A STUDY OF THE PRE-TREATMENT TEMPERATURE OF LIGNOCELLULOSIC BUTANOL AS AN ALTERNATIVE FUEL (BIOFUEL) PRODUCED FROM BAMBOO USING *Clostridium acetobutylicum*

BY

KOLAWOLE FUNSHO OLAITAN

A THESIS

SUBMITTED TO THE AFRICAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

ABUJA – NIGERIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

AWARD OF MASTER OF SCIENCE IN MATERIALS SCIENCE AND ENGINEERING

Supervisor: Prof. Wole Soboyejo

MAY, 2013

A STUDY OF THE PRE-TREATMENT TEMPERATURE OF LIGNOCELLULOSIC BUTANOL AS AN ALTERNATIVE FUEL (BIOFUEL) PRODUCED FROM BAMBOO USING *Clostridium acetobutylicum*

A THESIS APPROVED

BY

SUPERVISOR: **PROF. W. O. SOBOYEJO**

MEMBER

PROF. W. O. SOBOYEJO

MEMBER

Dr. Shola Odusanya

DECLARATION

I hereby declare that this thesis entitled "A Study of Pre-Treatment Temperature on Lignocellulosic Butanol as an Alternative Fuel (Bio-Fuel) Produced from Bamboo using *Clostridium Acetobutylicum*" was written by me and is the result of the investigation carried out by me under the supervision of Professor Wole Soboyejo at African University of Science and Technology, Abuja. It has not been presented in any previous application for the award of any degree or diploma.

In keeping with the general practice in reporting scientific observation, all sources have been duly acknowledged.

_____ _____

KOLAWOLE, Funsho Olaitan ***Date***

DEDICATION

This research thesis is dedicated to the Almighty God, the giver of all things, without whom, this thesis and my completion of a successful stay in this university would not have been possible.

ACKNOWLEDGEMENT

I would like to express my sincere appreciation to those who provided me help and inspiration in the conduct of this study.

First and foremost, I would like to acknowledge the contributions and support of my supervisor, Prof. Wole Soboyejo, whose kind and warm suggestions helped in bring out this piece of work to light. I also acknowledge Dr. Shola Odusanya my co-supervisor, Mr. Godwin A. Etuk-Udo and Mrs Stella Dozie for assisting me in the laboratory.

I am indebted to my parent Prof. & Mrs. Kolawole for their moral and financial support through the course of my study in this great university. I also wish to acknowledge other members of my family, Shola, Dipo and Bukola. Not forgetting my classmates especially Jacob Fortunatus, they were like my brothers and sisters. I also acknowledge Pastor Segun and his wife and sister Gloria, they supported me spiritually and they did not cease to pray for my success during my thesis work.

I will also like to appreciate Toyin Aladeusi, my fiancée, who stood by me and encouraged me in difficult times.

Lastly, nothing would have been possible without the help of our Lord and Saviour, Jesus Christ, He provided strength and emotional support when there was none in sight, when forging ahead proved difficult. My faith has increased a hundred fold as I have been a witness to His divine intervention during my course and thesis work.

ABSTRACT

Conversion of lignocellulosic biomass from bamboo (*Bambusa vulgaris*) to butanol is an important alternative energy source. In this work, bamboo was used as biomass feedstock for the production of butanol by the fermentation of sugars. Mechanical grinding was carried out, followed by pre-treatment with dilute sulfuric acid concentration of 0.5 and 1.0 (%v/v). This was done at temperatures of 25, 110, 120, 150 and 200^0C at time intervals of 2 and 4 hours. Prehydrolysate was later analyzed for total sugars by the use of UV-Visible Spectro Photometer. For the conditions considered, the maximum glucose yields were obtained at 200^oC. The yields after pre-treatment were 244.80 mg/g, at pre-treatment conditions of 200^0C and acid concentrations of 1%. Water insoluble solids obtained were subsequently hydrolysed with Celluclast (*Trichoderma reesi*) and β-glucosidase (Novozyme 188) for 72 hrs. Bacteria (*Clostridium acetobutylicum*) were then used to ferment the solubilized sugar into butanol. Raman spectroscopy was used to determine the butanol yield. Optical Microscope images of bamboo samples were obtained at various stages of pre-treatment and hydrolysis. These revealed the morphological changes that occur in the cellular structure of the bamboo during exposure to acid and enzymatic hydrolysis. The results show that, increasing temperature, time and acid concentration are associated with higher total sugar yields and cellulose conversion rates.

