PRODUCTION OF BIOETHANOL FROM EMPTY FRUIT BUNCH (EFB) OF OIL PALM

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BIOETHANOL PRODUCTION FROM EMPTY FRUIT BUNCH (EFB) OF OIL PALM

HAJAR HADYAN HANI BT ZAINI

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering

Faculty of Chemical and Natural Resources Engineering
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NOVEMBER 2006

I declare that this thesis entitled *"Bioethanol production from empty fruit bunch (EFB) of oil palm"* is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Special Dedication to my…

Beloved parents

ZAINI BIN OTHMAN
ZAKIAH BT YAZID

For Their Love, Support, Advices, Help and Best Wishes.

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ABSTRACT

There are several methods to produce bioethanol. The bioethanol can be produced from cellulose and hemicelluloses that originate from many sources of biomass. The studies focused on the production of ethanol from oil palm waste using Saccaromyces cerevisie as fermentation aide to expand the usage of the oil palm waste. The purposes of this study were to determine the yield of ethanol produced from certain amount of biomass waste (oil palm wastes). Other than that the effects of different glucose concentration to the yield of bioethanol were studied. Besides, the effects of the different concentration of Saccaromyces cerevisae used to the yield of glucose production were studied too. Two sets of experiments were performed; in first set, the sample was hydrolyzed with pretreatment with sodium hydroxide then being subjected to sulfuric acid. The oil palm empty fruit bunch (EFB) is hydrolyzed using different concentrations of acid. Pretreatment is for break the lignin seal. At the second set the fermentation with Saccaromyces cerevisae was done in anaerobic condition. Different concentration of yeast was used in this experiment. The result showed that, the inoculums concentration did not have pronounced effect on the final ethanol concentration but the duration of fermentation decreased with the increase of the yeast concentration. It also showed that as the concentration of glucose increased, the ethanol concentration also increased where the highest final ethanol concentration with 15mg/ml of glucose was 13.8 % (w/w)

ABSTRAK

Pada masa kini terdapat pelbagai kaedah untuk menghasilkan ethanol. Antara kaedah yang digunakan adalah penghasilan ethanol menggunakan hemiselullosa dan selullosa daripada hasil buangan biojisim. Kajian terhadap penghasilan ethanol daripada hasil buangan pokok kelapa sawit menggunakan kaedah penapaian menggunakan yis, dilakukan untuk memperluaskan penggunaan bahan buangan pokok kelapa sawit tersebut. Tujuan kajian ini dilakukan adalah untuk menentukan ethanol yang terhasil daripada bahan buangan pokok kelapa sawit. Kesan kepekatan glukosa yang berbeza terhadap ethanol terhasil juga dikaji Selain itu juga, kesan menggunakan kepekatan yis yang berbeza dalam penghasilan ethanol juga turut dikaji. Di dalam kajian ini, hasil buangan yang digunakan adalah tandan kosong buah kelapa sawit (EFB). Dua set eksperimen telah dilakukan; untuk set yang pertama, sampel dihidrolisiskan melalui prarawatan menggunakan natrium hidroksida kemudian ditindakbalaskan dengan asid sulfurik. Proses hidrolisis yang dilakukan ke atas tandan kosong buah kelapa sawit (EFB) tersebut menggunakan kepekatan asid sulfurik yang berlainan. Prarawatan adalah untuk memecahkan lignin dalam struktur. Kemudian pada set kedua penapaian menggunakan yis Saccaromyces cerevisae dijalankan dalam keadaan anaerobik. Proses penapaian dilakukan menggunakan kepekatan yis yang berbeza. Pada akhir eksperimen, didapati kepekatan yis yang berbeza pada sample tidak memberi kesan pada kepekatan ethanol yang terhasil. Masa proses penapaian didapati berkurangan dengan bertambahnya kepekatan yis. Selain itu didapati dengan bertambahnya kepekatan glukosa, kepekatan ethanol terhasil juga bertambah. Kepekatan tertinggi ethanol didapati ialah 13.8 % (w/w) iaitu pada kepekatan glukosa 15mg/ml.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Ethanol or ethyl alcohol (C_2H_5OH) is a flammable, colorless liquid with a boiling point of 78.5° C. Its low melting point of -114.5° C allows it to be used in antifreeze products. Its density is 789 g/L, about 20% less than that of water [1]. It is biodegradable, low in toxicity and causes little environmental pollution if spill. Figure 1.1 shows the formula structure of ethanol where ethanol is the second member of the aliphatic alcohol series. The aliphatic alcohols are a series of homologous series organic compounds containing one or more hydroxyl groups [-OH] attached to an alkyl radical.

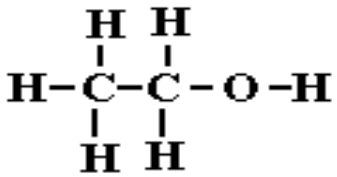

Figure 1.1: Formula Structure of Ethanol

Ethanol is one form of renewable energy that is becoming widely used, especially as fuel in automotive engine. Unlike gasoline, which is refined through distilling crude oil, ethanol can be synthesized from the starchy parts of plants. Microscopic yeast cells break down the starch and water, creating ethanol and carbon dioxide gas. Similar to gasoline, ethanol burns to produce carbon dioxide and water in complete combustion. It also a high octane fuel, subsequently has replaced lead as an octane enhancer in petrol.

Bioethanol is an ethanol synthesized from biomass and it is renewable. Therefore bioethanol has some advantages over petrol as fuel. As the biomass grows, it consumes as much carbon dioxide as it forms during the combustion of bioethanol, which makes the net contribution to the green house effect zero. By encouraging bioethanol's use, the rural economy would also receive a boost from growing the necessary crops. In addition, using bioethanol in older engines can help to reduce the amount of carbon monoxide produced by the vehicle thus improving air quality. Another advantage of bioethanol is the ease with which it can be easily integrated into the existing road transport fuel system. In quantities up to 5%, bioethanol can be blended with conventional fuel without the need of engine modifications [2].

Bioethanol is produced using familiar methods, such as fermentation, and it can be distributed using the same petrol forecourts and transportation systems as before.Two reactions are key to understanding how biomass is converted to bioethanol:

i. Hydrolysis is the chemical reaction that converts the complex polysaccharides in the raw feedstock to simple sugars. The feedstock must first be hydrolyzed into glucose molecules before ethanol production can begin [3]. In the biomass-to-bioethanol process, acids and enzymes are used to catalyze this reaction.

ii. Fermentation is a series of chemical reactions that convert sugars to ethanol. The fermentation reaction is caused by yeast or bacteria, which feed on the sugars. Ethanol and carbon dioxide are produced as the sugar is consumed. The simplified fermentation reaction for the 6-carbon sugar, glucose, is:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2$$

Glucose → ethanol + carbon dioxide (1.0)

The sugar formed in the hydrolysis reaction is fermented into bioethanol. The most common microorganism for this purpose is *Saccharomyces cerevisiae*, ordinary baking yeast [4]. Various species of *Saccharomyces* are examined for ethanol production processes because they are very efficient in converting sugars into ethanol [5]. Besides glucose, *Saccharomyces cerevisiae* has the ability to ferment manosse as well.

In this study empty fruit bunch (EFB) of oil palm has been chosen to be the substrate for the fermentation here in this research due to its abundant and low cost rather than using other source it also clean, a nontoxic and renewable.

1.2 Objective

As a bioethanol has a huge potential to substitute fossil gasoline as fuel in car engine, this research is carried out with the objective of producing bioethanol from empty fruit bunch (EFB) of oil palm

1.3 Scope of Study

In order to achieve the above objective, the following scopes have been identified:

i. To study the effect of different size of inoculumn in bioethanol production.
ii. To study the effect of different glucose concentration to the yield of bioethanol.
iii. To determine yield of bioethanol that can be produced from EFB

1.4 Problem Statements

The combustion of fossil fuels such as coal, oil, and natural gas has increased the concentration of carbon dioxide in the earth's atmosphere. The carbon dioxide and other so-called greenhouse gases allow solar energy to enter the Earth's atmosphere, but reduce the amount of energy that can re-radiate back into space, trapping energy and causing global warming.

One environmental benefit of replacing fossil fuels with biomass-based fuels is that the energy obtained from biomass does not add to global warming. All fuel combustion, including fuels produced from biomass, releases carbon dioxide into the atmosphere. Nonetheless, as because plants use carbon dioxide from the atmosphere

to grow (photosynthesis), the carbon dioxide released during combustion is balanced by that absorbed during the annual growth of the plants—unlike burning fossil fuels which releases carbon dioxide captured billions of years ago. Producing ethanol from cellulosic material involves generating electricity by combusting the non-fermentable lignin. The combination of reducing both gasoline use and fossil electrical production can mean a greater than 100% net greenhouse gas emission reduction.

Petroleum diesel and gasoline consist of blends of hundreds of different hydrocarbon chains. Many of these are toxic, volatile compounds such as benzene, toluene, and xylenes, which are responsible for the health hazards and pollution associated with combustion of petroleum-based fuels. Carbon monoxide, nitrogen oxides, sulfur oxides and particulates, are other specific emissions of concern. A key environmental benefit of using biofuels as an additive to petroleum-based transportation fuels is a reduction in these harmful emissions.

Bioethanol is used as fuel oxygenates to improve combustion characteristics, as extra oxygen atoms results in more complete combustion. This will reduce the content of carbon monoxide emissions. As such, it is another environmental benefit of replacing petroleum fuels with biofuels. Lastly, the production of bioethanol from empty fruit bunch can spur economic growth because it expands the usage of oil palm and also can reduces the cost in producing fossil fuels.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

Worldwide, biomass is the fourth largest energy resource after coal, oil, and natural gas. Biomass is material that comes from plants. Plants use the light energy from the sun to convert water and carbon dioxide to sugars through a process called photosynthesis. Organic waste is also considered to be biomass, because it began as plant matter. Some plants, like sugar cane and sugar beets, store the energy as simple sugars. These are mostly used for food. Other plants store the energy as more complex sugars, called starches. These plants include grains like corn and are also used for food.

Another type of plant matter, called cellulosic biomass, is made up of very complex sugar polymers, and is not generally used as a food source. This type of biomass is under consideration as a feedstock for bioethanol production. Specific feedstocks under consideration include:

i. Agricultural residues (leftover material from crops, such as the stalks, leaves, and husks of corn plants)
ii. Forestry wastes (chips and sawdust from lumber mills, dead trees, and tree branches)
iii. Municipal solid waste (household garbage and paper products)
iv. Food processing and other industrial wastes (black liquor, a paper manufacturing by-product)
v. Energy crops (fast-growing trees and grasses) developed just for this purpose

Biomass energy currently contributes 9–13% of the global energy supply—accounting for 45±10 EJ per year [6]. Biomass energy includes both traditional uses (e.g., firing for cooking and heating) and modern uses (e.g., producing electricity and steam, and liquid biofuels). Use of biomass energy in modern ways is estimated at 7 EJ a year, while the remainder is in traditional uses. With proper management and technologies, biomass feedstocks can be produced sustainably.

Oil palm production is a major agricultural industry in Malaysia. It contributes about US$ 7.3 billion in export earnings each year, mostly from the export of palm oil. Currently, there are more than three million hectares of oil palm plantations [7]. In total, about 90 million MT of renewable biomass (trunks, fronds, shells, palm press fiber and the empty fruit bunches) are produced each year.

The empty fruit bunches (EFB) represent about 9% of this total [7]. They are the residue left after the fruit bunches are pressed to extract oil at oil mills. The oil mills are located near or in the plantation itself [8]. EFB is a suitable raw material for recycling

because it is produced in large quantities in localized areas. In the past, it was often used as fuel to generate steam at the mills [8]. From this, liquid wastes are generated. The solid residues, mainly EFB, constitute more than 20% of the fresh fruit weight [9]. More than 500 kg (around 0.5 m^3) of liquid wastes, mainly in the form of palm oil mill effluent (POME), are discharged during the processing of 1.0 MT of fresh fruit bunches [10]. By expectation, more than 20 MT of EFB and more than 50 m^3 of POME will be generated from a mill after processing of 100 MT of fresh fruit bunches.

