

PRODUCTION OF BIOETHANOL FROM BIOMASS WASTE (SUGARCANE)

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**A thesis submitted in fulfilment of the requirements for the award of the degree
of Bachelor of Chemical Engineering**

**Faculty of Chemical & Natural Resources Engineering
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NOVEMBER 2006

I declare that this thesis entitled “**PRODUCTION OF BIOETHANOL FROM BIOMASS WASTE (SUGARCANE)**” 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.

Signature :

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Date : 20 November 2006

DEDICATION

Special Dedication of This Grateful Feeling to My...

Beloved Parents;
Mr. Yaziz B. Ibrahim
Mrs Musalmah Bt. Amad

Loving sister;
Nohidayah Bt. Yaziz
Norhidayu Bt Yaziz
Norhidayanty Bt Yaziz
Norhidahanis Bt Yaziz

Supportive and understanding friends;
Mohd Hisham B. Yusop
Mohd Shahril B. Mohamad
Mohd Farridd B. Termizi
Jedidiah Johnny

For Their Love, Support and Best Wishes.

ACKNOWLEDGEMENT

Bismillahirrahmanirahim,

I am so thankful to Allah S.W.T for giving me patient and spirit throughout this project and the research is successfully complete. With the mercifulness from Allah therefore I can produce a lot of useful idea to this project.

To my beloved father and mother, Yaziz B. Ibrahim and Musalmah Bt Amad. I am grateful to have both of you in my life and giving me full of support to through this life. I pray and wish to both of you are always in a good health and in Allah mercy. You are the precious gift from Allah to me.

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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 study was focus on the production of ethanol from sugarcane baggase using *Saccharomyces cerevisiea* as fermentation aide to expand the usage of the sugarcane baggase. The objectives of this study are to determine the yield of ethanol produced from certain amount of biomass waste (sugarcane baggase), the effects of the temperature and agitation speed in fermentation process. Pretreatment was used for breakdown the lignin seal to produce the highest yield of glucose. Enzymatic process is purposed to convert the cellulose to glucose. At the end of this study, the yield of ethanol produced was increase in a little with the temperature and agitation speed when reached the optimum point in fermentation process. Theoretically, every gram of glucose can theoretically yield 0.51 g of ethanol. The result given by the fermentation process where the ethanol composition at optimal temperature and agitation speed, 35°C and 200 rpm is 5.1 in weight percent and 6.5 in volume percent.

ABSTRAK

Pada masa kini terdapat pelbagai kaedah untuk menghasilkan ethanol. Antara kaedah yang digunakan adalah penghasilan ethanol menggunakan hemiselulosa dan selulosa daripada hasil buangan biojisim. Kajian terhadap penghasilan ethanol daripada hasil buangan hampas tebu menggunakan kaedah penapaian menggunakan yis, dilakukan untuk memperluaskan penggunaan bahan buangan pokok tebu tersebut. Tujuan kajian ini dijalankan adalah untuk menghasilkan ethanol daripada bahan buangan hampas tebu. Selain itu juga, kesan suhu dan kelajuan pengocakan dalam kaedah penapaian untuk penghasilan ethanol juga turut dikaji. Di dalam kajian ini, hasil buangan yang digunakan adalah hampas tebu. Prarawatan adalah untuk memecahkan lignin dalam struktur dalam menghasilkan jumlah glukosa yang tinggi. Hidrolisis yg melibatkan enzim dijalankan bertujuan untuk menukarkan struktur cellulosa kepada glukosa. Pada akhir eksperimen, penghasilan ethanol meningkat sedikit apabila suhu dan kelajuan pengocakan mencapai titik optimum di dalam kaedah penapaian.. Secara amnya setiap satu gram glukosa. Secara teorinya akan mendapat 0.51g ethanol. Hasil daripada proses penapaian yang dilakukan, pada suhu yang optima iaitu pada suhu dan kelajuan pengocakan, 35°C and 200 rpm komposisi ethanol yang dihasilkan ialah 5.1 dalam peratus berat dan 6.5 dalam peratus isipadu.

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LIST OF SYMBOLS

g	-	gram
ml	-	mililiter
L	-	liter
Tg	-	tonnegram
Mm ²	-	megameter square
Mg ha ⁻¹	-	megagram per hectar
GL	-	gigaliter
kg	-	kilogram
°C	-	°Celcius
%	-	percent
µg/ml	-	microgram per mililiter
mm	-	milimeter
rpm	-	rotation per minute
L/h	-	liter per hour
g/ml	-	gram per mililiter
vol/vol	-	volume per volume
wt %	-	weight percent

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Ethanol (Ethyl Alcohol or Grain Alcohol, C_2H_5OH) can be defined as clear, colorless flammable oxygenated hydrocarbon with boiling point 173.5°F in the anhydrous [1] (figure 1.1). Production of ethanol from renewable lignocelluloses resources may improve energy availability, decrease air pollution, and diminish atmospheric CO_2 accumulation [2]. It is biodegradable, low in toxicity and causes little environmental pollution if spill. 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.

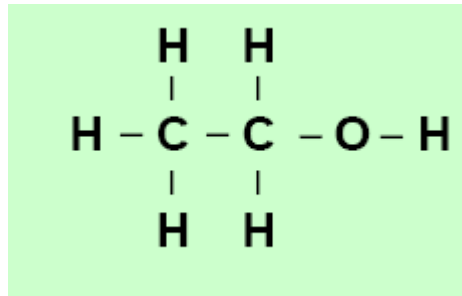


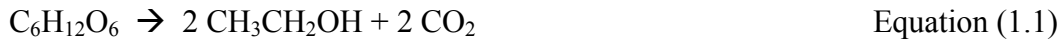
Figure 1.1: Ethanol Structure

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 as an alternative motor fuel has been steadily increasing around because of their characteristic. Bioethanol have a big potential to usable in all petrol-based engines without user having to make modification or adjustment to the engines at all. It also has a very potential energy source to replace or to complement the fossil based energy source to fuel transportation also in industries.

The example of biomass waste where is commercial to produce ethanol is sugarcane bagasse, rice husk, and corn starch. Each material contains different types of carbohydrate. Cellulose materials are cheap and easy to get in Malaysia so clearly it definitely can help to reduce cost in production of ethanol.

