

PRODUCTION OF BIO-ETHANOL FROM BIOMASS WASTE (SUGARCANE)

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**A thesis submitted in fulfillment for the award of the degree
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DECLARATION

I declare that this thesis entitled “*Production of Bio-Ethanol from Biomass Wastes (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 : 27 November 2006

DEDICATION

*Special dedication to my mum and family members that always love me,
my supervisor, my beloved friends, my fellow colleague,
and all faculty members*

For all your love, care, support, and believe in me.

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ABSTRACT

The purposes of this study are to produce ethanol from waste sugarcane using pre-treatment with alkali pre-treatment, enzymatic hydrolysis and anaerobic fermentation. The studies will focus on optimize various alkali pre-treatment condition with respect to final ethanol yield using sugarcane bagasse as feedstock. Different pre-treatment parameters (time and concentration) will be evaluated with intention of finding conditions which generate high sugar yields. In this experiment there are three stages to be completed in order to produce ethanol. In first stage, the sample will be through sodium hydroxide pre-treatment with certain temperature and time. At the second stage, the sample will be exposed to enzymatic hydrolyzes by a blend of Cellulast 1.5L and Novozym 188 cellulase mixture. Finally process fermentation with *Saccharomyces cerevisiae* will be done in anaerobic condition. At the end of the research, the sample pre-treated with sodium hydroxide at 2% concentration for 2 hour gives the higher concentration of glucose and ethanol which obtained 16.58 mg/ml and 14.59 by volume % .

ABSTRAK

Tujuan kajian ini adalah untuk menghasilkan ethanol daripada buangan tebu menggunakan kaedah pra-rawatan dengan pra-rawatan alkali, hidrolisis enzim dan penapaian aneorobik. Kajian ini akan menekankan kepada optimasi pelbagai keadaan pra-rawatan alkali terhadap hasil akhir ethanol dengan menggunakan hampas tebu sebagai bahan mentah. Parameter pra-rawatan yang berbeza (masa dan kepekatan) akan dinilai dengan tujuan mencari keadaan yang mana hasilkan gula tertinggi. Dalam eksperimen ini terdapat tiga peringkat yang perlu dilakukan untuk menghasilkan ethanol. Dalam peringkat pertama sampel akan melalui pra-rawatan natrium hidroksida pada kepekatan dan masa tertentu. Pada peringkat kedua sampel akan melalui proses hidrolisis enzim dengan campuran Cellulast 1.5L dan Novozym 188. Akhir sekali proses penapaian dengan yis *Saccaromyces cerevisiae* dilakukan dalam keadaan aneorobik. Pada akhir kajian sampel dengan 2% kepekatan natrium hidroksida pada 2 jam memberikan hasil kepekatan tertinggi glukosa dan ethanol sebanyak 16.58 mg/ml dan 14.59 % oleh isipadu.

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LIST OF SYMBOLS

g	-	gram
ml	-	milliliter
kg	-	kilogram
°C	-	degree Celsius
%	-	percent
μl	-	micro liter
mm	-	millimeter
min	-	minutes
g/ml	-	gram per milliliter
v/v	-	volume solute per volume solution
μmol	-	micromole
ABS	-	Absorbance

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CHAPTER 1

INTRODUCTION

1.0 Introduction

Since the 20th century, our major energy demand has been supplied by fossil fuels such as oil, coal and natural gas. Fossil fuels originate from deceased organisms that lived several million years ago and by time have been embedded in the earth's crust. Incineration of this fossil remains results in a net increase of today's carbon dioxide levels. Environmental issues such as the threatening increase in temperature caused by the greenhouse effect and the fact that fossil fuels are nonrenewable resources, has increased the interest in producing fuels from renewable resources such as biomass.

Ethanol as well as other bio-fuels produced from plant biomass is an alternative to fossil fuels. Ethanol does not add to a net carbon dioxide atmospheric increase thus there is in theory no contribution to global warming. Combustion of ethanol results in relatively low emissions of volatile organic compounds, carbon monoxide and nitrogen oxides (Bailey, 1990).