TABLE OF CONTENTS

Pages

CHAPTER THREE: MATERIALS AND METHODS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX

CHAPTER ONE

INTRODUCTION

1.1. BACKGROUND

The main purpose of fuel is to store energy, which should be in a stable form and can be easily transported to the place of production. Almost all fuels are chemical fuels. The user employs this fuel to generate heat or perform mechanical work, such as powering an engine. It may also be used to generate electricity, which is then used for heating, lighting or electronics purposes. As the population of the world continues to grow past the 7 billion mark, the demand for energy is becoming an ever more critical challenge for the world's energy leaders. Governments are looking for sustainable solutions that provide the most competitive energy supplies from secure sources, whilst at the same time trying to balance the long-term, and in some cases, short-term needs of the environment. Companies and state enterprises are seeking the most efficient solutions to meet the needs of shareholders and the national treasury to support growth. Innovators are looking at the latest trends that will enable them to capitalize on developing markets and the public simply want access to energy so that they can go about their business and prosper. These are the challenges that the World Energy Council addresses every day.

Nigeria produces an average of about 2.18 million barrels per day of crude oil, and exports 1.73 million barrels per day [1]. Crude oil has severed as Nigeria's major source of revenue, which is not enough to meet the needs of Nigerians. In view of this it became necessary for Nigerian to begin to seek alternative source of fuel, which could be cheaper and with less manufacturing processes which will reduce the dependence rate of the country on crude oil [2].

According to International Energy Agency (IEA) data from 1990 to 2008, the average energy use per person increased 10% while world population increased 27%. Regional energy use also grew from 1990 to 2008: the Middle East increased by 170%, China by 146%, India by 91%, Africa by 70%, Latin America by 66%, the USA by 20%, the EU-27 block by 7%, and world overall grew by 39%.

In 2008, total worldwide energy consumption was 474 exajoules (474×10^{18} J=132,000 TWh). This is equivalent to an average power use of 15 terawatts (1.504×10^{13} W) [3]. The potential for renewable energy is: solar energy 1600 EJ (444,000 TWh), wind power 600 EJ (167,000 TWh), geothermal energy 500 EJ (139,000 TWh), biomass 250 EJ (70,000 TWh), hydropower 50 EJ (14,000 TWh) and ocean energy 1 EJ (280 TWh) [4].

Energy consumption in the G20 increased by more than 5% in 2010 after a slight decline of 2009. In 2009, world energy consumption decreased for the first time in 30 years, by −1.1% (equivalent to 130 Megatonnes of oil), as a result of the financial and economic crisis, which reduced world GDP by 0.6% in 2009 [5].

This evolution is the result of two contrasting trends: Energy consumption growth remained vigorous in several developing countries, specifically in Asia (+4%). Conversely, in OECD, consumption was severely cut by 4.7% in 2009 and was thus almost down to its 2000 levels. In North America, Europe and the CIS, consumptions shrank by 4.5%, 5% and 8.5% respectively due to the slowdown in economic activity. China became the world's largest energy consumer (18% of the total) since its consumption surged by 8% during 2009 (up from 4% in 2008). Oil remained the largest energy source (33%) despite the fact that its share has been decreasing over time. Coal posted a growing role in the world's energy consumption: in 2009, it accounted for 27% of the total.

Most energy is used in the country of origin, since it is cheaper to transport final products than raw materials. In 2008 the share export of the total energy production by fuel was: oil 50% (1,952/3,941 Mt), gas 25% (800/3,149 bcm), hard coal 14% (793/5,845 Mt) and electricity 1% (269/20,181 TWh) [6].

Most of the world's energy resources are from the conversion of the sun's rays to other energy forms after being incident upon the planet. Some of that energy has been preserved as fossil energy, some is directly or indirectly usable; for example, via wind, hydro- or wave power. The amount of energy is measured by satellite to be roughly 1368 watts per square meter, [7] though it fluctuates by about 6.9% during the year due to the Earth's varying distance from the sun. This value is the total rate of solar energy received by the planet; about half, 89 PW, reaches the Earth's surface [8].