2.2 Bioethanol as a Fuel

Ethanol is the most widely used biofuel today. In 2003, more than 2.8 billion gallons were added to gasoline in the United States to improve vehicle performance and reduce air pollution [11]. Ethanol is an alcohol, and most is made using a process similar to brewing beer where starch crops are converted into sugars; the sugars are fermented into ethanol, and then the ethanol is distilled into its final form. Ethanol syntesized from cellulosic biomass materials instead of traditional feedstocks (starch crops) is called bioethanol.

Although ethanol production from corn can still expand greatly, its primary use is for animal feed and food products such as beverage sweeteners, and it may not always be in surplus. Advanced biotechnology cellulosic ethanol will supplement rather than replace corn ethanol, but it will also provide diversity, possible cost savings, and a vastly greater choice of potential feedstocks. Starches, such as that in corn kernels, and sugars are only a very small portion of available biomass materials; cellulose and hemicellulose form the bulk of most plant materials. Making ethanol from cellulose and hemicellulose dramatically expands the types and amount of available feedstock. This includes many

materials now regarded as wastes requiring disposal, as well as biomass residues such as corn stalks and wood chips or "energy crops" of fast-growing trees and grasses.

Ethanol is high-quality transportation fuel that has long been recognized. It can be used in a mixture with gasoline (3-22% ethanol), with no modification of the engine [12]. Ethanol is used to increase octane and improve the emissions quality of gasoline. The CO emission fir E10 is approximately 20% lower than that of gasoline in older vehicles. Also the emission of VOC (volatile organic compounds) decreases with E10.

The Clean Air Act Amendments of 1990 mandated the sale of oxygenated fuels in areas of the country with unhealthy levels of carbon monoxide. Since that time, there has been strong demand for ethanol as an oxygenate blended with gasoline. In some areas of the United States today, ethanol is blended with gasoline to form an E10 blend (10% ethanol and 90% gasoline), but it can be used in higher concentrations such as E85 or in its pure form [13]. All automobile manufacturers that do business in the United States approve the use of certain ethanol/gasoline blends. Fuel ethanol blends are successfully used in all types of vehicles and engines that require gasoline. Approval of ethanol blends is found in the owners' manuals under references to refueling or gasoline.

Ethanol has an octane number of 113, allowing a higher compression ratio in the gasoline engine. Ethanol can also be used in reformulated gasoline [14]. The blending octane value of ethanol can actually be much higher than that of neat ethanol, and the blending octane value increases with lower octane-base gasoline. Therefore, ethanol is an excellent additive to prevent engine knock and improve engine performance.

2.3 Empty Fruit Bunch (EFB)

Table 2.1: Composition of EFB

Parameter	Dry matter basis		Fresh wt. basis (mean)
	Range	Mean	
Ash (%)	4.8-8.7	6.3	2.52
Oil (%)	8.1-9.4	8.9	3.56
C (%)	42.0-43.0	42.8	17.12
N (%)	0.65-0.94	0.80	0.32
P_2O_2 (%)	0.18-0.27	0.22	0.09
K_2O (%)	2.0-3.9	2.90	1.16
MgO (%)	0.25-0.40	0.30	0.12
CaO (%)	0.15-0.48	0.25	0.10
B (mg/l)	9-11	10	4
Cu (mg/l)	22-25	23	9
Zn (mg/l)	49-55	51	20
Fe (mg/l)	310-595	473	189
Mn (mg/l)	26-61	48	19
C/N ratio	45-64	54	54

From the previous analysis done, one tonne of EFB (fresh weight) would have a fertilizer equivalent of 7 kg urea, 2.8 kg rock phosphate, 19.3 kg muriate of potash and 4.4 kg kieserite. At current fertilizer prices, this would have a monetary value of RM12.00. Based on the 1997 palm oil production of 9.07 million tonnes [11], it is estimated that 10.8 million tonnes of EFB was produced in Malaysia with a potential value of RM130 million [15].

2.4 Lignocelluloses Conversion Technology

The conversion of starch to ethanol by yeast is a well-established fermentation process. However, the fermentation of lignocellulosic materials to ethanol presents several new technical problems which arise mainly as a consequence of the physical and chemical characteristics of the particular lignocellulosic substrate under consideration. The inability of yeast to directly ferment cellulose posed another additional complication. To obtain ethanol from lignocellulosic materials, it requires development of either certain microorganisms that are able to ferment cellulose to ethanol or a chemical or biochemical means of hydrolyzing cellulose to a water-soluble sugar that can be fermented by yeast. The challenge lies in a way to develop a process that accepts a variety of potential feedstock and efficiently converts them to ethanol in concentrations that allow economical product recovery. Efforts to solve the problems in fermenting lignocellulosic materials have given rise to a wide variety of proposed routes to ethanol production.

The principal process elements which are generic to almost all lignocellulose-to-ethanol conversion process are as shown in Table 2.2. All the processes can be generally characterized by the permissible feedstock and the pretreatment and microorganisms employed to produce the ethanol. The design of the fermentation process is based on the characteristics of the fermentation culture and the physical and chemical properties of the lignocellulosic feedstock.

TABLE 2.2: Key process elements in the conversion of lignocelluloses to ethanol

Feedstock	-carbohydrates -lignocelluloses
Pretreatment	-chemical -physical -thermal -solvent -acid hydrolysis
Microorganisms	-yeast -Bacterial Co-culture -Bacterium -Enzyme Plus Yeast -Enzyme Plus Bacterium
Product Recovery	-Conventional Distillation -Vacuum Distillation -Membrane -Solvent Extractoin
Fermentation Substrate	-Glucose -Cellubiose -Xylose -Starch -Sucrose
Enzyme Production	-In situ -Separate Fermentation -Purchased

2.5 Bioethanol Synthesis

Most lignocellulosic materials require some degree of processing to expose or transform its constituent carbohydrate to a form that can be readily degraded and or taken up by microorganisms for fermentation to ethanol. The producers for pretreating lignocellulosic materials may conveniently be classified according to the physical or chemical modification introduced.

Comminution, irradiation, liquid ammonia, strong alkali, and acid decrystallize cellulose and dramatically enhance its susceptibility to acid and enzymatic hydrolysis. The relatively low yield of fermentable carbohydrates and the excessive consumption of energy or reagent have thus far precluded the utility of such methods.

In order to produce sugars from the biomass, the biomass is pre-treated with acids or enzymes in order to reduce the size of the feedstock and to open up the plant structure. The cellulose and the hemi cellulose portions are broken down (hydrolysed) by enzymes or dilute acids into sucrose sugar that is then fermented into ethanol. The lignin which is also present in the biomass is normally used as a fuel for the ethanol production plants boilers.

Biomass wastes contain a complex mixture of carbohydrate polymers from the plant cell walls known as cellulose, hemi cellulose and lignin. Figure 2.1 shows the structure of cellulose where cellulose $(C_6H_{10}O_5)_n$ is a long-chain polymer polysaccharide carbohydrate, of beta-glucose. It forms the primary structural component of plants and is not digestible by humans. Cellulose monomers (beta-glucose) are linked together through 1,4 glycosidic bonds by condensation. Cellulose is a straight chain (no coiling occurs).

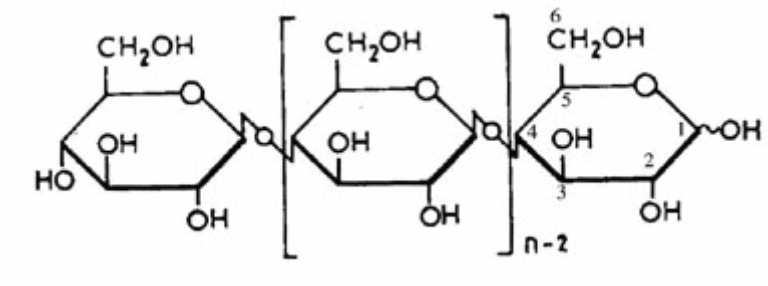

Figure 2.1: Structure of cellulose

In *microfibrils*, the multiple hydroxide groups hydrogen bonded with each other, holding the chains firmly together and contributing to their high tensile strength. This strength is important in cell walls, where they are meshed into a carbohydrate *matrix* that helps keeps the plants rigid. Cellulose is a linear crystalline polymer of (1-4)-β-D-glucose [4]. Cellulose is the most common form of carbon in biomass, accounting for 40%-60% by weight of the biomass, depending on the biomass source. It is a complex sugar polymer, or polysaccharide that synthesized from the simple sugar.. Its crystalline structure makes it resistant to hydrolysis, the chemical reaction that releases simple, fermentable sugars from a polysaccharide.

A hemicellulose can be any of several heteropolymers (matrix polysaccharides) present in almost all cell walls along with cellulose. Their molecular weights are usually lower than that of cellulose and they have a weak undifferentiated structure compared to crystalline cellulose. But the chains form a 'ground' - they bind with pectin to cellulose to form a network of cross-linked fibres. Hemicelluloses include xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan. The hemicellulose is primarily composed of the 5-carbon sugars and xylose [17] . Hemicellulose is an amorphous and partly crystalline polymer, which mostly consist of (1-4)-β-xylose. Hemicellulose is also a major source of carbon in biomass, at levels of between 20% and 40% by weight. It is relatively easy to hydrolyze into simple sugars but the sugars are difficult to ferment to ethanol.

Lignin is a complex polymer, which provides structural integrity in plants. It makes up 10% to 24% by weight of biomass. It remains as residual material after the sugars in the biomass have been converted to ethanol. It contains a lot of energy and

can be burned to produce steam and electricity for the biomass-to-ethanol process. Lignin, a

phenolic polymer, binds the fibres together. Figure 1.2 shows typical cell wall arrangements of cellulose, lignin and hemicelluloses that involve in ethanol synthesize.

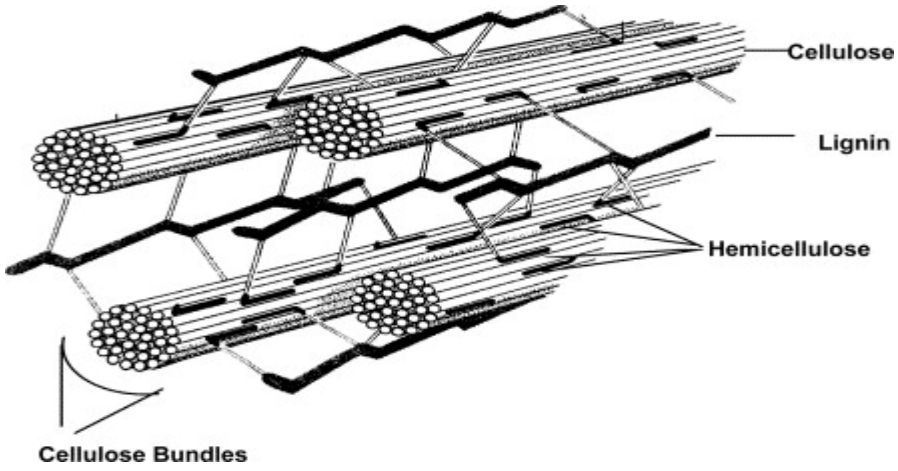

Figure 2.2: Typical plant cell wall arrangements

Previous study showed that the dilute acid hydrolysis process is one of the oldest, simplest and most efficient methods of producing ethanol from biomass. Dilute acid is used to hydrolyse the biomass to sucrose. The first stage uses 0.7% sulphuric acid at 190^0C to hydrolyse the hemi cellulose present in the biomass. The second stage is prefered to hydroliyse the more resistant cellulose fraction. This is achieved by using 0.4% sulphuric acid at 215^0C. The liquid hydrolates are then neutralized and recovered from the process [2]. The process needs high temperature and thus not suitable to be carried out in lab.

The hydrolysis process breaks down the cellulostic part of the biomass into sugar solutions that can then be fermented into ethanol. Yeast is added to the solution, which is then heated. The yeast contains an enzyme called invertase, which acts as a catalyst and helps to convert the sucrose sugars into glucose and fructose (both $C_6H_{12}O_6$). The sugar formed in the hydrolysis is fermented into ethanol.

