OPTIMISATION OF ETHANOL PRODUCTION BY FERMENTATION OF AGRICULURAL RAW METERIALS.

LIM SYLY

A project report submitted in partial fulfilment of the
requirements of the award of the
Bachelor of Engineering (Hons.) Chemical Engineering

Faculty of Engineering and Science
Universiti Tunku Abdul Rahman

MAY 2011

DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

Signature : _____

Name : ____LIM SYLY_____

ID No. : ____07 UEB 05125_____

Date : ____10th May 2011_____

APPROVAL FOR SUBMISSION

I certify that this project report entitled **"OPTIMISATION OF ETHANOL PRODUCTION BY FERMENTATION OF AGRICULTURAL RAW MATEIALS"** was prepared by LIM SYLY has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Engineering (Hons.) Chemical Engineering at Universiti Tunku Abdul Rahman.

Approved by,

Signature : _____

Supervisor : Prof. Dr. LOW CHONG YU__

Date : _____

Specially dedicated to
my beloved grandmother, father and mother

ACKNOWLEDGEMENTS

I would like to thank everyone who had contributed to the successful completion of this project. Throughout this training, I am very fortunate to be blessed with the guidance and encouragement from my research supervisor, Prof. Dr. Low Chong Yu. I would like to thanks for his invaluable advice, guidance and his enormous patience throughout the development of the research. He had shared a lot of ideas to me during my research period, it is means a lot to me.

In addition, I would also like to express my deep sense of gratitude to my loving parent and friends who had helped and given me encouragement throughout my four year course of studies.

Last but not least, I would like to Thanks Mr. Voon, Mr. Kho and Miss Leong for the guidance in the laboratory work.

OPTIMISATION OF ETHANOL PRODUCTION BY FERMENTATION OF CASSAVA

ABSTRACT

With the world's fossil fuel depleting in supply, agricultural materials were examined for the bio-ethanol production. Bio-ethanol has proven will reduce in greenhouse gases emissions. In humid tropical Malaysia, cassava is one of the options as the raw material for bio-ethanol production. Brazil is the world lead bio-ethanol production country which had been started from 40 years ago. Sugarcane is the main raw material for the bio-ethanol production in Brazil. In this research, raw cassava was bought from the local market and the cassava flour was prepared by drying and grinding. Industrial high strain yeast was used as the microorganism for the fermentation process. Different initial reducing sugar was used for the fermentation of cassava to produce bio-ethanol. 20 g/L, 40 g/L, 60 g/L and 80 g/L of initial reducing sugar contents were used. pH value, reducing sugar profile, cell pellet concentration and ethanol concentration were tested for each of the samples. Finally, 60 g/L of initial reducing sugar gives the highest yield that is 0.669128 g ethanol/ g reducing sugar where 80 g/L of initial reducing sugar gives the highest productivity of 1.005807 g ethanol/L/hr. Modified Gompertz equation was used for the kinetic modelling by using the Excel Solver Add-in Tools and Data Analysis Add-in Tools. According to the correlation coefficient (R^2), Modified Gompertz equation showed good agreement with the experimental data obtained, which means it is suitable to describe the ethanol fermentation process from cassava.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS / ABBREVIATIONS

T	Temperature, °C
%	Percent
μ	Micro
P_m	Maximum product concentration (g/L)
P	Product concentration (g/L)
$r_{p,m}$	Maximum product formation rate (g/(L·h))
t_L	lag phase/time to attain exponential product formation (h)
α	Alpha
R^2	Correlation Coefficient

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background

Nowadays, ethanol production from renewable resources has received great attention because of the petroleum shortage (Amuha and Gunasekaran, 2001) and the increases of the population throughout the world. According to Ayhan Demirbas (2009), the larger part of petroleum and natural gas reserves is located within a small group of countries. For example, the Middle East countries have 63% of global reserves and are the dominant supplier of petroleum. This energy system is unsustainable because of equity issues as well as environmental, economic, and geopolitical concerns that have far reaching implication. However, the renewable energy resources are more evenly distributed than fossil or nuclear resources.

Ethanol or ethyl alcohol which produced by starch hydrolysis (liquefaction and saccharification) and sugar fermentation processes from biomass is called as bio-ethanol or called as bio-fuel (Demirbas, 2008). Bio-fuel is the fuels which derived from the biomass. Bio-fuel is the renewable resources since it was mainly derived from the agricultural raw materials. Normally bio-fuel is produced by living organisms such as plants, animals and microorganisms or from metabolic by-products of such organisms.

With the growing of environmental awareness, bio-fuel had discovered to be the sustainable-source and eco-friendly energy that able to solve the energy crisis - depletion of fossil fuel. Bio-fuel is the ideal fuel to substitute for petrol. Such

solution will able to maintain the current transport infrastructure well in the future and it is good for our future generation. According to Christiana N. Ogbonna and Eric C. Okoli (2009), bio-fuel is produced commercially in various countries and methods as the renewable transportation fuel.

Based on the weather in Malaysia, climate conditions are too wet and difficult to obtain sugarcane with high sugar content. In addition, if Malaysia using sugarcane or corn as the raw material for production of bio-ethanol, the additional crop demand will raises thus causing the price of the sugar which obtain from sugar cane and the food items like sweet corn as the animal feed be more expensive. As a result, starch-containing root crops like sweet potato and cassava (figure 1.1) are more suitable to be the raw material of the bio-ethanol production in the subtropics zone.

Figure 1.1: Field-growth cassava plant

1.2 Problems or issues statements

Nowadays, world is approaching the “peak oil” which defined by the geologists, where is the points which daily demand will exceed the supply of the oil. The problem of the depletion of the energetic resources mainly based on non-renewable fuels caused the price of the oil shot up tremendously within these few years. For instance, Óscar J. Sánchez point out that permanent crisis in the Middle East and the speculation in the stock exchange, have caused the oil price to reach such elevated values of 100 dollars per barrel.

From the environmental point of view, when the burning of fossil fuel has substantially increased the emission of the greenhouse gases to the atmosphere. For example, carbon monoxide, carbon dioxide, methane and nitrous oxide will be emitted. These greenhouse gases actually trapped the heat in the atmosphere, caused the global warming and results the melting of Antarctic ice cores and raising the sea level. Moreover, the loss of coral reefs, earthquakes, volcano and the tsunamis will be happen more frequently.

Climate changes, as a result of global warming caused by greenhouse gases, mainly carbon dioxide (CO_2) produced during the burning of fossil fuel, have been causing significant changes in the ecosystems and leading to nearly 150,000 additional deaths every year (Teske.S, 2007). The constant rise in Earth's average temperature, threatens millions of people with the growing risk of hunger, floods, water shortage and diseases such as such as malaria.

1.3 Aims and Objectives

1) To optimize of bio-ethanol production by batch fermentation of cassava roots as the raw material by changing the initial reducing sugar for the fermentation.
2) To analyze bio-ethanol concentration by using gas chromatography.
3) To develop the kinetic model for bio-ethanol production from cassava roots.
4) To fully understand the current situation of bio-fuel production in Malaysia.
5) To obtain the yield of the bio-ethanol produced from the cassava roots and compare it with the production of bio-ethanol from other biomass.

1.4 Learning Outcomes

1) The bio-ethanol was produced by the batch fermentation of cassava roots. The ethanol production had been optimized by changing the initial reducing sugar content. 60 g/L of initial reducing sugar will gives higher ethanol yield then 80 g/L of initial reducing sugar will gives higher productivity.

2) The bio-ethanol which obtained from the batch fermentation was tested by the gas chromatography.
3) Modified Gompertz equation was used as the kinetic model for the batch fermentation for the bio-ethanol production from cassava roots.
4) The current situation of bio-fuel production in Malaysia had been studied and understood.
5) The maximum ethanol yield from cassava as raw materials had been compared with the other raw materials such as rice, wheat, maize

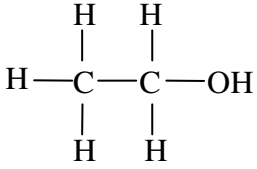
CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to ethanol and bio-ethanol

Ethanol (ethyl alcohol, bio-ethanol) is falls under the category on alcohol group. Table 2.1 shows the physical and chemical properties of ethanol. During the early years, ethanol was served as alcoholic beverages. Later on in the year 1826, ethanol was used as lamp fuel and a few decades after that, ethanol fuel was used to run automobiles. There are two kinds of ethanol, that are synthetic ethanol and other is the bio-ethanol. Synthetic ethanol is the petroleum product which able be produced by convert ethylene using steam and catalyst. However, bio-ethanol is produced from the bio-fermentation of sugars, which is the process that will be done throughout this research. Ethanol forms carbon dioxide and water when it burns in air with an almost invisible blue flame.

Table 2.1: Physical and Chemical Properties of Ethanol (Columbia Electronic Encyclopedia (6th edition) (2007).

Property	***Value***
Molecular formula	C_2H_5OH
Molecular structure	
Physical state	Clear colourless liquid, flammable
Melting point	-117.3 ^oC
Boiling Point	78.5 ^oC
Water solubility	Very miscible

According to S.L. Tan (2010), Brazil is a shining example of the success of bio-ethanol throughout the world. The world ethyl alcohol production has reached about 51,000 mill liters (Renewable Fuels Association, 2007), being the USA and Brazil the first producer (see Table 2.2). Currently, ethanol is the alcohol of choice and petrol cars can take up to 10 % ethanol (E10) without the need to modify their engines. In Brazil, the bio-ethanol is used interchangeably with petrol in specially modified car engines called Flex car. In addition, Bio-ethanol had been commercially produced in many countries as a renewable transportation fuel. According to Low (2009), USA and Canada use corn as their feedstock for bio-fuel production, China using cassava and molasses and Thailand using cassava. Many countries have implemented or are implementing programs for addition of ethanol to gasoline (see Table 2.3).

Table 2.2: World production of ethyl alcohol (mill liter) (Adapted from Ó.J. Sánchez, C.A. Cardona/Bio-resource Technology 99 (2008) 5270-5295.

Country	***2006***	***2007***
USA	18,376	16,139
Brazil	16,998	15,999
China	3,849	3,800
India	1,900	1,699
France	950	908
Germany	765	431
Russia	647	749
Canada	579	231
Spain	462	352
South Africa	386	390
Thailand	352	299
United Kingdom	280	348
Ukraine	269	246
Colombia[a]	269	27
Poland	250	220
Total	51,056	45,988

[a] These data correspond to the fuel ethanol produced in new distilleries whose construction started in 2005 (Londono, 2007); industrial and beverage alcohol are not include, although their share is significantly lower. Modified from Renewable Fuels Association, 2007.

Table 2.3: Fuel ethanol programs in some countries (Adapted from Ó.J. Sánchez, C.A. Cardona/Bio-resource Technology 99 (2008) 5270-5295.

Country	***Feedstock***	***Percentages of ethanol in gasoline blends, % (v/v)***	***Remarks***
Brazil	Sugar cane	24	ProAlcool program; hydrous ethanol is also used as fuel instead of gasoline
USA	Corn	10	Oxygenation of gasoline is mandatory in dirtiest cities; tax incentives; some states have banned MTBE; 85 % blends are also available.
Canada	Corn, wheat, barley	7.5 - 10	Tax incentives; provincial programs aimed to meet Kyoto Protocol
Colombia	Sugar cane	10	Began in November 2005; total tax exemption
Spain	Wheat, barley	-	Ethanol is used for ETBE production; direct gasoline blending is possible.
France	Sugar beet, wheat, corn	-	Ethanol is used for ETBE production; direct gasoline blending is possible.
Sweden	Wheat	5	85 % blends are also available; there is no ETBE production.
China	Corn, wheat	-	Trial use of fuel ethanol in central and north-eastern regions.
India	Sugar cane	5	Ethanol blends are mandatory in 9 states.
Thailand	Cassava, sugar cane, rice	10	All gasoline stations in Bangkok must sell ethanol blends; ethanol blends will be mandatory from 2007.

Adapted from Murray (2005) and Berg (2004).

From figure 2.1 shows the trend of production of ethanol. In average, 73 % of produced ethanol worldwide corresponds to fuel ethanol, 17 % to beverage ethanol and 10 % to industrial ethanol. It is obviously shows that the production of bio-ethanol is increased dramatically and is believed that this trend will be kept increasing in the future.

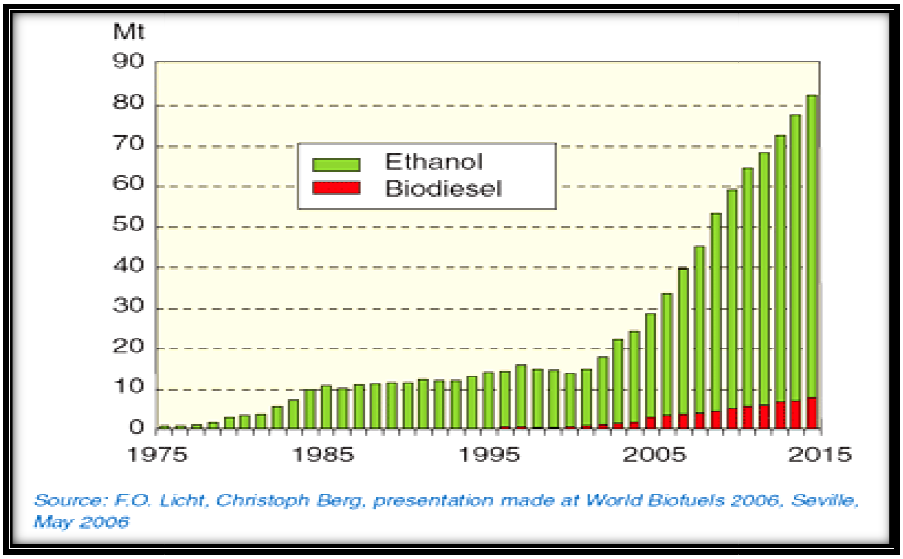


Figure 2.1: Trends of production of bio-fuel.

(Source:F.O. Licht, Christoph Beng, presentation made at World Biofuel 2006)

The first vehicle which completely powered by a bio-fuel made from cassava roots is shows in figure 2.2. This vehicle was already on the move in the department of Valle del Cauca, Colombia. The test run was being carried out using a CIAT pick-up truck. CIAT, together with Clayuca, a consortium that supports cassava research and development in Latin America and the Caribbean, recently inaugurated a pilot small-scale processing plant that produces hydrated ethanol using cassava, sugar sorghum, or sweet potato as raw material. This fuel contains 4 % to 5 % water, hence its name of hydrated ethanol (CIAT, International Centre for Tropical Agriculture, 2009).

Figure 2.2: First vehicle which completely powered by cassava-based bio-fuel.

(Source: CIAT, International Centre for Tropical Agriculture, 2009).

2.2 The Current Situation for Petrol

Based on Figure 2.3, the prices for the RON 97 and RON 95 in Malaysia were kept increasing from July 2010 till April 2011. This is mainly due to the depletion of the non-renewable resources throughout the world. The numbers of available oil well for extracting petroleum in Malaysia are greatly decreased recently and this caused us to find another renewable resource to replace current crude oil for the transportation used. Besides, the current situation in Egypt could disrupt the supply of oil and caused the price of crude oil goes up (Figure 2.4), resulting in a RON 97 hike. If the price of the petrol is keeping increasing in the future, most of the Malaysian will not able to afford the petrol price.

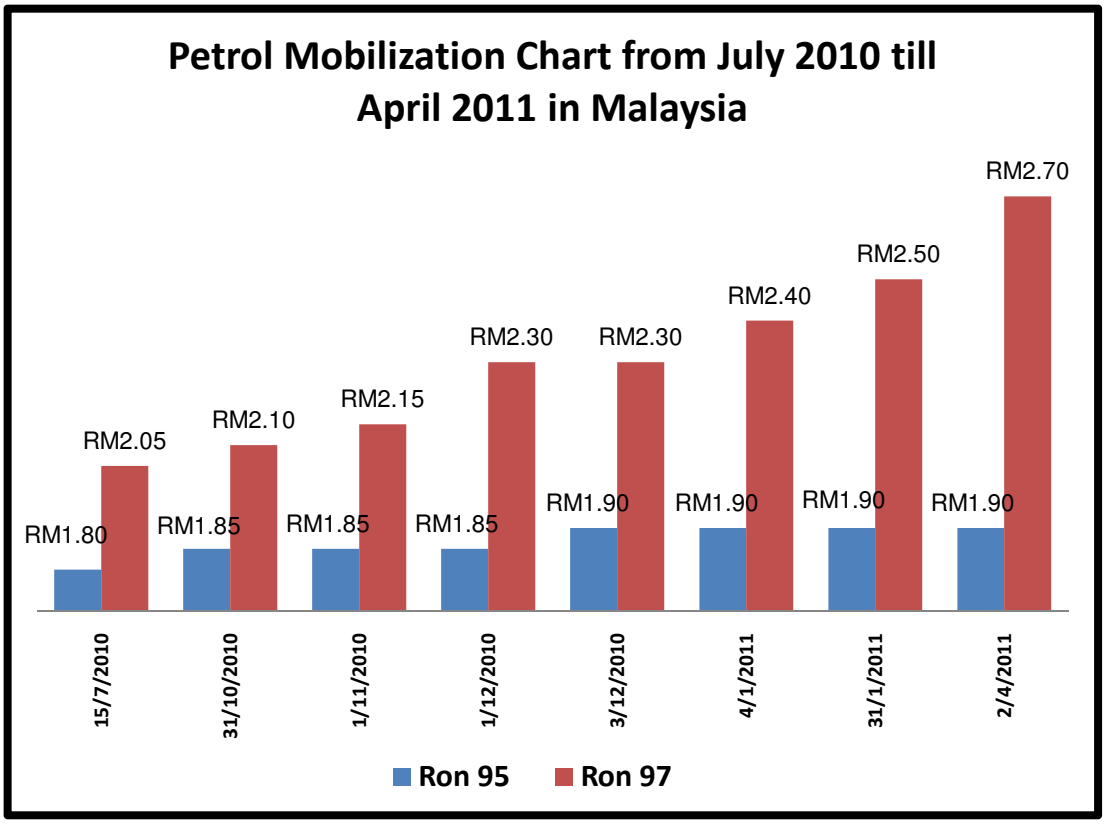


Figure 2.3: Petrol Price Mobilization for RON 95 and RON 97 in Malaysia. (Oriental Daily, 8th Feb 2011 and Nanyang Daily, 2nd April 2011).

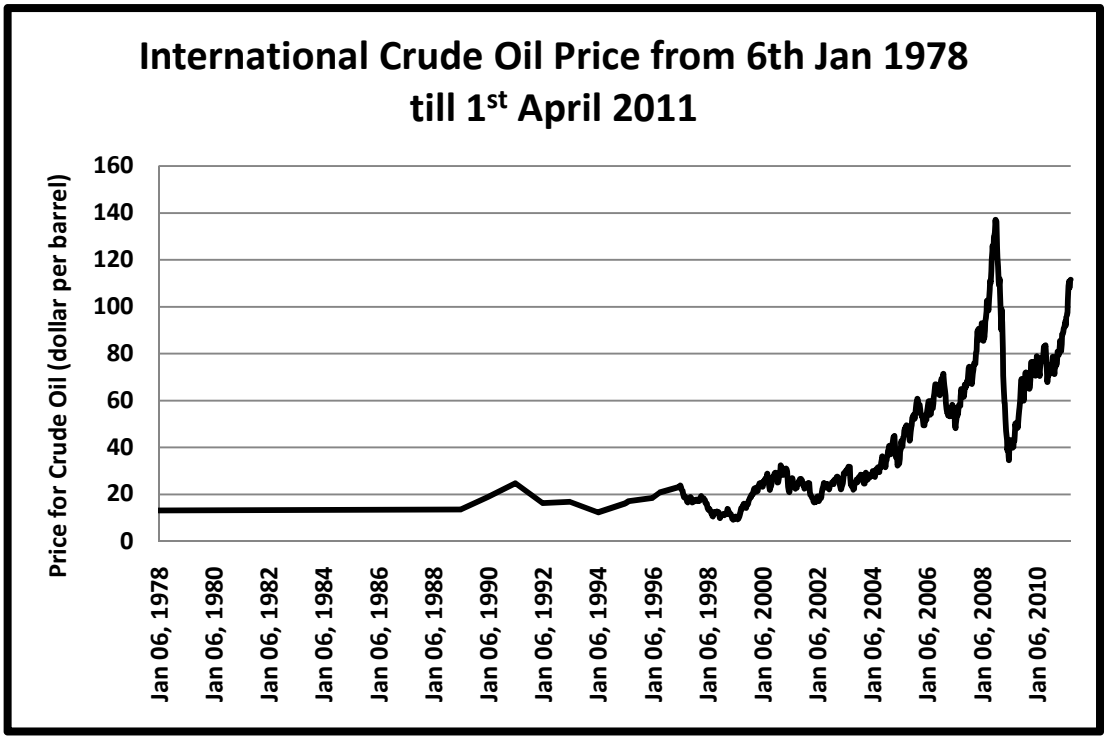


Figure 2.4: Price for Crude Oil from 6th Jan 1978 till 1st April 2011.