Until the beginning of the nineteenth century biomass was the predominant fuel, today it has only a small share of the overall energy supply. Electricity produced from biomass sources was estimated at 44 GW for 2005. Biomass electricity generation increased by over 100% in Germany, Hungary, the Netherlands, Poland, and Spain. A further 220 GW was used for heating (in 2004), bringing the total energy consumed from biomass to around 264 GW. The use of biomass fires for cooking is excluded [9].

World production of bioethanol increased by 8% in 2005 to reach 33 billion litres (8.72 billion US gallons), with most of the increase in the United States, bringing it level to the levels of consumption in Brazil [9]. Biodiesel increased by 85% to 3.9 billion litres (1.03 billion US gallons), making it the fastest growing renewable energy source in 2005. Over 50% is produced in Germany [9].

Crude oil is the dominant source of commercial energy use in Nigeria, accounting for over 70% of national commercial energy consumption, of this, the transport sector accounts for about 70% of commercial energy consumption. In Nigeria, crude oil has been a major economic growth determinant. For the past three decades it has claimed the topmost position in the export list of the country (National Bureau of statistics, 2006). Oil is Nigeria's major sources of revenue used for development. The majority of reserves are found along the country's coastal Niger Delta. Due to the Niger Delta crises, the oil producing companies are producing below capacity.

However, as a member of the Organization of Petroleum Exporting Countries (OPEC), Nigerian oil attracts very huge buyers in the international market. The major reason for this is because Nigerian oil is of high quality and most environmental friendly relative to oil from other countries. The local consumption of oil in the country is low. With only 3 of 4 refineries at work in Nigeria, there is inadequate capacity to meet the increasing demands for petroleum products. Nigeria's four refineries have a total capacity of 445,000 barrels of oil per day but these refineries are currently unable to meet domestic demand. This is as a result of inadequate maintenance and a general declining technical inefficiency causing incessant shortages, hoarding and long queues at petrol filling stations [2]. The dependence on crude oil has brought about a lot of corruption and poverty into the country.

Since there has been a heavy demand on the use of fuel, it became necessary for researchers to produce fuels from renewable feedstocks, including corn, sugar cane and sorghum. Thus far, corn-based ethanol has been the predominant biofuel produced in the United States, increasing by a factor of eight from 2000 to 2010. In fact, for the first time in history, more U.S. corn is devoted to ethanol than to livestock. This shift has also contributed to the rising corn prices and created economic, political and food-security issues [10]. Lignocellulosic biomass,

such as agriculture waste, wood, grass and bamboo are renewable resources that do not divert feedstock from food streams [10]. While much attention has been given to cellulosic ethanol production, ethanol is an imperfect fuel because it requires the modification of infrastructure equipment such as pumps and pipelines, and it can only be added as an oxygenate to fuel at 10% of the blend with gasoline.

As a fuel, butanol has a number of advantages over ethanol. First, butanol has a higher caloric value of 29.2MJ/L than ethanol of 21.2 MJ/L, although both are less than gasoline of 32.5 MJ/L [11]. Second, butanol is less corrosive than ethanol, so no infrastructure modifications to tanks, pipelines, pumps, filling stations, etc. are necessary. Third, during the combustion of butanol, no sulphur or nitrogen oxides, which are present in fossil fuels, are released. Fourth, butanol is less miscible in water and less volatile than ethanol [11]. However, a comparison by Pfromm et al. found cellulosic ethanol has a better yield per volume of biomass feedstock, and in order to be competitive, butanol production must be improved upon [11]. Using biological processes, butanol can be produced by bacterial fermentation at mild conditions with little additional energy inputs. Since then, biobutanol production is becoming an attractive method for renewable fuel technology [11]. Bamboo is a taxonomic group of large woody grasses, which grows naturally in subtropical and temperate zones on all over the world. Bamboo species have high growth rates, the ability to grow in a wide range of climates, sequester carbon and have low ash content and alkali index [11]. In mitigating atmospheric carbon levels, the management of bamboo stands has been of particular interest given the fast growth rate of bamboo and its ability to sequester and store carbon. According to the United Nations Food and Agriculture Organization, subtropical bamboo can grow up to an average of 20-30cm on a daily basis. Unlike tree root systems, which die once the tree has been cut, the rhizome and root system of bamboo

continues to live and give rise to new culms. A study of bamboo in North East India showed that the gross carbon stock in a bamboo plantation (in culm, branch, leave and floor mass) accounted for 120 tons carbon per hectare. Of the 36 million hectares of bamboo around the world, the distribution of bamboo resources by continent is as follows: 65% Asia, 28% America and 7% Africa [10]. For the production of fuel from biomass, it is important to find optimal treatment conditions specific to each feedstock material, and prior research with bamboo has been limited. Until now, only lignocellulosic ethanol has been produced from bamboo feedstock.