Production of ethanol is historically a well known process and in principal it is carried out by fermentation of plant sugars into ethanol using strains of yeast. However, plant biomass is made of sugar polymers, ordered in a matrix called lignocelluloses, which is not as easily fermented. In order to produce ethanol, this material must undergo degradation for the yeast more accessible components. For example mono and dimers of

sugars. This degradation can be made by hydrolysis of biomass using enzymes called enzymatic hydrolysis (EH).

The aim of this study is to optimize the alkali pre-treatment in order to release cellulose for production of ethanol using sugarcane bagasse as feedstock. Enzymatic hydrolyses were performed in lab scale to evaluate pre-treatments that were made. To include the level of fermentability, material from pre-treatments was fermented in batch process. Total ethanol and sugar yields for the tested conditions were evaluated to eventually find the most satisfying pre-treatment condition.

1.1 Problem statement

Malaysia is looking forward in industrial and development sector that need sustainable energy resources. The available fossil fuels sources now only can survive for another 20 to 30 years. Due to this scarcity and cost of it are likely to increase in the future. A substitute fuel must be developed, so both of the sectors above can be implementing effectively and these studies proposed bio-ethanol as substitutes to fossil fuel in the future. Producing bio-ethanol from biomass wastes will reduce its cost because the raw material is renewable and also cheaper than crude oil. Although the cost of process is high in small scale but it is worth if it's done in large scale. Furthermore, compare to the cost of fossil fuels that increasing by year in Malaysia, there is a strong reason to produce bio-ethanol using cheaper raw material.

For the past decade Malaysia is dealing with serious environmental problem. Among of the pollutions that occur is air pollution that mainly causes by transportation and factories. The air quality is poor particularly in urban areas such as Kuala Lumpur and Johor Bahru where traffic was most congested. This problem not only brings negative impact to the people but also affecting the health whole population. Using bio-ethanol as a fuel can improve air quality by reducing carbon monoxide emission levels from engines. This will make safer environmental to live in.

Along with the "green" campaign, this study contributes zero effect to the greenhouse. In order to reduce the carbon dioxide (CO₂) emission, it is safer to use bio-ethanol rather than fossil fuels. Biomass that is used as raw material consumes as much CO₂ as they contributes during the combustion of the bio-ethanol. This made a healthy recycle, where CO₂ from transportation that is released to the environment is actually consumed by the biomass that is used. Using biomass to produce bio-ethanol will help to reduce Malaysia CO₂ emissions in the future

1.2 Objective

The aim of this study is to:

- i. Optimize the pre-treatment to produce high yield of ethanol from biomass waste (sugarcane)

1.3 Scope of study

To achieve the objective, there are three scopes that have been identified:

- i. To produce bio-ethanol from bagasse using pre-treatment with alkali pretreatment, enzymatic hydrolysis and anaerobic fermentation
- ii. To study the effect of concentration agent to the alkali pretreatment
- iii. To study the effect of resident time to the alkali pretreatment

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Biomass energy currently contributes 9–13% of the global energy supply accounting for 45 ± 10 EJ per year (Thomas, 2000). Biomass energy includes both traditional uses such as a ring for cooking and heating and modern uses such as producing electricity and steam, and liquid bio-fuels. Use of biomass energy in modern ways is estimated at 7 EJ a year, while the remainder is in traditional uses. Biomass energy is derived from renewable resources. With proper management and technologies, biomass feedstock's can be produced sustainable. Ethanol derived from biomass, one of the modern forms of biomass energy, has the potential to be a sustainable transportation fuel, as well as a fuel oxygenate that can replace gasoline (Wang, 2000). Shapouri et al. (1995,2002) concluded that the energy content of ethanol was higher than the energy required producing ethanol. Kim and Dale (2002) also estimated the total energy requirement for producing ethanol from corn grain at 560 kJ MJ^{-1} of ethanol, indicating that ethanol used as a liquid transportation fuel could reduce domestic consumption of fossil fuels, particularly petroleum. The world ethanol production in 2001 was 31 GL (Berg, 2001). The major producers of ethanol are Brazil and the United States, which account for about 62% of world production.