Source: Energy Information Administration available at

http://tonto.eia.gov/dnav/pet/hist/LeafHandler.ashx?n=PET&s=WTOTWORLD&f=W

2.3 Ethanol Derivatives

There are few processes which use ethanol as the starting point and produce the ethanol derivatives. Biologically produced hydrocarbons are excellent substitutions to petrochemical products when renewable feedstock is desired. Bio-ethanol is the renewable feedstock since it able to produce from the starch agricultural material. The following are the derivatives which can produce from ethanol:

a) Acetic Acid

The ethanol is taken to the acetic acid plant, and is first converted to acetaldehyde, thereafter to acetic acid. Catalyst in the acetic acid reactor is manganese acetate, which is consumed at very low rates. Acetic acid is raw material for vinylacetate, acetic anhydride, cellulose acetate, acetic esters, glycol ether acetates, chloroacetic acid, PTA and pharmaceuticals.

b) Acetaldehyde

The acetaldehyde reactors have silver gauze catalyst. The silver is not consumed, but about every 12 months the gauze is sent back to the manufacturer for remarking. Acetaldehyde is formed mainly by the oxidation of ethanol and partly by dehydration. Acetaldehyde is raw material for acetic acid, crotonaldehyde, pyridine, pentaerythritol, peracetic acid and vinylacetate.

c) Acetic Anhydride

The acetic acid is cracked to ketone in a reactor by help of a catalyst. Absorption of ketone gas in acetic acid to crude acetic anhydride is followed by distillation to pure acetic anhydride. Systems for concentration of dilute acetic acid and recovery of distillation residues are included. Acetic Anhydride is raw material for cellulose acetate (fibers, films, plastics, cellulose lacquers), aspirin, agricultural chemicals, fragrances, pharmaceuticals and explosives.

d) Ethyl Acetate

Ethyl Acetate is solvent for paints, extraction agent, and raw material for pharmaceuticals, cosmetics and polishes.

e) Ethylene

Ethanol is converted to ethylene by letting vaporised ethanol pass through a catalyst reactor system. The hot ethylene/water vapour mixture leaving the last reactor bed enters a Waste Heat Boiler where some of the heat is recovered as steam. The ethylene is cooled and any acetic components are removed with the condensed water. A small quantity of caustic is added to neutralise any acids. In order to use the produced ethylene for polyethylene production it is purified using a caustic scrubber, a fixed bed absorber dryer and distillation and stripper columns. The liquid product ethylene obtained from the bottom of stripper is used to cool a number of process streams and is then delivered at battery limits as a vapour. Ethylene is raw material for PVC, polyethylene, ethylene oxide etc.

2.4 Raw materials for production of bio-ethanol

Bio-ethanol is renewable energy source (produced from crops). Hence, there will be no worry about the depletion of this source. By referring to J. C. Escobar et al., 2008, bio-ethanol is defined by the US DOE as an alternative fuel based on alcohol, produced by the fermentation and distillation of raw materials with high content of sugars and starches for example the cassava and sweet potato. Besides these raw materials, ethanol can be obtained from lingo-cellulosic biomass or called as non-edible biomass from tress and some herbs. In general, there are few different technologies for producing fuel ethanol, they are from sucrose-containing feed stocks (mainly sugar canes), starchy materials, lignocellulosic biomass and macroalgae.

According to Wyman (1999), the present commercial bio-ethanol production are relies on the fermentation of sucrose from sugar cane and molasses or glucose derived from starch-based crops such as corn, wheat and cassava, and there is a growing need for the industry to improve technology and expand the production. However, the use of such crops to obtain the bio-ethanol will affect the food security through competition with food for agricultural land (Christiana N. Ogbonna and Eric C. Okoli, 2009). As a result, technology to produce the bio-fuel from other raw materials is occurred by utilize lignocellulosic materials such as crop residues, grasses, sawdust, wood chips, animal and industrial residues (Prasad.S and Singh A., 2007). Even in this research cassava was use as the raw material for the production of bio-fuel, it is not the staple food in Malaysia. There will be no worry about the competition between the fuel and food.

Unfortunately, the cost of bio-ethanol production from lignocellulosic material is relatively high based on current available technologies. Collins K. (2011) stated that the cost of ethanol production from lignocellulosic substrate is approximately twice as high as that from starchy substrates. In addition, the production of bio-ethanol from this kind or raw material are having lower yield, high cost of hydrolysis and its need longer time for the bio-ethanol production. Then, the production technology of bio-ethanol by using the algae as the raw material is still considered as the new technology and is still under the research phase.

In this study, cassava was used as the raw material to produce bio-ethanol. Cassava or called tapioca, in particular, is considered a very attractive raw material for bio-ethanol production, as it is inexpensive and is not affected by food and feed shortage concerns (Hu Z., et. al, 2006). Besides the cost to produce a ton of bio-ethanol is approximately $ 40 to $ 60 less than using corn or wheat, which has been the common starch used for bio-ethanol production (Rubo L., et. al, 2008)

Based on Figure 2.5, bio-ethanol production able be produce from different types of raw materials, the bio-fuels that derive from edible (starch-based bio-fuel) biomass, primarily corn and soybeans (in the U.S.) and sugarcane (in Brazil) are low-hanging fruits in the forest of possible bio-fuel, given that the technology to convert these feed stocks into fuels already exists (180 refineries currently process corn into bio-ethanol in the U.S.) (N.Nair, M. Mel, M.I.A. Karim and R.M Yunus, 2009).

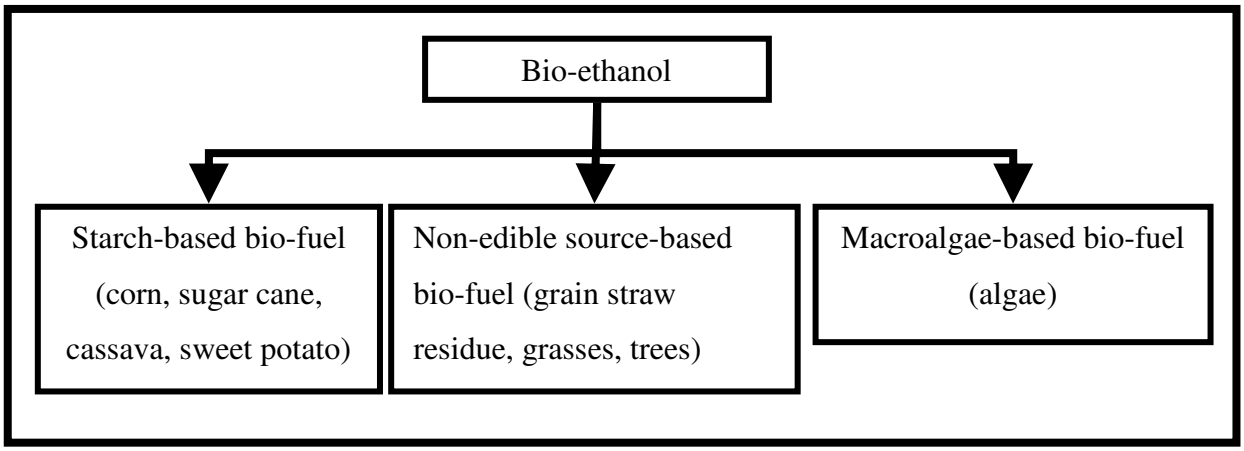


Figure 2.5: Bio-fuel production from different types of raw material

2.5 Ethanol fermentation

Ethanol fermentation is a biological process in which the glucoses ($C_6H_{12}O_6$) are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products. Generally, any raw material which consists of sugar is able to produce ethanol. The general reaction for ethanol production during fermentation is

$$\text{Sugar} \xrightarrow{\text{Microorganisms}} \text{Ethanol} + \text{By-products}$$

There are two type of microorganism which used to convert the glucoses to form ethanol. *Saccharomyces cerevisiae* is the commonly used microorganism that being used due to its capability to hydrolyze starch to glucose and fructose. Whereas, recombinant bacteria such as *Escherichia coli, Klebsiellaoxytoca, and Zymomonas mobilis. E. coli and K. oxytoca* are the microorganism which able to convert biomass to ethanol especially lignocellulosic biomass. Industrial's yeast *(ATCC 36858 S. Cerevisiae)* was the microorganism that being used throughout this research, the yeast was cultivated before being used in the fermentation process. The ethanol fermentation in this research was classified as an aerobic process because fermentation by using yeast required the present of oxygen. Conversion of starch and sugars to ethanol through fermentation is shown below:-

$$(C_6H_{10}O_5)n + nH_2O \rightarrow nC_6H_{12}O_6$$

starch or cellulose → glucose

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2C_6H_{12}O_6$$

maltose → glucoses

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

glucose(hexose) → ethanol

Different raw materials consists of sugar will undergo the same fermentation process and produce ethanol but they will have different production method. Ahindra (2007) found out that for the starchy materials such as cassava, sweet potato and wheat are necessary to undergo the hydrolysis (liquefaction and saccharification) process. Based on C. F. Gonzalez, J. I. Farina, L. I. C. de Figueroa (2008), cassava starch is composed of unbranched amylase (20 ± 5 %) and branched amylopectin (80 ± 5 %) both of wish can be hydrolyzed either with acids or enzymatically (either with pure enzymes or amylase-producing microorganisms) to release glucose and maltoologosaccharides (reducing sugar). Thereafter, both products are able to transport across the cell membrane and metabolized by yeasts. In shorts, hydrolysis is the process to hydrolyze the polymer of glucose to the fermentable sugars by the action of enzymes.

$C_6H_{12}O_6$	→	$2C_2H_5OH$	+	$2CO_2$
(glucose)		(ethanol)		(carbon dioxide)

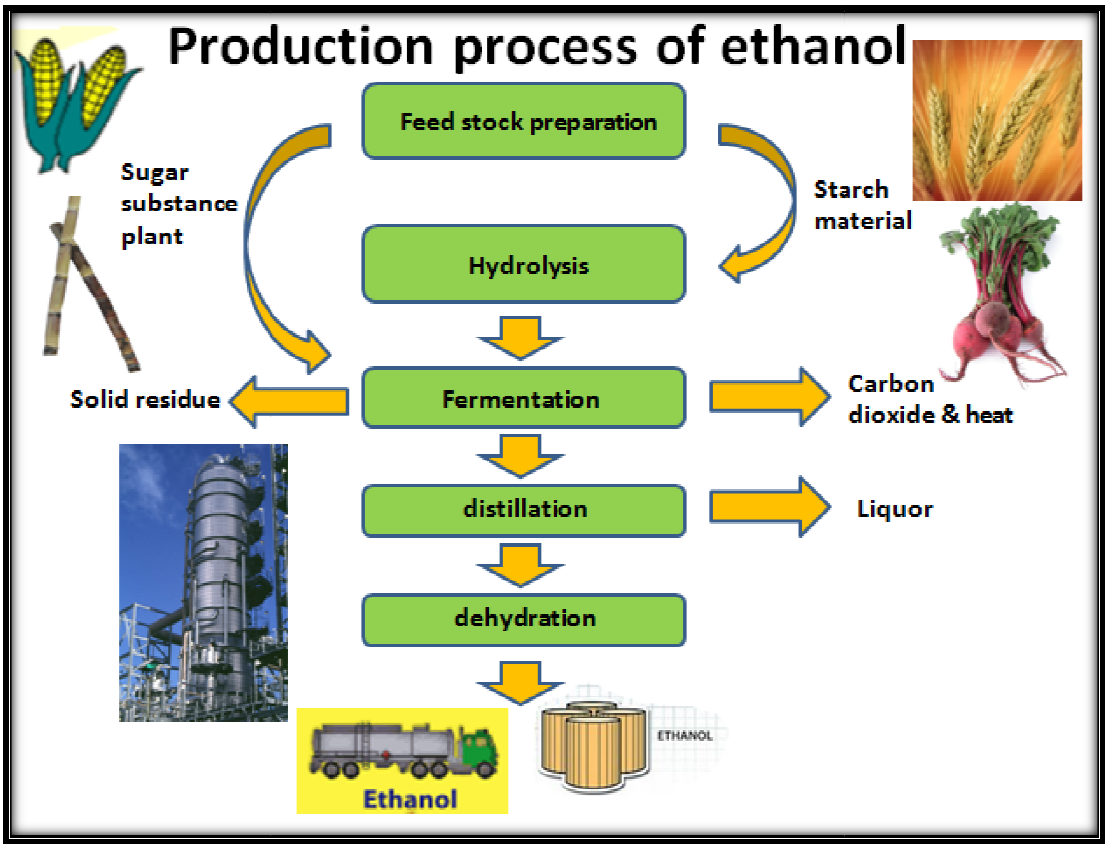


Figure 2.6: Industrial production process of ethanol

(Source: Peter & Jos, 2008)

After the fermentation process, the separation and the purification of the ethanol will be done. The distillation process will be carried out as separate the unwanted compound from the ethanol, the dehydration is to eliminate the water molecule that contained in the ethanol because the water molecule will caused the corrosion problem in the vehicle's engine. It is necessary to produce high purity of bio-ethanol from the distillation process.

2.6 Pros and Cons of bio-fuels

From the point of view on social and environmental concerns, bio-ethanol may seem as the solution for a world facing a future with ever-diminishing sources of fossil fuels. Bio-ethanol is non-toxic, water soluble and is effectively mixed with the gasoline and form "anhydrous ethanol".

Bio-ethanol environmentally friendly clean-burning renewable fuel which the oxygen molecule will add to gasoline when the ethanol presented, so the oxygen actually will helps bio-ethanol to have the complete combustion and significantly reduced emission of carbon monoxide (CO) by 30 % and smog-forming volatile organic compounds (VOC) emissions by 12 % (Low., 2009). Based on Rakesh. A and N. R. Singh (2010), bio-ethanol as the fuel will prevent the climate change and provide us the potential to maintain the current transport infrastructure well for the future generation.

From the environmental point of view, bio-ethanol fuel is completely biodegradable. Although carbon dioxide (CO_2) will be produced while the bio-ethanol is burn as the fuel energy for transportation usage, carbon dioxide will be utilized by the cassava plant for the photosynthesis. By compared with the petroleum, the CO_2 actually is trapped inside the atmosphere and caused the global warming. By refer to Figure 2.7, a full carbon cycle could be attained for the bio-fuel as the transportation use.

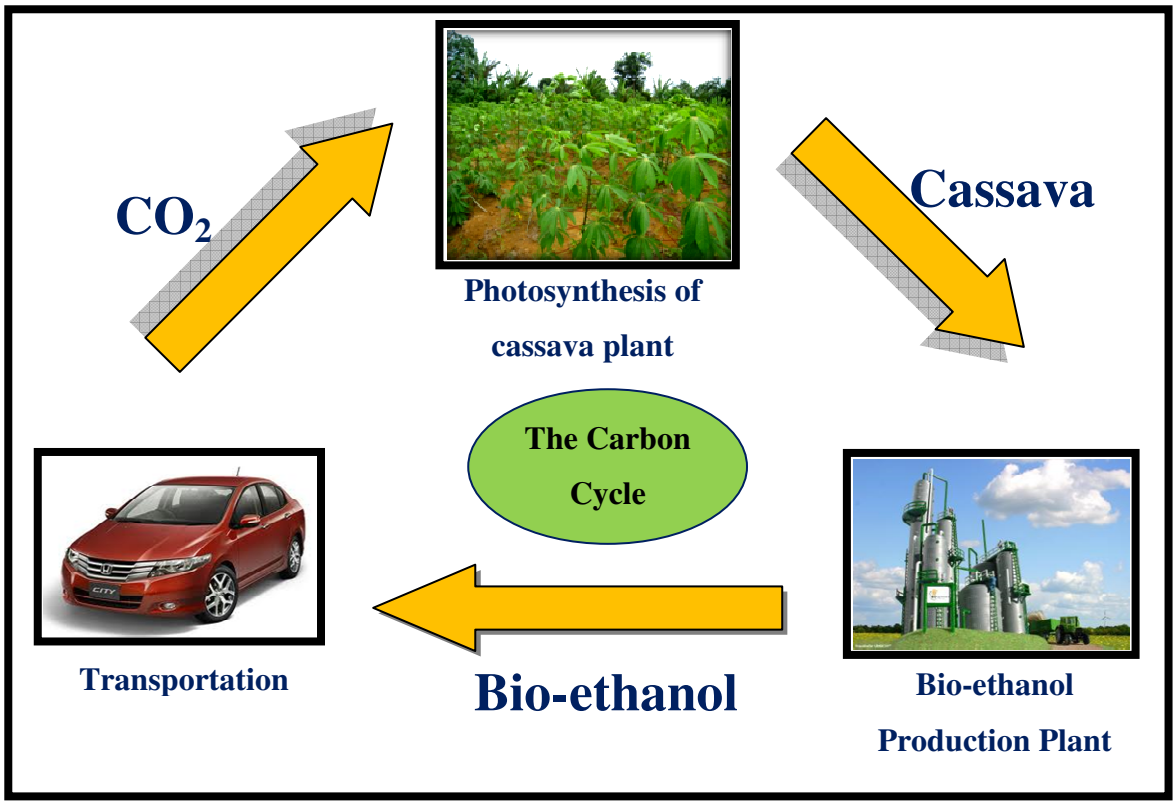


Figure 2.7: The carbon cycle for bio-ethanol production

The production and use of bio-ethanol will reduce the formation of green house gases significantly. Bio-ethanol is able to replace and eliminate the need for the MTBE (Methyl Tertiary Butyl Ether) which used as the additive to gasoline as oxygenate and raise the octane rating of the fuel during the past few years. However, MTBE is a hazardous liquid that will bring harm to biosphere while it diffused to the underground water, this will caused water pollution and may caused unwanted disease to the human being. Other than that, bio-ethanol is able to reduce particulate matter (PM) emissions by more than 25 %. Particulate matter will penetrate and accumulates deeply into human lungs and caused the side effect for the human health.

Furthermore, production of bio-ethanol increases the demand for the crops such as sugar cane, cassava and sweet potato. The agricultural transportation and economic growth in developing country will be improved. It helps reduce the need for oil imports and the production of bio-ethanol increases the value added from the

use of cane juice and molasses. In addition, the utilization of the farm land for the plantation of the crops for raw material of bio-ethanol will improve the air quality of the main cities. Plantation of the crops for the bio-ethanol productions will helps to fight against hunger in the world goes through sustainable development of rural regions, which would allowed the access to jobs and income for millions of people.

Nevertheless, there are number of issues which beg attention (S.L.Tan, 2010):

1) Bio-ethanol has only 67 % of the energy content of petrol.
2) Growing crops to produce bio-fuels will reduce the amount of arable land available for food and feed crops.
3) Already, the effects of channelling corn into bio-ethanol production are apparent. As example, the supply of corn for animal feed has been significantly reduced, leading to a world-wide shortage and to rising feed prices.
4) In the earlier part of 2008, the price of palm oil shot up tremendously because demand outstripped supply – probably as a result of palm oil being diverted from use as cooking oil to bio-diesel production.

However, these scenarios will change when the world eventually runs out of petroleum sources, and the human still need the fuel to run the vehicles. As a result, it is a matter of supply and demand. Moreover, the cassava is not the staple food for the Malaysian. The demand of cassava for food processing is not that high if compared with palm oil, so the price of cassava will not shot up tremendously due to the demand outstripped supply.