Although non-fuel applications of bamboo biomass may be more profitable than energy recover, there is potential for co-production of sugar or fuel with other bamboo processing. As bamboo continues to be developed as a substitute for wood in pulp and paper manufacturing as well as in construction of furniture and buildings, there is inevitably going to be bamboo waste, which can be processed into higher value products, such as fuel.

1.2. RESEARCH STATEMENT

Fuels for transportation and other domestic uses are very effective, but the cost and the availability is becoming a problem due to the high dependence on the fuel, this has then made people to start thinking of alternative fuels.

Researchers have produced fuels from renewable feedstock, thereby creating a high competition for survival which has brought about an increase in food price and also high demand. Due to rising price in food items, it became necessary to seek non-food products such as bamboo, palm and food/agricultural waste to produce ethanol and butanol. This study investigates the pretreatment temperature for the production of biobutanol using clostridium bacteria, of strain *C. acetobutylicum*, which have the innate ability to convert both C5 and C6

sugars into desirable products through acetone-butanol-ethanol fermentation. Experiments were designed to find optimal pretreatment conditions for locally grown bamboo by measuring sugar concentrations after pretreatment and hydrolysis.

Although bamboo has been used to produce bio-ethanol, and only of recent when Amanda M. Rees, produced bio-butanol from bamboo as far as literature is concern, because butanol has a higher caloric value of 29.2MJ/L than ethanol of 21.2 MJ/L, although both are less than gasoline of 32.5 MJ/L [11]. It is less corrosive than ethanol, so no infrastructure modifications to tanks, pipelines, pumps, filling stations, etc. are necessary. During the combustion of butanol, no sulphur or nitrogen oxides, which are present in fossil fuels, are released. It is less miscible in water and less volatile than ethanol.

This present study looks at the effect of pre-treatment temperature on the production of bio-butanol from bamboo, because bio-butanol was found to have a higher yield as was observed by Amanda M. Rees (2012) that at pretreatment temperature of 200^0C, 0.5%v/v acid/base concentration and time of 4 hours, had the highest yield of butanol.

1.3. AIM AND OBJECTIVES

1. To produce butanol from bamboo
2. To examine the effect of temperature on the yield of butanol
3. Compare the obtained results with standard results
4. Provide alternative bio-fuel.

1.4. SCOPE OF WORK

This study examines the effect of temperature, concentration and time on the yield of butanol from bamboo.

The study includes the following stages/components:

1. The pretreatment of bamboo with acid;

2. The enzymatic hydrolysis of the pretreated bamboo;

3. Material characterization before or after pre-treatment and after enzymatic hydrolysis:

i. X-ray diffraction (XRD) analysis of the bamboo;

ii. Moisture absorption of the pre-treated bamboo

4. The measurement of the sugar produced

i. glucose

5. The estimation of butanol yield

1.5. HYPOTHESIS

Increasing the temperature, acid concentration and residence time above 200 ^{O}C, 0.5% (v/v) and 4 hours would increase the glucose yield and the yield of butanol.

REFERENCE

[1]. "AccessScience |Encyclopedia Article | Alcohol fuel".Accessscience.com. Retrieved 2008-11-06.

[2]. Gbadebo, Olusegun Odularu;. Chinedu Okonkwo,. Does energy consumption contribute to economic performance? Empirical evidence from Nigeria. Journal of Economics and International Finance. 2009, 1, 2.

[3]. Energy – Consumption'!A1 "Consumption by fuel, 1965–2008" (XLS). Statistical Review of World Energy 2009, BP. 31 July 2006. Retrieved 24 October 2009.

[4]. State of the World 2009, Worldwatch Institute, 2009

[5]. Global Energy Review in 2011, Enerdata Publication

[6]. IEA Key energy statistics 2010 and IEA Key energy statistics 2009 oil page 11, gas p.13, hard coal (excluding brown coal) p. 15 and electricity p. 27

[7]. "Solar Radiation and Climate Experiment". National Aeronautics and Space Administration. Retrieved. 21 December, 2011.