Bioethanol is produced using familiar methods, such as fermentation. Two reactions are hint 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. 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 with sugars. Ethanol and carbon dioxide are produced as the sugar is consumed. The reaction for the 6-carbon sugar, glucose to produce ethanol shown in equation 1.1 and 1.2



The sugar formed in the hydrolysis reaction is fermented into bioethanol. The most common microorganism for this purpose is *Saccharomyces cerevisiae*, ordinary baking yeast [3]. Besides glucose, *Saccharomyces cerevisiae* has the ability to ferment manose as well.

1.2 Objective

The objective of this study /research is to produce bioethanol from biomass waste (sugarcane Baggase) by using fermentation process.

1.3 Scope of Study

The scopes for this study were:

- i. To determine yield of bioethanol that can be produced from sugarcane baggase
- ii. To optimize the temperature and agitation speed in fermentation process to produce the higher yield of ethanol.

1.4 Problem Statements

The hiking of fossil fuels price in Malaysia recently makes the consumers under pressure. This problem happened because of the depleting crude oil where only hold out approximately for 30 or 40 years from now. Due to this situation it can predict that are likely to increase in the future. There is the strong reason to produce the ethanol as substitutes to fossil fuels in the future. The lower cost to produce ethanol is come from biomass waste because the raw materials are renewable and cheap.

The arguments in favor of cellulosic ethanol as a replacement for gasoline in cars and trucks are compelling. Cellulosic ethanol will reduce depending on imported oil, increase energy security and reduce the trade deficit. Rural economies will benefit in the form of increased incomes and jobs. Growing energy crops and harvesting agricultural residuals are projected to increase the value of farm crops, potentially eliminating the need for some agricultural subsidies. Finally, cellulosic ethanol provides positive environmental benefits in the form of reductions in greenhouse gas emissions and air pollution [2]. Ethanol is biodegradable where it low in toxicity and caused a little environmental pollution if split. Ethanol fuel is a mixture of conventional gasoline and ethanol. The result is a much cleaner burning fuel that is just as efficient as standard fuels [2].

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Ethanol is substitutes for transportation fuel when oil crisis arise. It considered as one of alternative way to overcome this problem. Fuel ethanol still taking place at a research institute but in Brazil it already produced on a large scale for the market. From research ethanol performs well as a fuel in cars it also friendly environmental. Another reason to be mentioned the world demand for fuel in this era is predicted to be more than the capacity of global oil production.

Ethanol made from cellulosic biomass is called bioethanol. A major challenge is developing biocatalysts capable of fermenting lignocellulosic biomass for efficient industrial application. In the coming years it is believed that cellulosic biomass will be the largest source of bioethanol. The broad category of biomass for the production of ethanol includes agricultural crops & residues and wood. Biomass resources are abundant and have multiple application potential. Among the various competing processes, bioethanol from lignocellulosic biomass appears to have near-term economic potential. The crops residues such sugarcane bagasse where has are high content of sugar also easy to get in our place will be used as our raw materials, it also used to derive desired economic and environmental benefits and thus they could be important resource bases for

bioethanol production.

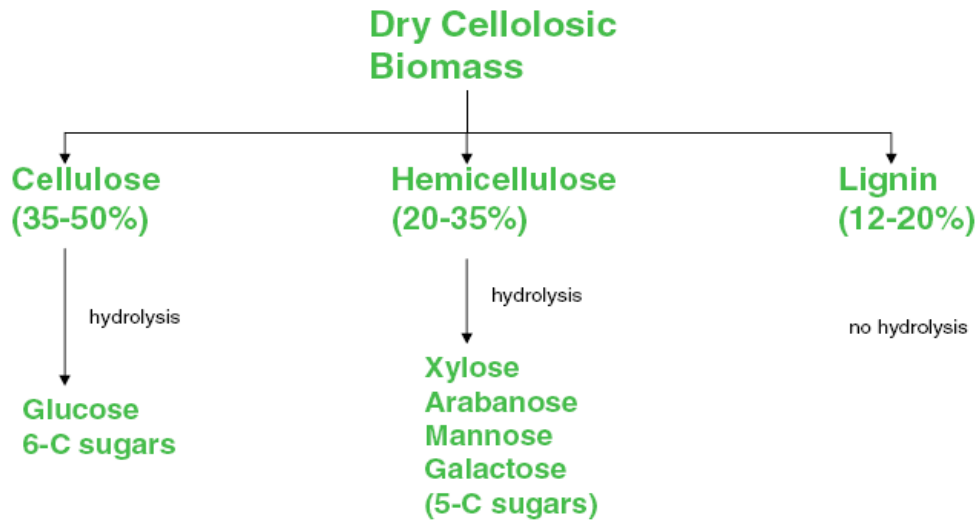


Figure 2.1: Composition of Dry Cellulosic Biomass. (Sources: Adapted from Bioalcohol Fuel Foundation, BAFF 2003)

2.1.1 Cellulose

Cellulose is a long-chain polymer with repeat unit of D-glucose (Figure 2.2). In native state, cellulose chains are held together by intermolecular hydrogen bonds where they form between glucose units of the same chain [4] (Figure 2.2). The additive effect of the bonding energy of the hydrogen bonds increases the rigidity of cellulose and causes it to be highly insoluble as well as highly resistant to most organic solvents [5]. In cellulose, the C1 (acetal linkage) oxygen is in the β -configuration (cellulose is poly [β -1,4-D-anhydroglucopyranose]). The β -configuration causes cellulose molecule a good fiber-forming polymer. Even though it often has long molecular chains, amylose is not a good fiber-former where amylose is a related polymer of glucose, but with the C-1 oxygen's in a α -configuration (Figure 2.3). Cellulose is the main reservoir of glucose, the desired fermentation substrate.