2.3 Cassava as bio-ethanol crop

Cassava and sweet potato are popular root crops of the tropical countries (FAO, 2006). Although, their primary use is as food crops, both the crops are widely used for the production of starch, their role has been increasingly recognized as industrial crops for the production of bio-ethanol, glucose, HFS (high fructose syrup) etc. (Baskar et al., 2008; Berghofer & Sarhaddar, 1998; Gorinstein, 1993; Shetty, Chotani, Gang & Bates, 2007).

Cassava is a starch-accumulating crop (figure 2.8), which is well utilized in various industries producing starch and starch derivatives. Based on the studies by Tan and Khatijah (2010), one of the earliest records of cassava in Asia was in 1786, when it was introduced into Ceylon (present-day Sri Lanka) from Mauritius. The increase in the prices of petroleum based fuels, strict government regulations on exhaust emission and future depletion of worldwide petroleum reserves encourage studies searching for alternative fuels (Hashem and Darwish, 2010).

Figure 2.8: Root of cassava

Cassava is a far more environmental-friendly crop which using less chemical inputs and it are less demanding on water. In addition, the cassava was proven contains more net energy ratio and efficient than corn in producing bio-ethanol. The high yields of starch and total dry matter in spite of drought conditions and poor soil, together with low argo-chemical requirements, results in energy input that represents only 5-6 % of the final energy content of the total cassava biomass (C. Jansson., ect.al, 2009). This translates to an energy profit of 95 %, by assuming complete utilization of the energy content in the total biomass. As a result, the bio-fuel crops that will be used in the research are the root of the cassava plant. A direct comparison of bio-ethanol production from different energy crops was reviewed by Wang (Table 2.4).

Table 2.4: Comparison of Bio-ethanol Production from Different Energy Crops. (Source: Wang.W)

Crops	*Yield (tonne ha^{-1} $year^{-1}$)*	*Conversion rate to bioethanol (L $tonne^{-1}$)*	*Bioethanol yield (L ha^{-1} $year^{-1}$)*
Sugarcane	70	70	4900
Cassava	40	150	6000
Sweet sorghum	35	80	2800
Maize	5	410	2050
Wheat	4	390	1560
Rice	5	450	2250

From figure 2.9 below, cassava is popular root crops of tropical countries (R. Johnson, G. Padmaja and S.N. Moorthy, 2009). Although, their primary use is as food crops for certain tropical country, cassava is widely used for the production of starch and of late, their role has been increasing recognized as industrial crops for the bio-ethanol (Baskar et al., 2008; Berghofer & Sarhaddar, 1988; Gorinstein, 1993; Shetty, Chotani, Gang & Bates, 2007). As a result, those countries are able to build the bio-ethanol plant in their region, this is because they able to provide the feed stock for the bio-ethanol production easily.

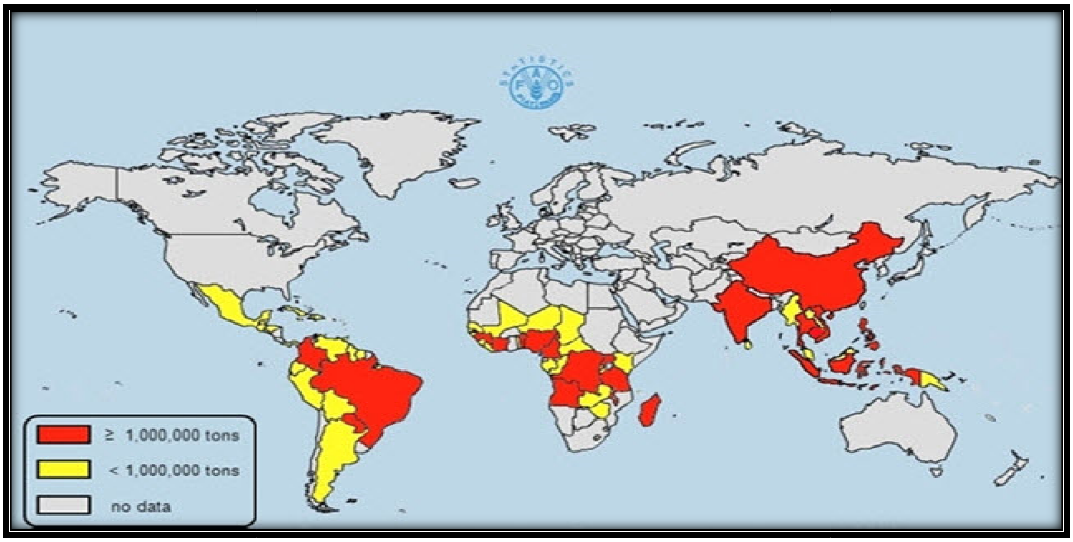


Figure 2.9: Cassava production in different countries in the world 2008. (Source: FAO 2008)

Tropical countries like Thailand are developing cassava-based ethanol plants. Cassava is one of the most important cash crops in Thailand (KAPI, 2003). With the production capacity improvement, cassava supply is expected to exceed the demand. Thus utilization of cassava root as raw material for ethanol production will stabilize the price of cassava tubers and enhance the rural economy. Anon, 1996 did a survey on the main area of cassava production has been Perak state in Peninsular Malaysia, which accounts for more than 40 % of the total production area.

2.4 Current situation of Bio-fuel in Malaysia

Based on Gordo H Chin, in Kota Kinabalu, Sabah, a major project between South Korean companies Jusin Group and Gaiax Energy Co. Ltd. is able to turn Malaysia into a hub for bio-energy. A big scale cassava plantation project in Sabah had been done to produce bio-ethanol, bio-diesel, bio-energy and bio-fuel. The new company name is Jsin Cenox (M) Sdn. Bhd.

This new company will assist the State Government to create more employment opportunities as well as helping cassava planters in the state to market their products. Before start this project, over USD 100 million had been invested by the companies from Japan and the United States. Currently, Jusin Group through its subsidiary companies Jusin Enterprise (M) Sdn. Bhd. and Hanal (M) Sdn. Bhd. has 4,000 hectares of land on Banggi Island, and planning to have another 30,000 hectares to have the cassava plantation. According to Jusin Group Chariman, Lee Ki Nam, one hectare of cassava plantation is able to produce about 200-250 tonnes of cassava. And they can start harvesting the cassava ten months after they are planted. But, 60 percent of the total harvest will be processed into bio-ethanol and the rest will be turned into pellet fuel.

The quality of fuel that Jusin Cenox can produce will be equivalent to RON 102, in other words it is better than RON 95 and RON 97 which currently can easily get from local petrol station. Based on Lee, they had conducted the tests of bio-fuel that they can produce from their cassava, and they found out that it will not only be

of higher quality compared to current available fuel, but it will be having higher octane number, more powerful and even cheaper. The reason why the Jusin Group choose Sabah, Malaysia as their production base not only due to good soil condition and climate, but also the peace and harmony prevailing in this nation

2.5 Cassava Farm Visitation on 5th November 2010

In order to fully understand the current situation on the cassava plantation, my final year project partner, Mr. Koh Cin Cong and I had visited the cassava farm which located in Kong Kong, Masai, Johor.

Figure 2.10: Photo Taking with the Owner of Cassava Plantation, Mr. Lee Hao Tong in Kong Kong, Masai, Johor on 5th November 2010.

The total plantation area for the cassava in Kong Kong, Masai, Johor is around 350 acres. Mr. Lee had explained the plantation process for the cassava. Figures below showed how the workers planted the cassava by covered the steam of the cassava under the soil. Besides, Mr. Lee had given us some of the cassava roots for our research purpose.

Figure 2.11: Entrance for the Cassava Plantation Area

Figure 2.12: Cassava Plantation Area

Figure 2.13: Cassava Planting

Figure 2.14: The sprout of the cassava

Figure 2.15: Mr. Lee and his worker were harvest the cassava crops

Figure 2.16: The machine which used for the cassava planting.

CHAPTER 3

METHODOLOGY

3.1 Sample collection and preparation steps

Cameron Highland, Pahang's cassava roots were purchased from the local hypermarket. The cassava roots were thoroughly washed with tap water to remove dirt and adhere particles.

After that, the peel of the cassava was hand peeled off by using the cutting knife. Then, the clean cassava roots were sliced to pieces and spread evenly on the aluminium coil. The purpose of this procedure is to provide the large surface area of the cassava for the drying process. Since the smaller the cassava shreds, the larger the surface area will be exposed to the air and hence more effective and faster for the drying process.

Sun drying and mild drying of the cassava shreds were used for the drying purpose. Since the starch will start denatured when it exposed to the heat around 40 ^{o}C. The cassava shreds were put under the sun in the open environment for the sun drying and the continuous flipping step was necessary (Figure 3.1). The sun drying process was carried out for at least 7 days with the clear weather or until the weight of cassava shreds kept at a constant.

Figure 3.1: Cassava Shreds

After the 7 days of sun drying, the cassava shreds were undergoing the mild drying process in the oven under temperature of 35 ^{o}C. The cassava shreds were continuously flipped manually.

When the dried cassava shreds were obtained, grinding process will be carried out. The grinder that used for my research is shows in figure 3.2. The cassava shreds were grinded in the batch mode that was 30 g of the cassava shreds for 20 seconds. Then the powder of the cassava was kept inside the clean plastic bag and stored inside the desiccators before the fermentation process (Figure 3.3).

Figure 3.2: Outlook of the Grinder

Figure 3.3: Powder of Cassava and the Cassava Powder in the Desiccators

3.2 Glucose Profile

The main objective for doing the glucose profile is to obtain the duration for the starch of the cassava convert to the simplest sugar that is glucose. Then the second objective is to determine the reducing sugar which contain inside the sample and the dilution factor which will be useful while undergo the ethanol fermentation.

3.2.1 Gelatinisation

Different percentages of starch content were prepared by measuring the quantity of cassava powder that needed to add into with the 200 ml of distilled water. 5 % (w/v), 10 % (w/v) and 15 % (w/v) of starch content were prepared. Each percentage of starch content was done in triplicate to increase the accuracy of the experiment.

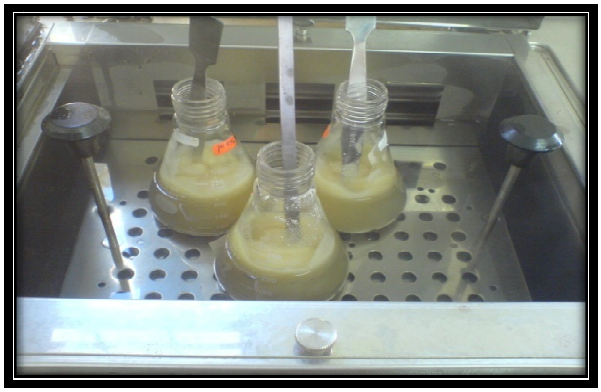


Figure 3.4: Gelatinisation process

In order to make sure the starch was well mixed with the distilled water, the conical flask which contained the cassava powder and the distilled water was put into the 80 ^{o}C water bath and stirred continuously then the starch gelatinisation process will be started (figure 3.4). The mixture will be stirred and heated until becomes gel like solution. The samples were heated and the water bath's temperature was let it keep increasing until 90 ^{o}C. After that, the samples were taken out and ready for the liquefaction and saccharification process.

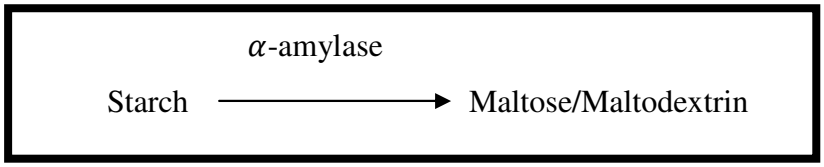
3.2.2 Starch Hydrolysis (Preliminary study)

The amount of reducing sugar content that able to produce by the cassava flour will be obtained after liquefaction and saccharifcation process. In other words, this is a preliminary study on the amount of reducing that will be produce by different amount of initial starch content. 5ml of the sample was taken for every 15 minutes for 2 hours of liquefaction and 3 hours of saccharification for 5 % (w/v) and 10 % (w/v) of starch content. But the duration of the saccharification will be increased while doing the 15 % (w/v) of starch content. This is due to increasing of initial starch will directly produced more amount of reducing sugar. To obtain a stable reducing sugar profile for 15 % (w/v) of starch content, 4 hours of sample taking is necessary.

The Termamyl 120L, Type L (thermostable α-amylase) and Dextrozyme DX (glucoamylase) were purchased from Novozymes, China and these two enzyme were used in the liquefaction and saccharification respectively in this research. Termamyl is a thermostable α-amylase, which produced from a strain of *Bacillus Lichenifornis.* This enzyme is in a liquid preparation, it is stable in starch solution at high temperature. It has an optimum pH at 5.5 with a broad pH tolerance and the activity of this enzyme is the amount of enzyme that hydrolyzes 5.26 mg starch/hour. Dextrozyme is a mixture of glucoamylase and pullulanase which obtained from genetically modified strains of *Aspergillus Niger* and *Bacillulus Deramificans.* The activity of this enzyme is defined as the amount of enzyme that splits 1 μ mole of maltose per minute at 25 ºC. It have an approximately density of 1.15 g/ml, and have

activities based on amyloglucosidase units (AGU/g), which represents the amount of enzyme which able to hydrolyze 1 μ mole of maltoseper minute at 37 ºC with pH at 4.3. The optimum temperature of this enzyme is 65 ºC.

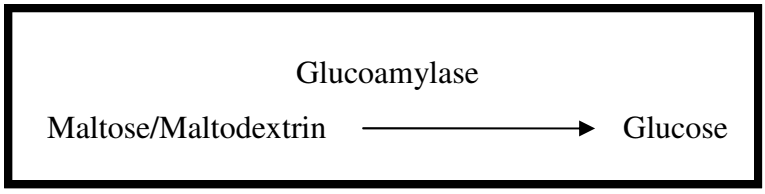
Before the liquefaction process start, 0.04 ml of α-amylase (Figure 3.5) was added into each of the samples and mixed well. We observed that the gel which formed by the gelatinisation will become less viscous after α-amylase had been added. This is due to α-amylase is the enzyme which used to cleave the starch to maltose. And the maltose is less viscous then the cooked starch. The reaction step of liquefaction is shows below:



Then the samples were put back into the water bath again with the temperature of 90 ^{o}C and the temperature was maintained throughout liquefaction process. Based on Regy Johnson, G. Padmaja*, S.N. Moorthy, 90 ^{o}C is the optimum operating temperature for α – amylase. After that, stirred the samples every 15 minutes and let it incubate for 2 hours. During the incubation, 5 ml of each sample was taken for 15 minutes time interval to test for the reducing sugar concentration by using the *DNS method*. After the 2 hours incubation, the liquefied starch will be used for subsequent saccharification study.

The saccharification step was started when the reducing sugar concentration in the reaction mixture reached a maximum concentration. Before the saccharification process, every liquefied starch had to undergo the pH control process. Here, every liquefied starch's pH was adjusted to 4.5 by using 0.1 M NaOH or 0.1 M of HCl. Based on Regy Johnson, G. Padmaja*, S.N. Moorthy the optimum pH for Glucoamylase is 4.5. After that, 0.066 ml of Glucoamylase (Figure 3.5) was added into each of the samples. Glucoamylase is the enzyme use to further cleavage of the maltose and form the glucose. Since the glucose is the simplest sugar which

can be consume by the yeast to produce the ethanol. The reaction step of saccharification is shows below:



After the Glucoamylase had been added, stirred the samples well and put it back to the water bath for the heating process. Here, the optimum operating temperature for Glucoamylase is 65 ^{o}C (Regy Johnson, G. Padmaja*, S.N. Moorthy). Then, every 15 minutes the samples will be stirred well and 5 ml of the sample was taken from each of the conical flask for the reducing sugar concentration analysis. Saccharification process was continued until further increased in glucose concentration was not detected.

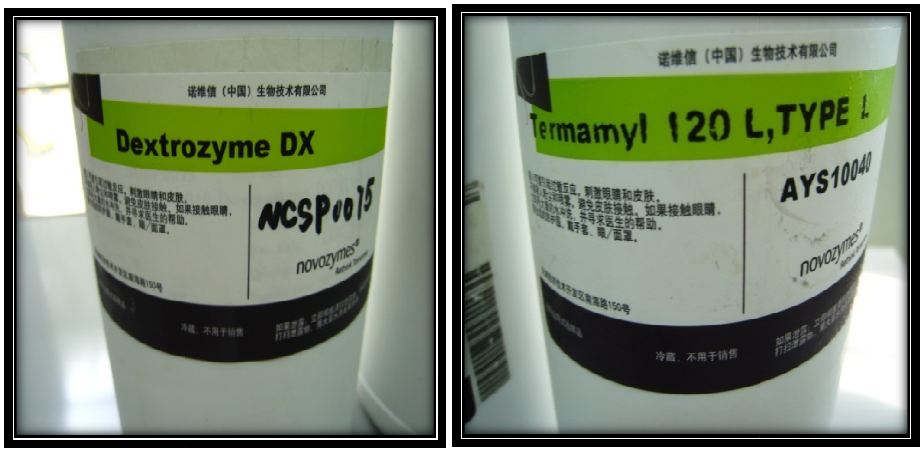


Figure 3.5: Enzymes for liquefaction and saccharification

The difficulty that we faced during the liquefaction and saccharification process was mainly due to the time arrangement of the experiment. In order to complete one experiment with an amount of starch content, at least 6 hours were needed. Besides, the sampling time was 15 minutes of interval time. Hence, a good time arrangement for the experiment is necessary.

3.3 Yeast Preparation

Industrial's yeast *(ATCC 36858 Saccharomyces cerevisiae)* was used in this research throughout the whole fermentation. It is different with the baker's yeast which easily found from the market. Even the price of the industrial's yeast is much higher than the baker's yeast yet the amount that used in this research is very little (around 0.01 µl) and this amount of industrial's yeast can be cultured.

Table 3.1: Comparison of Industrial's Yeast *(ATCC 36858 S. Cerevisiae)* with Baker's Yeast

Parameters	**Industrial's Yeast *(ATCC 36858 S. Cerevisiae)***	**Baker's Yeast**
Strength	High	Low
Productivity	High	Low
Condition	Liquid	Solid
Price	Expensive	Cheap
Cultivation	Able to be cultivate	Not able to be cultivate

In order to know the amount of the yeast which need to be added into each of the sample, single colony of the industrial yeast was taken from the petri dish and then deep inside the YP medium (Mixture of Casein Peptone, Yeast Extract and Dextrose) which contained the nutrient for the yeast to growth in liquid form (figure 3.6 and 3.7). Then the yeast will incubate inside the shaker for 24 hours before added into the samples.

Even the industrial yeast is able to be cultivated, it is not necessary confirmed it will be success for every cultivation. The yeast must be cultivated in the laminar air flow machine where the air is filtered and the environment is clean. Yeast is a sensitive microorganism which will die if exposed to the unpleasant environment condition (bad cultivation temperature). Besides, contamination problem will cause the failure on the cultivation.

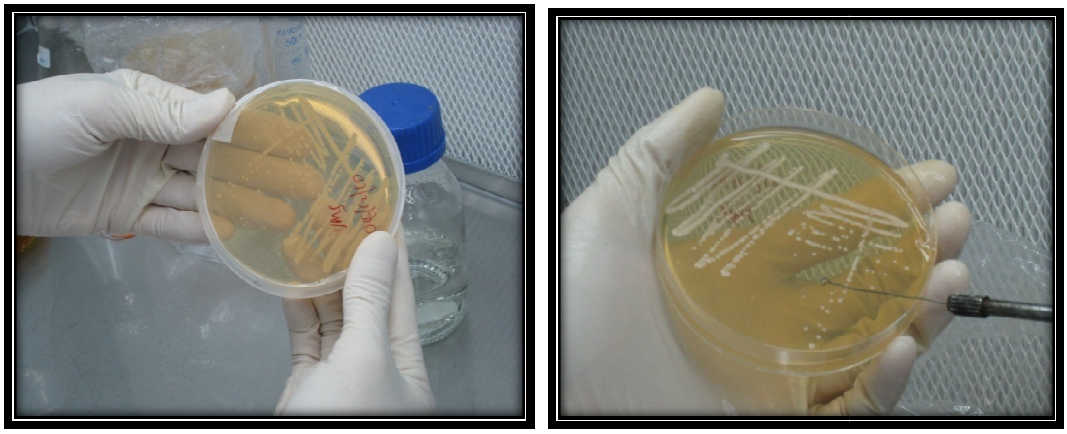


Figure 3.6: Cultivation of industrial yeast *(ATCC 36858 S. Cerevisiae)* in the petri dish

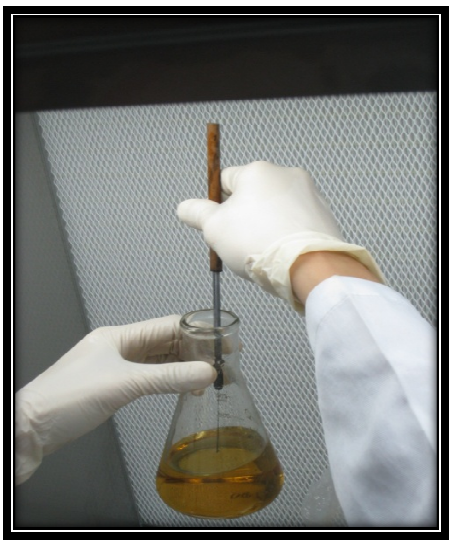


Figure 3.7: Cultivation of industrial yeast *(ATCC 36858 S. Cerevisiae)* into YP medium in Erlenmeyer flask.