[8]. Energy in Sweden 2010, Facts and figures Table 55 Regional energy use, 1990 and 2008 (kWh per capita)

[9]. "Renewables, Global Status Report 2006" (PDF). Renewable Energy Policy Network for the 21st century. 2006. Retrieved 3 April 2007.

[10]. http://www.businessdayonline.com/NG/index.php/economic-watch/30814-q3-2011-nigeria-records-218-mbd-in-crude-oil-production.

[11]. Amanda M. M. Rees: Bamboo to Butanol: Production of Lignocellulosic Butanol through Fermentation by Clostridia, Senior Thesis, Department of Chemical and Biochemical Engineering, Princeton University, Princeton, New Jersey, USA. 2012.

CHAPTER TWO

LITERATURE SURVEY

2.1. FUEL

Fuels are materials that store energy that can later be extracted to perform mechanical work in a controlled manner. Most fuels used by humans undergo combustion, a redox reaction in which a combustible substance releases energy after it ignites and reacts with the oxygen in the air. Other processes used to convert fuel into energy include various other exothermic chemical reactions and nuclear reactions, such as nuclear fission or nuclear fusion. Fuels are also used in the cells of organisms in a process known as cellular respiration, where organic molecules are oxidized to release usable energy. However, hydrocarbons are by far the most common source of fuel used by humans, although many other substances, such as radioactive metals, are currently used as well [1].

Fuels can be divided into solid fuels, liquid fuels and gaseous fuels. However, in this study, we will like to focus on liquid fuels. Liquid fuels are those combustible or energy-generating molecules that can be harnessed to create mechanical energy, usually producing kinetic energy. They also take the shapes of their containers. Most liquid fuels in widespread use, are or derived from fossil fuels. However, there are several types, such as hydrogen fuel (for automotive uses), which are also categorized as liquid fuels [1].

2.1.1. LIQUID FUELS FOR TRANSPORTATION

Most transportation fuels are liquid. This is because vehicles usually require high energy densities, as occurs in liquids and solids [1]. High power density can be provided most inexpensively by an Internal Combustion Engine (ICE) [1]. ICE require clean burning fuels to

keep the engine clean and minimize air pollution [1]. The fuels that are easiest to burn cleanly are typically liquids and gases [1]. Thus, liquids (and gases that can be stored in liquid form) meet the requirements of being both portable and clean burning. Also, liquids and gases can be pumped, which means handling is easily mechanized, and thus less laborious [1].

2.1.2. GASOLINE

Gasoline is the most widely used liquid fuel. Gasoline, as it is known in United States and Canada, or petrol in Nigeria, India, Britain, Australia, New Zealand, South Africa and many other Commonwealth countries, is made of hydrocarbon molecules forming aliphatic compounds, or chains of carbons with hydrogen atoms attached. However, many aromatic compounds (carbon chains forming rings), such as benzene, are found naturally in gasoline and cause the health risks associated with prolonged exposure to the fuel [1].

The production of gasoline is achieved by distillation of crude oil [1]. The desirable liquid is separated from the crude oil in refineries [1]. Crude oil is extracted from the ground in several processes; the most commonly seen may be beam pumps. To create gasoline, petroleum must first be removed from crude oil [1].

Liquid gasoline itself is not actually burned, but its fumes ignite, causing the remaining liquid to evaporate and then burn. Gasoline is extremely volatile and easily combusts, making any leakage potentially extremely dangerous. Gasoline sold in most countries carries a published octane rating. The octane number is an empirical measure of the resistance of gasoline to combusting prematurely, known as knocking. The higher the octane rating, the more resistant the fuel is to auto-ignition under high pressures, which allows for a higher compression ratio. Engines with a higher compression ratio, commonly used in race cars and high-performance

regular-production automobiles, can produce more power. However, such engines require a higher octane fuel [1].

2.1.3. DIESEL

Conventional diesel is similar to gasoline in that it is a mixture of aliphatic hydrocarbons extracted from petroleum. Diesel may cost more or less than gasoline, but generally costs less to produce because the extraction processes used are simpler. Many countries (particularly in Europe, as well as Canada) also have lower tax rates on diesel fuels [1].

After distillation, the diesel fraction is normally processed to reduce the amount of sulphur in the fuel. Sulphur causes corrosion in vehicles, acid rain and higher emissions of soot from the tail pipe (exhaust pipe). Historically, in Europe lower sulfur levels than in the United States were legally required. However, recent US legislation reduced the maximum sulphur content of diesel from 3,000 ppm to 500 ppm in 2007 and 15 ppm by 2010. Similar changes are also underway in Canada, Australia, New Zealand and several Asian countries, which also uses Ultra-low-sulfur diesel [1].

