



UNIVERSITI PUTRA MALAYSIA

**CHEMICAL PRETREATMENT OF RUBBER WOOD FOR ENZYMATIC
SACCHARIFICATION IN BIOETHANOL PRODUCTION**

**ALHASSAN MUHAMMAD ALHASSAN
FS 2010 1**



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ALHASSAN MUHAMMAD ALHASSAN

**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

2010



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SACCHARIFICATION IN BIOETHANOL PRODUCTION**

By

ALHASSAN MUHAMMAD ALHASSAN

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Master of Science.**

January 2010



DEDICATION

This dissertation is dedicated to my parents;

Hon Justice Muhammad Alhassan and Hajiya Zainab Alhassan



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

**CHEMICAL PRETREATMENT OF RUBBER WOOD FOR ENZYMATIC
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January 2010

Chairman: Professor Dzulkefly Kuang Abdullah, PhD

Faculty: Science

The potentials of rubber wood to serve as raw material for bioethanol production was studied. The objective of this study was to investigate the effectiveness of sulphuric acid, nitric acid, sodium hydroxide and combinatorial nitric acid and sodium hydroxide pretreatments for conversion of rubber wood to ethanol. Sulfuric acid, nitric acid and sodium hydroxide, at concentrations of 1, 2 and 3% (w/v) were used to treat rubber wood sawdust samples at liquor to wood ratio of 1:6. Treatment temperatures of 80°C and 100°C were investigated for residence times of 30, 60, and 90 minutes. The combinatorial nitric acid and sodium hydroxide pretreatment was performed using nitric acid first followed by sodium hydroxide. Rubber wood sawdust was treated with dilute nitric acid (4.5 and 6% w/v) at temperatures of 90 or 100°C for 30, 45 and 60 minutes followed by dilute sodium hydroxide (0.5, 0.75 and 1%), at 90°C for 30 min. Lignin and carbohydrate, analyses were performed on the pretreated woods. The cellulose in the pretreated woods were then hydrolyzed using cellulases from *Trichoderma reesei* and β -glucosidase at



50°C for 72 hrs and the released sugars were converted to ethanol by fermentation using yeast (*Saccharomyces cerevisiae* D5A strain). The results showed that time, temperature and concentration were all significant ($p \leq 0.05$) factors in lignin and hemicellulose reduction. Sulfuric acid pretreatment resulted in small lignin and hemicellulose reduction of 10.9% and 29.4% respectively, and glucose yield of 22.5% during enzymatic hydrolysis. Sodium hydroxide pretreatment resulted in higher, lignin and hemicellulose reduction of 51.0% and 30.1% respectively, and a glucose yield of 38.0%. Nitric acid pretreatment resulted in lignin and hemicellulose reduction of 30.6% and 64.9% respectively, and a glucose yield of 46.1%. Combinatorial nitric acid and sodium hydroxide pretreatment removes almost all the lignin and hemicellulose which resulted in isolated cellulose (95.8%) and a maximum glucose yield of 99.5%. The ethanol yield obtained after fermentation was 89.5% which is equivalent to 182.16g of ethanol per kg of rubber wood. The results obtained demonstrate that combinatorial nitric acid and sodium hydroxide pretreatment is an effective method for improving the enzymatic hydrolysis of rubber wood at moderate reaction condition and it is better than, sulphuric acid, sodium hydroxide or nitric acid pretreatment. The results also demonstrate that rubber wood is a potential raw material for bioethanol production.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

RAWATAN KIMIA TERHADAP KAYU GETAH UNTUK SAKARIFIKASI ENZIM BAGI MENGHASILKAN BIOETANOL

Oleh

ALHASSAN MUHAMMAD ALHASSAN

Januari 2010

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Potensi kayu getah sebagai bahan mentah untuk penghasilan bioetanol telah dikaji. Objektif kajian ini adalah untuk mengkaji keberkesanan rawatan asid sulfurik, asid nitrik, natrium hidroksida dan kombinasi asid nitrik dan natrium hidrosiksida dalam prarawatan untuk menukarkan kayu getah kepada etanol. Kepekatan asid sulfurik, asid nitrik dan natrium hidrosiksida pada kepekatan 1, 2 dan 3% (berat/isipadu) telah digunakan untuk merawat serbuk kayu getah pada nisbah cecair kepada kayu 1:6. Suhu rawatan pada 80°C dan 100°C telah dikaji untuk rawatan selama 30, 60, dan 90 minit. Rawatan gabungan asid nitrik dan natrium hidroksida telah dilakukan dengan menggunakan asid nitrik cair dan diikuti pula dengan natrium hidroksida. Serbuk kayu getah dirawat dengan asid nitrik cair (4, 5, dan 6% berat/isipadu) pada suhu 90°C atau 100°C selama 30, 45, dan 60 minit diikuti dengan natrium hidroksida cair (0.5, 0.75 atau 1%) pada suhu 90°C untuk 30 minit. Analisis lignin dan kabohidrat, telah dilakukan