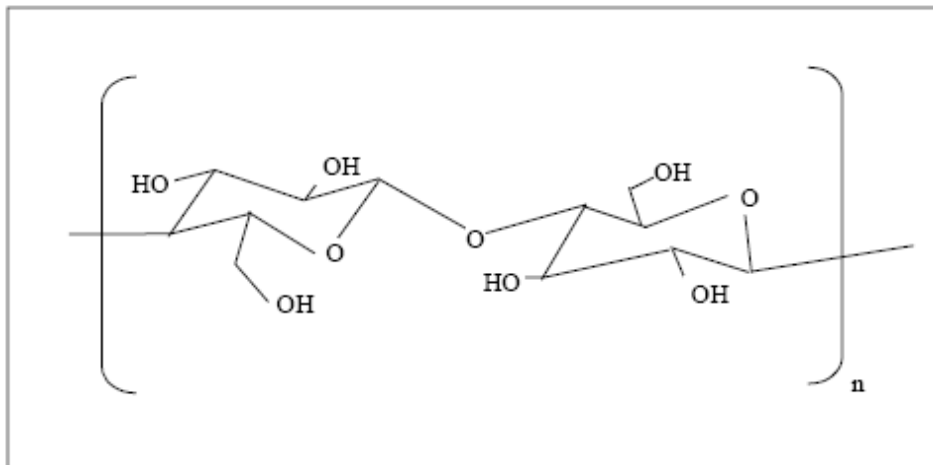


Figure 2.2: The structure of cellulose, showing $\beta(1-4)$ glycosidic bond.
(Sources: Adapted from SFE, Tina Jeoh, 1998)

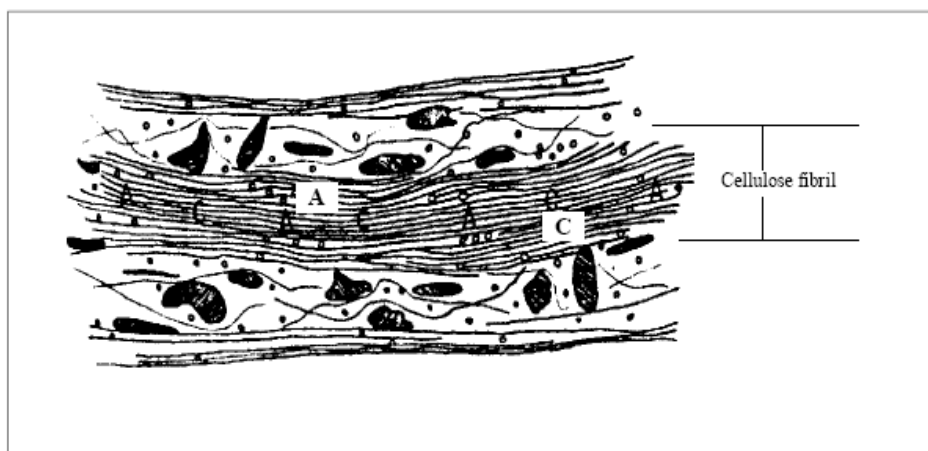


Figure 2.3: Longitudinal section of a microfibril. C designates crystalline region of Cellulose fibers; A designates the amorphous regions. (Adapted: from Bodig and Jayne 19820).

2.1.2 Hemicellulose

In contrast to cellulose, which is crystalline, strong and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength [6]. It is easily hydrolyzed by dilute acid or base, but nature provides an arsenal of hemicellulose enzymes for its hydrolysis [6]. Many different pentose are usually present in hemicellulose, however xylose always the predominating sugar. The pentoses are also present in rings that can be five- or six-member. [6]. In general, hemicellulose readily hydrolyzes into its constituent sugar under acidic condition [5].

2.1.3 Lignin

Lignin is biosynthesized by a free-radical coupling reaction [6]. Lignin is stable, high-molecular weight compound built on phenylpropane units (Figure 2.4). As part of the microfibrillar structure, lignin filling the space between around cellulose and hemicellulose and completing with the polymer [5]. Lignin is very rigid where the presence of lignin greatly limits accessibility to the cellulose and hemicellulose molecules. Lignin serves as a binding between the cells for structural support of plant.

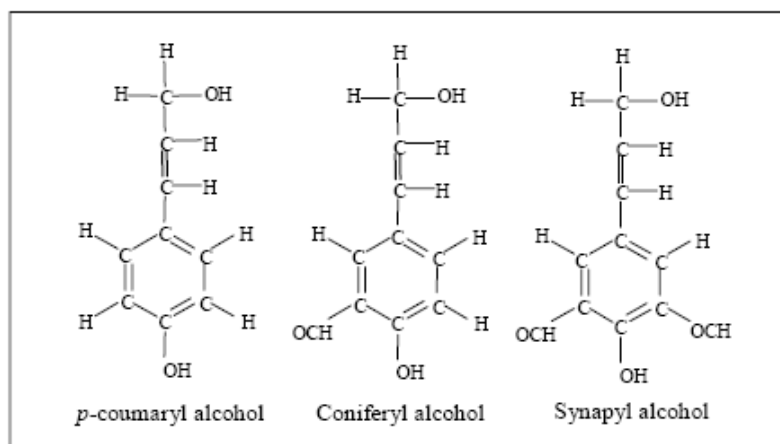


Figure 2.4: Phenylpropane units of hardwood and soft woods, the basic component

Lignin (Sources: Adapted from SFE, Tina Jeoh, 1985)

2.2 Background of Ethanol

Table 2.1: Sugar and Ash Composition of Various Biomass Feedstock-weights percent
(Sources adapted from <http://www.pcr.org>)

Material	Sugars	Lignin	Ash
Hardwoods	57-78%	15-28%	0.3-1.0%
Softwoods	49-69%	24-27%	0.1-0.4%
Ag Residues	42-81%	11-29%	2-18%

Table 2.2: Typical levels of cellulose, hemicelluloses and lignin in biomass (Sources adapted from <http://www.pcr.org>)

Feedstock	Gallons ethanol/dry ton
Bagasse	112
Corn Stover	113
Rice straw	110
Forest thinning	82
Hardwood sawdust	101
Mixed paper	116

2.2.1 Ethanol around the world

Other countries are either producing or using ethanol in large quantities or are providing incentives to expand ethanol production and use. Brazil and Sweden are using large quantities of ethanol as a fuel. Some Canadian provinces promote ethanol use as a fuel by offering subsidies of up to 45 cents per gallon of ethanol. India is initiating the use of ethanol as an automotive fuel. Based on experiments by the Indian Institute of Petroleum, a 10 percent ethanol blend with gasoline and a 15 percent ethanol blend with diesel are being considered for use in vehicles in at least one state. In France, ethanol is produced from grapes that are of insufficient quality for wine production. Prompted by the increase in oil prices in the 1970s, Brazil introduced a program to produce ethanol for use in automobiles in order to reduce oil imports. Brazilian ethanol is made mainly from sugar cane. Pure ethanol (100% ethanol) is used in approximately 40 percent of the cars in Brazil [7]. The remaining vehicles use blends of 24 percent ethanol with 76 percent gasoline. Brazil consumes nearly 4 billion gallons of ethanol annually. In addition to consumption, Brazil also exports ethanol to other countries. Sweden has used ethanol in chemical production for many years; As a result, Sweden's crude oil consumption has been cut in half since 1980 [7]. During the same time period, the use of gasoline and diesel for transportation has also increased. Emissions have been reduced by placing catalytic converters in vehicle exhaust systems which decrease carbon monoxide, hydrocarbon, and nitrogen oxide emissions [7].