3.4 Ethanol Fermentation and Sample Collection

Batch fermentation mode will be used to produce bio-ethanol throughout the project. The manipulated parameter was the initial reducing sugar content. Different initial reducing sugar with the amount of 20 g/L, 40 g/L, 60 g/L and 80 g/L were prepared in different Erlenmeyer flask. And each parameter was done in triplicate to increase the accuracy.

Figure 3.8: Fermentation process was done in the shaker.

5 ml of each sample was taken out from every conical flask for 6 hours interval time for three days (72 hours). Then each of the samples was ready to undergo for the analytical testing.

During the whole 72 hours of fermentation, contamination was the main problem. It is necessary to undergo the sample taking in the laminar air flow machine where the air is clean. If there was any contamination occurred in the sample, the sample will be taken out. Since the sample was taken in every 6 hours of interval time, so even in the mid-night the sample taking procedure was still continued.

3.5 Analytical Methodologies

pH of the sample, ethanol concentration, reducing sugar concentration and growth of yeast were tested in our research. There was only one manipulated parameter, which is by changing the different amount of initial reducing sugar content in the culture broth. Finally the yield of the ethanol was calculated and this able to find what is the optimum reducing sugar content which able to produce the highest yield of ethanol.

3.5.1 Determination of Reducing Sugar

Dinitrosalicylic acid (DNS) method was used to measure the amount of reducing sugar in the samples. Since the reducing sugar will be dissolved in the liquid and not the solid, so the liquid and solid need to be separate and test the reducing content inside the liquid.

Before the *DNS method*, each sample was put into the centrifuge machine (Figure 3.9) which able to separate the solid and liquid by the centrifugal force. The centrifuge machine was control at 9000 rpm, and the samples were continuing spin for 5 minutes. After the spinning, the samples inside the centrifuge tube will form 2 layers, the upper part is the clear solution which called as supernatant and the bottom part is the solid which called as cell pellet (Figure 3.10). The supernatant was transferred to the boiling tube for the dilution.

Reducing sugar content was determined by tested supernatant. To perform determination of reducing sugar in supernatant, 3, 5-dinitrosalicylic acid (DNS) method was used for the analysis. The DNS solution was prepared by dissolved 10.0 g of 3,5-DNS (SIGMA,AUSTRALIA), 2.0 g of phenol (R&M,UK) and 0.5 g of sodium sulphite (FISHER SCIENTIFIC,UK) in 500 mL of 2.0 % sodium hydroxide (COPENS ENTERPRISE, MALAYSIA) solution and then was diluted to 1 L with distilled water. The reagent was stored in dark colour bottle because DNS is a light sensitive solution.

Absorbencies were read at 540 nm using a Spectrophotometer (HACH, INDONESIA) (Figure 3.11). In order to let the spectrometer which used to test for the reducing sugars operate at the optimum range (<1.5 Abs), dilution should be done. Distilled water was used as blank solution. Glucose calibration standards were completed to estimate reducing sugars as glucose equivalents (Figure 3.12).

Figure 3.9: Centrifuge machine

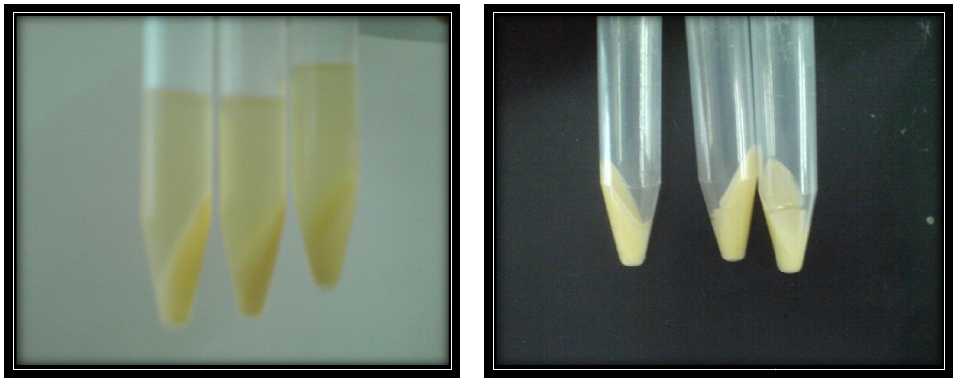


Figure 3.10: After spinning by centrifuge machine, upper layer is supernatant and lower layer is cell pellet.

Figure 3.11: Spectrophotometer (HACH, INDONESIA)

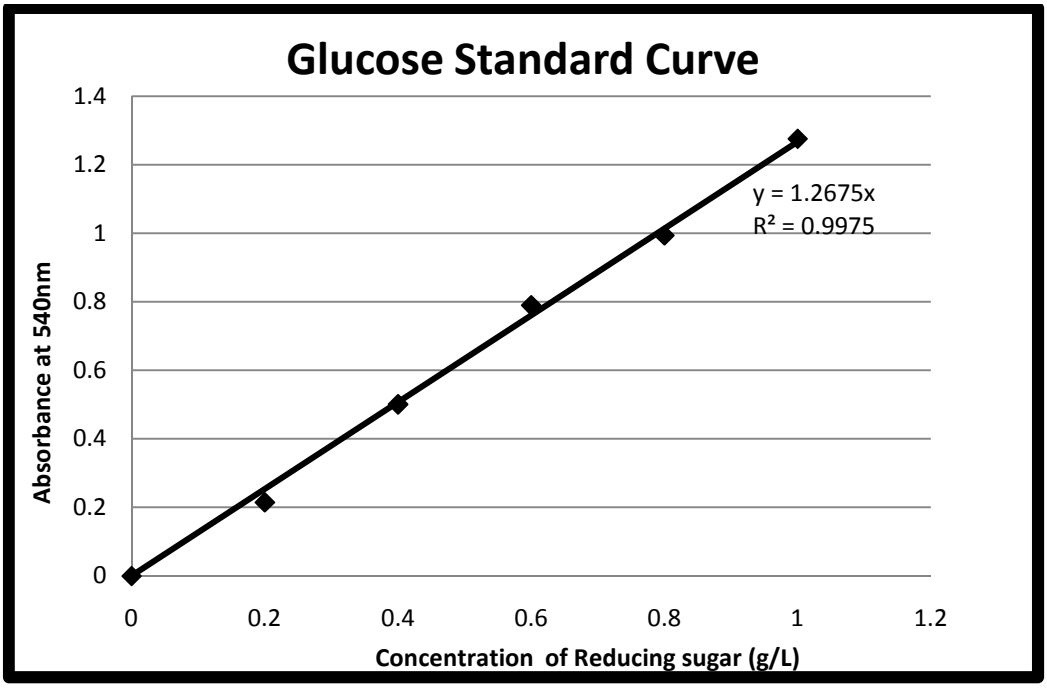


Figure 3.12: Standard Calibration Curve for Reducing Sugar Concentration

3.5.2 pH determination

The pH for each of the initial samples was measured by the pH meter (METTLER TOLEDO, UNITED STATES). The pH meter was first calibrated using pH 7.0 and pH 4.0 standards buffers solution prior to pH measurement.

3.5.3 Ethanol Concentration Determination

Ethanol content was tested by the Gas Chromatography (PERKINELMER, USA). A stainless steel column (1.5 m x 2 mm) was fitted into the instrument to provide the column injection. The detector and injector temperature was maintained at 280 ºC. The flow rate of the carrier gas (Nitrogen) was set at 30 mL/min. The injection sample volume is 1 µL. The gas chromatograph was connected to an integrator and computer system to determine the area under the graph of ethanol peak at residence time around 3.3 min. Table 3.2 shows the specification of the GC. 1 µl

of every sample was injected into the gas chromatography then the ethanol content will be obtained.

In my research, the bio-ethanol analysis by gas chromatography is considered as quantitative analysis. A pure ethanol was injected into the column and the retention time and the area under the peak were noted down. Then 1 µl of sample was injected into the column. By referring to the retention time of the pure ethanol, the ethanol peak of the sample could be determined. The area under the peak is proportional to the amount of ethanol present in the sample. By using the mathematical function of integration, the area under the peak could be found. Concentration of the bio-ethanol could be calculated using a calibration curve (Figure 3.13) by knowing the area under the peak.

Table 3.2: Specification of GC

Analytical Column	Zebron; ZB-WAXplus capillary column
Injection	Split 15.0 @ 140 °C , 1-2 µL
Carrier Gas	N_2/Air
Column Flow	140 mL/min
Oven Program	35 °C for 5 minutes to 200 °C @ 30 °C/min for 1 min.
Detector	Flame ionization detector @280 °C

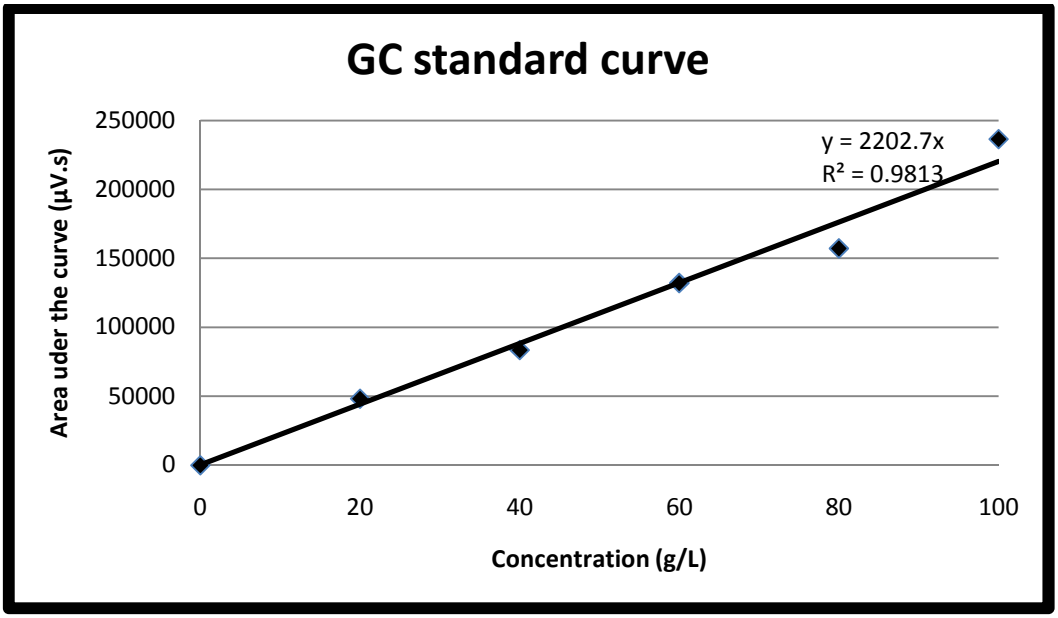


Figure 3.13: Standard Calibration Curve for Ethanol Determination

3.5.4 Growth of Yeast (Determination of Biomass Concentration)

The growth of yeast (biomass) could be determined by measured the absorbance of the cell pellet (suspended solid) and the procedures were showed below:

1) 5 ml of distilled water was added to the cell pellet.
2) The mixture was vortex properly by using the vortex mixer.
3) The mixture was centrifuged again with 9000 rpm for 5 minutes.
4) Supernatant (clear liquid) was discarded from the separation process.
5) Then 5 ml of distilled water was added into the cell pellet.
6) Well mixed the mixture with the vortex mixer and obtained the cell suspension.
7) Absorbance of the cell suspension was measured by using the spectrometer with 660 nm wavelength.

3.6 Proposed Kinetic Modelling

In this research, the Excel Solver Add-in and Data analysis add-in tools were used in this kinetic modelling to examine the experimental result for the ethanol production. The optimal values of the kinetic parameters were approximated by minimizing the discrepancy between the model predictions and corresponding experimental data.

3.6.1 Ethanol Fermentation (Production Formation)

In this study, the product for the fermentation is bio-ethanol then Modified Gompertz equation was used to modelling the product formation during the fermentation process. Experimental data obtained from batch fermentation studies with different initial reducing sugar (glucose) content were fitted to modified Gompertz equation .From the equation 3.1, the Modified Gompertz model is depend on the lag time, specific production rate and the maximum potential production concentration.

$$P = P_m exp\{-exp[\frac{r_{p,m}\, exp(1)}{P_m}(t_L - t) + 1]\} \quad (3.1)$$

Where P is the concentration of ethanol formation (g ethanol/L), P_m is the potential maximum ethanol concentration (g ethanol/L), $r_{p,m}$ is the maximum ethanol production rate (g ethanol/(L.h)) and t_L is the lag phase or the time to exponential ethanol production (h). The parameters of $r_{p,m}$ and t_L can be estimated using Microsoft Excel Add-in Tools:

a) Solver,
b) Data Analysis (Multiple Regressions).

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Reducing Sugar Profile during the Liquefaction and Saccharification with different Initial Starch Content (Preliminary Study)

Liquefaction and Saccharification were the preliminary study for the reducing sugar production which produced by different initial starch content. And the reducing sugar will produced ethanol by adding the industrial's yeast *(ATCC 36858 Saccharomyces cerevisiae)* into the sample. The cassava starch was hydrolyzed with α - amylase enzyme in liquefaction process and further hydrolyzed by glucoamylase enzyme in the saccharification stage. Then the reducing sugar was determined by the DNS method.

The total reaction time for the liquefaction process was 2 hours. From the observation from the experiment, actually the system reached at equilibrium condition around 30 minutes. Then for the saccharification stage, total reaction time was 3 hours for both 5 % (w/v) and 10 % (w/v) of cassava starch and 4 hours for the 15 % (w/v) of cassava starch. From the experiment, 5 % (w/v) and 10 % (w/v) starch content reached equilibrium around 3 hours whereby 15 % (w/v) of starch content reached equilibrium around 4 hours.

From Figure 4.1, reducing sugar increased while the initial starch concentration increased. Moreover, more time for hydrolysis for the higher initial starch concentration. While the system reached equilibrium, this implies that the enzyme (α - amylase and glucoamylase) successfully converted all available starch to the reducing sugars.

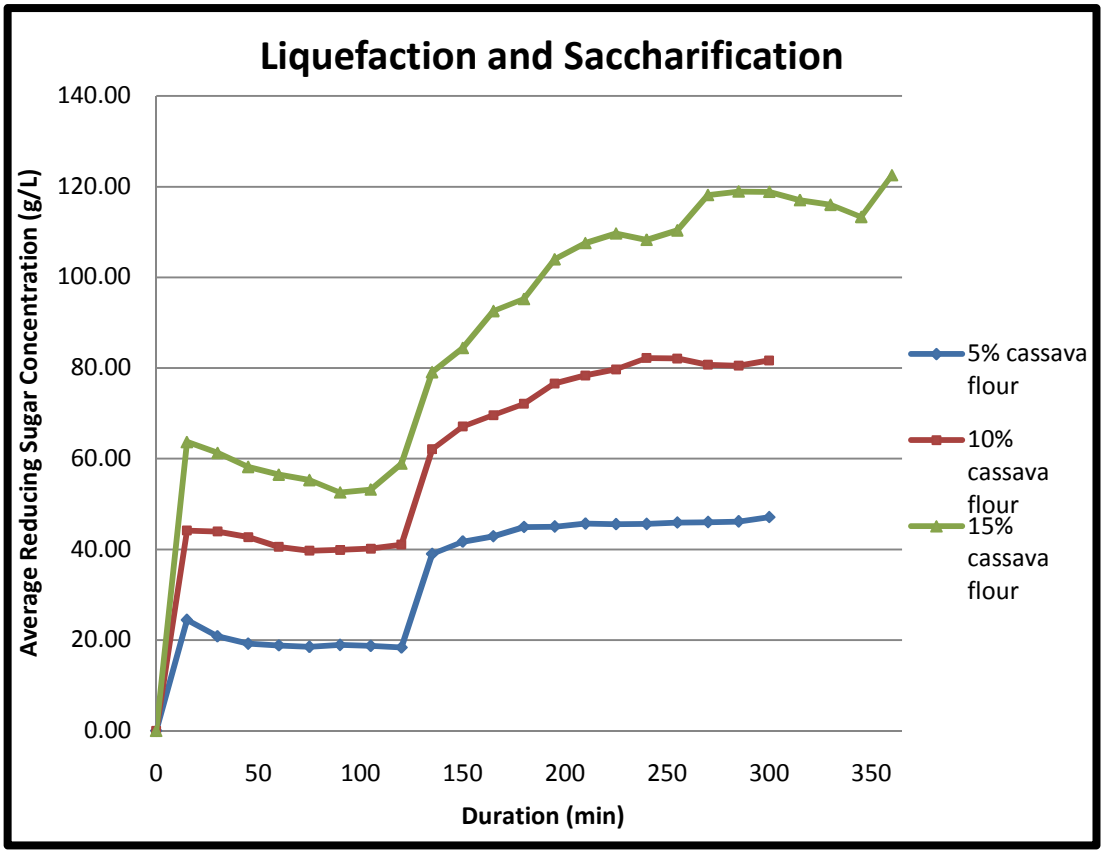


Figure 4.1: Profile of reducing sugar in medium with different concentration of initial starch.

From the observation, the higher the initial starch content, the higher the reducing will be obtained. This corresponds to higher productivity. However, too much increased of initial starch content will significantly lower the productivity and will not gives much increment for yield of the reducing sugar.

Based on Table 4.1, 15 % (w/v) of starch content shows the lower productivity of reducing sugar compared with 10 % (w/v) of starch content. This is mainly due to the higher the starch concentration will cause inhibition, which substantially lowers the yield of reducing sugar. Problem in mixing and mass transfer also arise with higher starch concentration. Besides, the ratio of enzyme to starch concentration is another factor that will cause the lower yield of reducing sugar.

Table 4.1: Kinetic parameter of production of reducing sugar

Initial Starch Concentration (%, w/v)	Maximum Reducing Sugar Concentration (g/L)	Time to attained maximum reducing sugar concentration (hr)	Reducing Sugar Yield (g/g)	Reducing Sugar Productivity (g/L.hr)
5	47.10	3.50	0.942	13.46
10	81.70	4.50	0.817	18.16
15	122.52	5.83	0.817	21.02

Sample calculation:

For 5 % (w/v):

Since,

volume of distilled water that will mixed with flour = 200 ml

Hence,

weight of flour that mixed in distilled water = 200 ml x 5 % (w/v) = 10 g

Hence,

initial reducing sugar (g/L) = 50 g/L

$$Yield\ \left(\frac{reducing\ sugar\ g}{starch\ g}\right) = \frac{Maximum\ Reducing\ Sugar\ Concentration\ (gL^{-1})}{Initial\ Sarch\ Concentration\ (gL^{-1})} \quad (4.1)$$

$$= \frac{47.10\ gL^{-1}}{50\ gL^{-1}}$$

$$= 0.942\ \frac{reducing\ sugar,g}{starch,g}$$

$$Productivity\ \left(\frac{g}{L.hr}\right) = \frac{Maximum\ Reducing\ Sugar\ Concentration\ (gL^{-1})}{Time\ to\ attained\ maximum\ reducing\ sugar\ concentration\ (hr)} \quad (4.2)$$

$$= \frac{47.10\ gL^{-1}}{3.50\ hr}$$

$$= 13.46\ \frac{g}{L.hr}$$

4.2 Ethanol Fermentation

The batch fermentation was done in this research to produce the bio-ethanol from the cassava starch. The fermentation was carried out in triplicate for 72 hours (3 days) with different initial reducing sugar concentration. There are 20 % (w/v), 40 % (w/v), 60 % (w/v) and 80 % (w/v). The total working volume was 250 ml in a conical flask. Then all samples were incubated in the shaker for 3 days (72 hours) with temperature of 30 ℃ and 250 rpm.