The sucrose reacts with enzyme called invertase and produce Fructose and Glucose as well.

The chemical reaction is shown below:

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

Sucrose + Water → Fructose + Glucose (2.0)

The fructose and glucose sugars then react with another enzyme called zymase, which is also contained in the yeast to produce ethanol and carbon dioxide.

The chemical reaction is shown below:

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

Fructose/Glucose Ethanol Carbon dioxide (2.1)

Usually the fermentation process takes around three days to complete and is carried out at a temperature between 250^{o}C and 300^{o}C.

From the previous study, the fermentation can be done after hydrolysis process and preparation of yeast cultures. One liter of production medium was prepared according to the requirements of *Saccharomyces cerevisiae*, containing 22 % sucrose, 0.3 % dry yeast extract, 0.5 % peptone, 0.15 % $(NH_4)_2$, HPO_4 and 0.3 % $MgSO_4$ in tap water. Then preparation of inoculums is done before fermentation process is started. Dry baker's yeast was used. In the fermentations with free cells, 50 ml of stock culture solution containing the given amounts of nutrients was transferred into a 250 ml Erlenmeyer flask, the pH was adjusted to pH 5.0 with 0.1 M phosphoric acid and the solution was autoclaved at 121^{o}C for 20 min. 0.5 g of dry baker's yeast was added after sterilization. The mixture was then incubated at 28^{o}C on an Infors AG-CH-4103 Bottmingen-type shaker working at 200 rpm for a period of 4 hours before inoculation into the production vessel. After that 400 ml fermentation medium was inoculated with 50 ml inoculum and the pH was adjusted to pH 5.0. Fermentation was carried out in a rotary shaker at 200 rpm and 28^{o}C. The weight decrease was measured every 2 hours in order to determine the amount of ethanol. Fermentation was terminated after 96 hours [18].

The focus of this study is to produce bioethanol using empty fruit bunch as raw material. The empty fruit bunch must be hydrolyzed first before fermentation process. Previous study indicates that batch fermentation is better than the other operation mode [19]. Therefore, the experiment in this research is carried out using the batch fermentation as operation mode.

CHAPTER 3

MATERIALS & METHODOLOGY

3.0 Introduction

Empty fruit bunch (EFB) of oil palm was used as raw material in this experiment. To produce bioethanol from EFB, two steps processing are required, via acid hydrolysis followed by fermentation. Acid hydrolysis was employed to hydrolyze the EFB into simple sugar (glucose) while fermentation was carried out subsequently to turn the glucose into bioethanol.

3.1 Acid hydrolysis

Based on Figure 3.1, the sample (oil palm EFB) as shown in Figure 3.2, which is procured from an oil palm plantation at *Felda Lepar Hilir 2* is prepared.

The EFB was washed and dried naturally.

↓

The EFB was grounded using grinder to about 0.5 mm

Figure 3.1: The EFB preparation

The oil palm empty fruit bunch (EFB) shown in Figure 3.2 was cut into small pieces. Then it was washed and left to dry naturally. After that, EFB was grounded to 0.5 mm size using fiber grinder. Previous study has shown that washed and grounded samples provided better results compared to unwashed and ungrounded samples [20].

Figure 3.2: Oil palm EFB

The grounded sample was then treated with solution of 1 % sodium hydroxide (NaOH) for two hours at temperature of about 100˚C before pretreated with the 75 % sulfuric acid (H_2SO_4). The ratio of sample to NaOH used was 1:50 (w/v), as this ratio was found to work well on fibers of the oil palm fruit [18]. In this work, 27 g of EFB was treated with 1.35 L of NaOH. The NaOH treated sample was then thoroughly washed and dried to constant weight before being subjected to H_2SO_4 pretreatment.

The pre-treated sample was then pretreated with a 75 % H_2SO_4 solution for one hour at 50˚C. The ratio of acid to the sample was controlled at 15:1 (w/w). This ratio was found to work well on fibers of the oil palm fruit, bagasse and rice husk [21]. As such, in this experiment, 27 g of EFB was treated by 405 g of H_2SO_4.

After the pretreatment, the sample was then treated with dilute solution of H_2SO_4 in a water bath at 80˚C for two hours. The steps were repeated with the last procedure being carried out using different concentration of H_2SO_4 (3.0-6 %).

The solid-solution mixture was filtered through a *Whatman 12 mm* filter paper and neutralized with a solution of 2.5 M NaOH. Subsequently, it was analyzed for the glucose content. The glucose was analyzed using UV-Visible Spectrophotometer. The preparation of glucose method is summarized in Figure 3.3

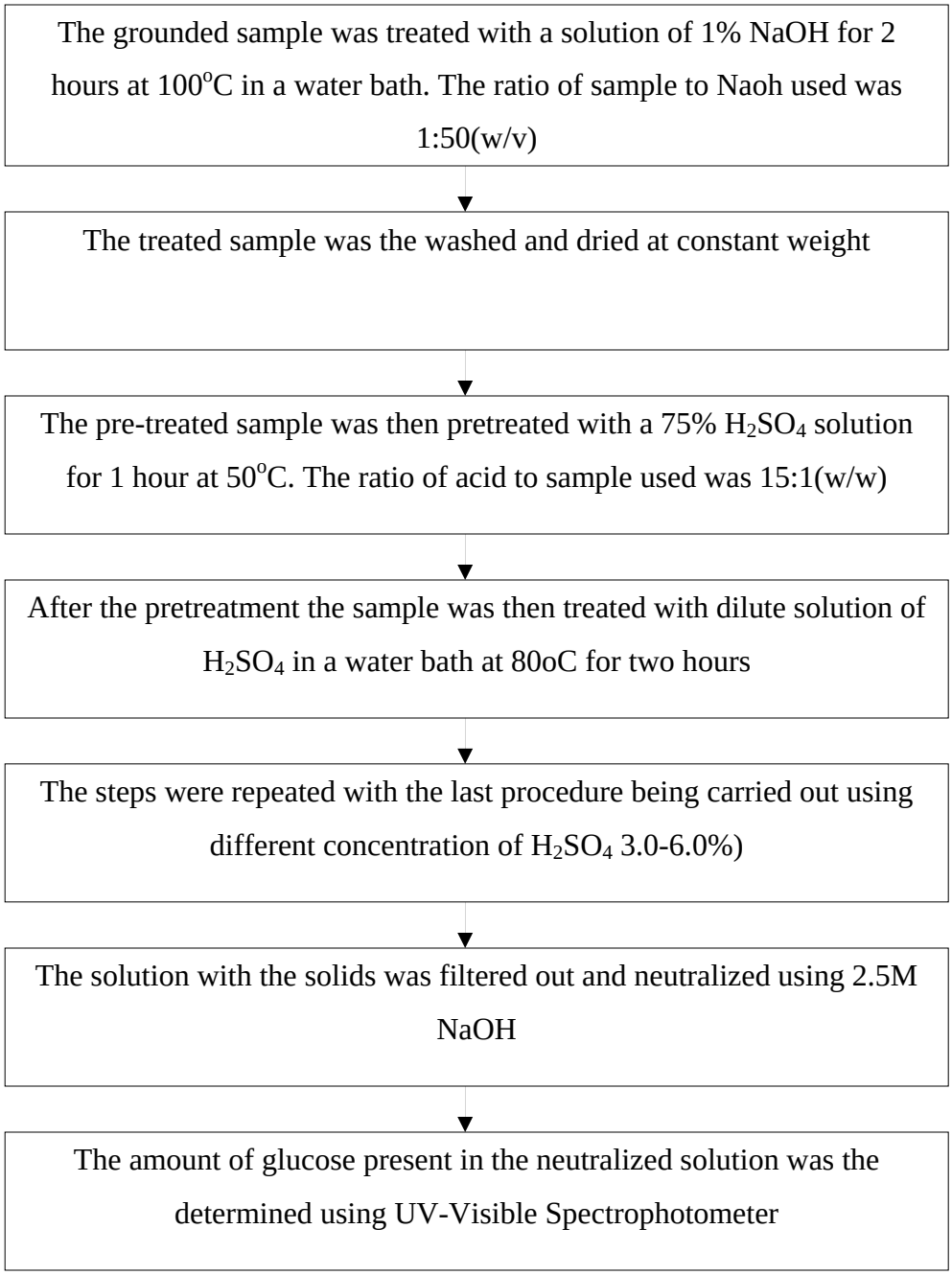

Figure 3.3: The Glucose preparation

3.2 Fermentation preparation

Before fermentation process was carried out, stock cultures were prepared. Previous study showed that the growth and dominance of the yeast *Saccharomyces cerevisiae* must be encouraged to ensure complete fermentation and to avoid ethanol aroma defects. [22]

3.2.1 Preparation of Growth media and Inoculums

A seed culture of *Saccaromyces cerevisiae* was grown in a media of 6 mg of Saboured Dextrose (SDB) up a to a total volume of 300 ml of distilled water. SBD powder was weighed in a weighing boat and poured into the Schott bottle. The bottle was hand shaked to mix the solution. The media was then sterilized at 121^{o}C for 15 min. After that, the broth solution was cooled to the room temperature and it was transferred into three different autoclaved serum bottles. The stock culture of the microorganisms was transferred to the broth media for preparation of seed culture. During the transferring process, the inoculating loop was flamed with a Bunsen burner until it was red hot for sterilization. The test tube cap that contains the stock culture was removed and the lip of the test tubes was flamed using the Bunsen burner. The inoculating loop was inserted to the stock culture test tube and a small portion of the stock culture was removed. After that, the test tube lip was flamed again and thereafter closed tight. The inoculating loop that contains the stock culture was inserted into a serum bottle that contains the broth solution. The inoculating loop was flamed again after completing the procedure. The same procedure was repeated for another serum bottle. One of the serum bottles was used as controller.

All the procedures outlined above were done in a laminar flow cabinet in order to prevent any contamination. The serum bottles then were incubated in Double Stack Shaking Incubator, orbital shaker at 30^{o}C and 180 rpm for 48 hours. After two days, as shown in Figure 3.4, the universal bottles that have the microorganisms have different color from the controller. Both the bottle were taken out from the incubator and kept in the refrigerator at -4^{o}C. The growth media preparation method is summarized in Figure 3.5.