2.2.2 Future of Ethanol

By replacing existing vehicles with new technology such as like the E-85 vehicles. Auto manufacturers are responding to the technical challenges of meeting the new standards. Since 1996, new model vehicles have on-board diagnostic monitoring systems capable of monitoring tailpipe and evaporative emissions. New computer technology makes this possible. New E-85 vehicle models are being produced each year. Programs are also in place to reduce emission levels by updating engine technology in

mass transit city buses and over-the-road trucks. Buses in several cities are powered by converted diesel engines that burn 100 percent ethanol. “E-Diesel,” a blend of ethanol and diesel fuel, is being introduced for use in large trucks, tractors and construction equipment. Ethyl tertiary butyl ether (ETBE) is being tested and used as a motor fuel additive in reformulated gasoline [7]. Ethanol and ETBE blends in gasoline are approved by the Environmental Protection Agency (EPA) for mandated winter-time oxygenated fuel programs where the objective is to lower vehicle carbon monoxide emissions from vehicles. This Renewable Fuel Standard would serve to expand the production and use of ethanol nationally and would provide the impetus for producing ethanol from a wide variety of renewable feed stocks.

2.3 Ethanol as a Fuel

Ethanol is an alternative energy source. It is an alcohol made by fermenting sugarcane baggase or other similar biomass material. There are three primary ways that ethanol can be used as transportation fuel:

- i. As a blend of 10 percent ethanol with 90 percent unleaded gasoline called “E-10 Unleaded”
- ii. as a component of reformulated gasoline, both directly and/or as ethyl tertiary butyl ether(ETBE)
- iii. As a primary fuel with 85 parts of ethanol blended with 15 parts of unleaded gasoline called “E-85.”[7]

Introduction and operation of variable fuel vehicles in 1990s where the vehicles are capable of operating on unleaded fuel with ethanol mixtures up to 85 percent without having to make any engine adjustments [7]. These vehicles were introduced in 1992 and have been used extensively in federal and state fleets and in some city governments. They became commercially available shortly thereafter.E-85 vehicles have been designed for versatility. The key component in a variable fuel vehicle is a sensor that determines the

percentage of ethanol in the fuel. With the help of a computer, the vehicle automatically adjusts for best performance and emissions. Chrysler began offering E-85 minivans in the 1998 model year and Ford continues to offer the Taurus and added Wind star and Ranger to the E-85 flexible fuel vehicles in the 1999 model year. Ford, GMC, Chevrolet and Daimler-Chrysler are now offering E-85 variable fuel vehicles [7].

2.3.1 Engine performances and Ethanol

Auto manufacturers today are recommending ethanol-blended gasoline for the vehicles they sell. A recent survey revealed that nine out of ten auto dealers use ethanol-blended gasoline in their personal vehicles. Over half of the dealerships surveyed indicated their customers reported benefits that included: reduced knocking and pinging, improved gas mileage, better acceleration, and improved starting qualities. Independent automotive technicians also trust their family cars to ethanol blends. A 1997 Iowa survey indicated that nine out of ten technicians used ethanol in their personal vehicles and reported the same benefits as the auto dealers. E-10 Unleaded (10% ethanol / 90% gasoline) is approved under the warranties of all domestic and foreign automobile manufacturers marketing vehicles in the United States. In fact, the nation's top three automakers, Daimler-Chrysler, Ford and General Motors, recommend the use of oxygenated fuels such as ethanol blends because of their clean air benefits and performance qualities [7]. Ethanol is a good cleaning agent. In newer vehicles it helps keep the engine clean. In older vehicles it can sometimes loosen contaminants and residues that have already been deposited in a vehicle's fuel delivery system. Occasionally, these loosened materials collect in the fuel filter, and can then be removed simply by changing the fuel filter. All alcohols have the ability to absorb water. Condensation of water in the fuel system is absorbed and does not have the opportunity to collect and freeze. Since ethanol blends contain at least 10 percent ethanol, they are able to absorb water and eliminate the need for adding gas-line antifreeze in winter [8]. Ethanol is a fuel for old and new engine technology. Automotive engines older than 1969 with non-hardened valve seats may need a lead substitute added to gasoline or ethanol

blends to prevent premature valve seat wear. Valve burning is decreased when ethanol blends are used because ethanol burns cooler than ordinary unleaded gasoline [7]. Some of the chemicals used to manufacture gasoline, such as olefins, have been identified as a cause of deposits on port fuel injectors. Today's gasoline contains detergent additives that are designed to prevent fuel injector and valve deposits.

2.3.2 E-85

The driving forces for this type of automotive fuel technology are: air pollution from fossil fuels such as gasoline, dependence on foreign suppliers, and the dim prospects for gasoline as the world's oil supply dwindles. The National Ethanol Vehicle Coalition [8] helped introduce E-85, an ethanol-based fuel comprised of 85 percent ethanol and 15 percent unleaded gasoline. E-85 is environmentally friendly. It has the highest oxygen content of any fuel available today, making it burn cleaner than ordinary gasoline. The use of E-85 reduces pollutants such as ozone and carbon monoxide and air toxins like benzene. Testing shows the E-85 cars perform well with significant reductions in emissions when compared to vehicles using ordinary unleaded gasoline [8]. Reductions in carbon monoxide and hydrocarbons, two particularly troublesome pollutants, are reduced significantly. Ethanol is one of only two liquid fuels available that combats global warming because of its raw material source. As sugarcane grows, it converts carbon dioxide into oxygen. Since E-85 is a cleaner burning fuel, it is expected that the life of a flexible fuel vehicle will be somewhat longer than that of a comparable gasoline vehicle. A gallon of E-85 blended gasoline contains about 2/3 the energy of a gallon of gasoline [8].

