concentration of Glucose**
Pulp 24 Hours	**A**	1.33700	0.48129
	B	1.33714	0.48232
	C	1.33704	0.48158
	D	1.33710	0.48202
Pulp 48 Hours	**Banana**	**Refractive Index**	**Concentration of Glucose**
	A	1.33730	0.48349
	B	1.33728	0.48335
	C	1.33732	0.47542
	D	1.33730	0.47594

Table 9 : Concentration of Glucose for Banana Peel

	Banana	Refractive Index	Concentration of Glucose
Peel 24 Hours	**A**	1.33625	0.47579
	B	1.33619	0.47535
	C	1.33620	0.47542
	D	1.33627	0.47594
	Banana	**Refractive Index**	**Concentration of Glucose**
Peel 48 Hours	**A**	1.33643	0.47711
	B	1.33629	0.47608
	C	1.33637	0.47667
	D	1.33630	0.47616

As calculated in Table 8 and Table 9, both the pulp and peel of banana which undergo 48 hours of fermentation produced higher concentration of glucose. It is concluded that higher concentration of glucose can be produced by having a longer time of fermentation process.

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4.6 Chemical Analysis of Banana

Five different concentration of sulphuric acid of 0M, 0.25M, 0.5M, 0.75M, and 1.0M were used for the hydrolysis of banana and analyzed using refractometer. Using the same formula to calculate the concentration of glucose from refractive index, the resulted concentration of glucose for five samples of pulp and peel bananas were tabulated in Table 10 and Table 11.

Table 10 : Concentration of Glucose for Pulp of Banana

Pulp					
Sample (%H2SO4)	**Refractive Index**				**Concentration (% glucose)**
	Sample 1	**Sample 2**	**Sample 3**	**Average**	
0.00	1.33657	1.33672	1.33660	1.33663	0.47858
0.25	1.33793	1.33818	1.33801	1.33804	0.48892
0.50	1.34042	1.34048	1.34044	1.34045	0.50658
0.75	1.34334	1.34351	1.34332	1.34339	0.52817
1.00	1.34354	1.34366	1.34357	1.34359	0.52964

Table 11 : Concentration of Glucose for Peel of Banana

Peel					
Sample (%H2SO4)	**Refractive Index**				**Concentration (% glucose)**
	Sample 1	**Sample 2**	**Sample 3**	**Average**	
0.00	1.33476	1.33483	1.33477	1.33479	0.46505
0.25	1.33679	1.33677	1.33681	1.33679	0.47975
0.50	1.33853	1.33858	1.33863	1.33858	0.49288
0.75	1.34004	1.34001	1.34007	1.34004	0.50360
1.00	1.34147	1.34142	1.34145	1.34145	0.51392

Based on the graph in Figure 8 and Figure 9, sulphuric acid of concentration 1.0 M produced the highest concentration of glucose for both pulp and peel. We can clearly see the trend where higher concentration of sulphuric acid were able to produce higher concentration of glucose. As stated in the methodology, the concentration of sulphuric acid producing the highest glucose concentration will be used for the secondary pretreatment process.