A diesel engine is a type of internal combustion engine that ignites fuel by injecting it into a combustion chamber previously compressed with air (which in turn raises the temperature), as opposed to using an outside ignition source, such as a spark plug [1].

2.1.4. KEROSENE

Kerosene, once used in kerosene lamps as an alternative to whale oil, is today mainly used in fuel for jet engines (more technically Avtur, Jet A, Jet A-1, Jet B, JP-4, JP-5, JP-7 or JP-8). One form of the fuel known as RP-1 is burned with liquid oxygen as rocket fuel. These fuel

grade kerosenes meet specifications for smoke points and freeze points. In the mid-20th century, kerosene or "TVO" (Tractor Vaporising Oil) was used as a cheap fuel for tractors. The engine would start on gasoline, and then switch over to kerosene once the engine warmed up. A "heat valve" on the manifold would route the exhaust gases around the intake pipe, heating the kerosene to the point where it can be ignited by an electric spark [1].

Kerosene is sometimes used as an additive in diesel fuel to prevent gelling or waxing in cold temperatures. However, this is not advisable in some recent vehicle diesel engines, as doing so may interfere with the engine's emissions regulation equipment [1].

2.1.5. COMPRESSED NATURAL GAS

Natural gas, composed chiefly of methane, can be compressed to a liquid and used as a substitute for other traditional liquid fuels. Its combustion is very clean, compared to other hydrocarbon fuels. However, the fuel's low boiling point requires the fuel to be kept at high pressures to keep it in the liquid state. Though it has a much lower flash point than fuels such as gasoline, it is in many ways safer due to its higher auto-ignition temperature and its low density, which causes it to dissipate when released in air [1].

2.1.6. LIQUIFIED PETROLEUM GAS

LP gas is a mixture of propane and butane, both of which are easily-compressible gases under standard atmospheric conditions. It offers many of the advantages of compressed natural gas (CNG), but is denser than air, does not burn as cleanly, and is much more easily compressed. Commonly used for cooking and space heating, LP gas and compressed propane are seeing

increased use in motorized vehicles; propane is the third most commonly used motor fuel globally [1].

2.2. ALTERNATIVE FUELS

Alternative fuels, known as non-conventional or advanced fuels, are any materials or substances that can be used as fuels, other than conventional fuels. Conventional fuels include: fossil fuels (petroleum (oil), coal, propane, and natural gas), as well as nuclear materials such as uranium and thorium, as well as artificial radioisotope fuels that are made in nuclear reactors, and store their energy. Some well-known alternative fuels include: biodiesel, bioalcohol (methanol, ethanol and butanol), chemically stored electricity (batteries and fuel cells), hydrogen, non-fossil methane, non-fossil natural gas, vegetable oil, and other biomass sources [12].

2.2.1. AMMONIA

Ammonia can be used as fuel. A small machine can be set up to create the fuel and it is used where it is made. Benefits of ammonia include, no need for oil, zero emissions, low cost, and distributed production reducing transport and related pollution [13].

2.2.2. HYDROGEN FUEL

Hydrogen is an emissionless fuel. The byproduct of hydrogen burning is water, although some mono-nitrogen oxides NOx are produced when hydrogen is burned with air [13].

2.2.3. HYDROGEN COMPRESSED NATURAL GAS (HCNG)

HCNG (or H2CNG) is a mixture of compressed natural gas and 4-9 percent hydrogen by energy. HCNG is a vehicle fuel which is a blend of compressed natural gas and hydrogen, typically 8-50% hydrogen by volume. Global HCNG testing to date has demonstrated the fuel's potential to reduce nitrous oxide (NO_x), carbon dioxide (CO_2), and carbon monoxide (Co) vehicle emissions compared to traditional CNG. Existing natural gas engines can be used with HCNG, although higher hydrogen blends require re-tuning of the engines for optimal performance. Studies indicate that HCNG mixture with 20 - 30% hydrogen by volume are optimal for vehicle performance and emissions reduction [14].