Figure 3.4: Preparation of inoculums (right) and controller (left)

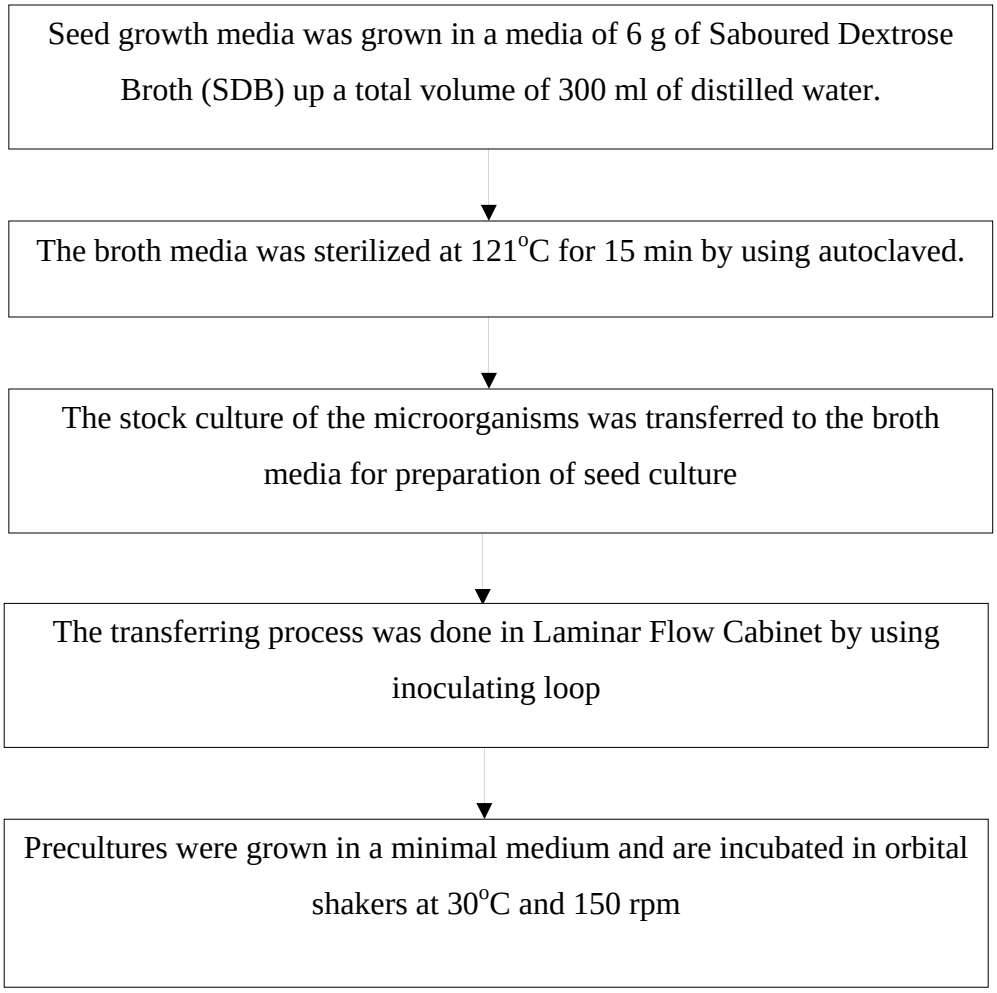

Figure 3.5: Preparation of Growth Media and Inoculums

3.2.2 Agar preparation

100 ml of agar solution was prepared in a 500 ml Schott bottle. The Schott bottle was filled with distilled water until 100 ml. Approximately 6.5 g of Saboured Dextrose Agar (SDA) was weighed and poured into the Schott bottle. Next, the bottlewas shaked until the SDA solution mixed well. The solution in the bottle was then sterilized for 15 minutes at 121°C. After that, 20 ml of the agar solution was poured into three different test tubes. Each of the test tubes was then held at slanted position until it hardened. After that, the bottles were kept in a refrigerator at -4°C.

3.2.3 Stock Culture Preparation

Stock culture is important to keep a pure culture within a long period of time without lacking of nutrients. A nutrients broth could only last for weeks and the culture started to decay based on nutrient depletion and accumulation of toxic by-products. The stock culture also can last up to six month in the refrigerator. To prepare stock culture, firstly the inoculating loop was flamed with a Bunsen burner until red hot to sterilize it. The inoculating loop was inserted into the serum bottle and small portion of pure culture was taken out. The serum bottle lip was flamed again and capped. The inoculating loop that has little pure culture was inserted into the test tube that contained the agar. The inoculating loop was flamed again after finished using it. The same procedures were repeated for the next test tube. One of the test tubes that contain the agar was kept as a controller. The entire test tubes were then incubated in the Double Stack Shaking Incubator at 30^{o}C and 150 rpm for two days. After two days, both the test tubes that have the microorganisms have different condition from controller. The white colonies that appeared in the two tubes are sign that the microorganism was growing. After two days the test tubes were taken out and kept in the refrigerator at -4^{o}C. The stock culture preparation is summarized in Figure 3.6

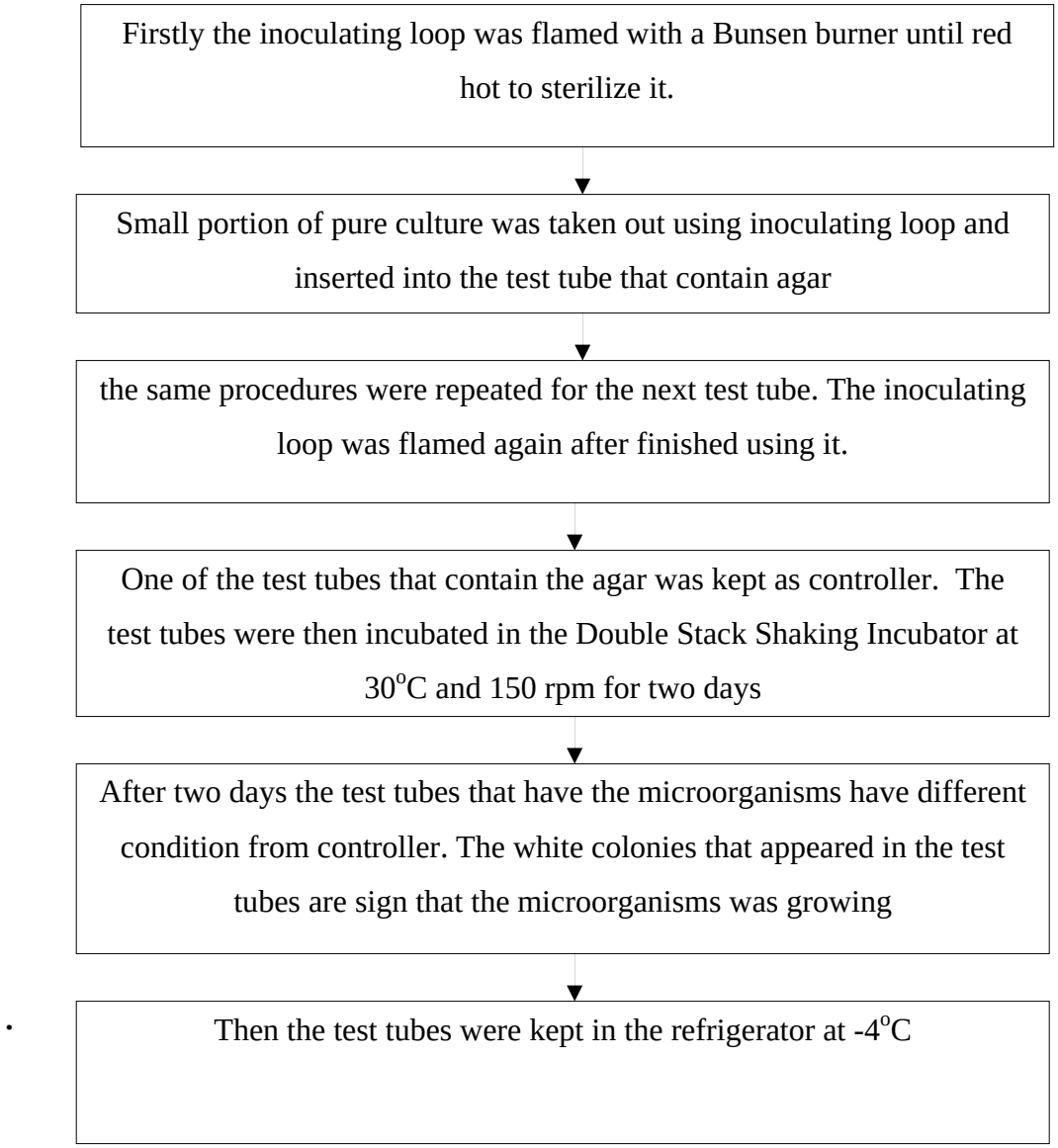

Figure 3.6: Preparation of Stock Culture

3.2.4 Preparation of Acetate Buffer Solution (pH 4.8)

One liter Erlenmeyer flask was washed clean and filled up with distilled water until it reached 500-litre. Sodium acetate anhydrous was weighed for 4.59-gram in a weighing boat and poured into the Erlenmeyer flask. The solution was stirred until all the powder dissolved in the water. Next, five ml of glacial acid was poured into a clean measuring cylinder. The pipette was rinsed with distilled water and then 1.8 ml of glacial acetic acid was pipette out from the cylinder and transferred to the Erlenmeyer flask. The solution was stirred until it mixed well. The solution pH was checked using the pH meter. If the pH of the solution was higher than 4.8, glacial acetic acid was added slowly until the pH reached 4.8. The buffer solution was prepared for another set.

3.2.5 Fermentation Process with 2L fermenter

In batch fermentation, fermentation medium, glucose with buffer was autoclaved together with the fermenter. First step in using the 2L Biotron Fermenter (LiFlux Gx) is to disconnect all the power connection and any other connection that connect the CPU and the fermenter. The vessel was rinsed with de-ionized water. Then, the fermentation medium was poured into the vessel and the fermenter was close with top cover. Before autoclaving the fermenter, all the power supply and electronic gadgets must be disconnected. All of the autoclave-able tubes were wrapped with cotton wool and aluminum foil in order to increase the life span of the tubes.

After the fermenter was all set, it is put inside the autoclave to sterile the medium and the equipment. The fermenter was autoclave to sterile the medium and the equipment. The fermenter was autoclaved for 20 minutes at 121°C. After the temperature of the autoclaved dropped to 50°C, the fermenter was carried out and let cooled to the room temperature. Then, all the power supplies and all the connection to the CPU have to be connected. Next step was transferring the inoculums from the

serum bottle into the autoclaved feed bottled and connect the bottle to the fermenter. After the fermenter was ready, the condenser pump, jacket pump, compressor were switched on. The main CPU is switched on and the condition for the fermentation was set. Fermentation was carried out at 100 rpm and 30°C. The absorbance of the medium was measured every six hours in order to determine the amount of ethanol. The above experiment was repeated using different concentration of inoculums (5%, 10%, and 15%) for each glucose concentration. The fermentation process is summarized in Figure 3.7.

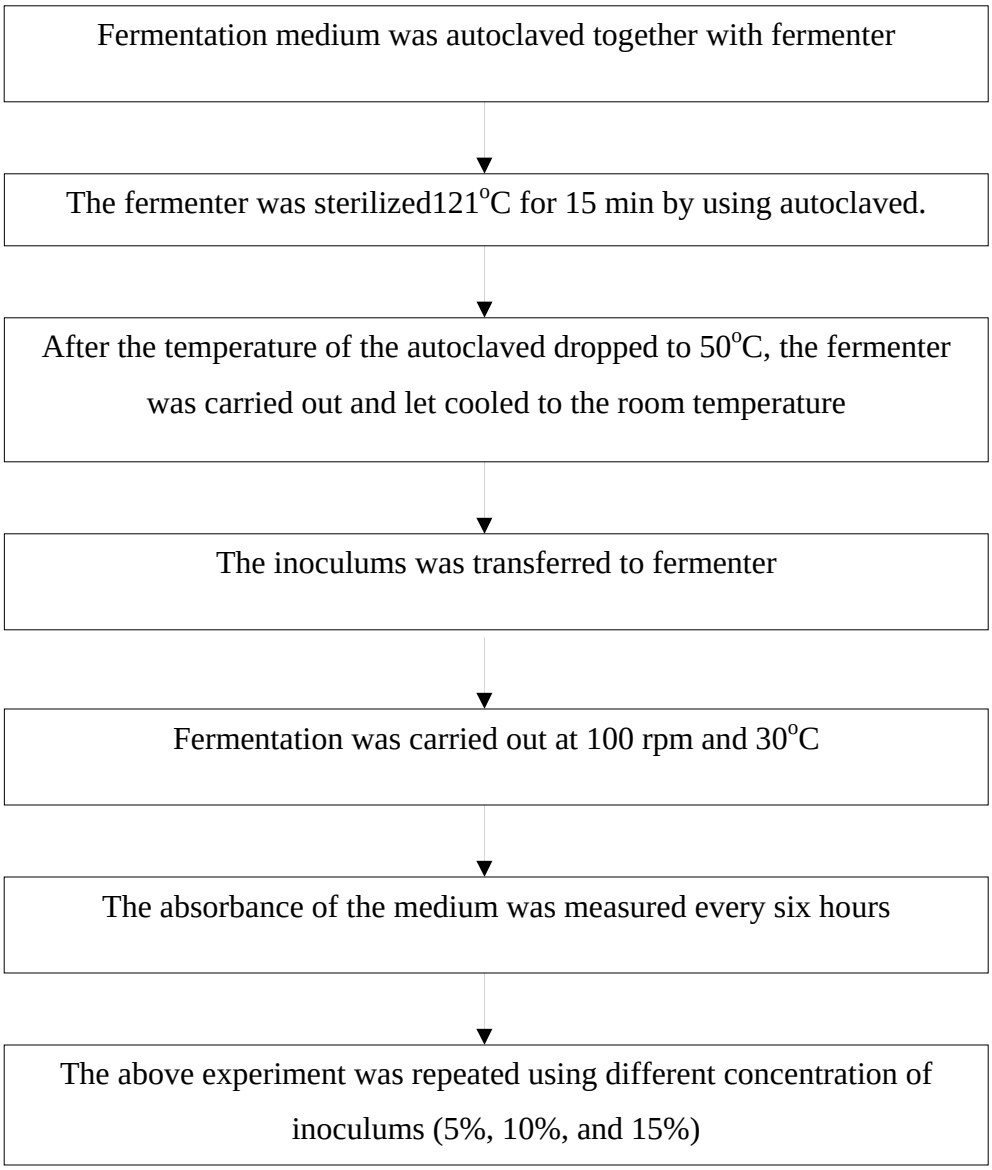

Figure 3.7: Fermentation process

3.3 Analytical procedures

The content of glucose after the acid hydrolysis treatment was to be determined using UV-Visible Spectrophotometer. The standard calibration curve of glucose was prepared using UV-Visible Spectrophotometer. For the fermentation process, the content of ethanol was determined using refractometer (METTLER TOLEDO Refracto 30 PX)