terhadap kayu getah yang telah dirawat. Selulosa dalam kayu terawat kemudian dihidrolisis menggunakan enzim selules daripada *Trichoderma reesei* dan β -glukusides pada suhu 50°C selama 72 jam penukaran glukosa yang dibebaskan kepada etanol menggunakan ragi (*Saccharomyces cerevisiae* D5A strain) dalam proses famentasi.. Keputusan menunjukkan masa, suhu, dan kepekatan adalah factor pentig ($p \leq 0.05$) dalam pengurangan lignin dan hemiselulosa. Rawatan dengan asid sulfuric dapat mengurangkan lignin dan hemiselulosa masing-masing sebanyak 10.9% dan 29.4%, dan menghasilkan glukosa sebanyak 22.5% semasa hidrolisis enzim. Sebaliknya rawatan dengan natrium hidroksida paling banyah mengurangkan lignin dan hemiselulosa masing-masing 51.0% dan 30.1% dan menghasilkan glukosa sebanyak 30.8% semasa hidrolisis enzim. Rawatan asid nitrik dapat mengurangkan lignin dan hemiselulosa lebih banya lagi iaitu 30.6% dan 64.9%. Serta menghasilkan glukosa sebanyak 46.1% semasa hidrolisis enzim. Rawatan gabungan asid nitrik dan natrium hidroksida dapat mengeluarkan semua lignin dan hemiselulosa dan menghasilkan selulosa talen sebanyak (95.8%) dan glukosa sebanyak 99.5%. Etanol diperolehi selepas fermentasi adalah 89.55% iaitu bersamaan degan 182.16g ethanol per kg kayu getah. Keputusan kajian menunjukkan bahawa kombinasi rawatan asid nitric dan natrium hidroksida adalah kaedah yang paling berkesan untuk memperbaiki hidrolisis enzim kayu getah pada keadaan tindakbalas serdahana dan ia adalah lebih baik jika dibandingkan dengan rawatan asid sulfuric, natrium hydroksida atau asid nitric secora individu. Keputusan kajian ini mennunjukkan bahawa kayu getah adalah satu bahan mentah yang potensi bagi penghasilan bioetanol.

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CERTIFICATION

I certify that an Examination Committee has met on 4th January, 2008 to conduct the final examination of Alhassan Muhammad Alhassan on his Master of Science thesis entitled “Chemical Pretreatment of Rubber wood for Enzymatic Saccharification in Bioethanol Production” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that, it has not been previously or concurrently submitted for any other degree in UPM or other institutions.

ALHASSAN MUHAMMAD ALHASSAN

Date: 14 January 2010



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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
AFEX	Ammonia fiber explosion
ARP	Ammonia recycled percolation
ATP	Adenosine triphosphate
CBU	Cellobiase Units
DI	Deionised
FID	Flame ionizing detector
FPU	Filter paper unit
FTIR	Fourier transform infrared
G-6-P	Glucose-6-phosphate
G-6-PDH	Glucose-6-phosphate dehydrogenase
HK	Hexose kinase
HPLC	High performance liquid chromatography
LAP	Laboratory analytical procedure
NADP	Nicotinamide adenine dinucleotide phosphate
NREL	National Renewable Energy Laboratory
SEM	Scanning electron microscope
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation
XRD	X-ray diffraction



CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Global energy consumption has increased steadily over the last century, as human population has grown and more cities have become industrialized (Sun and Cheng, 2002). Currently, fossil fuel is the major source of energy used worldwide, especially for the transportation sector (Wyman et al., 2005). The fossil fuel's reserve is limited in supply and could get depleted as the global fuel consumption keeps increasing. In addition, the use of fossil fuel in vehicles releases large amount of carbon dioxide to the atmosphere (green house gas emission) which is a major contributor to the potential global climate change. These problems lead to a worldwide interest in exploring clean and a renewable fuels of which bioethanol is viewed as one of the most promising options. (Rosillo-Calle and Walter, 2006).

Bioethanol is the most widely used renewable alternative transport fuel in the world and its demand has in recent years increased tremendously (Balat et al., 2008). Currently, bioethanol is produced mainly from sugar and starch containing feedstocks such as, sugar cane and corn, respectively. However, this method is faced with the problem of high cost of raw materials, which accounts for 40-70% of total ethanol production cost (Galbe and Zacchi, 2002). Furthermore, increased cultivation of sugar and starch containing crops for ethanol will likely compete for limited agricultural land for food and feed production.