2.4 Ethanol & the Environments.

The oxygenated fuel programs resulted in excellent program compliance during the first year. In the winter of 1992-93, seven programs in the western states realized 50 percent fewer CO violations compared to the previous year [7]. Eight new California programs experienced 80 percent reductions in violations. Use of oxygenated fuels is a quicker and more economical way of achieving reductions than implementing a vehicle emission maintenance program that requires every vehicle to be tested or repaired [7]. Ethanol is one of the best tools we have to fight air pollution. Ethanol reduces pollution through the volumetric displacement of gasoline and by adding oxygen to the combustion process which reduces exhaust emissions. The use of ethanol results in reductions in every pollutant regulated by the Environmental Protection Agency (EPA), including ozone, air toxins, carbon monoxide, particulate matter, and nitrogen oxides [7]. Advances in computer technology not only improve monitoring and control of emissions, but also make it possible to use blends of up to 85 percent ethanol [7]. These flexible fuel vehicles are able to sense the volume of ethanol in gasoline and make the necessary engine adjustments for best efficiency, performance, and emission levels.

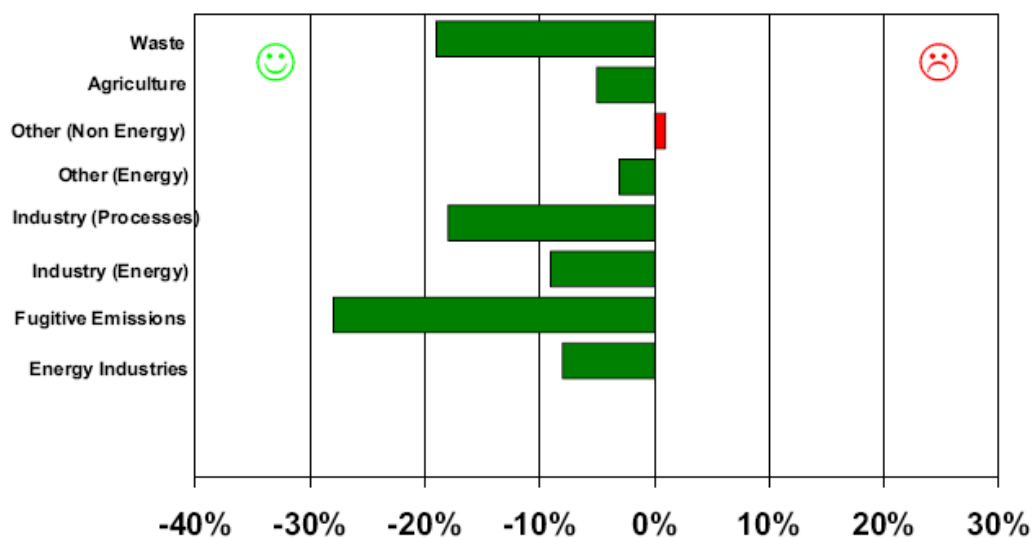


Figure 2.5: Change (%) in EU greenhouse gas emissions by sector and pollutant
(Sources: adapted from European Environment Agency 1990-99)

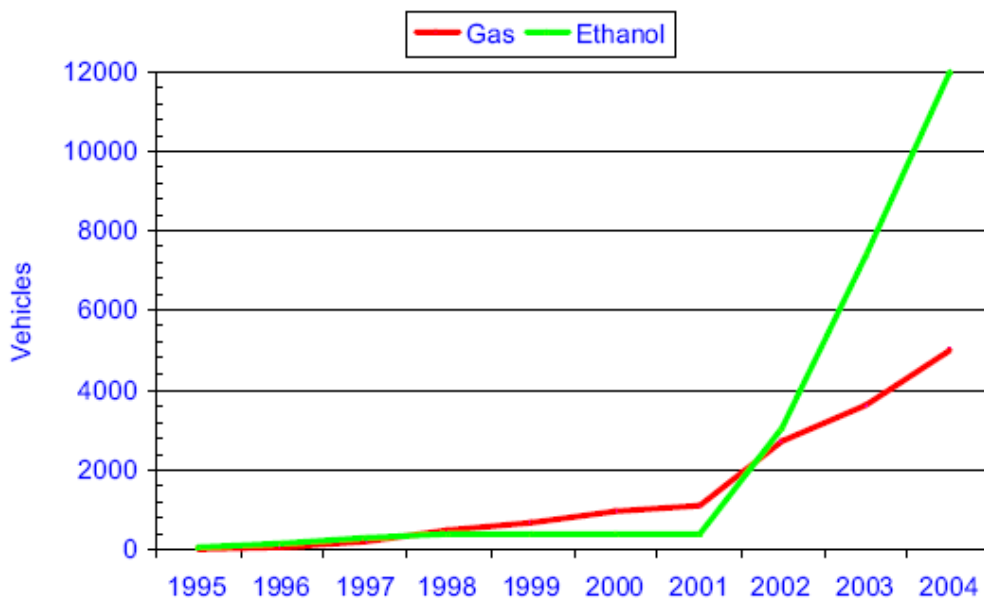


Figure 2.6: Flexi Fuel versus all the other environmental vehicles (Sources: adapted from,Bioalcohol Fuel Foundation,BFF 2003)

2.5 Ethanol Economics

Ethanol is made from farm-produced raw products which are usually in surplus. Sugarcane is the primary grain used in ethanol production, and it supplies most of the raw material needed. Ethanol production is the third largest user of sugarcane, behind domestic livestock feed and export uses. The conclusions of the report verify that the federal ethanol program is cost effective. The increase in production and price would raise gross farm income. The increase in farm expenditures and employment opportunities in the ethanol industry is projected to increase annually in relationship to additional ethanol production and use. As the domestic ethanol industry continues to grow, it is witnessing a surge in the construction of farmer-owned ethanol production facilities. Farmers are realizing the added benefits to the ethanol industry through ownership of manufacturing plants. Ethanol's importance to agriculture is evident like

added markets for farmers, stimulating rural economies by increasing sugarcane prices and rural income.