3.3.1 Preparation of Standard Calibration Curve for Glucose

The amount of reducing sugar was measured by the 3,5-dinitrosalicylic acid (DNS) procedure described by Miller (1959) with slight modification. DNS reagent (0.6ml) was added into the sample (0.6 ml) followed by 60 µl 0.1N NaOH. The mixture was then incubated at 100ºC for 5 minutes. Subsequently, the reaction was stopped by cooling on ice. Five ml distilled water was added and the colour intensity was measured at 540 nm. Distilled water was used as the blank. Preparation of DNS reagent was shown in Table 3.1 and the data was plot in graph as shown in Figure 3.8 below.

Table 3.1: Preparation of DNS Reagent

	Amount (g/l)
Dinitrosalicylic acid	10.0
Phenol	2.0
Sodium sulfite, Na_2SO_3	0.5
Sodium hydroxide, NaOH	10.0
Potassium sodium tartrate	182.0

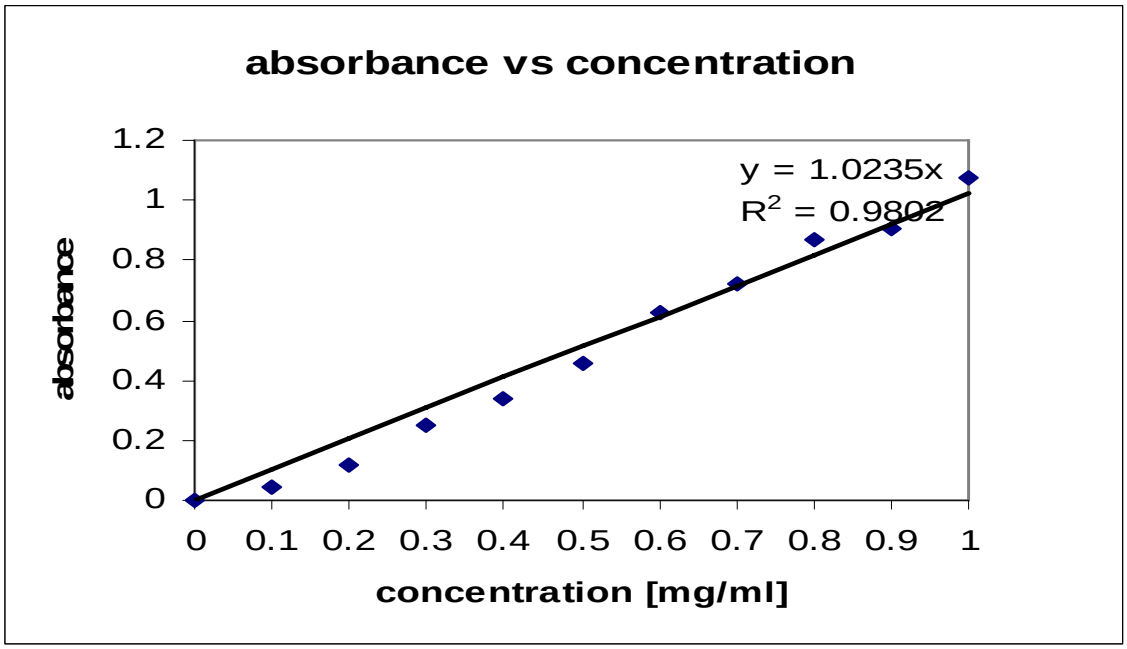

Figure 3.8: Standard Calibration Curve for Glucose.

From Figure 3.8, the concentration of glucose that was produced from this study can be calculated.

3.3.2 Preparation of Refractive Index (RI) Standard Calibration Curve for Bioethanol

Refractive index (RI) readings were used to determine the composition of bio-ethanol from anaerobic fermentation. A set of mixtures containing ethanol and water was prepared within a specified range of composition between pure water and pure ethanol. For each mixture, the refractive index readings were obtained using refractometer (METTLER TOLEDO Refracto 30 PX).The calibration curve of RI versus composition for the whole range of mixtures was plotted. The summary data of the mixtures was shown below in Table 3.2.

Table 3.2: Calibration Curve for Ethanol-Water Mixtures

Amount of Ethanol (g)	Amount of Water (g)	Ethanol Composition		Refractive Index (RI)
		wt%	vol%	
0	9.97	0		1.3332
0.746	9.023	7.63	9.5027391	1.3379
1.492	8.076	15.59	19.004587	1.344
2.237	7.129	23.89	28.496437	1.3492
2.983	6.181	32.55	38.001525	1.3537
3.729	5.234	41.6	47.502863	1.3568
4.475	4.287	51.07	57.00331	1.3594
5.22	3.34	60.98	66.4986	1.3613
5.966	2.393	71.37	75.998475	1.3619
6.339	1.919	76.76	80.752129	1.3618
6.712	1.446	82.28	85.49746	1.3612
7.085	0.972	87.93	90.251145	1.3613
7.308	0.688	91.4	93.099039	1.3591
7.458	0.499	93.73	94.995555	1.3583

Based on the data from Table 3.2, RI versus wt% and RI versus vol% were plotted in a graph. After the data has been plotted, the quantitative measurement based on wt% and vol% from the samples in anerobic fermentation can be obtained from the graph as shown in Figure 3.9 for wt% and Figure 3.10 for vol%.