2.2 Background of Ethanol

The principle fuel used as a petrol substitute for road transport vehicles is bio-ethanol. Bio-ethanol fuel is mainly produced by the sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. The main sources of sugar required to produce ethanol come from fuel or energy crops. These crops are grown specifically for energy use and include corn, maize and wheat crops, waste straw, willow and poplar trees, sawdust, reed canary grass, cord grasses, Jerusalem artichoke, and sorghum plants. There is also ongoing research and development into the use of municipal solid wastes to produce ethanol fuel. Ethanol or ethyl alcohol (C_2H_5OH) is a clear colorless liquid; it is biodegradable, low in toxicity and causes little environmental pollution if spilt. Ethanol burns to produce carbon dioxide and water. Ethanol is a high octane fuel and has replaced lead as an octane enhancer in petrol. By blending ethanol with gasoline we can also oxygenate the fuel mixture so it burns more completely and reduces polluting emissions. Ethanol fuel blends are widely sold in the United States. The most common blend is 10% ethanol and 90% petrol (E10). Vehicle engines require no modifications to run on E10 and vehicle warranties are unaffected also. Only flexible fuel vehicles can run on up to 85% ethanol and 15% petrol blends (E85).

Bio-ethanol has a number of advantages over conventional fuels. It comes from a renewable resource i.e. crops and not from a finite resource and the crops it derives from can grow well in the United Kingdom (UK) such as cereals, sugar beet and maize. Another benefit over fossil fuels is the greenhouse gas emissions. The road transport network accounts for 22% (www.foodfen.org.uk) of all greenhouse gas emissions and through the use of bio-ethanol, some of these emissions will be reduced as the fuel crops absorb the carbon dioxide they emit through growing. Also, blending bio-ethanol with petrol will help extend the life of the UK's diminishing oil supplies and ensure greater fuel security, avoiding heavy reliance on oil producing nations. By encouraging bio-ethanol's use, the rural economy would also receive a boost from growing the necessary crops. Bio-ethanol is also biodegradable and far less toxic than fossil fuels. In addition,

by using bio-ethanol in older engines can help reduce the amount of carbon monoxide produced by the vehicle thus improving air quality. Another advantage of bio-ethanol is the ease with which it can be easily integrated into the existing road transport fuel system. In quantities up to 5%, bio-ethanol can be blended with conventional fuel without the need of engine modifications. Bio-ethanol is produced using familiar methods, such as fermentation, and it can be distributed using the same petrol forecourts and transportation systems as before.

2.2.1 Bio-ethanol Production from Waste Sugarcane

Wasted sugar cane could produce 1:6 GL of bio-ethanol, replacing 1:1 GL of gasoline when ethanol is used in E85 fuel. Sugar cane bagasse is a co-product in sugar cane food manufacture, and the yield of bagasse is about 0.6 dry kg per 1 dry kg of sugar cane used in food manufacture (producing about 120 Tgrams of sugar). Globally about 180 Tgrams of dry sugar cane bagasse is produced and can be utilized and could produce about 51 GL of bio-ethanol. Furthermore, lignin-rich fermentation residues from bagasse could generate 103 TWh of electricity and 593 PJ of steam. Wasted sugar cane and sugar cane bagasse could produce globally about 53 GL of bio-ethanol, replacing 38 GL of gasoline in an E85 midsize passenger vehicle, or about 3.4% of the global gasoline consumption. Asia can produce about 22 GL of bio-ethanol. The regional potential bio-ethanol production is shown in Table 2.1.