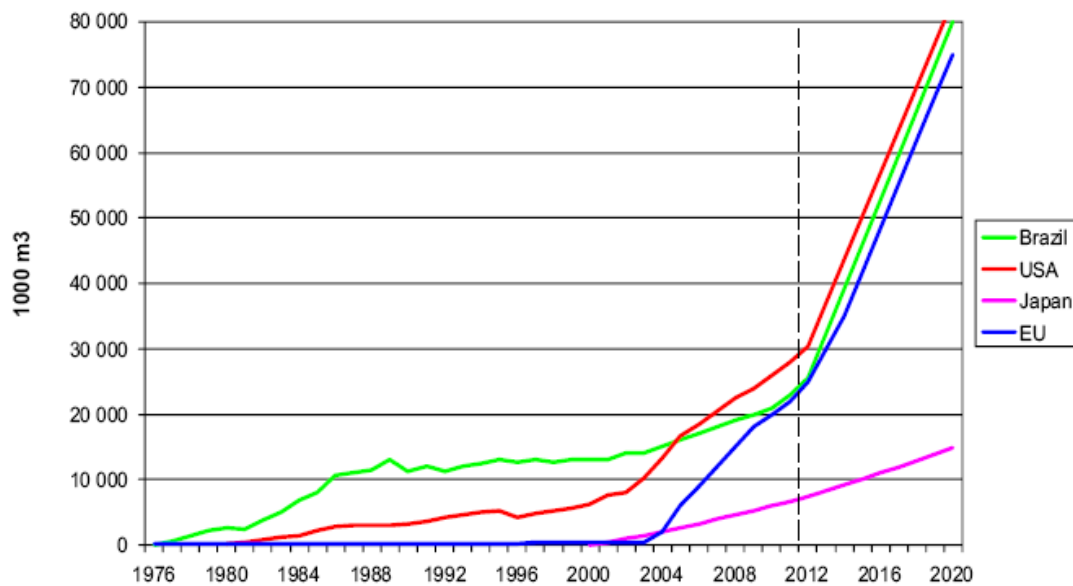


Figure 2.7: Ethanol markets develop rapidly (Sources: Adapted from Bioalcohol Fuel Foundation, BAFF, 2003)

2.6 Ethanol Production Process

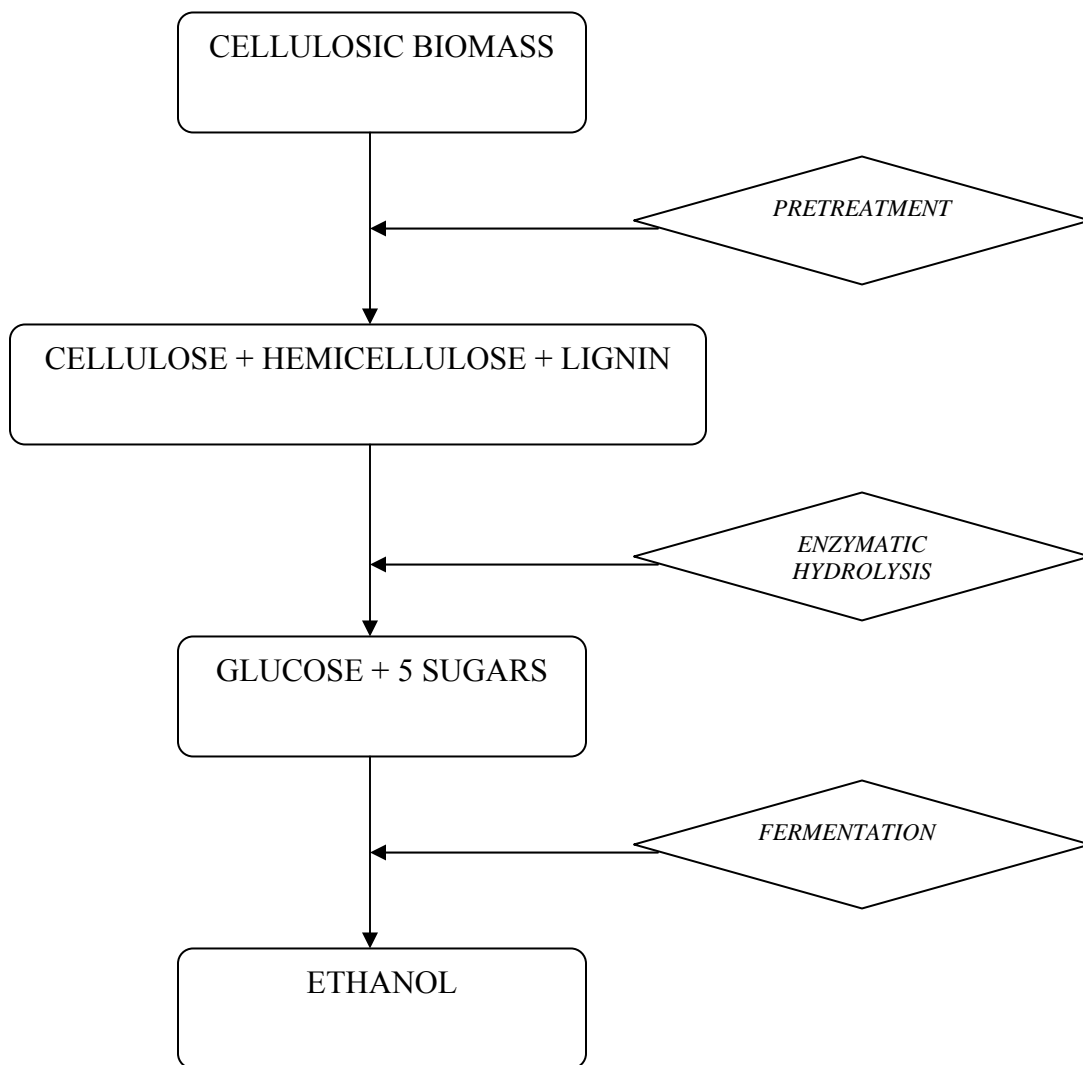


Figure 2.8: Overall Process in Production Ethanol through Fermentation Process

2.7 Pre-Treatment Process

Sugarcane baggase can be used as a fermentation feedstock only after being subjected to an effective pretreatment. To qualify as effective, the pretreatment must meet the following criteria:

- 1) Maximize fermentable sugar yields,
- 2) Avoid, or minimize degradation of carbohydrates,
- 3) avoid, or minimize the formation of microbial growth-inhibiting byproducts,
- 4) Be energetically, and most importantly, economically efficient.