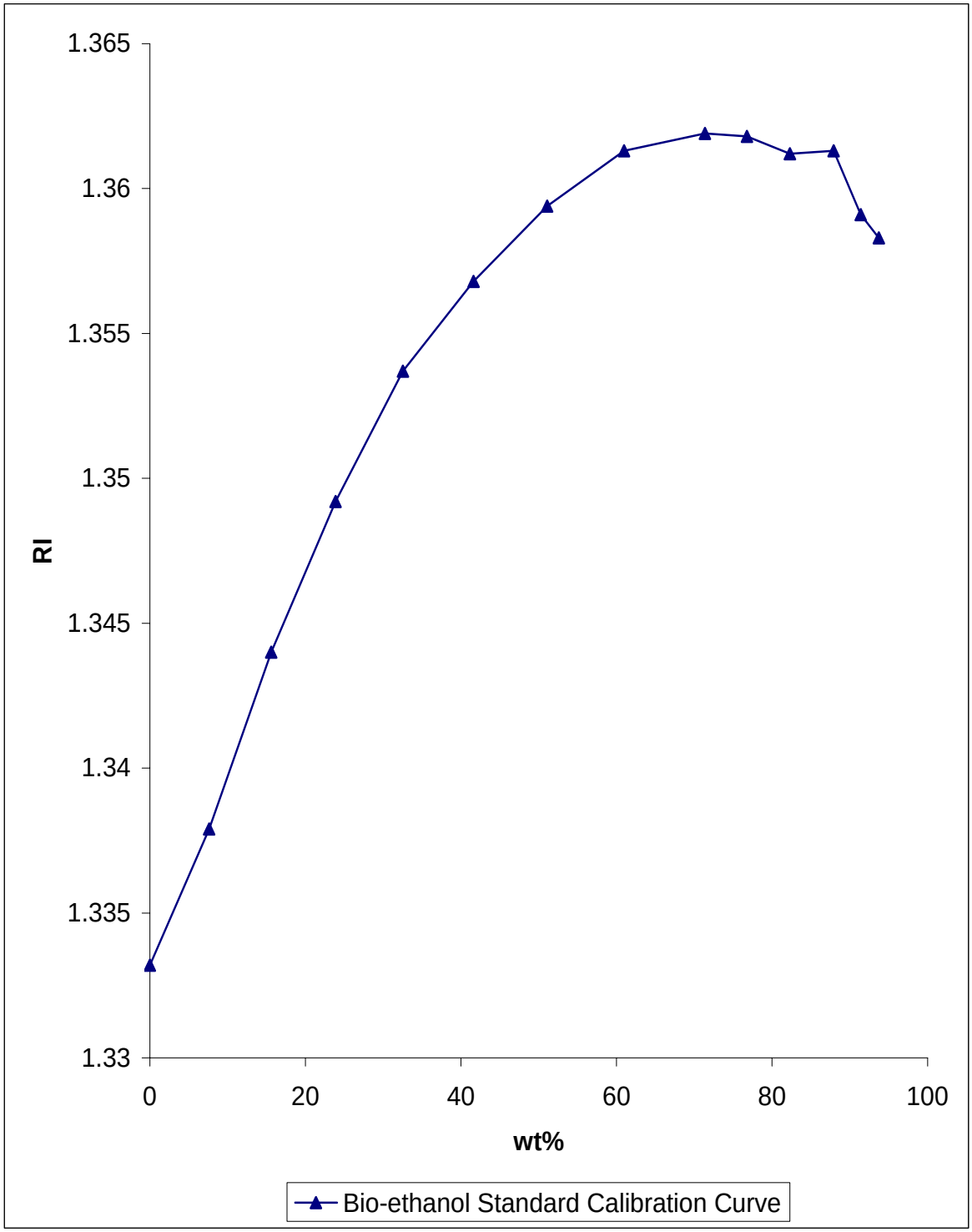

Figure 3.9: The effect of RI on the wt% of bioethanol

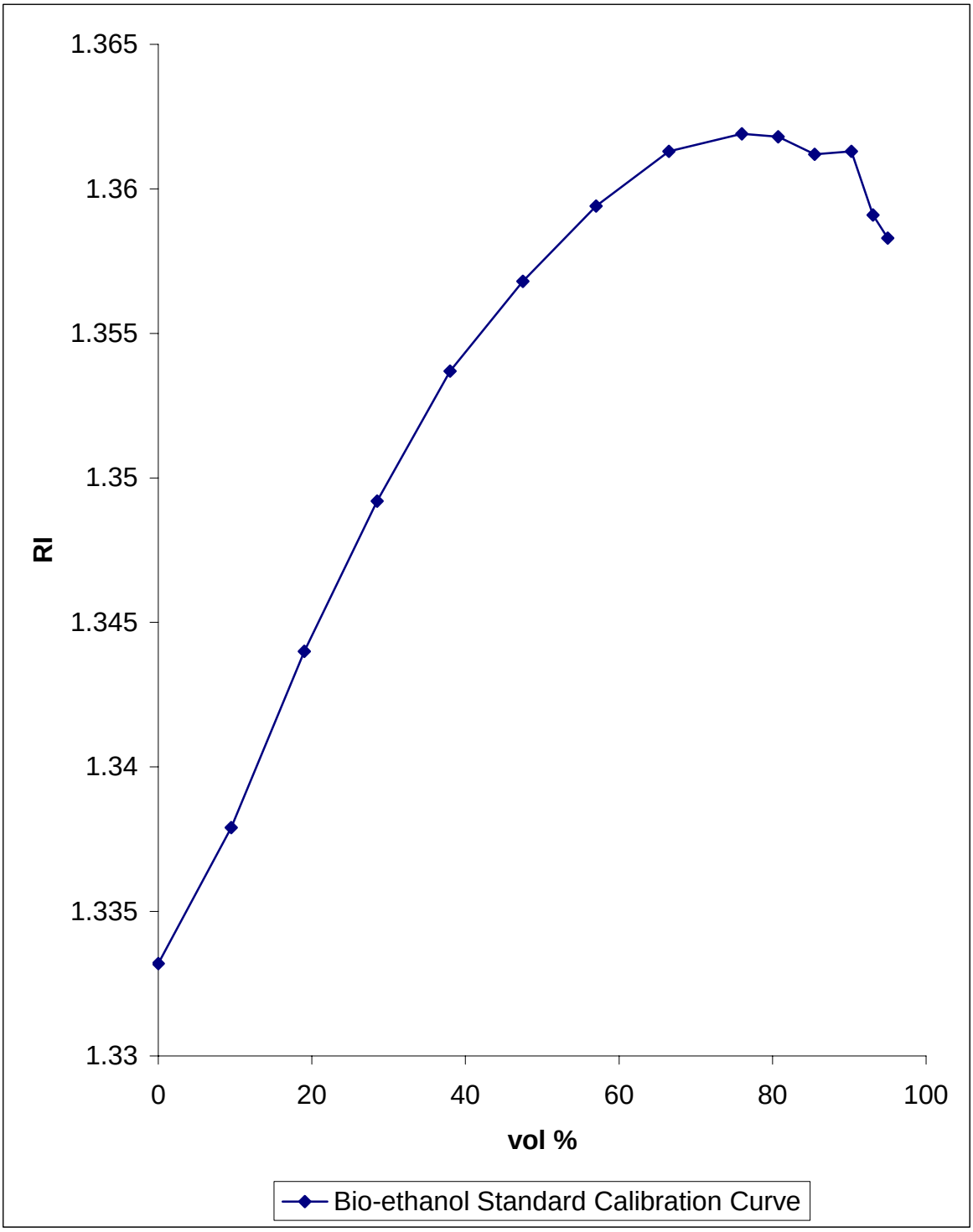

Figure 3.10: The effect of RI on the vol% of Bioethanol

3.3.3 Optical Density

Five ml of the sample of ethanol was collected from the fermenter every six hours and put into a vial. Optical density was determined using UV-Visible Spectrometer. The wavelength for the microorganism cell is 600 nm. The purpose of optical density study was to determine whether the microbe grew constantly or not.

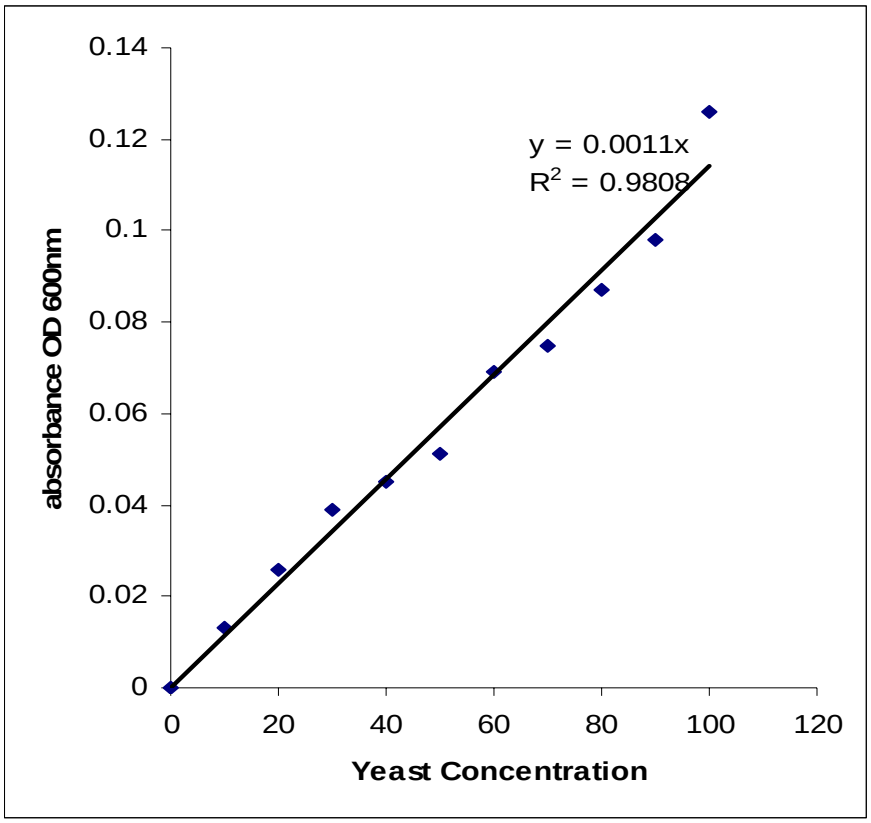

Figure 3.11: Standard calibration of Optical Density for Saccaromyces cerevisiae

From Figure 3.11, the concentration of yeast that was produced from this study can be calculated.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

There are many factors that can affect the fermentative production of ethanol, such as temperature, pH, speed of agitator, fermentation time and many more. However this study was carried out to study the effect of different size of inoculums in bioethanol production and the effect of different glucose concentrations on the yield of bioethanol.

4.2 Glucose Production

In order to produce glucose; two stage hydrolysis of EFB is done on three different set of sample. For the first set of sample, the hydrolysis was carried out using 5.0 mm sample where the sample was treated with 3% of H_2SO_4. In order to produce 2 L of glucose, 27 g of EFB was used. The experiment was repeated using 4% and 6% of H_2SO_4. In this experiment, total of 5.0 mm EFB used was less than 100g.

4.2.1 Effect of Sulfuric Acid Concentration on Glucose

The two-stage acidic hydrolysis of oil palm EFB that had been carried out for two hours using water bath at 80ºC would produce a dark color hydrolyzates as shown in Figure 4.1.

Figure 4.1: Samples of EFB after the two-stage hydrolysis at 80ºC using water bath for two hours

The concentration of glucose for three different acid sulfuric concentrations is shown in Table 4.1. From the table, the results showed that, by using low H_2SO_4 concentration, the concentration of glucose produced was low. At higher acid sulfuric concentration, the glucose concentration was high.

Table 4.1: Glucose concentration for different sulfuric acid concentration

Sulfuric acid concentration (%)	**Absorbance**	**Glucose concentration (mg/ml)**
0.0	0	0
3.0	0.575	5.6
4.0	1.2	11.7
6.0	1.479	15.1

The concentration of glucose was found to be rather sensitive to the acid concentration between the ranges of 4.0-6.0 %, achieving maximum yield of glucose of 11.7 and 15.1 mg/ml

Based on Figure 4.2, the glucose yield using 3 % of H_2SO_4 was 5.6 mg/ml.

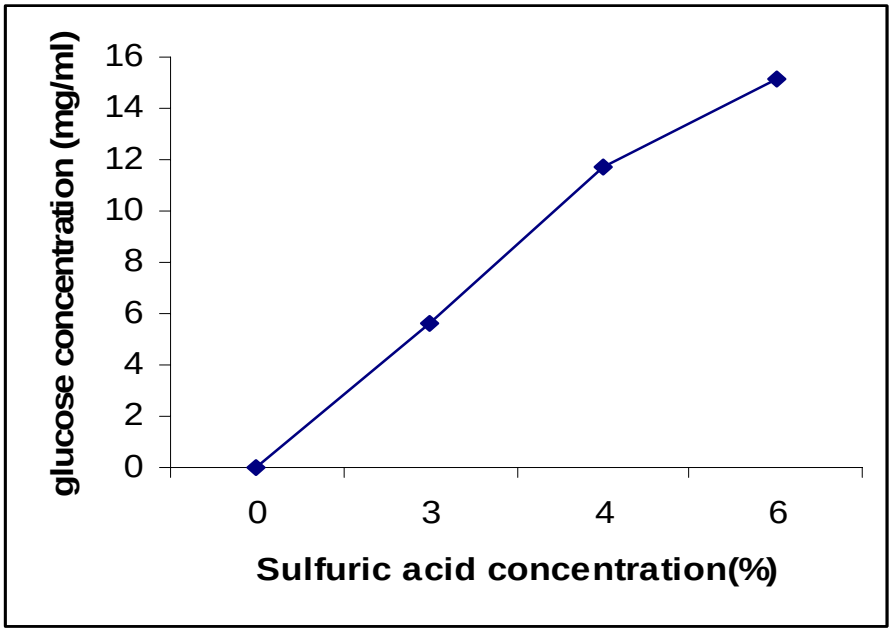

Figure 4.2: Glucose yield at different acid concentrations

By using 4.0 % of H_2SO_4, the glucose yield suddenly increased up to 11.7 mg/ml. Meanwhile, by using 6 % of H_2SO_4, the glucose yield was highly increased up to 15.1 mg/ml

The increase in the yield of glucose with the increment of H_2SO_4 concentration is attributed to the H_2SO_4. H_2SO_4 acts as a hydrolysis agent to hydrolyze the hemicelluloses and cellulose present in the oil palm EFB into glucose. The breakage of hemicelluloses and cellulose bonds were caused by the chemical decomposition process that uses water to split chemical bond of hemicelluloses and cellulose. This resulted in the yield of glucose.

4.3 Fermentation Process

The fermentation of glucose into ethanol by a *Sachcaromyces cerevisiaes* was investigated in a 2L Fermenter under controlled condition. The fermentation was held at 30^{o}C, agitation rate of 100 rpm and pH at 4.8. The fermenter was also continuously purged with N_2 to create anaerobic condition.

Fermentation process was done on three different set of sample. For the first set of sample, the fermentation was carried out using 15 mg/ml of glucose concentration with 5% and 15% of size of inoculums. Experiments for the second set of samples were done using 11.7 mg/ml of glucose concentration with 10% and 15% size of inoculums. Finally, for the third set of sample, the fermentation was carried out using 11.7 mg/ml of glucose concentration with 15% and 10% of size of inoculums. Table 4.2 shows summary of parameters involved in this experiment.

Table 4.2: Parameters in fermentation process

	Set 1		Set 2		Set 3	
Concentration of glucose (mg/ml)	15		5.6		11.7	
Size of inoculums (%)	10%	15%	10%	15%	5%	15%
Agitation rate (rpm)	100		100		100	
pH	4.8		4.8		4.8	
Temperature (^{o}C)	30		30		30	

4.3.1 Cell Growth

Cell growth determination in inoculation process plays a crucial role determining the growth of microbe. There are four phase in cell growth [23] as shown in Figure 4.6. The first phase was lag phase, where during this period it appears that no growth takes place and may be considered as a time of adaptation. The length of the lag phase should be reduced as much as possible and this may be achieved by using suitable inoculums. The second phase was log phase or exponential phase where during this period, the growth rate of the cells gradually increase, constantly and at maximum specific growth rate. The third phase is deceleration phase where the growth of the organism has resulted in the depletion of substrate to a growth limiting concentration. The last phase was stationary phase where during this period the growth rate has declined to zero.