HCNG refueling sites require sources of hydrogen and natural gas, which are blended and pressurized on-site for vehicle fueling. Gas is supplied through the existing natural gas infrastructure while hydrogen is provided though electrolysis or natural gas reformation. Electrolysis is generally performed on-site using electricity from the local grid. Hydrogen from the reformation of natural gas is supplied to sites by truck or produced on-site using small-scale reformers. All these options have been tested in field trials [14].

2.2.4. LIQUID NITROGEN

Liquid nitrogen is another type of emissionless fuel. Liquid nitrogen is generated by cryogenic or reversed Stirling engine [15], coolers that liquefy the main component of air, nitrogen (N_2). The cooler can be powered by electricity or through direct mechanical work from hydro or wind turbines [16].

Liquid nitrogen is distributed and stored in insulated containers. The insulation reduces heat flow into the stored nitrogen; this is necessary because heat from the surrounding

environment boils the liquid, which then transitions to a gaseous state. Reducing inflowing heat reduces the loss of liquid nitrogen in storage. The requirements of storage prevent the use of pipelines as a means of transport. Since long-distance pipelines would be costly due to the insulation requirements, it would be costly to use distant energy sources for production of liquid nitrogen. Petroleum reserves are typically a vast distance from consumption but can be transferred at ambient temperatures.

Liquid nitrogen consumption is in essence production in reverse. The Stirling engine or cryogenic heat engine offers a way to power vehicles and a means to generate electricity. Liquid nitrogen can also serve as a direct coolant for refrigerators, electrical equipment and air conditioning units. The consumption of liquid nitrogen is in effect boiling and returning the nitrogen to theatmosphere.

In the Dearman Engine the nitrogen is heated by combining it with the heat exchange fluid inside the cylinder of the engine [17].

2.2.5. COMPRESSED AIR

The air engine is an emission-free piston engine using compressed air as fuel. Unlike hydrogen, compressed air is about one-tenth as expensive as fossil oil, making it an economically attractive alternative fuel.

2.2.6. COMPRESSED NATURAL GAS

Compressed natural gas (CNG) is a cleaner burning alternative to conventional petroleum automobile fuels. The energy efficiency is generally equal to that of gasoline engines, but lower compared with modern diesel engines. CNG vehicles require a greater amount of space for fuel

storage than conventional gasoline power vehicles because CNG takes up more space for each GGE (Gallon of Gas Equivalent). Almost any existing gasoline car can be turned into a bi-fuel (gasoline/CNG) car. However, natural gas is a finite resource like all fossil fuels, and production of natural gas is expected to peak soon after oil, this has been expected since 2011 [18].

2.2.7. NATURAL GAS

Natural gas, like hydrogen, is another fuel that burns cleanly; cleaner than both gasoline and diesel engines. Also, none of the smog-forming contaminants are emitted, seen substantially by the latter. Around the world, this gas powers more than 5 million vehicles, and just over 150,000 of these are in the U.S [18].

2.2.8. RADIOTHERMAL GENERATORS

Radioisotopes have been used as alternative fuels, on both land and in space. Their use on land is declining due to the danger of theft of the isotope and environmental damage than can occur if the unit is opened. The decay of radioisotopes generates both heat and electricity in many space probes, particularly probes to outer planets where sunlight is weak and low temperatures is a problem. Radiothermal generators (RTGs) which use such radioisotopes as fuels do not sustain a nuclear chain reaction, but rather generate electricity from the decay of a radioisotope which has (in turn) been produced on Earth as a concentrated power source (fuel) using energy from an Earth-based nuclear reactor [18].

2.3. BIOFUEL

Biofuels are also considered a renewable source. Although renewable energy is used mostly to generate electricity, it is often assumed that some form of renewable energy or a percentage is used to create alternative fuels. A **biofuel** is a type of fuel whose energy is derived from biological carbon fixation. Biofuels include fuels derived from biomass conversion, as well as solid biomass, liquid fuels and various biogases [12]. Similarly, bio-fossil fuels also have their origin in ancient carbon fixation. Biofuels are gaining increased public and scientific attention, driven by factors such as oil price hikes, the need for increased energy security, concern over greenhouse gas emissions from bio-fossil fuels, and support from government subsidies [12].