In simpler terms, the purpose of a pretreatment is to breakdown the lignocellulosic structure to its monosaccharide components for use as fermentation substrates. The three main factors on the ease of lignocellulose breakdown to fermentable monosaccharide are pore size, cellulose crystallinity and the removal of lignin. Enhanced cellulose accessibility can be achieved by hemicellulose removal because the relative ease of hemicellulose hydrolysis provides an ideal avenue for creating larger pores in the microfibrils. [9] shows that increased enzyme digestibility is directly proportional to hemicellulose removal. [9] showed that positive correlation exists between pore volume (and available surface area) to glucose yields from enzyme hydrolysis; (i.e. greater pore volumes corresponded to higher percentages of glucose yield)

Cellulose crystallinity is the second deterministic factor for glucose yields. Higher a degree of crystallinity is proportional to slower hydrolysis rates [10] demonstrated that chemical and thermal treatments have a tendency to increase the relative crystallinity index (RCI) of amorphous cellulose. The same study showed that no significant increase in RCI is seen for crystalline cellulose. Thirdly, because access to cellulose microfibrils is highly restricted by the surrounding lignin matrix, removal of the lignin will largely enhance polysaccharide accessibility. There are several types of biomass pretreatment procedures to convert lignocellulosic biomass to fermentable sugars. These include alkali and dilute acid pretreatments, acid hydrolysis, ammonia steam explosion (AFEX), steam

explosion, enzyme hydrolysis etc. However, this review is confined to acid hydrolysis because they show more promise than the others.

2.7.1 Acid Hydrolysis

Acid hydrolysis has been the traditional pretreatment for lignocellulosic fermentation [11] that treating wood with concentrated sulfuric acid yields glucose. The "American" process, also known as the Simonsen method, was used between 1910 and 1922. Southern yellow pine sawmill waste was hydrolyzed by a batch process using 0.5% sulfuric acid and steamed at 912 kPa. The ethanol yield from the overall process, at 22 gal/ton, proved to be uneconomical. A German process, developed a few years later by Heinrich Scholler produced improved yields at 52-58 gal/ton of ethanol in 13-20 hour hydrolysis time. The Scholler process utilized a "pulse percolation" method where batches of 0.8% sulfuric acid were percolated through a column of compressed wood waste at temperatures of 120°C to 180°C. Peak operation of the Scholler process was during World War II in Germany. The U.S. Forest Products Laboratory improved the Scholler process, increasing ethanol yields to 64.5 gal/ton in a mere 3-hour hydrolysis time. The improvement seen in the Forest Products Laboratory's Madison Wood Sugar

Process was due to the continuous flow of the dilute acid as well as the continuous removal of the hydrolysate, minimizing monosaccharide degradation. The Madison Process was never truly established commercially on the account of its inability to compete effectively with ethanol derived from petroleum sources.

2.7.2 Acid Hydrolysis Mechanism

Initially, acid hydrolysis appears to be a relatively efficient means of accessing and breaking down cellulose. The hydrogen ion, therefore, does not face the problem of accessibility compared to cellulase enzymes. Furthermore, the basic mechanism of the

hydrolysis of glycosidic bonds is relatively simple the mechanism is similar to the hydrolysis of other glycosides such as starch (α 1-4 linked glucose chains, with α 1-6 branches). Step 3 is the rate-limiting step of the process because of the formation of the high energy half-chair configuration by the cyclic carbonium ion. Initial hydrolysis rates are typically very rapid performed experiments to show that in the initial stages of the hydrolysis reaction, larger pore volumes do correspond to faster reaction rates. However, after limited hydrolysis, the reaction rate slows down considerably. The glycosidic bonds most susceptible to hydrolysis are those either at the surfaces or in the amorphous regions of cellulose. Rapid hydrolysis rates reflect hydrolysis activity in these regions and can be seen as a decrease in the degree of polymerization (DP) from several thousand to about 200. This point is referred to as the leveling off degree of polymerization (LODP). Further hydrolysis is much more difficult beyond the LODP because of the high crystallinity of the remaining cellulose molecules.

2.8 Enzymatic Hydrolysis Process

Either using acids or enzymes can be hydrolyzing the cellulose material to get the glucose. In this study enzymes will be used because of many disadvantages by using acids hydrolysis. Cellulases are a group of enzymes that act synergistically to hydrolyze cellulose. At present, the actual mechanism of cellulase hydrolysis and the interactions between the components are not completely understood and are still under investigation. According to current understanding, the components of cellulase include endoglucanases, exoglucanases (cellobiohydrolases), and β -glucosidases (cellobiases) [11]. β -glucosidases, however, are under separate genetic controls and are often not considered to be a cellulase. Current literature on cellulose systems no longer recognizes a separate C1 component. Although the vote is not unanimous, many now consider exoglucanase (cellobiohydrolase) as the "C1 component". There is agreement, however, that crystalline cellulose needs to be hydrated and rendered amorphous before the hydrolysis of its glycosidic bonds can occur.

Endo-glucanase splits the long chain of cellulose to shorter. Cellobiohydrolase cleaves units to two linked glucose molecules (cellobiose) from the ends of the cellulose chain. Finally β -glucosidase cleaves cellobiose into glucose molecules. Most cellulases and β -glucosidase have an optimum at 50 ± 5 °C and pH 4.0- 5.0[12]. The enzymes used in the this study is were from commercial enzymes mixture from Novozymes ,Cellulast 1.5 L and Novozyme188. Material from pretreatment was dissolved to a final concentration of 2% by weight with addition of acetate buffer solution.