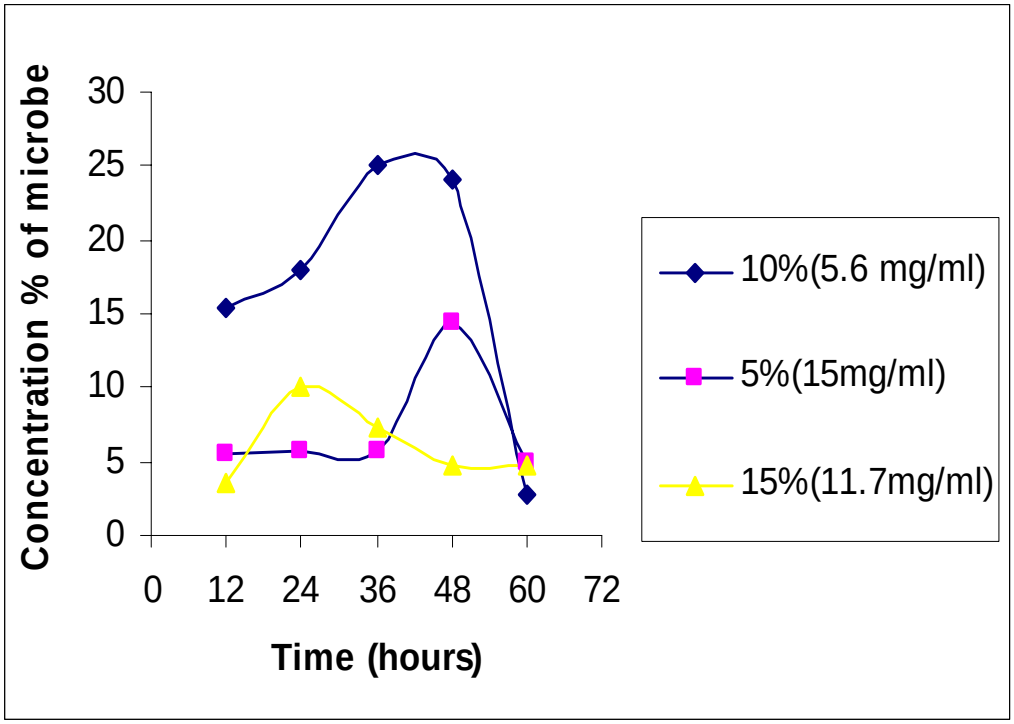

Figure 4.3: Growth curve of optical density for different concentration of glucose and different inoculums

From the Figure 4.3, it was observed that optical density was initially low but increased gradually with time where at this period microorganisms reorganize their molecular constituents when they are transferred to a new environment. A long lag phase was because of low concentration of some nutrients and growth factors. Hence to minimize the duration of lag phase, the inoculum size should be large. The optical density tended to increase as time progress as utilization of the glucose substrate by the yeast for growth occurred. The biomass concentration would then be expected to level off as steady state is reached. This situation occurs because all glucose in the substrate has been used. The patterns of cell growth are also influenced by environmental condition such as temperature, Ph, and dissolved-oxygen concentration.

4.3.2 Glucose Concentration

The figure 4.4 is the result for the samples taken from concentration for 15% size inoculums only. UV-Visible spectrometer is used for analysis.

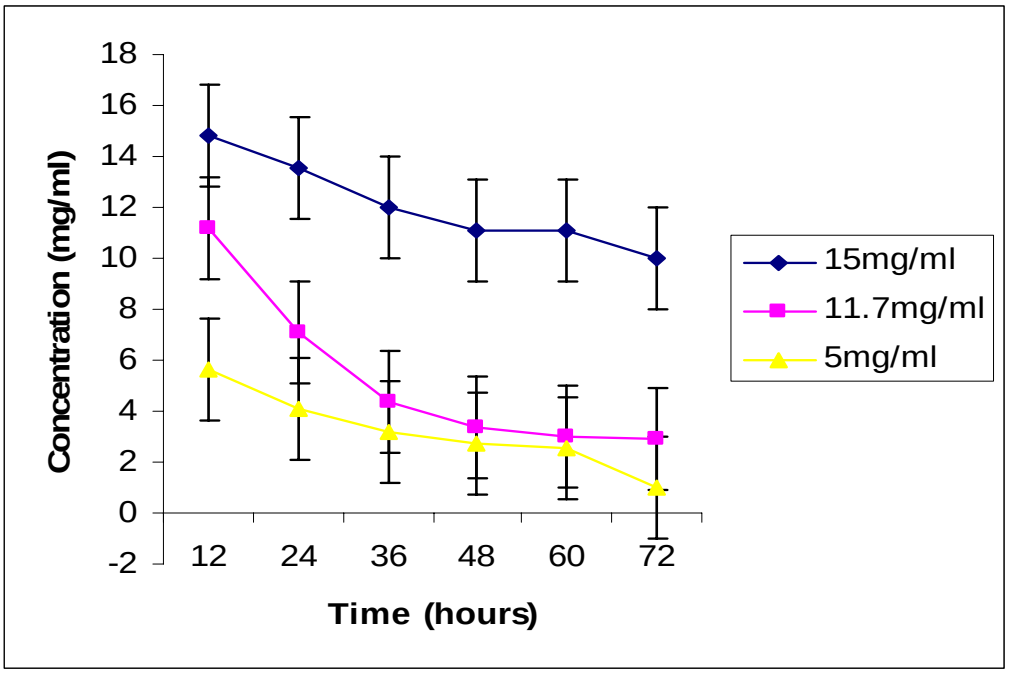

Figure 4.4: Glucose concentrations during fermentation process

It shows that concentration is decreased during the fermentation process. This reduction in glucose concentration is expected through, Glick and Pasternaks's experiment (1994) showing that 96% of sugars were converted to ethanol and other byproduct. Other than that it shows that the *Saccaromyces cerevisiae* is react with glucose where it converts the glucose into ethanol. Since the fermentation process is in anaerobic condition, the yeast only depends on glucose as it nutrients.

Under anaerobic condition, the glucose concentration in the media was possibly too low and result in lower concentration of ethanol compared to under aerobic condition. This result maybe caused of *Saccaromyces cerivisia* was using ethanol as a carbon and energy source for growth. Previous study indicates that *Saccaromyces cerevisiae* can continue growth when ethanol is the limiting carbon and energy sources [22].

4.3.3 Effect of Substrate Concentration on Ethanol Yield

Ethanol concentration is determined by using Figure 3.4 in Chapter 3 where the standard curve that is obtained from the analysis is used in the equation by the straight line of standard calibration curve for ethanol. Summarized of ethanol concentration %(w/w) at different glucose concentration within 54 hours duration time shown in Table 4.3.

Table 4.3 Ethanol concentration % (w/w) at different glucose concentration

	Concentration of glucose					
	5.6 mg/ml		11.7 mg/ml		15 mg/ml	
Time	Size of inoculums					
duration	15%	10%	5%	15%	10%	15%
0 hr	0	0	0	0	0	0
6 hr	6.1	5.9	2.3	5.5	10.3	9.9
12 hr	6.7	6.3	4.1	9	11.2	12.1
18 hr	7.2	6.9	5.8	9.8	12.8	13
24 hr	8	7.8	8	10.4	13.3	13.9
30 hr	8.1	8.3	9	10.6	13.4	13.8
36 hr		8.2	9.8	10.4	13.7	
42 hr		8.4	10		13.7	
48 hr			10.4			
54 hr			10.6			

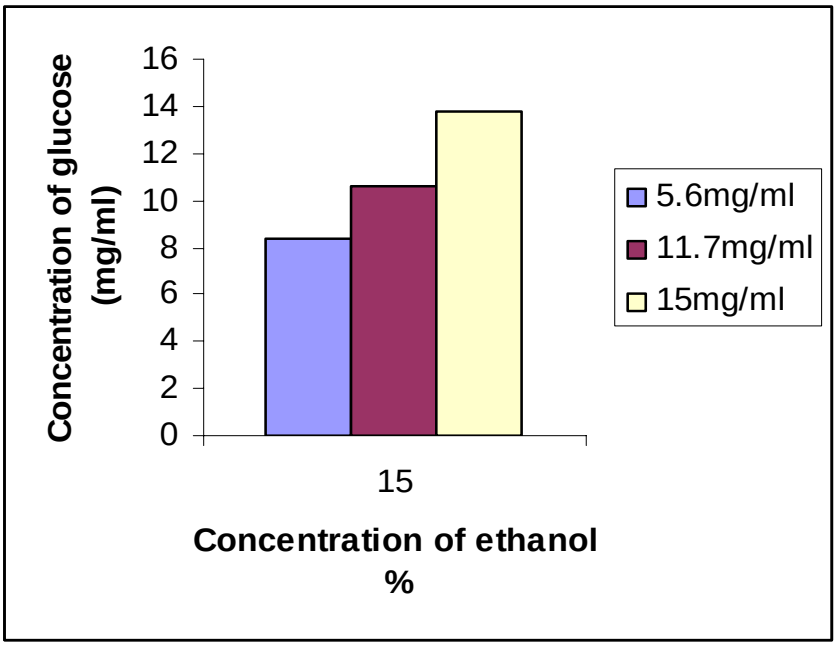

Figure 4.5: Concentration of ethanol at different glucose concentration

Figure 4.5 shows the ethanol concentration at different glucose concentration. The final ethanol concentration with 15mg/ml of glucose was 13.7%. While for 11.7mg/ml of glucose produce 10.6% ethanol concentration. However at lower concentration of glucose, 5.6mg/ml produce ethanol concentration 8.1%. It showed that as the concentration of glucose increased, the ethanol concentration is increased. The main carbon sources in this fermentation were glucose where glucose became nutrients to yeast. In anaerobic fermentations, a large fraction of substrate carbon is converted to ethanol [23]. Hence the production of ethanol was depending on carbon sources, glucose. The inoculums concentration did not have pronounced effect on the final ethanol concentration. This is because in this fermentation, glucose was growth- limiting factor hence inoculums concentrations only affect the duration time of fermentation process.

4.3.4 Effect of Size of inoculums

The time course of the ethanol fermentation of EFB glucose was performed with various inoculums concentrations of *Saccaromyces cerevisiae* and the results are presented in Fig. 4.6 to 4.8.

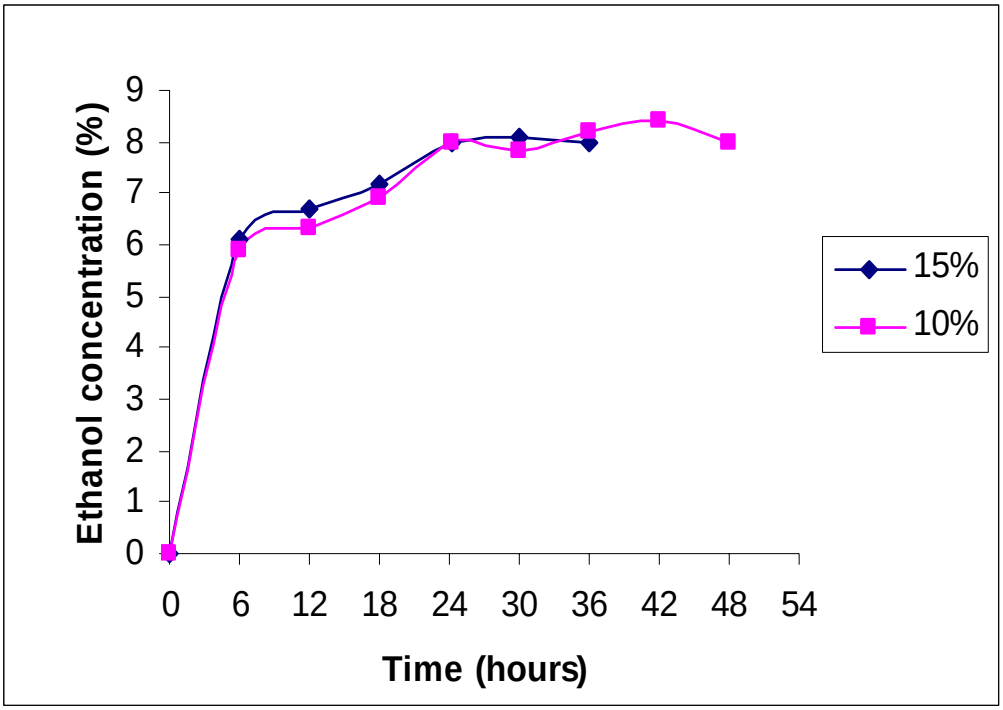

Figure 4.6: Effect of the *Saccaromyces cerevisiae* concentration on the fermentation of substrate (5.6mg/ml)

The final ethanol yields obtained by fermentation with 10% and 15% (w/w) of inoculums were 8.1% and 8.4% of the theoretical yield, respectively. However, the duration of fermentation decreased with the increase of the inoculums concentration. Thus, fermentation with 10% (w/w) of yeast lasted 48 hours, while with 15% (w/w) of yeast the fermentation was accomplished in 36 hours. As the glucose was growth-limiting factor, the yeast in higher concentration of inoculums need shorter time to consume the glucose rather than in lower size of inoculums.