Therefore, to achieve a lower cost of production and at the same time meet the increased bioethanol demand, alternative raw materials have to be considered (Galbe and Zacchi, 2002; Silverstein et al., 2007). Attention has now turned towards lignocellulosic biomass, especially residues from agricultural and forestry operations, to provide a cheaper source of carbohydrate for fermentation (Pan et al., 2005). Lignocellulosic biomass is the most abundant renewable organic material on earth with an annual supply of approximately 200 billion metric tons (Zhang et al., 2006; Ragauskas et al., 2006). Lignocellulosic waste generated from forestry and agricultural practices has been considered as promising raw material for bioethanol production, because it is renewable, cheap and abundant in large quantity. One of such lignocellulosic waste is rubber wood sawdust.

Rubber tree (*Heavea brasiliensis*) is a major industrial crop grown in South East Asia with an estimated plantation area of 1.8 million hectares (20% of global plantation) in Malaysia alone (Srinivasakannan and Abu Bakar, 2004). The trees are logged off after 25 to 30 years and utilized for down stream processing such as, making of furniture. The logging operations and lumber processing generate large amount of residual biomass as waste. The biomass generated from these processes does not have significant value addition but for its usage in the making of products like briquetted fuel and compressed powder boards (Srinivasakannan and Abu Bakar, 2004). However, due to its high cellulose content, rubber wood waste represents a potential raw material for bioethanol production.

1.2 Definitions

Bioenergy refers to energy derived from biological sources. In general bioenergy sources are categorized into three. Solid biomass (e.g. wood pellets), liquid biofuel (e.g. bioethanol and biodiesel) and biogas (e.g. methane) fuels. Among the three energy sources, liquid biofuel is the most important and the most widely studied because of its potential to be used as alternative to petroleum products (gasoline and diesel) in automobiles.

Bioethanol refers to ethanol (C_2H_5OH) that is produced from biomass (mainly plant materials). Chemically, bioethanol is identical to synthetic ethanol. Bioethanol is the most widely used biofuel in the world. Bioethanol can be produced from any organic material containing glucose.

Lignocellulosic biomass refers to plant materials which are made up of three major components; cellulose, hemicellulose and lignin. Examples lignocellulosic biomass includes waste generated from forestry and agricultural practices (e.g. corn stover, rice hulls, and sawdust), municipal solid waste (e.g. yard waste and papers) and dedicated energy crops (e.g. switch grass). Lignocellulosic biomass is generally considered as renewable bioresource because it is available in recurring bases and it can continually be grown or regenerated.

1.2 Fuel Ethanol



The use of ethanol as transport fuel goes right back to the origin of automobile industry. For example, Henry Ford's Model T also called Quadricycle developed in 1908 was aimed at ethanol. Ford's vision was to develop a vehicle affordable to working family and powered by a fuel that would boost the rural farm economy (Berg, 2004). Ethanol became established as fuel in the early 1970s when the global oil crisis generated a worldwide interest in alternative transport fuels, of which ethanol was viewed as the most promising option (Ragauskas et al., 2006; Rosillo-Calle and Walter, 2006). The interest in fuel ethanol decreased in the decade that followed as the fuel shortage abated. However, issues regarding environmental pollution (emission of green house gasses), increased fuel prices and fear associated with inevitable depletion of fossil fuel's reserve, stimulated a renewed interest in fuel ethanol, in the mid 1990s (Ragauskas et al., 2006). This interest has grown tremendously in recent years.

Today, bioethanol is being used as fuel in many countries. Ethanol has a number of favorable properties that are desirable for use as fuel. Firstly, the use of bioethanol as fuel provides a reduction in carbon dioxide emission. In addition, bio-ethanol has a higher octane number, broader flammability limits, higher flame speeds and higher heats of vaporization. These properties allow for a higher compression ratio and shorter burn time, which lead to theoretical efficiency advantages over gasoline in an automobile engine (Wyman, 1996). Octane number is a measure of the gasoline quality for prevention of early ignition, which leads to cylinder knocking. The fuels with higher octane numbers are preferred in spark-ignition internal combustion engines (Balat et al.,



2008). An oxygenate fuel such as bio-ethanol provides a reasonable antiknock value. Bio-ethanol is an oxygenated fuel that contains 35% oxygen, which reduces particulate and nitrogen oxides (NO_x) emissions from combustion (Wang et al., 1999). Using bio-ethanol blended fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission. Bioethanol has lower energy density than gasoline (about 66% of energy that gasoline has). However, this disadvantage is partly compensated for by higher engine efficiency. Even though ethanol has only two third of the volumetric energy content of gasoline, it will still be possible to drive 75-80% distance on a given volume of ethanol (Wyman, 1996).