2.9 Fermentation Process

The success of fermentation depends upon the existence of defined environmental conditions for biomass and product formation. To achieve this goal it is important to understand what is happening during the fermentation process and how to control it to obtain optimal operating conditions there are many parameters should be concern like temperature, agitation speed, pH value, dissolved O₂ and nutrient. In this research it concern about temperature and agitation speed. For the temperature this is a very important factor in maintaining a healthy growth for the microorganisms being cultivated. If the temperature of the fermenter goes outside a predefined band, the culture could be lost otherwise produce the side product. For the agitation control, the purpose of agitation is two-fold. First, it is to maintain a homogenous mixture within the vessel. By Using shake flask the working volume must be $\frac{1}{4}$ from flask volume. The fermentation process in produce ethanol must be done in anaerobic conditions (inhibited by the presence of oxygen).The presence of oxygen make that cell just produce CO₂ and water only not ethanol [3] In this fermentation process the *S.Cerevisae* will be used. This cell can produce the large amount of ethanol in temperature range around 30-40°C and the agitation speed around 150-200 rpm. [13]

CHAPTER 3

MATERIALS & METHOD

3.0 Introduction

In order to produce ethanol, the simplest way, like pretreatment, enzymatic hydrolysis must be done before fermentation process. In this experiment the batch operation using bioreactor will be used. Sugarcane baggase is used as raw material in this experiment. This experiment will use bioreactor in batch operation.

3.1 Grinding

The material was thoroughly washed, dried and grinding to 1 cm particle size using wet grinding. The bagasse was washed with tap water in the sieve tray and autoclaved at 121°C for 20 minute. After autoclaving, the treated bagasse was washed with tap water again and then dried at in the oven at 30°C between 10 to 15 hours before the sample sealed in the seal bag and stored at ambient condition. The grinding process purposed to dissolve 'lignin' to get the cellulose where in a plant; cellulose is encased in a substance called lignin. This is an alternative method to dissolve lignin besides to reduce the cellulose material to as fine as possible compared using strong acids where convert and destroy the glucose.

3.2 Pretreatment

There are many ways of pre-treating the material where with chemically, biologically, physically or their combination. This purposed is to make it easier for enzymatic hydrolysis process. These cellulose materials were treated with pre-treatment to decompose lignin. In this experiment, the pretreatment with chemically method has been choosing. The pretreatment involved is with using 4 % Natrium Hydroxide at ambient temperature for 10 days [14].

First step in this process is diluting the sodium hydroxide (NaOH) to known the concentration. A 1000 ml Beaker is filled with distilled water 750 ml. Then the beaker was added with 30-gram sodium hydroxide (NaOH) pellets in a weighing boat. The solution is stirred until the entire pallet completely dissolved. The sample (sugarcane baggase) from seal bag is taken out and weighed for 50-gram in a weighing boat. After that the sample is poured into the beaker. The solution is then stirred using the glass rod and covered with paraffin paper. The beaker containing the baggase and NaOH solution stored for 10 days at ambient condition

After that time, the remaining solution is filtered out by the sieve trays and the pretreated baggase is washed with tap water to neutralize the alkali. The baggase dried at 40°C for 8 hour then stored inside a seal bag and labeled.

3.3 Preparation of Standard Calibration Curve for Glucose

This preparation is important in order to find the concentration of tested. A 1000 μ g/ml standard glucose solution is prepared by adding 100mg of D (+)-glucose anhydrous into 100 ml of distilled water. The solution is stirred until all glucose dissolved in the water. Next, 5 ml glucose solution of 5 different concentrations is prepared from 1000 μ g/ml glucose solution. The first concentration is 50 μ g/ml is prepared by adding

0.25ml of glucose solution into 4.75 ml of distilled water. The procedure repeated for the next 9 concentrations.

After all of the glucose solution is ready, 3ml of each glucose solution from different concentration is spurge out using micro Spurger and put it in a lightly capped test tube. Next, all the solution is being test with **DNS method**.

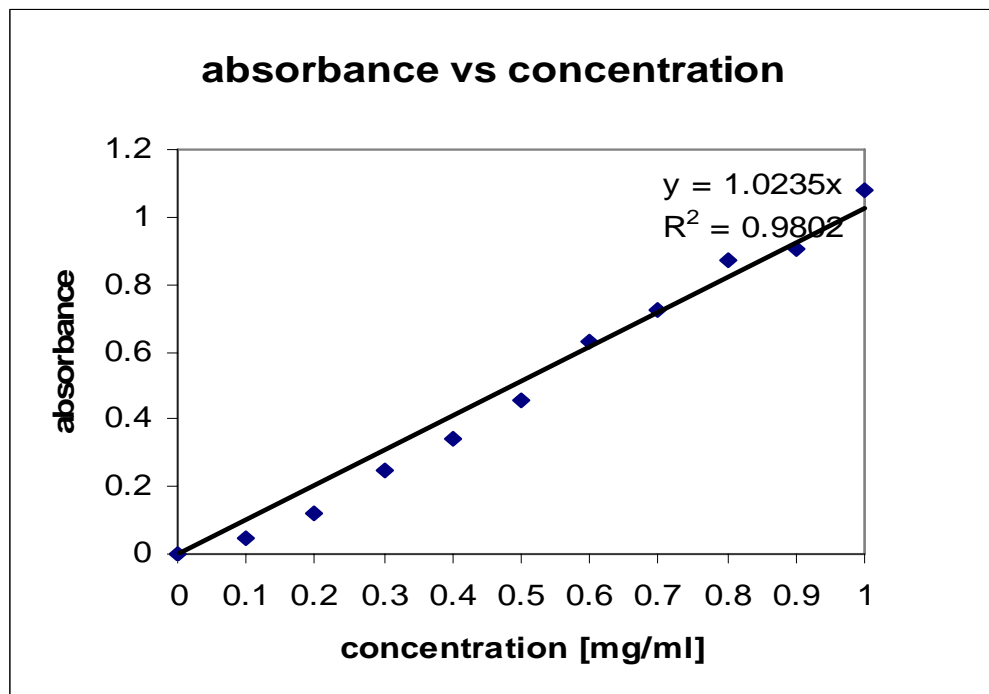


Figure 3.1: Standard Calibration Curve of Glucose

3.4 Preparing Acetate Buffer (pH=4.8)

A 1L Erlenmeyer flask is filled up with distilled water to 1 L. Sodium acetate anhydrous is weighed for 16.4-gram in a weighing boat and poured inside the Erlenmeyer flask. The solution is stirred until all the powder dissolved in water. Next, 11.5ml of glacial acetic acid poured in a clean measuring cylinder. That contains 988.5mL distilled water. The solution is stirred until it mixed well. Measure the pH using the pH meter until reaches 4.8 value. This buffer solution is prepared for another set with same procedure