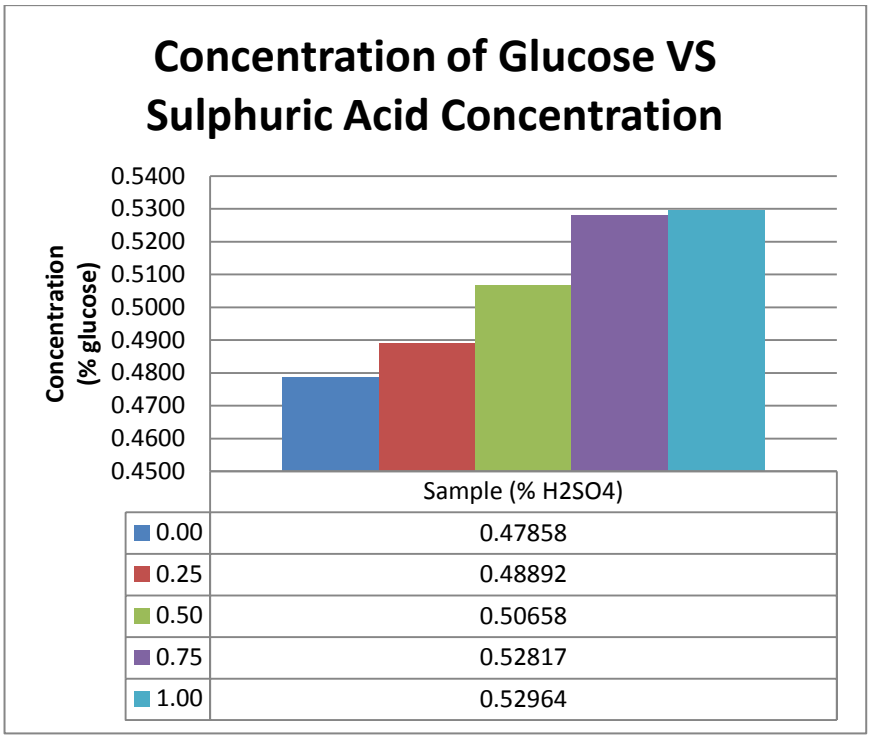

Figure 9 : Effect of Sulphuric Acid Concentration on Concentration of Glucose for Pulp of Banana

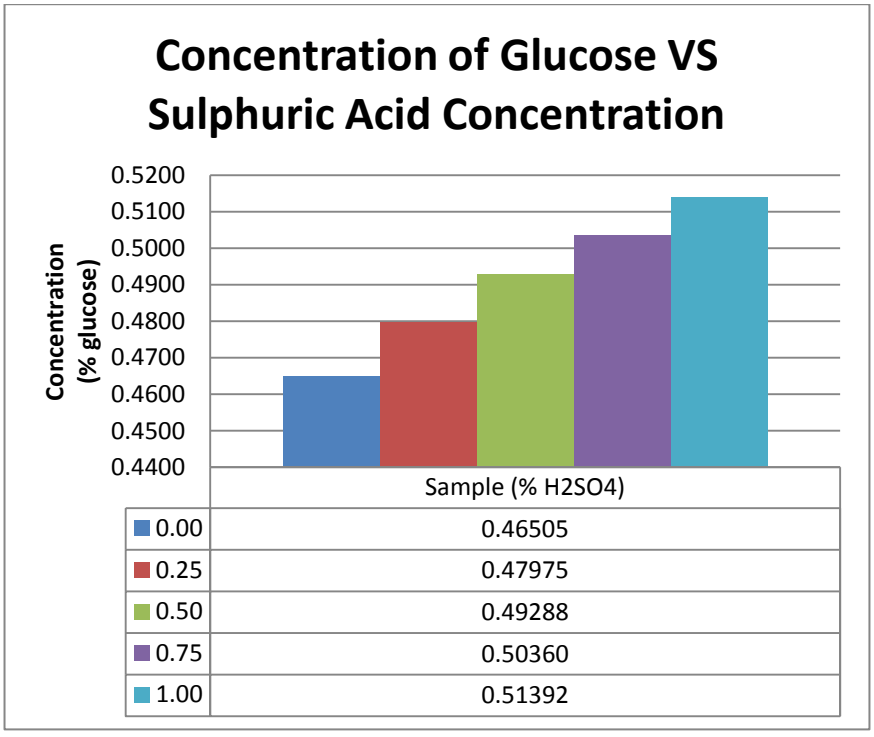

Figure 10 : Effect of Sulphuric Acid Concentration on Concentration of Glucose for Peel of Banana

4.7 Response Surface Methodology

Response Surface Methodology is used in order to increase the accuracy and precision of the results. Sequence of experimental were designed to obtain an optimal response by exploring the relationships between several explanatory or response variables. The experiment were conducted based on the data generated by the RSM. Table 12 shows the experimental design based on Response Surface Methodology for both pulp and peel of the banana.