The global bioethanol production has increased from 17.25 billion liters in 2000 to 46 billion (4% of world gasoline output) liters in 2006 and its demand is expected to grow beyond 125 billion liters by 2020 (Balat and Balat, 2009). Figure 1.1 shows the trend in global bioethanol production from 2000-2007. United States and Brazil are the two largest bioethanol producers in the world with an annual bioethanol production of 18.3 billion (approx. 47% of global production) and 17.5 billion (approx. 45% of global production) respectively in 2006. While other countries contribute less than 10% (Balat and Balat, 2009).

1.3 Classification of Bioethanol Production

Based on the feedstock that is used in production, bioethanol can be classified into first generation bioethanol and second generation bioethanol.



1.4.1 First Generation Bioethanol

Bioethanol produced from food crops is referred to as first generation bioethanol. This can be broadly classified into two; (1) Ethanol produced from Sucrose containing feedstocks (such as sugar cane and sugar beet) and (2) ethanol produced from starchy materials (such as corn, wheat, sweet potatoes and cassava).

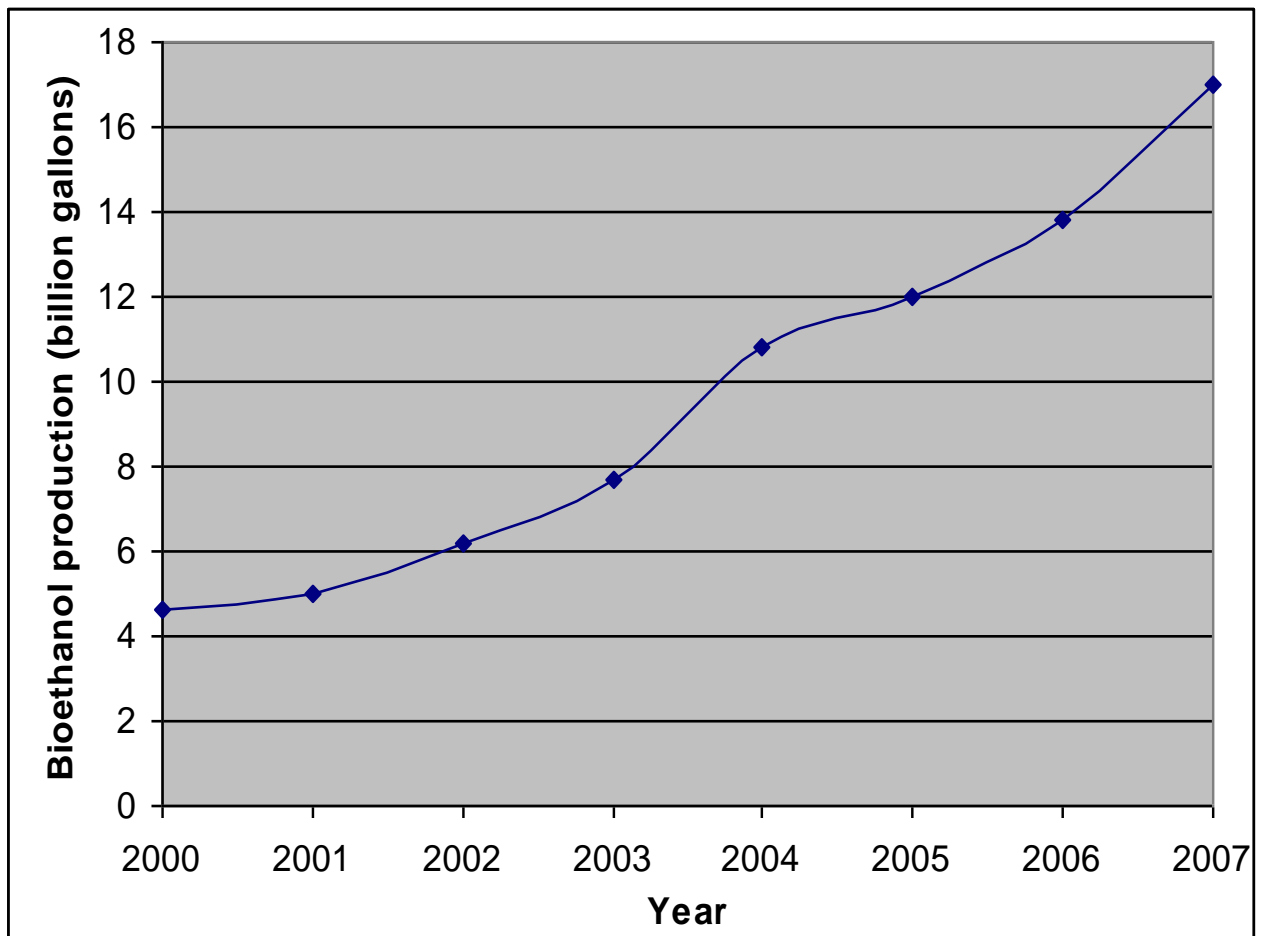


Figure 1.1: Global ethanol production from 2000 to 2007 (Balat & Balat, 2009).