4.2.1 pH measurement

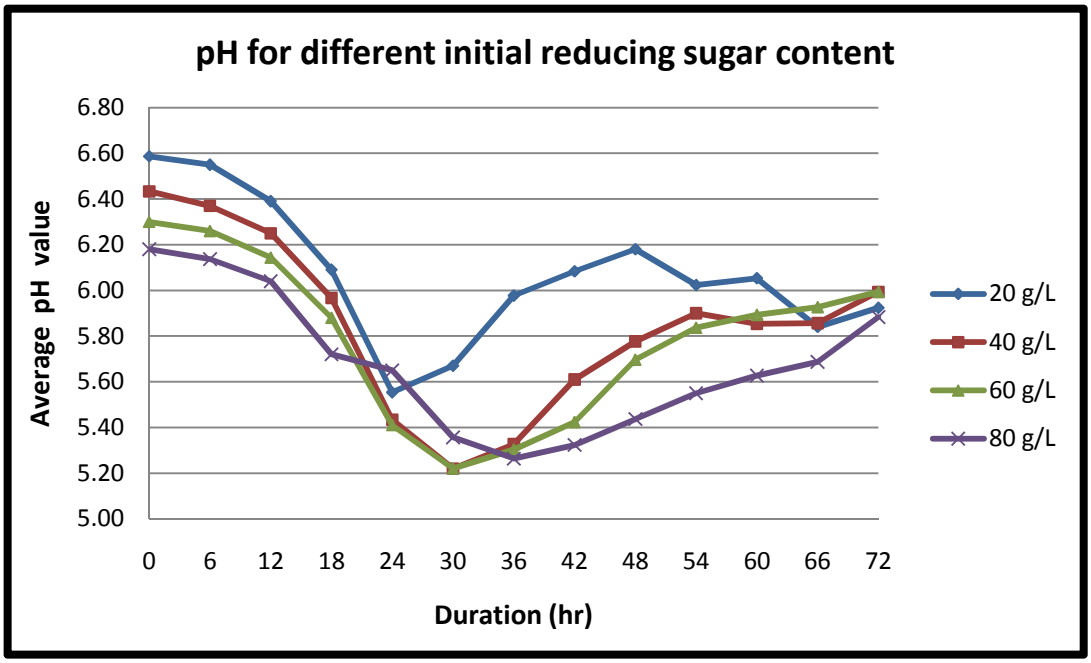


Figure 4.2: pH value for the samples with different concentration of initial reducing sugar content.

From figure 4.2, the observation shows that the pH value of the samples were reduced initially then increased after that. This is caused by the formation of ethanol, carbon dioxide (CO_2) and small amount of organic acids such as lactic acid. Loncar et al. (2006) also reported that the conversion of sucrose in organic acids promotes the decrease of pH value during the fermentation process.

4.2.2 Cell Growth

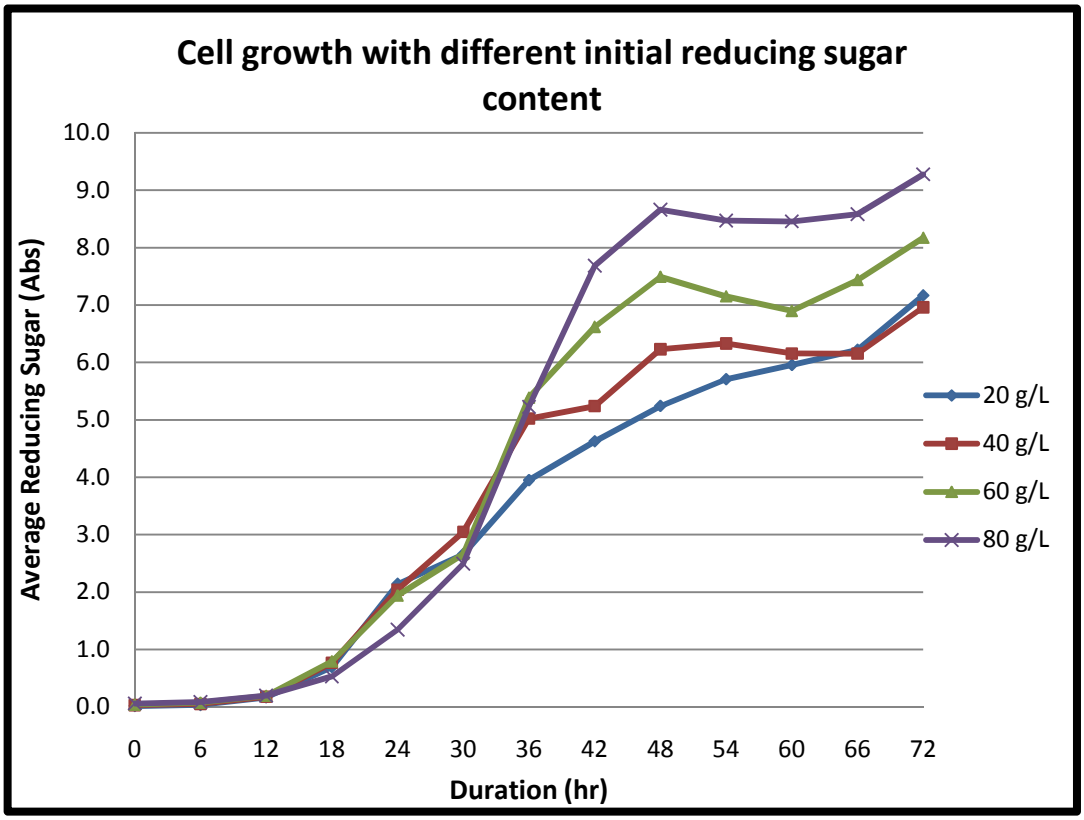


Figure 4.3: Cell growth profile with different concentration of initial starch.

From the observation, the cell (yeast) growth was increasing and kept around constant growth after 42 hours. Batch ethanol fermentation by *S. cerevisae* showed a classical growth trend (Figure 4.4), in which an initial lag phase was observed (0 hr – 6 hr). During the lag phase is the time of adaption of cell to the 'new' environment or medium. During this phase, cell mass may increase a little, without an obvious increase in cell number density. Once the necessary alternations are completed, the cells move into the growth phase or called as exponential phase.

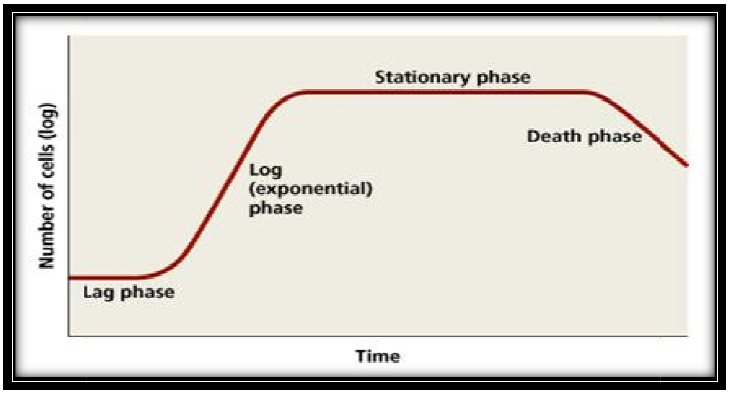


Figure 4.4: Classical cell growth curve

6 hour till 42 hour was the exponential phase for the batch fermentation. Exponential phase also known as logarithmic growth phase. During this phase, the cells can multiply rapidly where the *S. cerevisae* grow at a constant and maximum rate of the cell numbers increase exponentially with time. Eventually, a deceleration phase was achieved, which started at 42 hour and lasted until 48 hour. During this phase, the growth rate of the cell slowly decreased due to the consumption of nutrient becomes depleted and the accumulation of toxic by-product of growth. These changes occur over a very short period of time. From the observation, this phase only lasted for 6 hours.

After 48 hour of fermentation, due to the accumulation of toxic product that inhibit the growth, the cell enters the stationary phase where it starts at the end of deceleration phase. Stationary phase occurs when all the cells have stopped dividing or when viable cells have reached equilibrium with dead cells. It is a point where the growth rate has declined to zero and the cell concentration was almost kept constant with time.

4.2.3 Time Course of Ethanol Fermentation

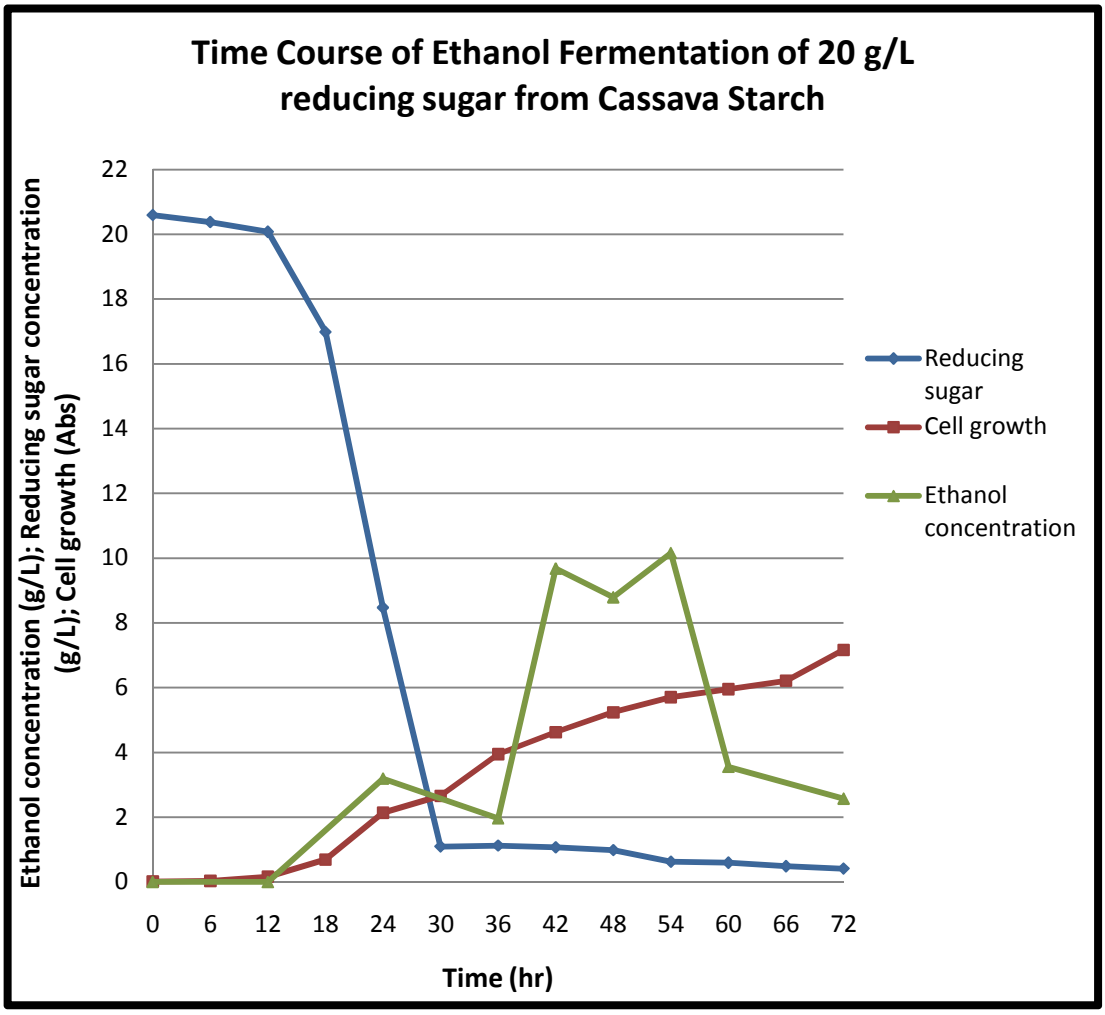


Figure 4.5: Time course of ethanol fermentation of 20 g/L reducing sugar from cassava starch.

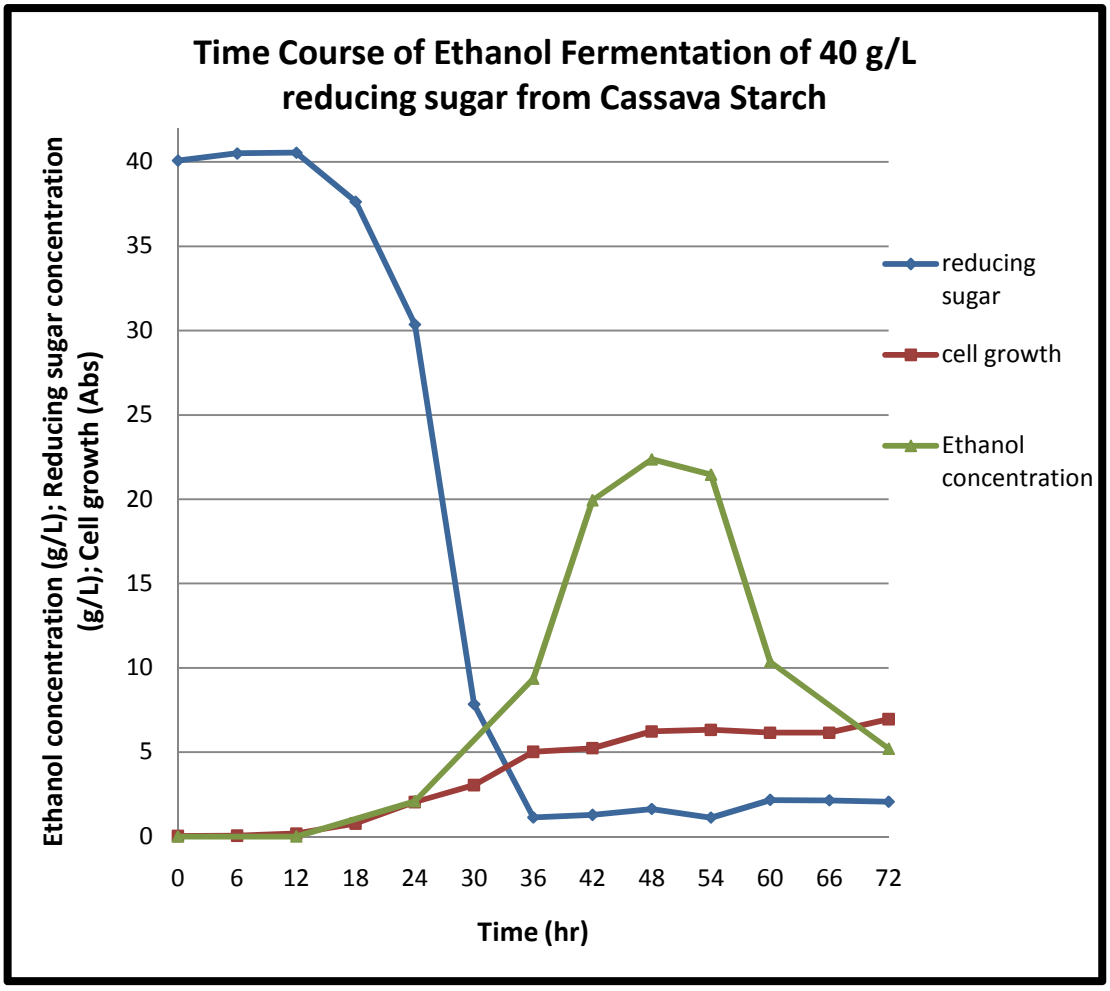


Figure 4.6: Time course of ethanol fermentation of 40 g/L reducing sugar from cassava starch.

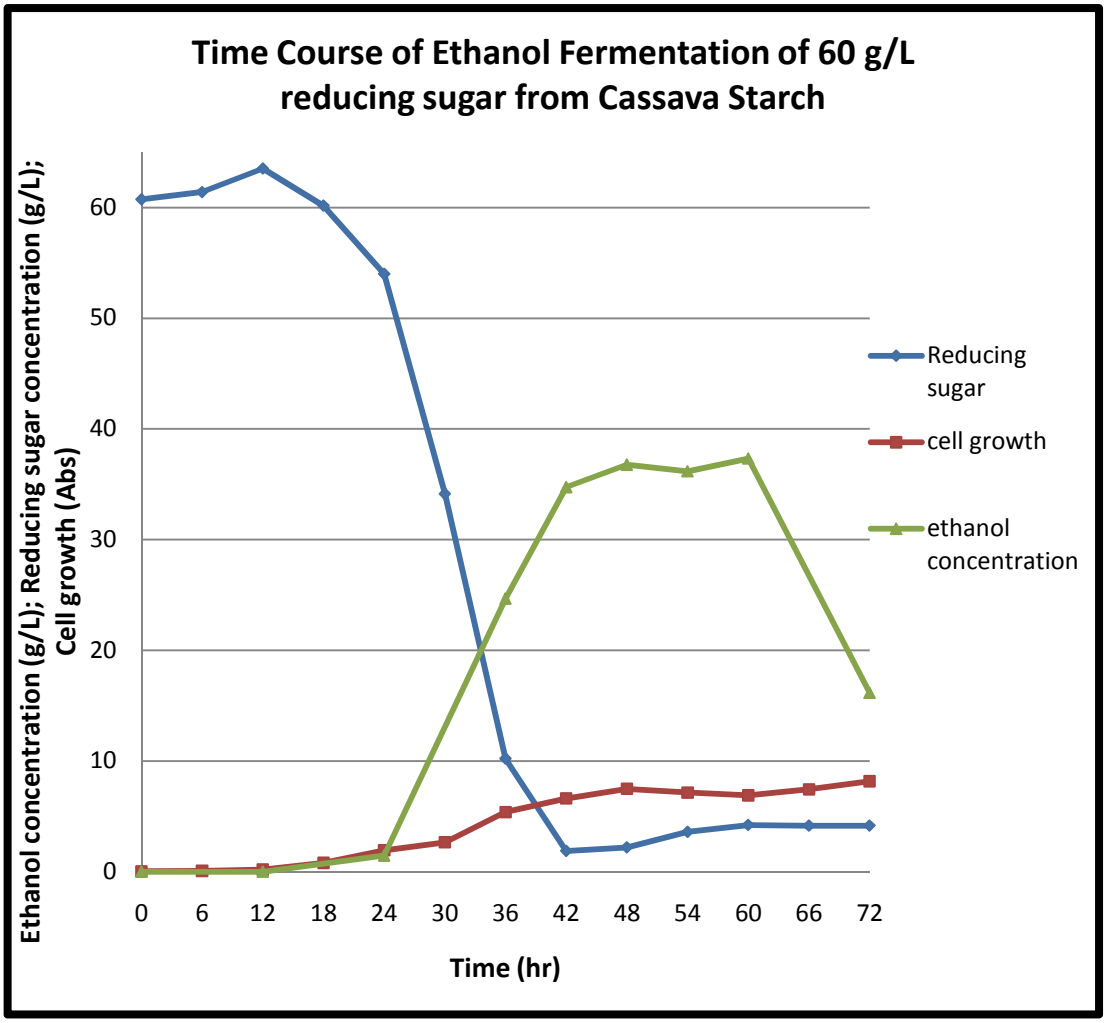


Figure 4.7: Time course of ethanol fermentation of 60 g/L reducing sugar from cassava starch.