Table 12 : Experimental Design based on Response Surface Methodology

Run	Factor 1 A : Temperature deg C	Factor 2 B: Yeast Concentration w/v	Response for Pulp Ethanol Concentration g/L	Response for Peel Ethanol Concentration g/L
1	40.00	0.10	0.4773	0.4558
2	35.00	0.30	0.4638	0.4534
3	35.00	0.30	0.4641	0.4536
4	40.00	0.50	0.4754	0.4539
5	30.00	0.10	0.4876	0.4573
6	35.00	0.30	0.4674	0.4564
7	30.00	0.50	0.4753	0.4565
8	35.00	0.58	0.4665	0.4568
9	42.07	0.30	0.4784	0.4592
10	27.93	0.30	0.4661	0.4565
11	35.00	0.02	0.4802	0.4580
12	35.00	0.30	0.4678	0.4563
13	35.00	0.30	0.4671	0.4561
14	35.00	0.30	0.4657	0.4543

The RSM analyzed the data and fit the data into various models such as linear, two-factorial, quadratic, and cubic. The resulted analysis of variance or ANOVA suggested that the quadratic model is the most suitably described for this kind of interaction.

Table 13 : Analysis of Variance for Pulp of Banana

Source	Sum of Squares	Mean Square	F-Value	Prob > F
Model	5.103×10^{-4}	1.021×10^{-4}	4.40	0.0392
A	6.547×10^{-6}	6.547×10^{-6}	0.28	0.6116
B	1.408×10^{-4}	1.408×10^{-4}	6.07	0.0432
AB	2.746×10^{-5}	2.746×10^{-5}	1.18	0.3125
A^2	1.612×10^{-4}	1.612×10^{-4}	6.95	0.0336
B^2	2.000×10^{-4}	2.000×10^{-4}	8.63	0.0218
Lack of Fit	1.521×10^{-4}	5.069×10^{-5}	19.89	0.0072
Pure Error	1.019×10^{-5}	2.549×10^{-6}		

Based on Table 13, the model F-Value of 4.40 shows that the model is significant. There is only 3.92% chance that a 'Model F-Value' this large could occur due to noise. Values of 'Prob > F' less than 0.0500 indicate model terms are significant. Model A shows the value for temperature while Model B shows the value for yeast concentration. In this case, B, A^2, and B^2 are significant model terms. The 'Lack of Fit F-Value' of 19.89 implies the Lack of Fit is significant. There is only a 0.72% chance that a 'Lack of Fit F-Value' this large could occur due to noise. The significant terms contributed to a quadratic model in terms of coded factor and actual factor. For coded factor,

$$\mathbf{Y = 0.47 - 4.195 \times 10^{-3} B + 4.671 \times 10^{-3} A^2 + 5.204 \times 10^{-3} B^2}$$

While for the actual factors,

$$\mathbf{Y = 0.7341 - 0.19074 B + 1.86858 \times 10^{-4} A^2 + 0.1301 B^2}$$

Table 14 : Summary of ANOVA for Pulp of Banana

Model	Standard Deviation	R^2
Quadratic	4.815×10^{-3}	0.7587

The P-Value obtained from the analysis was very low, 0.0072 indicating a good reproducibility of experimental data. While the R^2 value is 0.7587 as shown in Table 14.

While for the peel of banana, the 'Model F-Value' of 1.12 from Table 15 implies the model is not significance relative to the noise. There is a 42.83% chance that a 'Model F-Value' this large could occur due to noise. For this case, there are no significant model terms. Values greater than 0.100 indicate model terms are not significant. The 'Lack of Fit F-Value' of 1.41 implies the Lack of Fit is not significance relative to the pure error. There is a 36.2% chance that a 'Lack of Fit F-Value' this large could occur due to noise.

Table 15 : Analysis of Variance for Peel of Banana

Source	Sum of Squares	Mean Square	F-Value	Prob > F
Model	1.339×10^{-5}	2.678×10^{-6}	1.12	0.4283
A	1.389×10^{-8}	1.389×10^{-8}	0.058	0.9413
B	2.502×10^{-6}	2.502×10^{-6}	1.05	0.3400
AB	3.025×10^{-7}	3.025×10^{-7}	0.13	0.7323
A^2	7.092×10^{-6}	7.092×10^{-6}	2.97	0.1284
B^2	4.26×10^{-6}	4.26×10^{-6}	1.79	0.2231
Lack of Fit	8.598×10^{-6}	2.866×10^{-6}	1.41	0.3620
Pure Error	8.110×10^{-6}	$2.027\ 10^{-6}$		

As shown in Table 16, the P-Value obtained from the analysis was 0.362 while the R^2 value is 0.4449.