Table 2.1: Regional Production from Wasted Sugarcane Bagasse

	Potential bio-ethanol production (GL)		Total bio-ethanol	Gasoline equivalent
	From wasted sugar cane	From bagasse		
Africa	0.23	3.33	3.56	2.56
Asia	0.82	21.3	22.1	15.9
Europe	-	0.004	0.004	0.003
North America	-	1.31	1.31	0.94
Central America	0.18	5.46	5.64	4.05
Oceania	0.0001	1.84	1.84	1.32
South America	0.37	18.1	18.5	13.3
World	1.59	51.3	52.9	38

2.2.2 The Marketplace

Ethanol from biomass was projected to cost about \$1.58 per gallon for the enzyme process. A 1999 study projected that cost at \$1.16 per gallon. This cost assumes access to moderately priced feedstock's (at around \$25 per dry ton), and reflects a combination of the best results reported by various research groups in industry and in the private sector. The fuel ethanol market currently supports a price of anywhere from \$1.00 to \$1.40 per gallon. Though the high capital investment and higher risk of deploying new technology are still hurdles to be overcome, it is clear that the new bio-ethanol technology is poised for commercial introduction. In 2000 the fuel ethanol market accounted for 1.6 billion gallons per year in sales (2.0 billion capacities). This market is now constrained because of cost to the use of ethanol as blending agent in gasoline, with only limited sales of "E85" 85% ethanol fuel. As technology costs drop, bio-ethanol will add sales of anywhere from 6 to 9 billion gallons year. With the blend market saturated at this level of ethanol sales, we will then set our sites on the bulk fuel market competing head-to-head with gasoline at ethanol costs of around \$0.60 per gallon.

2.3 Overview Biomass in Malaysia

Malaysia has abundant biomass waste resource coming mainly from its palm oil, wood and agro-industries. A total of about 665 MW capacities can be expected if the estimated overall potential of about 20.8 millions tone of biomass residues from this main source in addition to 31.5 million m³ of palm oil mill effluent (POME) is used for power generation and cogeneration. In addition, there is a substantial amount of unexploited biomass waste resources in the form of logging wood residues, rice straw, palm kernel trunks and other residues. This biomass residue could further supplement future biomass-based power generation in the country if necessary.

Biomass fuels currently account for about 16% of the energy consumption in the country, of which about 51% is palm oil biomass waste and about 22% is wood waste. The present installed biomass-based power generation capacity in the country is 138 MW, about 100 MW of which are in the palm oil industry.

In Malaysia, biomass resources are mainly from palm oil mill residues, bagasse, rice husks and wood/forest residues. As shown in table 2.2 below, the bagasse residues accounts for the less biomass waste production in the country. This is because the bagasse residues are hardly available and are presently requiring cost effective means of disposal. Currently, most of these residues are disposed of through incineration and dumping. A small portion is used as fuel for the Millis's heat and power requirement in a very inefficient manner.

Table 2.2: Biomass Resource Potential in Year 1999.

Sector	Quantity kton / yr	Potential Annual Generation (GWh)	Potential Capacity (MW)
Rice Mills	424	263	30
Wood Industries	2177	598	68
Palm Oil Mills	17980	3197	365
Bagasse	300	218	25
POME	31500	1587	177
Total	72962	5863	665

(Source: MPOB, SIRIM, FRIM, Forestry Dept. and Ministry of Agricultural, 1999)

2.4 Ethanol Production

The central element in ethanol production biotechnology is the fermentation of sugars by microorganisms. In conventional ethanol production the sugars are directly derived from molasses or sugar cane or through hydrolysis (the conversion of starch to sugar) of starch containing crops such as corn. The production of sugars from this feedstock is a simple and effective process. The resulting sugars can be readily fermented by yeasts. Deriving fermentable sugars from lignocelluloses biomass is more difficult. Biomass is predominantly composed of cellulose, hemi-cellulose and lignin. Both the cellulose and hemi-cellulose fractions are a potential source of fermentable sugars which are less easily accessible and therefore require suitable pretreatment and hydrolysis steps. The hydrolysis produces a variety of sugars. The C5 sugars (mainly xylose) cannot be fermented with the standard yeast used in the ethanol industry. Ethanol production from these sugars requires the use of specially selected or genetically modified micro-organisms. Both in the conventional and the new process the ethanol product is recovered from the fermentation broth, and subsequently dehydrated by distillation and final deep dewatering by molecular sieves, to the required fuel specifications (< 0.1 wt% water). The non-fermentable, lignin-rich fraction and other organic wastes from the process (e.g. water treatment sludge) are used for Combined Heat & Power (CHP) production. The produced electricity and heat (steam) are used to a