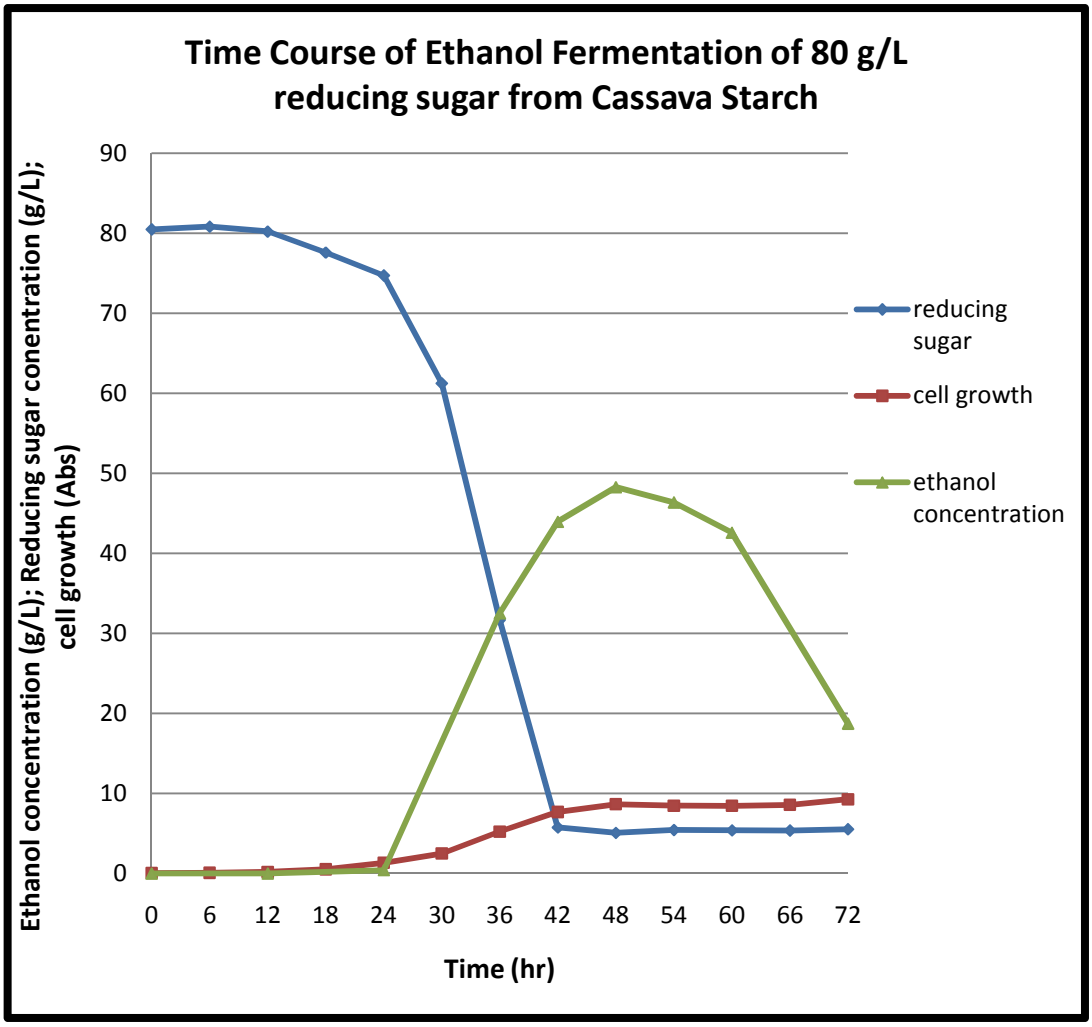


Figure 4.8: Time course of ethanol fermentation of 80 g/L reducing sugar from cassava starch.

From figure 4.5 till 4.8 shows the time course profile of the ethanol fermentation for 20 g/L, 40 g/L, 60 g/L and 80 g/L of initial reducing sugar content respectively. By comparing all of the four diagrams, the higher the initial reducing sugar content, the higher the ethanol concentration will be produced. After 12 hours, all data showed that the reducing sugar concentration started deplete. The reducing sugar will be utilized to produce the ethanol. Hence, the reducing sugar concentration decreased with the time but the ethanol concentration increased with the time. During 42 hour to 48 hour where the reducing sugar concentration nearly finish consumed by yeast, while the ethanol concentration was the highest at this point.

From the experimental data was known that the optimum duration for the ethanol fermentation was 48 hours. This is because, from the data shown, the ethanol concentration was the maximum at 48hours and after that the ethanol will decreased with time. This may caused by the ethanol evaporated while we were taking the samples.

4.2.4 Ethanol Yield and Productivity

From the experimental data shows in table 4.3, 60 g/L of initial reducing sugar able to produced higher yield than the 80 g/L of initial reducing sugar. But with the lower of reducing sugar longer fermentation time was required. This is proved by comparing the productivity of the 60 g/L with 80 g/L of reducing sugar content. In order to obtain higher productivity, 80 g/L of reducing sugar should be used for the fermentation of bio-ethanol. In general, normally 52.50 hr of fermentation process was needed to produce around 0.605190 product yields (g ethanol/ g reducing sugar).

The Product Yield was obtained by:

$$Product\ Yield\ \left(\frac{g\ ethanol}{g\ reducing\ sugar}\right) = \left(\frac{P_m - P_i}{S_i - S_m}\right) \quad (4.3)$$

Where P_m = Maximum Ethanol Concentration (g/L)

P_i = Initial Ethanol Concentration (g/L)

S_i = Initial Reducing Sugar Concentration (g/L)

S_m' = Reducing Sugar Concentration Attained at Maximum Product Concentration (g/L)

The Ethanol Productivity was obtained by:

$$Ethanol\ Productivity = \left(\frac{Maximum\ Ethanol\ Concentration}{Time\ taken\ to\ attained\ MAximum\ Ethanol\ Concentration}\right) \quad (4.4)$$

Table 4.2: Ethanol Yield and Productivity for Different Initial Reducing Sugar Concentration

Initial reducing sugar concentration (g/L)	Maximum Ethanol concentration (g/L)	Reducing sugar concentration attained at maximum product concentration (g/L)	Time to attain maximum ethanol concentration (hr)	Product Yield (g ethanol/ g reducing sugar)	Productivity (ethanol g/L/hr)
20	10.159707	0.623011	54.00	0.524318	0.188143
40	22.366676	1.628139	48.00	0.582893	0.465972
60	37.326015	4.216963	60.00	0.669128	0.622100
80	48.278712	5.082183	48.00	0.644422	1.005807
Average	-	-	52.50	0.605190	0.570506

4.3 Mass Balance for Ethanol Production

Two trays (A and B) which original contained 700 g of cassava chips were dried in oven for 5 days. The weight of the tray was 10 g and 9 g for tray A and B respectively.

Table 4.3: Weight of Cassava Chip throughout the Drying Process (Include Weight of Tray)

Number of Days	Weight of cassava chips (g)	
	Tray A	Tray B
0	700	700
1	300	247
2	256	227
3	253	224
4	253	224
5	253	224

Hence from the experimental obtained, the dry weight for cassava chips is,

$$Average\ dry\ weight\ for\ cassava\ chips\ (without\ weight\ of\ tray)$$

$$= \frac{(253g - 10g) + (224g - 9g)}{2}$$

$$= 229\ g$$

Then the cassava flour content is,

$$Flour\ content\ from\ cassava = \frac{229\ g}{700\ g} \times 100\ \%$$

$$= 32.70\ \%\ \text{(w/w)}$$

- Taking 60 g/L of initial reducing sugar concentration which able to produce the highest product yields (g ethanol/g reducing sugar) that is 0.669 g ethanol/g reducing sugar.

From experimental data (Table 4.3):

1 kg of raw cassava able to produce 32.70 % (w/w) of cassava flour,

1 g of flour will produce 0.559 g/L of reducing sugar,

Hence,

$$\frac{0.559\ g\ of\ reducing\ sugar}{1\ g.L\ of\ cassava\ flour} \times \frac{0.669\ g\ of\ ethanol}{g\ of\ reducing\ sugar} = \frac{0.374\ g\ ethanol}{g\ cassva\ flour}$$

Since, 1 g of cassava flour can produce 0.374 g ethanol, and 1 kg = 1000 g

Hence,

$$327.0\ g\ of\ cassava\ flour \rightarrow 327.0\ g\ cassava\ flour \times 0.374\ g\ of\ ethanol$$

$$\rightarrow 122.30\ g\ of\ ethanol$$

In other words, 1 kg of raw cassava can produce 122.30 g of ethanol.

Since the density of ethanol is 0.789 g/ml,

Hence,

$$1\ kg\ of\ raw\ cassava\ \rightarrow\ \frac{122.30\ g\ of\ ethanol}{\frac{0.789g}{ml}}$$

$$\rightarrow 155.00\ ml\ of\ ethanol$$

Finally, 1 kg of raw cassava can produce 0.155 L of ethanol.

OR,

6.45 kg of raw cassava can produce 1 L of ethanol,

OR,

1 ton of raw cassava able to produce 155 L of ethanol.

4.4 Kinetic Modelling on Product Formation

Kinetic Modelling for the product (Bio-ethanol) formation had been done by using Microsoft Excel Add-in Tools:

a) Solver,

b) Data Analysis (Multiple Regressions).

The kinetic model that used to represent the relationship between the ethanol mass production with the fermentation time was the Modified Gompertz Equation (equation 3.1). Different kinetic modeling was done on the ethanol formation for 20 g/L, 40 g/L, 60 g/L and 80 g/L of initial reducing sugar respectively.

Solver Add-in was used to minimize the error square between the experimental data with the calculated data from the Gompertz Equation. By providing the potential maximum ethanol concentration, P_m was known from the experiment results, maximum ethanol production rate and lag phase could be found by using the Solver. Then the comparison between experiment and calculated data could be done by the Data Analysis Add-in tool, that is by refer to the error square, smaller the error square was required. Besides, equation for the model, R2, F and P value will be obtained from Data Analysis Add-in tool. Figure 4.9 till 4.12 shows the

comparison of experimental with calculated production formation data for different initial reducing sugar content.

Since from the experimental data that obtained before (figure 4.5 till 4.8) shows the ethanol concentration dropped around 50 hour which due to the evaporation of the ethanol, so the data after 48 hour were ignored for the kinetic modeling.

4.4.1 Model of Product Formation with 20g/L of initial reducing sugar

The experimental data were fitted to the equation and the parameter values of the product formation model for 20 g/L as initial reducing sugar were listed in Table 4.4. The equation obtained after fitting the calculated parameters into equation 3.1 was shows below:

$$P = 4.222\exp\{-\exp\left[\frac{0.141\exp(1)}{4.222}(8.33 - t) + 1\right]\} \quad (4.5)$$

Table 4.4: Calculated Parameter using Modified Gompertz Equation for Ethanol Formation during Fermentation for 20 g/L of Initial Reducing Sugar Content.

Kinetic Parameter	Estimate
Potential Maximum Ethanol Concentration, P_m (g ethanol/L)	4.222
Maximum Ethanol Production Rate, $r_{p,m}$ (g ethanol/L.h)	0.141
Lag Phase, t_L (hr)	8.33

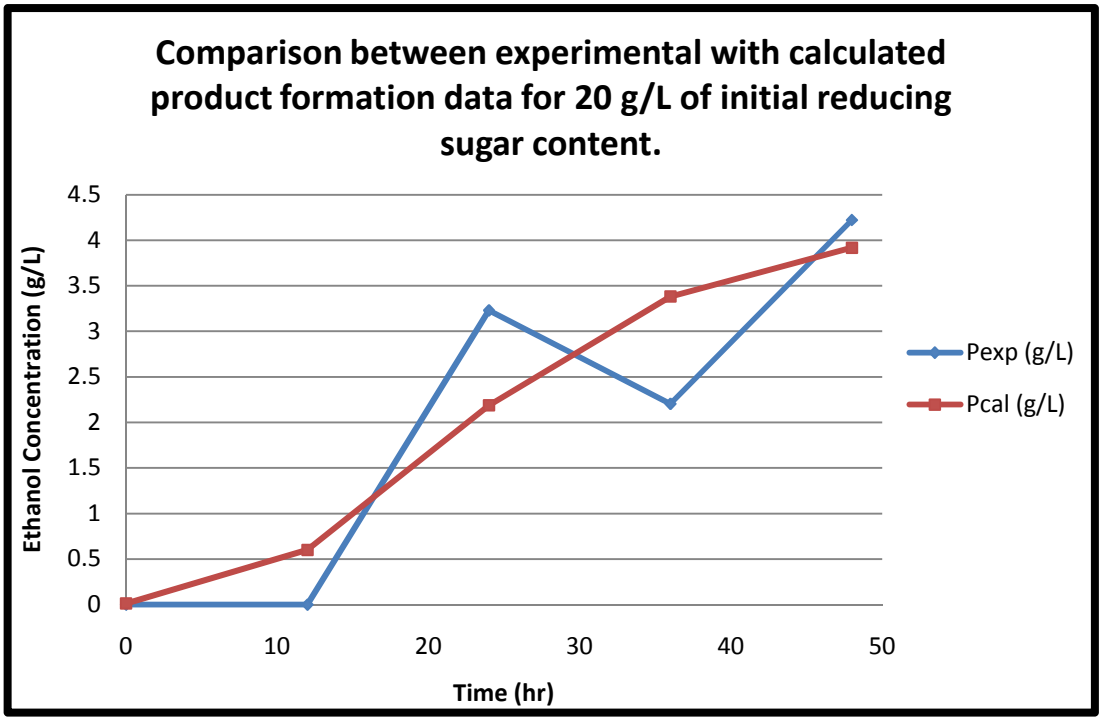


Figure 4.9: Comparison of experimental and calculated data of ethanol formation during the fermentation time for 20 g/L of initial reducing sugar content. Blue line represents experimental data and red line represents calculated data according to Gompertz Equation.

Figure 4.9 shows the kinetic of ethanol formation for 20 g/L as initial reducing sugar content. From the calculated data, it could be found that the ethanol concentration was gradually increased, but by refer to the slope of the calculated data, the maximum production rate would be within 10 hr till 35 hr. this is due to the steeper slope was found within 10 hr till 35 hr. Then the ethanol production rate was slowly decreased after 40 hr of fermentation.

Table 4.5: Comparison of experimental and calculated data using non-linear regression analysis for 20 g/L of initial reducing sugar content.

Kinetic Parameter	Ethanol Formation
Error Square (from Solver Add-in)	2.93000
Equation (from Data Analysis Add-in)	$P_{exp} = 1.0022P_{cal} - 0.0935$
R^2 value (from Data Analysis Add-in)	0.80032
F value (from Data Analysis Add-in)	12.0241
P value (from Data Analysis Add-in)	0.04040

4.4.2 Model of Product Formation with 40g/L of initial reducing sugar

The experimental data were fitted to the equation and the parameter values of the product formation model for 40 g/L as initial reducing sugar were listed in Table 4.6. The equation obtained after fitting the calculated parameter into equation 3.1 is shows below:

$$P = 15.117\exp\{-\exp\left[\frac{7.742\exp(1)}{15.117}(34.74 - t) + 1\right]\} \quad (4.6)$$

Table 4.6: Calculated Parameter using Modified Gompertz Equation for Ethanol Formation during Fermentation for 40 g/L of Initial Reducing Sugar Content.

Kinetic Parameter	Estimate
Potential Maximum Ethanol Concentration, P_m (g ethanol/L)	15.117
Maximum Ethanol Production Rate, $r_{p,m}$ (g ethanol/L.h)	7.742
Lag Phase, t_L (hr)	34.74

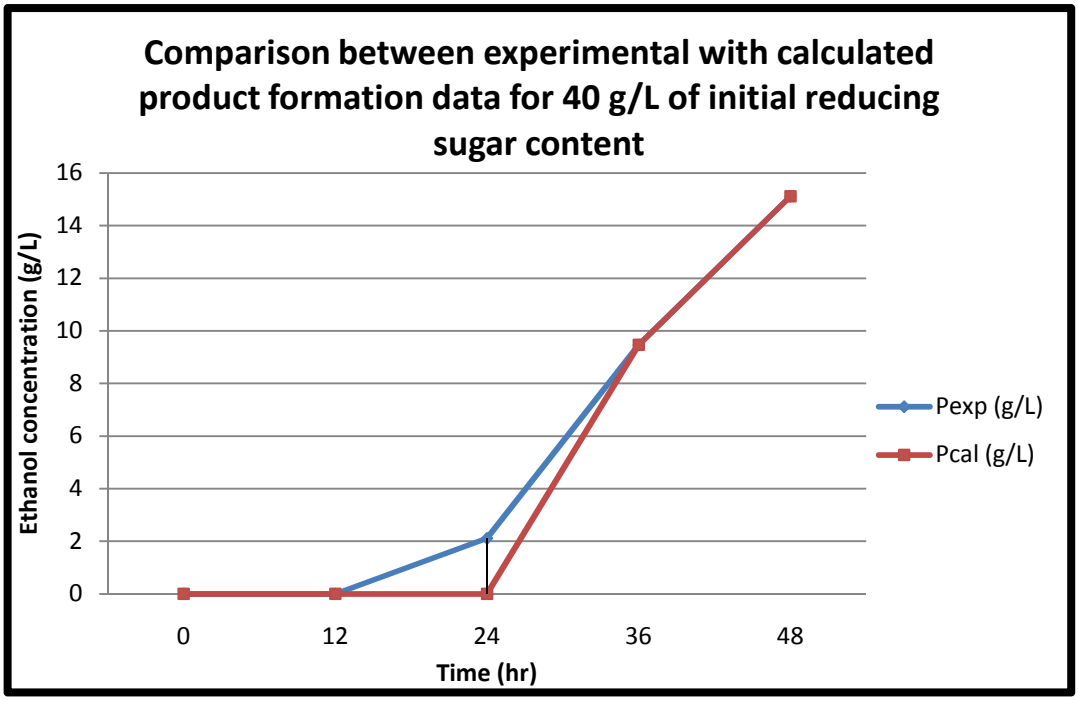


Figure 4.10: Comparison of experimental and calculated data of ethanol formation during the fermentation time for 40 g/L of initial reducing sugar content. Blue line represents experimental data and red line represents calculated data according to Gompertz Equation.

From Figure 4.10, the estimated model for the ethanol production (40 g/L of intial reducing sugar) was maintain at zero production from 0 hour till 24 hour. But the ethanol concentration incresed rapidly after 24 hour.

Table 4.7: Comparison of experimental and calculated data using non-linear regression analysis for 40 g/L of initial reducing sugar content.

Kinetic Parameter	Ethanol Formation
Error Square (from Solver Add-in)	4.47000
Equation (from Data Analysis Add-in)	$P_{exp} = 0.9473P_{cal} + 0.6818$
R^2 value (from Data Analysis Add-in)	0.98319
F value (from Data Analysis Add-in)	175.51233
P value (from Data Analysis Add-in)	0.00093

4.4.3 Model of Product Formation with 60g/L of initial reducing sugar

The experimental data were fitted to the equation and the parameter values of the product formation model for 60 g/L as initial reducing sugar were listed in Table 4.8. The equation obtained after fitting the calculated parameter into equation 3.1 is shows below:

$$P = 34.437\exp\{-\exp\left[\frac{19.417\exp(1)}{34.437}(34.38 - t) + 1\right]\} \quad (4.7)$$

Table 4.8: Calculated Parameter using Modified Gompertz Equation for Ethanol Formation during Fermentation for 60 g/L of Initial Reducing Sugar Content.

Kinetic Parameter	Estimate
Potential Maximum Ethanol Concentration, P_m (g ethanol/L)	34.437
Maximum Ethanol Production Rate, $r_{p,m}$ (g ethanol/L.h)	19.417
Lag Phase, t_L (hr)	34.38

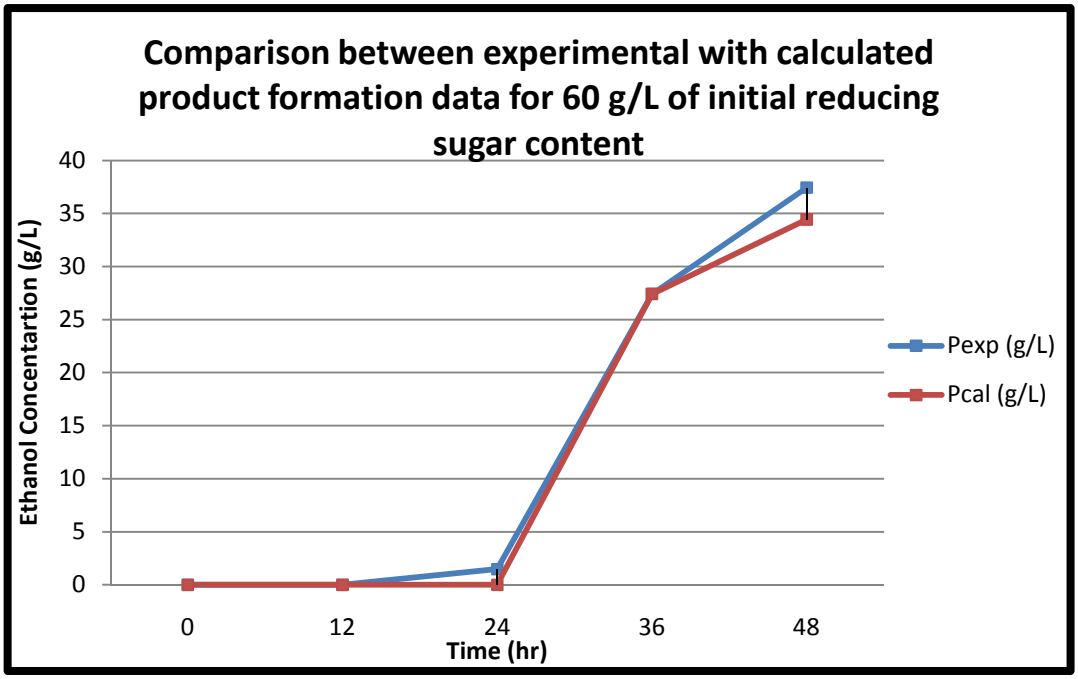


Figure 4.11: Comparison of experimental and calculated data of ethanol formation during the fermentation time for 60 g/L of initial reducing sugar content. Blue line represents experimental data and red line represents calculated data according to Gompertz Equation.