Table 16 : Summary of ANOVA for Peel of Banana

Model	Standard Deviation	R^2
Quadratic	1.545×10^{-3}	0.4449

The interaction between independent process factors and their respective response was then plot graphically based on the mathematical analysis of the experimental data. Figure 11 and Figure 12 shows the three-dimensional surface plot and contour plot for pulp of banana accordingly.

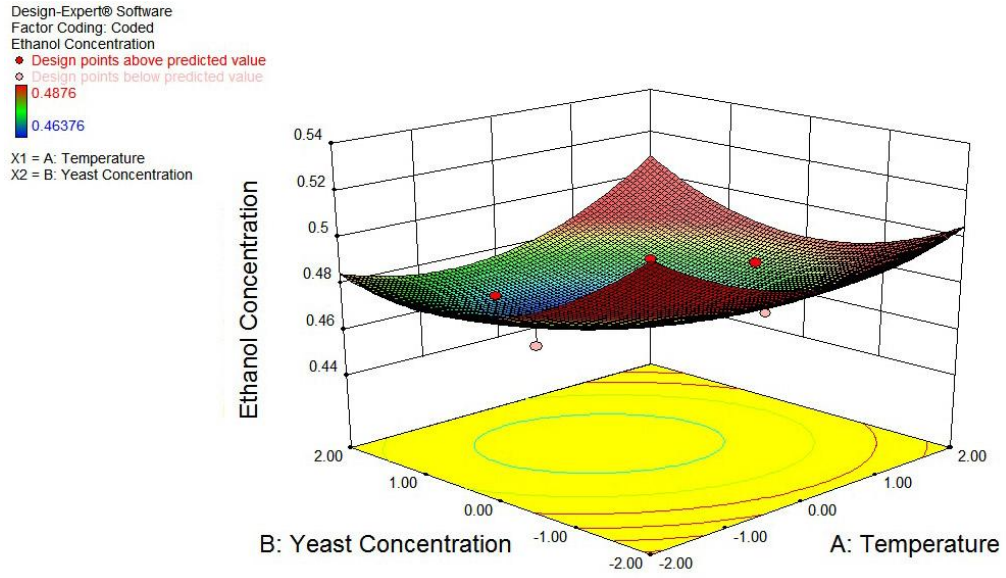

Figure 11 : 3D Surface Plot for Pulp of Banana

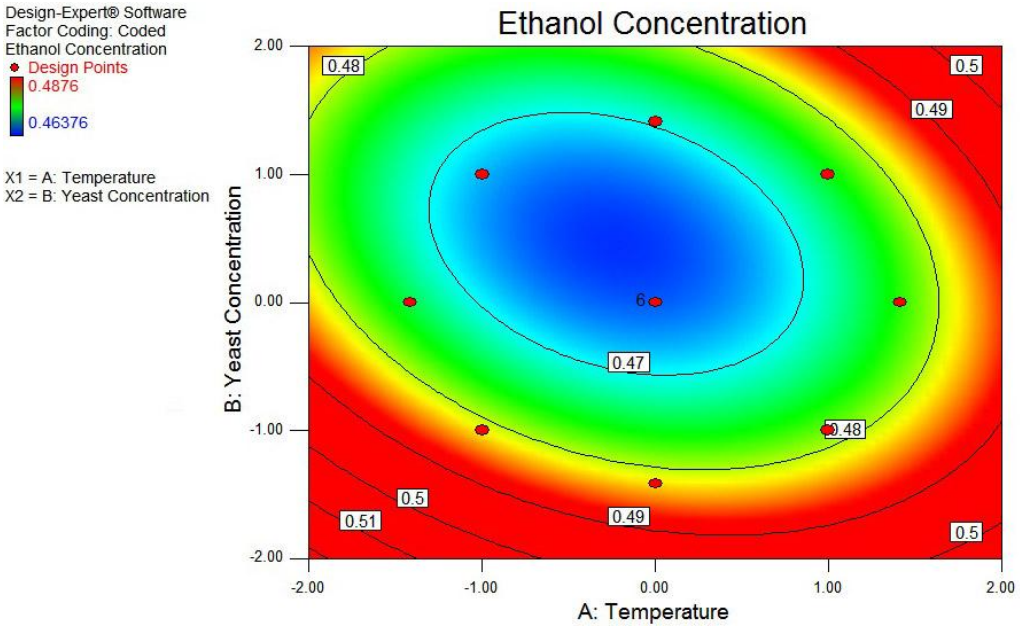

Figure 12 : Contour Plot for Pulp of Banana

For the pulp of banana in Figure 11 and Figure 12, the response surfaces based on the two parameters, temperature and yeast concentration are varied in the range of 30-40°C and 0.1-0.5% (w/v) respectively. While, the pH and fermentation time are fixed to 5 and 15 hours respectively. From the figure, it is clearly shows that ethanol concentration goes higher as the colour contour goes from blue to red.