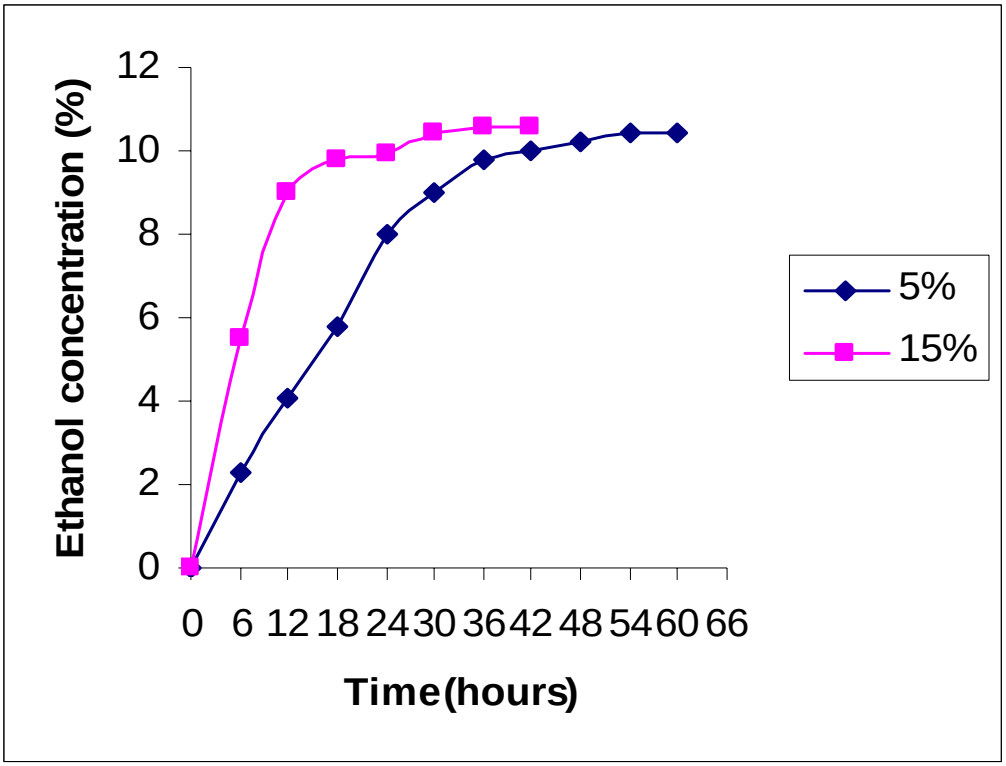

Figure 4.7: Effect of the *Saccaromyces cerevisiae* concentration on the fermentation of substrate (11.7 mg/ml)

The final ethanol yields obtained by fermentation with 5% and 15% (w/w) of inoculums were 10.4% and 10.6% of the theoretical yield, respectively. The figure 4.7 shows that, the duration of fermentation decreased with the increase of the inoculums concentration. Thus, fermentation with 5% (w/w) of yeast lasted 60 hours; while with 15% (w/w) of yeast require 42 hours. As the growth rate in 5% size of inoculums was low, the yeast takes time to consume the glucose compare to the higher size of inoculums. The growth rate was higher in high size of inoculums.

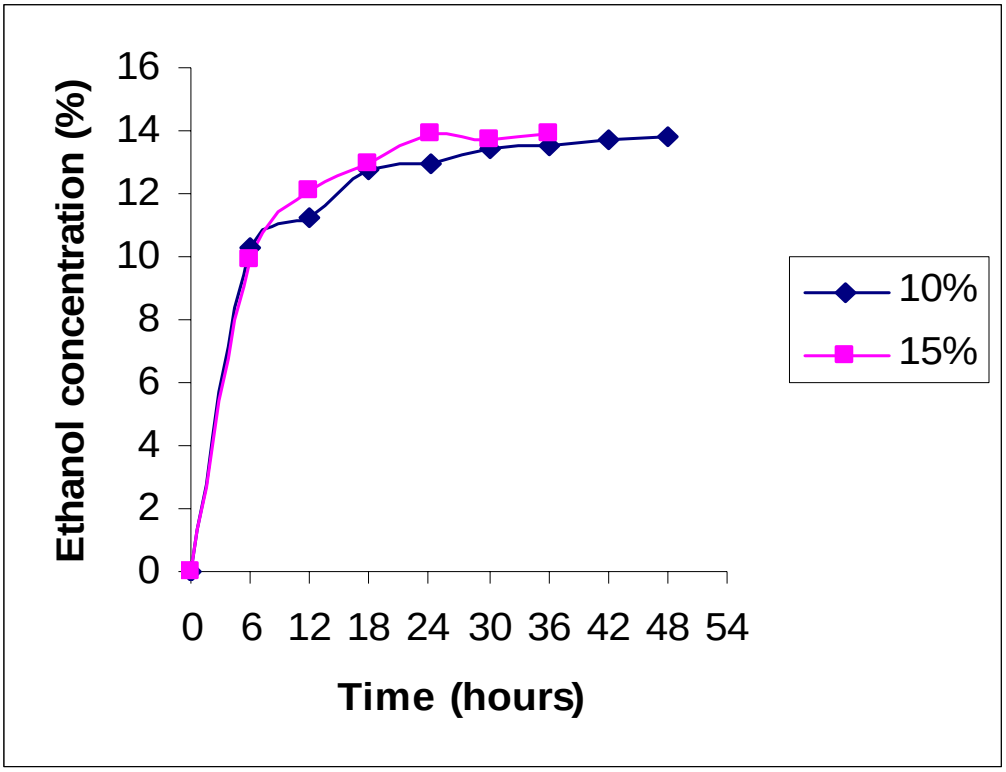

Figure 4.8: Effect of the *Saccaromyces cerevisiae* concentration on the fermentation of substrate (15mg/ml)

The final ethanol yields obtained by fermentation with 10% and 15% (w/w) of inoculums were 13.9% and 13.5% of the theoretical yield, respectively. The figure 4.8 shows that, the duration of fermentation decline with the increase of the inoculums concentration. Thus, fermentation with 10% (w/w) of yeast lasted 48 hours; while with 15% (w/w) of yeast require 36 hours.

The increase of inoculums concentration did not have pronounced effect on the final ethanol concentration. However the duration of fermentation decreased with the increase of the inoculums concentration. Hence, by increasing the inoculum's concentration from 5 to 15% w/w of *Saccaromyces cerevisiae*, the fermentation time could be reduced [21].

This situation happened because the growths of yeast were different for each size of inoculums. This phenomenon known as diauxic growth is caused by a shift in metabolic pathway in the middle of growth cycle. The process to consume the nutrients became shorter since the growth of yeast become dominant in higher size of inoculums as glucose was growth-limiting factor in the medium.

CHAPTER 5

CONCLUSION & RECOMMENDATIONS

5.0 Conclusion

The project was to study the feasibility of converting EFB into glucose which could then to produce ethanol. Based on the experiments that had been done, the results showed that two-stage hydrolysis of EFB could produce glucose with the help of acid hydrolysis which is H_2SO_4. At higher H_2S0_4 concentration, the glucose concentration was high.

Fermentation with *Saccharomyces cerevisiae* was done in anaerobic condition. Different size of inoculums was used in this experiment. The result showed that by increasing the inoculum's concentration from 5-15%, the fermentation time could be reduced. The result also showed as the concentration of glucose increased, the ethanol concentration is increased. The highest concentration that produced from the experiment was 13.8 % (w/w) by using 15mg/ml of glucose. Even though the concentration of ethanol produced from EFB is low, the project was economical since the process was only consumed few chemicals and biomass waste.

Bioethanol demonstrate promising potential as an alternative fuel to substitute fossil fuel. In a country like Malaysia, bioethanol offers a plenty of advantages, especially to the economic of the nation. In this research, empty fruit bunch (EFB) was used as a raw material to produce ethanol for mixing with gasoline thus this can

potentially reduce the dependency on fossil fuels and can expand the usage of oil palm since there are abundant of EFB nowadays. Since bioethanol has a lot of advantages as listed in previous chapters, the new breakthrough from this research will contribute significantly to the energy program of the nation. Beside, this research can become reference in development of bioethanol production in Malaysia in the future.

5.1 Recommendations

There are several recommendations made in order to produce a large amount and optimum yield of bioethanol using biomass wastes.

i. In the experiments, large quantities of H_2SO_4 and NaOH were used. Therefore, the feasibility of using the two-stage hydrolysis procedure for a large scale conversion of EFB to glucose needs further evaluation and consideration. The project might be feasible only if ways can be discovered to recycle the H_2SO_4 and NaOH used.

ii. The recovery and purification of ethanol in this study can be done since it is essential to any commercial process.

iii. Use other fermentation method such as immobilized cell technique in order to determine the best method in obtaining high yield of productivity

iv. Commercializing the production of bioethanol in Malaysia so that the demand of bioethanol is high. This will help saving the earth and a precaution steps due to the depletion of crude oil in our country.

v. Simplified the equipment that is needed in producing bioethanol from biomass wastes. Several methods are on research to combine the procedure in producing bioethanol so that it can safe time, money and energy.

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APPENDICES

Sulfuric acid concentration (%)	**Absorbance**	**Glucose concentration (mg/ml)**
0.0	0.00	0
3.0	0.575	5.6
4.0	1.200	11.7
6.0	1.479	15.1

Appendix A1: Glucose concentration for different sulfuric acid concentration

	Set 1		Set 2		Set 3	
Concentration of glucose (mg/ml)	15		5.6		11.7	
Size of inoculums (%)	10%	15%	10%	15%	5%	15%
Agitation rate (rpm)	100		100		100	
pH	4.8		4.8		4.8	
Temperature (^{o}C)	30		30		30	

Appendix A2: Parameters involve in fermentation process

Time(hours)	Absorbance	Concentration of glucose (mg/ml)
12	1.5451	15.10
24	1.3847	13.53
36	1.2241	11.96
48	1.1392	11.13
60	1.1361	11.10
72	1.0214	9.98

Appendix A3: Absorbance and concentration of glucose values for set 1

Time(hours)	Absorbance	Concentration of glucose (mg/ml)
12	1.1974	11.00
24	0.7266	7.10
36	0.4482	4.38
48	0.3438	3.36
60	0.3101	3.03
72	0.2978	2.91

Appendix A4: Absorbance and concentration of glucose values for set 2

Time(hours)	Absorbance	Concentration of glucose (mg/ml)
12	0.5731	5.60
24	0.4170	4.08
36	0.3232	3.16
48	0.2814	2.75
60	0.2589	2.53
72	0.1064	1.04

Appendix A5: Absorbance and concentration of glucose values for set 3

Appendix B1: The abundance of oil palm fruit bunches

Appendix B2: Washed and dried EFB

Appendix B3: Ground EFB (0.5 mm in size)

Appendix C1: Fermenter 2L

Appendix C2: UV-VIS spectrophotometer (Hitachi U-1800)

GANTT CHART FOR INDUSTRIAL PROJECT 2 (PSM 2)
(10 JULY 2006 - 19 NOVEMBER 2006)

TASK/WEEK	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Production of Bioethanol:																			
Sample Preparation for Pre-treatment																			
Acid hydrolysis																			
preparation of inoculum																			
Anaerobic Fermentation																			
Analysis of Collecting Samples																			
Editing First Draft																			
Final Draft 1 Submittion																			
Preparation for Progress Report Presentation																			
Progress Report Presentation																			
Technical Paper Preparation for Seminar 2																			
Seminar 2																			
Editing Final Draft 1																			
Final Draft 2 Submittion																			
Editing Final Draft 2																			
Thesis Submittion																			