Figure 4.11 shows that with the initial reducing sugar of 60 g/L, the estimated ethanol concentration will keep constant at 0 g/L from 0hr until 24 hr, but the ethanol production will increase after 24 hr. Furthermore, by comparing the slope of the graph of calculated data, the ethanol production rate is higher between 24 hr till 36 hr compared with the production rate after 36 hr.

Table 4.9: Comparison of experimental and calculated data using non-linear regression analysis for 60g/L of initial reducing sugar content.

Kinetic Parameter	Ethanol Formation
Error Square (from Solver Add-in)	11.2000
Equation (from Data Analysis Add-in)	$P_{exp} = 1.0409P_{cal} + 0.3896$
R^2 value (from Data Analysis Add-in)	0.99590
F value (from Data Analysis Add-in)	730.88353
P value (from Data Analysis Add-in)	0.00011

4.4.4 Model of Product Formation with 80g/L of initial reducing sugar

The experimental data were fitted to the equation and the parameter values of the product formation model for 80 g/L as initial reducing sugar were listed in Table 4.10. The equation obtained after fitting the calculated parameter into equation 3.1 is shows below:

$$P = 53.334\exp\{-\exp\left[\frac{11.620\exp(1)}{53.334}(33.28 - t) + 1\right]\} \quad (4.8)$$

Table 4.10: Calculated Parameter using Modified Gompertz Equation for Ethanol Formation during Fermentation for 80 g/L of Initial Reducing Sugar Content.

Kinetic Parameter	Estimate
Potential Maximum Ethanol Concentration, P_m (g ethanol/L)	53.334
Maximum Ethanol Production Rate, $r_{p,m}$ (g ethanol/L.h)	11.620
Lag Phase, t_L (hr)	33.28

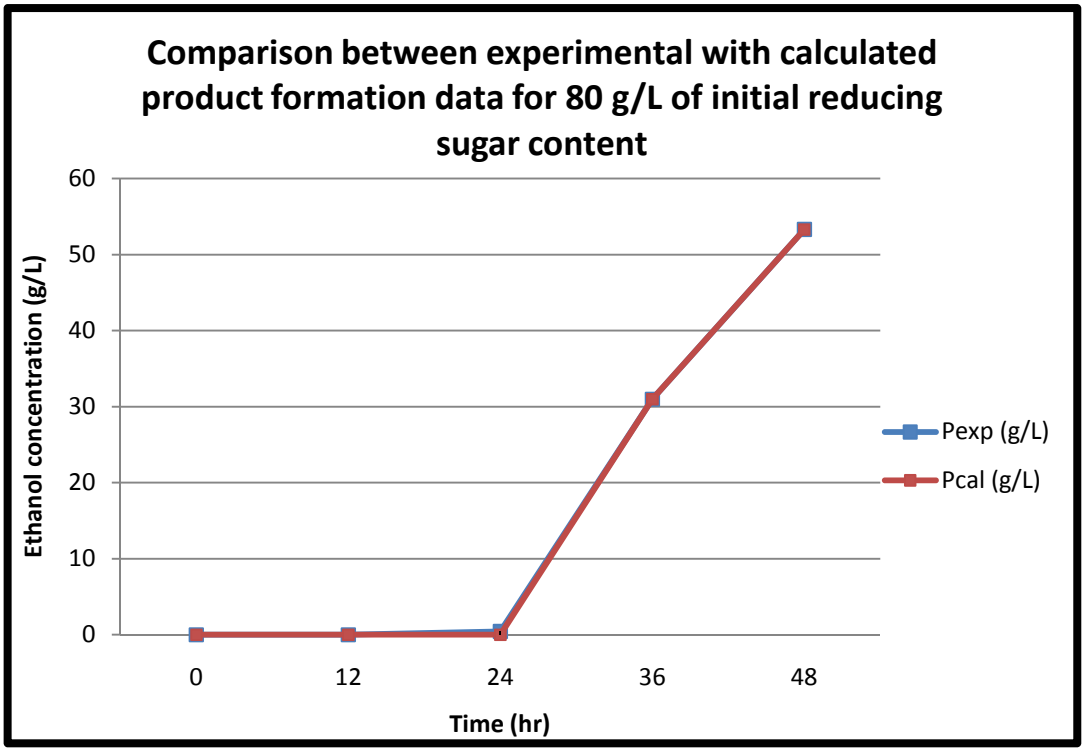


Figure 4.12: Comparison of experimental and calculated data of ethanol formation during the fermentation time for 80 g/L of initial reducing sugar content. Blue line represents experimental data and red line represents calculated data according to Gompertz Equation.

According to Figure 4.12, with the initial reducing sugar of 80 g/L, there will be no ethanol produced from 0 hr till 24 hr. However the ethanol production will be increased after 24 hr.

Table 4.11: Comparison of experimental and calculated data using non-linear regression analysis for 80 g/L of initial reducing sugar content.

Kinetic Parameter	Ethanol Formation
Error Square (from Solver Add-in)	0.185
Equation (from Data Analysis Add-in)	$P_{exp} = 0.9973P_{cal} + 0.1358$
R^2 value (from Data Analysis Add-in)	0.99994
F value (from Data Analysis Add-in)	55961.90676
P value (from Data Analysis Add-in)	1.66573E-07

By comparing the correlation coefficient (R^2 value) for each of the experiment, it was found that 80 g/L will gives the most fitted value that was 0.99994. In addition, the R^2 value for different initial reducing sugar was between 0.80032 till 0.99994 which showed the results were satisfactory between the calculated data and experimental data. In other words, suggested Modified Gompertze Equation is able to describe the ethanol production for cassava starch fermentation.

4.5 Comparison with Other Raw Material Used for Ethanol Production

The selection of the most appropriate feedstock for ethanol production strongly depends on the local conditions (Óscar and Carlos, 2007). Different feed stocks that can be utilized for bio-ethanol production and their comparative production potential are given in Table 4.12.

From Table 4.12, the highest bio-ethanol production will able be produce by rice followed by maize, wheat, barley, and cassava. However, it is not suitable to used rice, wheat and barley as the raw material for the bio-ethanol production. Rice, wheat and barley are the staple food for Malaysian. If these raw materials are use for the ethanol production, food crisis may happen and directly increased the food price. However, Malaysians do not highly depend on the cassava for the food production. Hence, cassava is recommended to be the raw material for the bio-ethanol production.

Table 4.12: Different feed stocks for bio-ethanol production and their comparative production potential

Raw Materials	*Bio-ethanol Production Potential (l/ton)*
Sugar Cane	70
Sugar beet	110
Potato	110
Wheat	340
Sweet Sorghum	60
Cassava**	155 (This Work)
Maize	360
Rice	430
Barley	250

Source (except Cassava): Mustafa et. al., 2008.

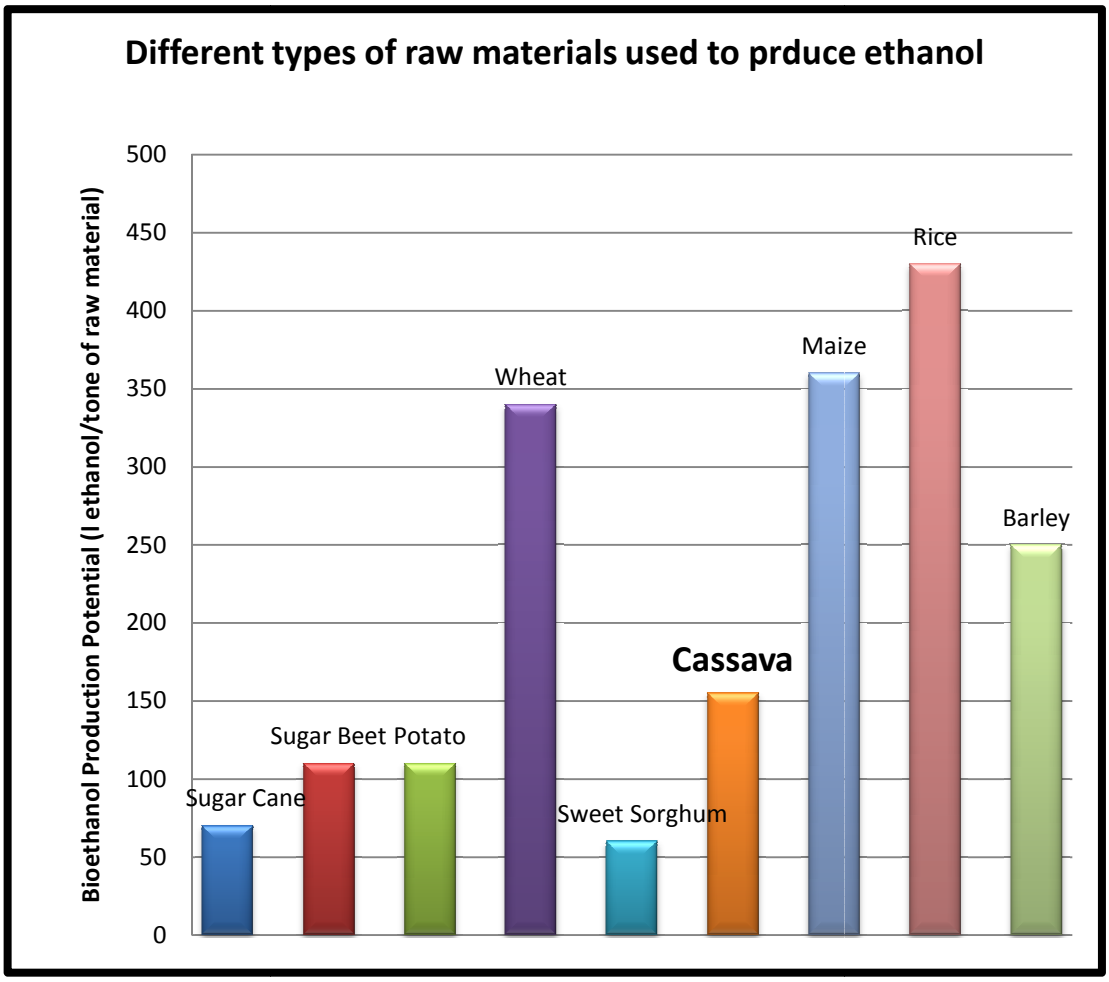


Figure 4.13: Comparison of the ethanol yield for different raw material used.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Ethanol Fermentation

Batch fermentation process was investigated in this research for the production of bio-ethanol from cassava by adding the industrial yeast *(ATCC 36858 S. Cerevisiae)* into the culture broth. The maximum ethanol yield which able to obtained was produced by using 60 g/L of initial reducing sugar content and the yield was 0.669128 g ethanol/ g reducing sugar. However, 80 g/L of initial reducing sugar will gives the higher productivity that was 1.005807 ethanol g/(L.hr).

From the fermentation process, the pH value was decreasing with time due to the formation of carbon dioxide, cell pellet was increasing with time then kept at a constant, and the ethanol concentration was kept increasing and decreased at around 48 hour due to the ethanol evaporation.

By comparing the ethanol yield that able to produce by cassava with other agricultural raw material, cassava appears to be the potential raw material to produce bio-ethanol in Malaysia. This is due to cassava is not the staple food in Malaysia, hence there will be no worry for the food crisis.

In conclusion, cassava is a suitable crops which able to be the raw material for the bio-ethanol production in Malaysia.

5.2 Kinetic Modelling for Ethanol Formation

Throughout this study, Modified Gompertz equation was used to describe the modelling for the ethanol (product) production from the cassava root as the raw material. Overall, the correlation coefficient (R^2 value) was between 0.80032 and 0.99994, and showed good agreement with the experimental data. This proved that modified Gompertz equation is suitable and able to describe the product formation for ethanol production from cassava root.

By using the Modified Gompertz equation, the optimization design or control parameters could be done without doing the experiment. This will save a lot of energy, time and indirectly save the production cost for the bio-ethanol.

5.3 Recommendations

To improve the fermentation process, it is recommended to use the bench – top fermenter experiment instead of using the shake flask experiment. More parameters such as temperature, pH, agitation speed, substrate concentration and product formation will be examined and control by using the bench – top fermenter, this will increased the accuracy and the ethanol yield since the evaporation problem that faced in this research will be greatly minimized.

In the fermentation process, higher reducing sugar content can be further examined since the initial reducing sugar that used in this research were 20 g/L, 40 g/L, 60 g/L and 80 g/L, then 100 g/L and 120 g/L of initial reducing sugar could be done to obtain the optimum initial reducing sugar for the ethanol fermentation.

High Performance Liquid Chromatography (HPLC) is the new analytical machine that available in University Tunku Abdul Rahman. HPLC test could be done to test the characteristic for saccharified medium in terms of reducing sugar concentration and the type of reducing sugar that obtained after the liquefaction and saccharification.

REFERENCES

Ahindra, N. (2007). *Biofueal Refining and Performance*. Retrieved July 17, 2010 @ http://books.google.com.my/books?id=SHNg1o0xdv8C&printsec=frontcover&dq=Biofuel&hl=en&ei=sSJOTL2VMc2yrAfoo6G5Dg&sa=X&oi=book_result&ct=result&resnum=3&ved=0CDkQ6AEwAg#v=onepage&q=Biofuel&f=false

Amutha, R. and P. Gunasekaran, 2001. Production of ethanol from liquefied cassava starch using co-immobilized cells of *Zymomonas mobilis* and *Saccharomyces diastaticus.* J. Biosci. Bioeng., 92: 560-564.

Anon. (1996). Crop hectareage statistics, Peninsular Malaysia, Kuala Lumpur, Department of Agriculture.

Baskar, G., Muthukumaran, C., & Renganahan, C. (2008). Optimaization of enzymatic hydrolysis of Manihot esculenta root starch by immobilized α-amylase using response surface methodology. International Journal of Chemical and Biomolecular Engineering, 1, 3 @ www.waset.orgsummer 2008.

Berg, C., 2004. World Fuel Ethanol. Analysis and Outlook. http://www.distill.com/World-Fuel-Ethanol-A&O-2004.html.

Berghofer, E., & Sarhaddar, S. (1988). Production of glucose and high fructose syrup by enzymatic direct hydrolysis of cassava roots. Process Biochemistry, 32, 910-915.

Bernardo Ospina, Neil Palmer (2009). Centro Internacional de Agricultura Tropica: Car Fulled by Cassava is a World First. Available in: http://webapp.ciat.cgiar.org/newsroom/release_42.htm

Cassava Production in the Different Countries in the World 2008. Source: FAO 2008 adapted by Hoang Long. Available in http://foodcrops.blogspot.com/2010/05/current-situation-of-cassava-in-vietnam.html

Collins K, Economic issues related to biofuels: a written testimony for field hearing. Retrieved from http://www.usda.gov on 12th March 2011.

C.F. González, J.I. Fariña, L.I.C. de Figueroa (2008). Optimized amylolytic enzymes production in *Saccharomycopsis fibuligera* DSM-70554, An approach to efficient cassava starch utilization. Enzymes and Microbial Technology 42, 272-277.

Christiana N. Ogbonna, Eric C. Okoli (2009). Conversion of cassava flour to fuel ethanol by sequential solid state and submerged cultures. Process Biochemistry 45 (2010) 1196-1200.

C. Jansson, A. Westerbergh, J. Zhang, X. Hu, C. Sun (2009). Cassava, a potential biofuel crop in (the) People's Republic of China, Applied Energy 86, S95-S99.

Demirbas, A., 2008. Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. Energy Convers. Manage., 49: 2106-2116.

Dr. Low Chong Yu (2009). Research on Agricultural Materials for Ethanol as a Gasoline, Department of Chemical Engineering, Faculty of Engineering and Science, UTAR, Setapak, KL.

FO Licht's World Ethanol & Biofuels Report (2006) @ http://www.agra-net.com

FAO Production Year Book (2006), Rome, Italy.

George W. Huber and Bruce E. Dale (2009). Grassoline at the Pump, Scientific American, July 2009.

Gorinstein, S. (1993). Kinetic studies during enzyme hydrolysis of potato and cassava starches. Starch/Starke, 45, 91-95.

Hguyen TLT, Gheewala SH, Garivait S. Full chain energy analysis of fuel ethanol from cassava in Thailand. Environ Sci Technol 2007; 4135-42.

Hu Z, Tan P and P G, Multi-objective optimization of cassava-based fuel ethanol used as an alternative automotive fuel in Guangxi. China Appl Energy 83:819-840 (2006).

José C. Escobar, Electo S.Lora, Osvaldo J. Venturini, Edgar E. Yáñez, Edgar F Castillo, Oscar Almazan (2009). Biofuels: Environment, technology and food security, Renewable and Sustainable Energy Reviews 13, 1275-1287.

Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI) (2003). Executive Summary, Development of Raw Material Management Plan for the Ethanol Industry in Thailand. National Ethanol Committee, Ministry of Industry, KAsetsart University.

Klanarong Sriroth, Present Situation of Cassava Starch Industry in Thailand. Cassava and Starch Technology Research Unit Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, KAsetsart University, Bangkok, Thailand.

Mohamed HAshem, Sounia M.I. Darwish (2010). Production of bioethanol and associated by-products from poato starch stream by *Saccharomyces cerevisiae.* Biomass and Bioenergy 34, 953-959.

Murray, D., 2005. Ethanol's potential: Looking beyond corn. Eco-Economy Updates. Earth Policy Institute. http://earth-policy.org/Updates/2005/Updates49.htm.

Mustafa Balat, Havva Balat, Cahide Oz. (2008). Progress in Bioethanol Processing. Progress in Energy and Combustion Science, 34, 551-573.

N. Nadir, M. Mel, M.I.A. Karim and R.M. Yunus (2009). Comparison of sweet sorghum and cassava for ethanol production by using *Saccharomyces cerevisiae*. Journal of Applied Science 9 (17): 3068-3073.

Óscar J. Sánchez, Carlos A. Cardona (2007). Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresource technology 99 (2008) 5270-5295.

Peter, Z. and Jos, V. (2008). *Sugarcane ethanol*. The Netherlands: Wageningen Academic Publishers.

Prasad S, Singh A, Joshi HC. Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resour Conserv Recycl 2007; 50: 1-39.

Rakesh Agrawal and Navneet R. Singh (2010). New Biofuel Processing Method for Mobile Facilities, Biotechnology news. Available in http://www.lifesciencesworld.com/biotechnology-news/view/150551?page=25

Regy Johnson, G. Padmaja, S.N. Moorthy (2009). Comparative production of glucose and high fructose syrup from cassava and sweet potato roots by direct conversion techniques. Innovative Food Science and Engineering Technologies 10 (2009) 616-620.

Renewable Fuels Associaton. 2007. Industry statics. http://www.ethanolrfa.org/industry/statistics/.

Rubo L, Wang C, Zhang C, Dai D and Pu G, Life cycle inventory and energy analysis of cassava-based fuel ethanol in China. J Clean Prod 16:374-384 (2008).