While, for the peel of banana, similar range of values are used for the parameters but there are only small changes among the values of ethanol concentration against the changes of yeast concentration and temperature as shown in Figure 13 and Figure 14.

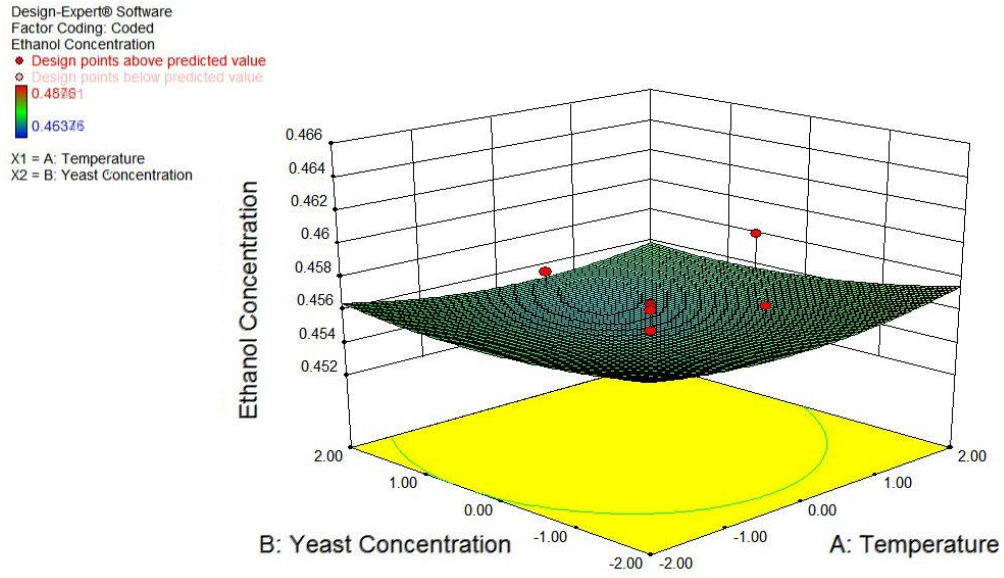

Figure 13 : 3D Surface Plot for Peel of Banana

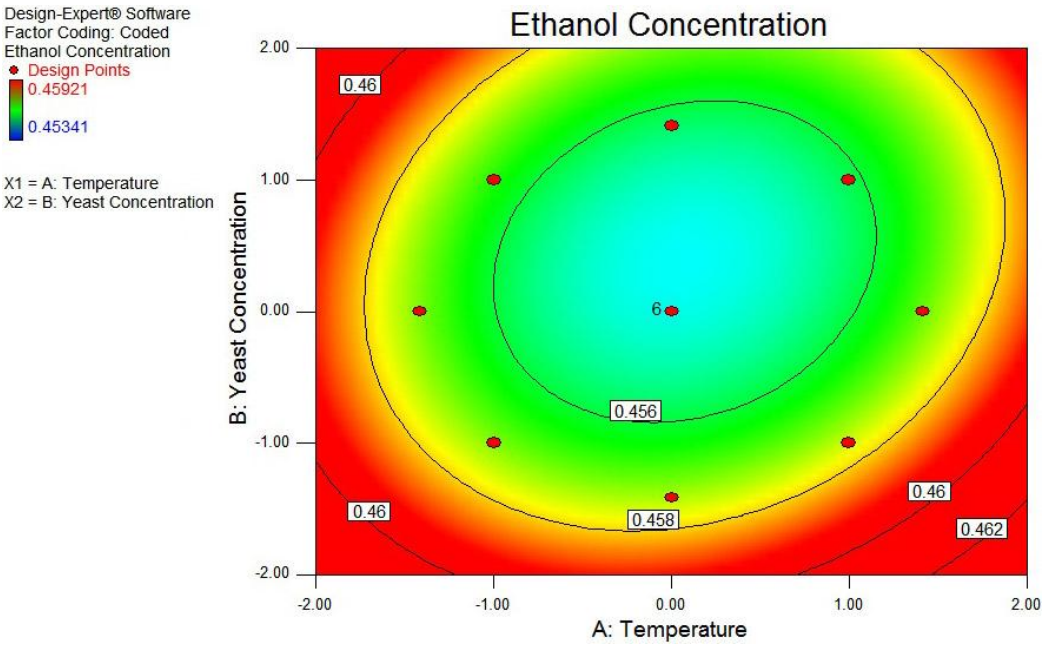

Figure 14 : Contour Plot for Peel of Banana

Numerical optimization are used in order to synthesize the maximum ethanol yield at the optimum temperature and yeast concentration. This numerical optimization gives the highest desirability which indicates the highest ethanol yield at the optimum condition of temperature and yeast concentration.

Table 17 : Numerical Optimization for Pulp of Banana

Reaction Condition	Temperature (°C)	Yeast Concentration % (w/v)	Predicted Yield (g/L)	Desirability
1	30.0	0.1	0.4818	0.755
2	40.0	0.1	0.4783	0.611
3	39.3	0.1	0.4773	0.567
4	40.0	0.5	0.4752	0.479
5	40.0	0.4	0.4721	0.346

Table 17 shows the numerical optimization for pulp of banana. The most desirable operating condition is at temperature of 30.0°C and 0.1% w/v of yeast concentration. While for peel of banana, in Table 18, the numerical optimization shows that the optimum condition of temperature is at 40.0°C and 0.1% w/v of yeast concentration.

Table 18 : Numerical Optimization for Peel of Banana

Reaction Condition	Temperature (°C)	Yeast Concentration % (w/v)	Predicted Yield (g/L)	Desirability
1	40.0	0.1	0.4575	0.711
2	39.3	0.1	0.4572	0.658
3	30.0	0.1	0.4570	0.630
4	30.0	0.5	0.4565	0.532
5	40.0	0.5	0.4559	0.423

CHAPTER 5

CONCLUSION AND RECOMMENDATION FOR FUTURE WORK

5.1 Conclusion

In conclusion, this project serves to enhance the research and development of the biofuel energy sources towards the sustainability of energy. In this context, banana biofuel is gaining more significance as an alternative renewable energy source in view of the global depletion of petroleum and natural gas.

The study on the characteristics of banana on different stages of ripeness shows that weight of banana decreased during the growth period of fourteen days. A drastic dropped of weight of banana during day nine to day eleven where the banana having a changes from green to yellow further conclude that the reduction in weight of bananas were due to losses associated with both drying of the banana and the internal metabolic activities (Dhabekar and Chandak, 2010).