large extent within the ethanol production process, whereas the surplus of electricity is fed to the public grid. See Figure 2.1 below.

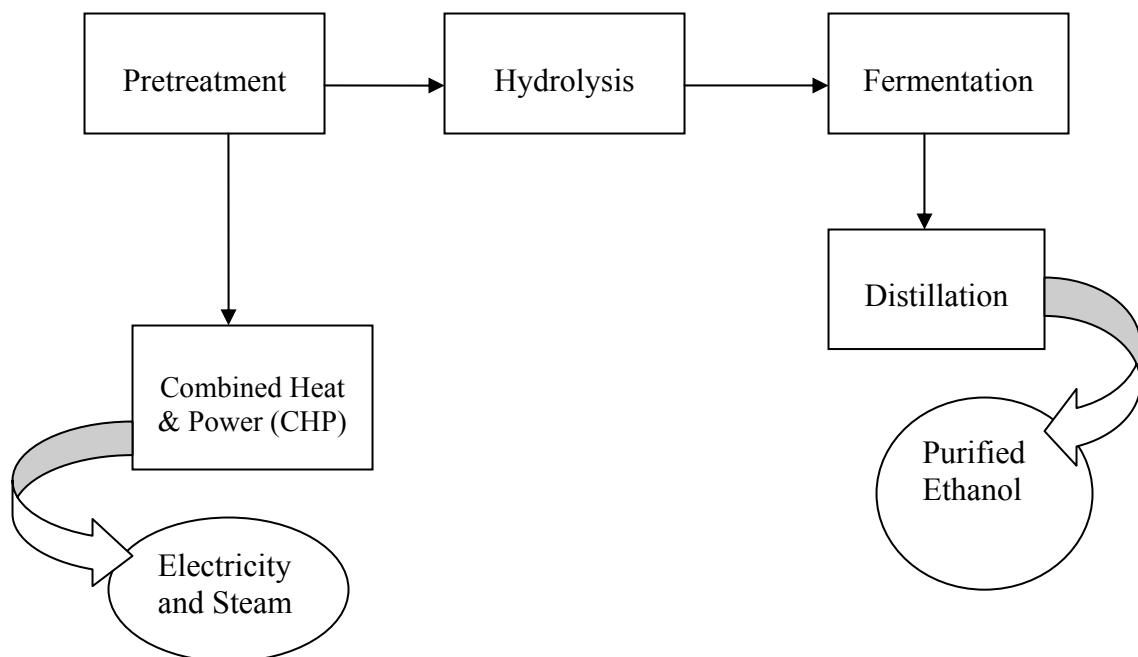


Figure 2.1: Overall Process to Produce Bio-ethanol

2.4.1 Sugarcane Bagasse

Sugarcane (*Saccharum officinarum*) is a grass that is harvested for its sucrose content. After extraction of sugar from the sugarcane, the plant material that remains is termed bagasse. Sugarcane bagasse found at sugar mills contain both relatively easy and hard to degrade materials. The easily degraded materials appear to be from the leaf matter and the hard to degrade from the rind (Fox et al, 1987). Bagasse has several advantages for use in ethanol production. Most importantly, unlike corn stover, bagasse is collected as part of the sugar production process, so it does not require a separate harvest. It is also physically ground as part of the extraction process (Fox et al, 1987). Furthermore, bagasse is cheap, readily available and has high carbon content (Martin et