Shetty, J., Chotani, G., Gang, D., & Bates, D. (2007). Cassava as an alternative feedstock in the production of renewable transportation fuel. International Sugar Journal, 109 (1307), 3-11.

S.L. Tan. (2008). Rice & Industrial Crops Research Centre: Are bio-fuels a red herring or a sustainable option? Journal of Science and Technology in the Tropics (2008) 4: 83-88.

Tan Swee Lian and Khatijah Idris (2010). Present Situation and Future Potential of Cassava in Malaysia.

Teske S, Schäfer O (2007). Energy revolution: a sustainable world energy outlook. Renewable Energy World. Available in http://www.renewable-energy-world.com. Acessed: 15/13/2007

The Borneo Post, Gordon H Chin, 13th April 2010.

The Columbia Electronic Encyclopedia, 6th edition. (2007). *ethanol: Properties*. Retrieved July 15, 2010 @ http://www.infoplease.com/ce6/sci/A0858037.html

Wang W. Cassava production for industrial utilization in (the) PRC – present and future perspective. In: Cassava research and development in Asia: exploring new opportunities for an ancient crop. Seventh regional cassava workshop, Bangkok, Thailand. October 28- November 1; 2002. P. 33-8.

Wyman CE (1999). Biomass ethanol: technical progress, opportunities, and commercial challenges. Annu Rev Energy Env 1999;24:189-226.

APPENDICES

APPENDIX A: Experimental Results

Experimental Result for Liquefaction and Saccharification with 5% Initial Starch Content

Reducing Sugar Profile (5%)	Time (Min)	Abs			Dilution Factor	Concentration (g/L)			Average Concentration (g/L)
		1st Sample	2nd Sample	3rd Sample		1st Sample	2nd Sample	3rd Sample	
Liquefaction	0	0	0	0	0	0.00	0.00	0.00	0.00
	15	1.240	1.234	1.243	25	24.46	24.34	24.52	24.44
	30	0.969	1.083	1.114	25	19.11	21.36	21.97	20.82
	45	0.909	0.981	1.029	25	17.93	19.35	20.30	19.19
	60	0.895	0.974	0.994	25	17.65	19.21	19.61	18.82
	75	0.889	0.946	0.980	25	17.53	18.66	19.33	18.51
	90	0.900	0.970	1.011	25	17.75	19.13	19.94	18.94
	105	0.876	0.988	0.983	25	17.28	19.49	19.39	18.72
	120	0.901	0.945	0.945	25	17.77	18.64	18.64	18.35
Saccharification	135	0.971	1.276	1.006	50	38.30	50.34	39.68	38.99
	150	1.062	1.066	1.043	50	41.89	42.05	41.14	41.70
	165	1.064	1.120	1.078	50	41.97	44.18	42.52	42.89
	180	1.114	1.153	1.149	50	43.94	45.48	45.33	44.92
	195	1.158	1.106	1.160	50	45.68	43.63	45.76	45.02
	210	1.161	1.146	1.166	50	45.80	45.21	46.00	45.67
	225	1.174	1.151	1.139	50	46.31	45.40	44.93	45.55
	240	1.163	1.166	1.139	50	45.88	46.00	44.93	45.60
	255	1.168	1.163	1.160	50	46.07	45.88	45.76	45.90
	270	1.162	1.171	1.166	50	45.84	46.19	46.00	46.01
	285	1.163	1.185	1.162	50	45.88	46.75	45.84	46.15
	300	1.224	1.190	1.168	50	48.28	46.94	46.07	47.10

Experimental Result for Liquefaction and Saccharification with 10% Initial Starch Content

Reducing Sugar Profile (10%)	Time (Min)	Abs			Dilution Factor	Concentration (g/L)			Average Concentration (g/L)
		1st Sample	2nd Sample	3rd Sample		1st Sample	2nd Sample	3rd Sample	
Liquefaction	0	0	0	0	0	0.00	0.00	0.00	0.00
	15	0.944	1.120	1.120	50	37.24	44.18	44.18	44.18
	30	0.849	1.110	1.119	50	33.49	43.79	44.14	43.96
	45	0.832	1.089	1.076	50	32.82	42.96	42.45	42.70
	60	0.806	1.065	0.992	50	31.79	42.01	39.13	40.57
	75	0.770	1.021	0.993	50	30.37	40.28	39.17	39.72
	90	0.770	1.011	1.012	50	30.37	39.88	39.92	39.90
	105	0.778	1.014	1.023	50	30.69	40.00	40.36	40.18
	120	0.791	1.057	1.025	50	31.20	41.70	40.43	41.07
Saccharification	135	1.007	1.073	1.068	75	59.59	63.49	63.20	62.09
	150	1.141	1.130	1.131	75	67.51	66.86	66.92	67.10
	165	1.194	1.176	1.160	75	70.65	69.59	68.64	69.63
	180	1.214	1.181	1.261	75	71.83	69.88	74.62	72.11
	195	1.320	1.271	1.293	75	78.11	75.21	76.51	76.61
	210	1.321	1.308	1.345	75	78.17	77.40	79.59	78.38
	225	1.362	1.360	1.320	75	80.59	80.47	78.11	79.72
	240	1.394	1.373	1.401	75	82.49	81.24	82.90	82.21
	255	1.433	1.383	1.347	75	84.79	81.83	79.70	82.11
	270	1.386	1.333	1.374	75	82.01	78.88	81.30	80.73
	285	1.370	1.363	1.350	75	81.07	80.65	79.88	80.53
	300	1.375	1.401	1.366	75	81.36	82.90	80.83	81.70

Experimental Result for Liquefaction and Saccharification with 15% Initial Starch Content

Reducing Sugar Profile (15%)	Time (Min)	Abs			Dilution Factor	Concentration (g/L)			Average Concentration (g/L)
		1st Sample	2nd Sample	3rd Sample		1st Sample	2nd Sample	3rd Sample	
Liquefaction	0	0	0	0	0	0.00	0.00	0.00	0.00
	15	0.947	0.952	1.200	75	56.04	56.33	71.01	63.67
	30	0.856	0.950	1.121	75	50.65	56.21	66.33	61.27
	45	0.824	0.846	1.121	75	48.76	50.06	66.33	58.20
	60	0.796	0.844	1.065	75	47.10	49.94	63.02	56.48
	75	0.812	0.831	1.038	75	48.05	49.17	61.42	55.30
	90	0.855	0.817	0.960	75	50.59	48.34	56.80	52.57
	105	0.796	0.798	1.000	75	47.10	47.22	59.17	53.20
	120	0.838	0.859	1.133	75	49.59	50.83	67.04	58.93
Saccharification	135	1.013	0.939	1.055	100	79.92	74.08	83.23	79.08
	150	1.051	0.990	1.169	100	82.92	78.11	92.23	84.42
	165	1.192	1.099	1.229	100	94.04	86.71	96.96	92.57
	180	1.241	1.137	1.243	100	97.91	89.70	98.07	95.23
	195	1.315	1.215	1.424	100	103.75	95.86	112.35	103.98
	210	1.334	1.267	1.489	100	105.25	99.96	117.48	107.56
	225	1.394	1.309	1.467	100	109.98	103.27	115.74	109.66
	240	1.420	1.337	1.360	100	112.03	105.48	107.30	108.27
	255	1.444	1.307	1.445	100	113.93	103.12	114.00	110.35
	270	1.525	1.524	1.444	100	120.32	120.24	113.93	118.16
	285	1.268	1.122	1.228	125	125.05	110.65	121.10	118.93
	300	1.235	1.166	1.214	125	121.79	114.99	119.72	118.84

Experimental Result for Fermentation Process

A) Reducing Sugar Concentration with different initial Reducing Sugar concentration

Time (hr)	Abs															
	20 g/L				40 g/L				60 g/L				80 g/L			
	Sample 1	Sample 2	Sample 3	Average	Sample 1	Sample 2	Sample 3	Average	Sample 1	Sample 2	Sample 3	Average	Sample 1	Sample 2	Sample 3	Average
0	1.0500	1.0640	1.0190	1.0443	1.0320	0.9990	1.0180	1.0163	1.0670	1.0040	1.0090	1.0267	1.0300	1.0230	1.0080	1.0203
6	1.0340	1.0420	1.0240	1.0333	1.0270	1.0260	1.0280	1.0270	1.0570	1.0190	1.0370	1.0377	1.0280	1.0480	0.9980	1.0247
12	1.0290	1.0340	0.9910	1.0180	1.1030	0.9930	0.9880	1.0280	1.0820	1.0690	1.0700	1.0737	1.0160	1.0600	0.9750	1.0170
18	0.8550	0.8430	0.8860	0.8613	0.9320	0.9300	1.0010	0.9543	1.0540	1.0310	0.9660	1.0170	0.9920	1.0330	0.9260	0.9837
24	0.4160	0.5140	0.3590	0.4297	0.7630	0.7780	0.7680	0.7697	0.9340	0.9130	0.8910	0.9127	0.9720	0.9280	0.9420	0.9473
30	0.1000	0.0790	0.0990	0.0927	0.4210	0.1750	0.1490	0.2483	0.6520	0.5090	0.8360	0.6657	0.8840	0.8610	0.8430	0.8627
36	0.0790	0.1240	0.0820	0.0950	0.0460	0.0300	0.0310	0.0357	0.0220	0.0150	0.5610	0.1993	0.4590	0.4320	0.4480	0.4463
42	1.1580	1.3430	1.5680	1.3563	0.2850	2.0850	2.4960	1.6220	2.6420	2.8280	1.6610	2.3770	0.2980	0.2860	0.2920	0.2920
48	0.7380	1.5010	1.5030	1.2473	2.0970	2.1770	1.9170	2.0637	2.8750	2.6540	2.8110	2.7800	0.2590	0.2570	0.2570	0.2577
54	0.4810	1.2500	0.6380	0.7897	1.5830	1.5660	1.1060	1.4183	2.1070	1.9800	2.7550	2.2807	0.6850	0.6890	0.6950	0.6897
60	0.4500	1.3450	0.4760	0.7570	1.6600	1.6240	0.8310	1.3717	0.9650	0.9280	1.3140	1.0690	0.6760	0.6940	0.6790	0.6830
66	0.6000	0.7980	0.4600	0.6193	1.7620	1.5560	0.7570	1.3583	1.0180	0.8960	1.2450	1.0530	0.6760	0.6890	0.6770	0.6807
72	0.5150	0.5430	0.5180	0.5253	1.5660	1.6810	0.6710	1.3060	1.0570	0.9510	1.1560	1.0547	0.6830	0.7030	0.7220	0.7027

B) pH value of each sample with different initial Reducing Sugar concentration

Time	pH															
	20 g/L				40 g/L				60 g/L				80 g/L			
	Sample 1	Sample 2	Sample 3	Average	Sample 1	Sample 2	Sample 3	Average	Sample 1	Sample 2	Sample 3	Average	Sample 1	Sample 2	Sample 3	Average
0	6.58	6.59	6.59	6.59	6.43	6.44	6.43	6.43	6.30	6.30	6.30	6.30	6.18	6.18	6.18	6.18
6	6.54	6.55	6.56	6.55	6.38	6.35	6.38	6.37	6.23	6.29	6.26	6.26	6.12	6.17	6.12	6.14
12	6.40	6.39	6.38	6.39	6.25	6.24	6.26	6.25	6.15	6.15	6.13	6.14	6.03	6.04	6.05	6.04
18	6.13	6.07	6.07	6.09	5.93	5.98	5.99	5.97	5.89	5.88	5.87	5.88	5.94	5.93	5.29	5.72
24	5.56	5.54	5.56	5.55	5.11	5.58	5.61	5.43	5.60	5.60	5.03	5.41	5.67	5.65	5.63	5.65
30	5.64	5.63	5.74	5.67	4.89	5.43	5.34	5.22	5.38	5.35	4.93	5.22	5.37	5.35	5.35	5.36
36	5.94	5.95	6.04	5.98	5.02	5.50	5.46	5.33	5.43	5.48	5.00	5.30	5.24	5.27	5.28	5.26
42	5.77	6.16	6.32	6.08	5.38	5.67	5.78	5.61	5.55	5.77	4.95	5.42	5.28	5.35	5.34	5.32
48	5.65	6.40	6.49	6.18	5.51	6.07	5.75	5.78	5.75	6.12	5.22	5.70	5.40	5.49	5.42	5.44
54	5.80	6.55	5.72	6.02	5.78	6.18	5.74	5.90	5.86	6.14	5.51	5.84	5.48	5.62	5.55	5.55
60	5.87	6.54	5.75	6.05	5.82	6.24	5.50	5.85	5.90	6.12	5.66	5.89	5.56	5.70	5.62	5.63
66	5.81	5.95	5.76	5.84	5.80	6.30	5.47	5.86	5.98	6.14	5.66	5.93	5.60	5.80	5.66	5.69
72	5.96	5.76	6.05	5.92	5.82	6.53	5.63	5.99	6.12	6.20	5.66	5.99	5.78	6.00	5.87	5.88

C1) Cell Pellet Concentration of each sample with 20g/L Reducing Sugar Concentration

Time	Absorbance				
	20 g/L				
	Sample 1	Sample 2	Sample 3	Dilution Factor	Average
0	0.018	0.015	0.016	0	0.0163
6	0.033	0.037	0.037	0	0.0357
12	0.153	0.177	0.169	0	0.1663
18	0.689	0.63	0.765	0	0.6947
24	1.05	1.016	1.146	2	2.1413
30	1.335	1.287	1.367	2	2.6593
36	0.764	0.783	0.822	5	3.9483
42	0.484	0.446	0.458	10	4.6267
48	0.554	0.479	0.539	10	5.2400
54	0.605	0.497	0.61	10	5.7067
60	0.641	0.535	0.611	10	5.9567
66	0.595	0.607	0.663	10	6.2167
72	0.671	0.718	0.761	10	7.1667

C2) Cell Pellet Concentration of each sample with 40g/L Reducing Sugar Concentration

Time	Absorbance				
	40 g/L				
	Sample 1	Sample 2	Sample 3	Dilution Factor	Average
0	0.037	0.03	0.027	0	0.0313
6	0.052	0.052	0.05	0	0.0513
12	0.174	0.169	0.191	0	0.1780
18	0.817	0.757	0.734	0	0.7693
24	1.176	0.952	0.934	2	2.0413
30	1.348	1.599	1.628	2	3.0500
36	0.95	0.989	1.075	5	5.0233
42	0.515	0.537	0.52	10	5.2400
48	0.595	0.616	0.658	10	6.2300
54	0.603	0.592	0.704	10	6.3300
60	0.61	0.592	0.645	10	6.1567
66	0.546	0.627	0.673	10	6.1533
72	0.643	0.681	0.764	10	6.9600

C3) Cell Pellet Concentration of each sample with 60g/L Reducing Sugar Concentration

Time	Absorbance				
	60 g/L				
	Sample 1	Sample 2	Sample 3	Dilution Factor	Average
0	0.046	0.045	0.036	0	0.042
6	0.087	0.066	0.074	0	0.076
12	0.216	0.193	0.178	0	0.196
18	0.63	0.686	1.071	0	0.796
24	0.901	0.973	1.038	2	1.941
30	1.369	1.511	1.116	2	2.664
36	1.226	1.25	0.756	5	5.387
42	0.694	0.741	0.551	10	6.620
48	0.781	0.795	0.671	10	7.490
54	0.731	0.773	0.641	10	7.150
60	0.716	0.779	0.574	10	6.897
66	0.741	0.834	0.656	10	7.437
72	0.818	0.914	0.719	10	8.170

C4) Cell Pellet Concentration of each sample with 80g/L Reducing Sugar Concentration

Time	Absorbance				
	80 g/L				
	Sample 1	Sample 2	Sample 3	Dilution Factor	Average
0	0.06	0.066	0.06	0	0.062
6	0.086	0.088	0.088	0	0.087
12	0.193	0.194	0.204	0	0.197
18	0.491	0.558	0.543	0	0.531
24	1.546	1.253	1.238	0	1.346
30	1.263	1.238	1.241	2	2.495
36	1.014	1.036	1.087	5	5.228
42	0.737	0.828	0.741	10	7.687
48	0.854	0.875	0.869	10	8.660
54	0.829	0.853	0.859	10	8.470
60	0.939	0.851	0.747	10	8.457
66	0.834	0.874	0.867	10	8.583
72	0.984	0.915	0.884	10	9.277

D1) Ethanol Concentration of each sample with 20g/L Reducing Sugar Concentration

Time (hr)	Samples							
	1		2		3		Average	
	Area under the graph	concentration (g/l)	Area under the graph	concentration (g/l)	Area under the graph	concentration (g/l)	Area under the graph	concentration (g/l)
0	0.00	0.00	-	-	0.00	0.00	0.00	0.00
12	0.00	0.00	-	-	0.00	0.00	0.00	0.00
24	7765.29	3.53	-	-	6291.86	2.86	7028.58	3.19
36	4363.55	1.98	4294.34	1.95	-	-	4328.95	1.97
42	22625.48	10.27	-	-	19996.80	9.08	21311.14	9.68
48	12811.98	5.82	25912.30	11.77	-	-	19362.14	8.79
54	27050.12	12.28	-	-	17693.23	8.04	22371.68	10.16
60	5127.79	2.33	-	-	10527.73	4.78	7827.76	3.55
72	3784.33	1.72	-	-	7549.81	3.43	5667.07	2.57

D2) Ethanol Concentration of each sample with 40g/L Reducing Sugar Concentration

Time (hr)	Samples							
	1		2		3		Average	
	Area under the graph	concentration (g/l)	Area under the graph	concentration (g/l)	Area under the graph	concentration (g/l)	Area under the graph	concentration (g/l)
0	0.00	0.00	0.00	0.00	-	-	0.00	0.00
12	0.00	0.00	0.00	0.00	-	-	0.00	0.00
24	2864.34	1.30	6334.50	2.88	-	-	4599.42	2.09
36	15353.76	6.97	25863.48	11.75	-	-	20608.62	9.36
42	39118.25	17.76	48714.17	22.12	-	-	43916.21	19.94
48	42823.72	19.45	-	-	55679.12	25.29	49251.42	22.37
54	44427.27	20.18	50088.38	22.75	-	-	47257.83	21.46
60	35442.85	16.10	10194.98	4.63	130880.00	-	22818.92	10.36
72	11263.44	5.12	11658.51	5.29	-	-	11460.98	5.20

D3) Ethanol Concentration of each sample with 60g/L Reducing Sugar Concentration

Time (hr)	Samples							
	1		2		3		Average	
	Area under the graph	concentration (g/l)	Area under the graph	concentration (g/l)	Area under the graph	concentration (g/l)	Area under the graph	concentration (g/l)
0	0.00	0.00	0.00	0.00	-	-	0.00	0.00
12	0.00	0.00	0.00	0.00	-	-	0.00	0.00
24	2841.02	1.29	3586.67	1.63	-	-	3213.85	1.46
36	-	-	52451.09	23.82	56171.91	25.51	54311.50	24.66
42	72548.49	32.95	80451.01	36.54	-	-	76499.75	34.74
48	-	-	80370.77	36.50	81532.84	37.03	80951.81	36.76
54	80435.93	36.53	78866.19	35.82	-	-	79651.06	36.17
60	91481.32	41.54	72902.45	33.11	-	-	82191.89	37.33
72	44590.13	20.25	26546.39	12.06	-	-	35568.26	16.15

D4) Ethanol Concentration of each sample with 80g/L Reducing Sugar Concentration

Time (hr)	Samples							
	1		2		3		Average	
	Area under the graph	concentration (g/l)	Area under the graph	concentration (g/l)	Area under the graph	concentration (g/l)	Area under the graph	concentration (g/l)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	-	-	493.27	0.22	1376.34	0.63	934.81	0.42
36	69427.40	31.53	-	-	73773.36	33.50	71600.38	32.52
42	-	-	104393.43	47.41	89189.47	40.50	96791.45	43.96
48	86817.91	39.43	98881.14	44.91	133230.12	60.50	106309.72	48.28
54	-	-	101302.42	46.00	102954.42	46.75	102128.42	46.38
60	-	-	95473.04	43.36	92108.32	41.83	93790.68	42.59
72	-	-	35849.06	16.28	46645.62	21.18	41247.34	18.73

Appendix B: Selected Chromatograms for Ethanol Concentration Analysis by Gas Chromatography (GC)