Different parameters such as temperature and yeast concentration associated with ethanol production from banana are being studied. The results are then optimized using Response Surface Methodology in order to fully utilized the banana wastes. From the experiment, the second objective is proven, different parameters of temperature and yeast concentration affect the production of ethanol from banana.

Other than that, the RSM successfully generate optimum condition of yeast concentration and temperature for both, the pulp and peel of banana. The optimum yeast concentration and temperature based on numerical optimization for production of bioethanol from banana pulp is at 30°C and 0.10% w/v yeast concentration while for peel is at 40°C and 0.10% w/v yeast concentration, respectively.

5.2 Recommendation for Future Work

For the first recommendation, it is highly recommended to use the High Performance Liquid Chromatography or also known as HPLC for analyzation in order to get a more accurate results. Early booking should be made and we should avoid the equipment failure problem which frequently results in the failure problem of getting a more accurate results.

Banana peel was found to be less dependent on yeast concentration and temperature during fermentation compared to the banana pulp. It is recommended to study other parameters such as pH and fermentation time in order to increase the ethanol concentration.

Besides that, in order to prevent errors, the method should be followed accordingly and measurement made are done correctly. Since the main parameter that needs to be studied are related to the measurement technique, small changes might affect the whole results of the experiment.

Last but not least, the optimum condition that have been studied for ethanol production can be applied in large scale industry. This can be done by increasing the scale of consumption in order to produce the desired amount of ethanol.

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