In 2010, the worldwide biofuel production reached 105 billion liters (28 billion gallons US), up 17% from 2009, and biofuels provided 2.7% of the world's fuels for road transport, a contribution largely made up of ethanol and biodiesel [11]. Global ethanol fuel production reached 86 billion liters (23 billion gallons US) in 2010, with the United States and Brazil as the world's top producers, accounting together for 90% of global production. The world's largest biodiesel producer is the European Union, accounting for 53% of all biodiesel production in 2010 [11]. As of 2011, mandates for blending biofuels exist in 31 countries at the national level and in 29 states/provinces [18]. According to the International Energy Agency, biofuels have the potential to meet more than a quarter of world demand for transportation fuels by 2050 [12].

2.4. BIOMASS

Biomass in the energy production industry is living and recently dead biological material which can be used as fuel or for industrial production.

2.5. FIRST GENERATION BIOFUELS

First-generation biofuels or conventional biofuels are made from sugar, starch, vegetable oil, or animal fats using conventional technology. These are generally produced from grains high in sugar or starch fermented into bioethanol; or seeds that which are pressed into vegetable oil used in biodiesel. Common first-generation biofuels include vegetable oils, biodiesel, bioalcohols, biogas, solid biofuels, syngas [12].

2.5.1 BIOALCOHOLS

Biologically produced alcohols, most commonly ethanol, and less commonly propanol and butanol, are produced by the action of microorganisms and enzymes through the fermentation of sugars or starches (easiest), or cellulose (which is more difficult).

Butanol (C_4H_9OH) is formed by ABE fermentation (acetone butanol ethanol) and experimental modifications of the process show potentially high net energy gains, with butanol as the only liquid product. Butanol will produce more energy and allegedly can be burned "straight" in existing gasoline engines (without modification to the engine or car) [19]. It is less corrosive and less water soluble than ethanol, and could be distributed via existing infrastructures. It also has a higher calorific value than ethanol [11].

DuPont and BP are working together to help develop Butanol. E. coli have also been successfully engineered to produce butanol by hijacking their amino acid metabolism [20]. Biobutanol (also called biogasoline) is often claimed to provide a direct replacement for gasoline, because it can be used directly in a gasoline engine (in a similar way to biodiesel in diesel engines).

Ethanol can be used in petrol engines as a replacement for gasoline; it can be mixed with gasoline to any percentage. Most existing car petrol engines can run on blends of up to 15% bioethanol with petroleum/gasoline. Ethanol has a lower energy density than gasoline; this fact means that it takes more fuel (volume and mass) to produce the same amount of work. An advantage of ethanol (CH_3CH_2OH) is that it has a higher octane rating than ethanol-free gasoline available at roadside gas stations. This allows for an increase of an engine's compression ratio for increased efficiency. Also, in high altitude (thin air) locations, some states mandate a mixture of gasoline and ethanol as a winter oxidizer to reduce atmospheric pollution emissions.

Bioethanol is an alcohol made by fermentation. It is produced mostly from carbohydrates in sugar or starch crops, such as corn or sugarcane. Cellulosic biomass, derived from non-food sources such as trees and grasses, is also being developed as a feedstock for ethanol production. Bioethanol is widely used in the USA and in Brazil. Current plant design does not provide for converting the lignin portion of plant raw materials to fuel components by fermentation.

According to a joint research agenda conducted through the U.S. Department of Energy [21], the fossil energy ratios (FER) for cellulosic ethanol, corn ethanol, and gasoline are 10.3, 1.36, and 0.81, respectively. Even dry ethanol has roughly one-third lower energy content per unit of volume compared to gasoline, so larger / heavier fuel tanks are required to travel the same distance, or more fuel stops are required. With large current unsustainable, non-scalable subsidies, ethanol fuel still costs much more per distance traveled than current high gasoline prices in the United States [22].

Methanol is currently produced from natural gas, a non-renewable fossil fuel. It can also be produced from biomass as biomethanol. The methanol economy is an alternative to the hydrogen economy, compared to today's hydrogen production from natural gas.

Table 2.1.: Calorific Value for some fuels [23].

Fuels	Calorific Value Mass (MJ/Kg)	Calorific Value Volume (MJ/L)
Gasoline	43.9	32.7
Methanol	20.0	16.0
Ethanol	27.0	21.2
Butanol	33.22	26.9
Biodiesel	37.8	32.5
Diesel	42.6	36.7
Fisher-Tropsch Diesel	43.0	32.0

2.5.2. BIODIESEL

Biodiesel is made from animal fats or vegetable oils, renewable resources that come from plants such as, soybean, sunflowers, corn, olive, peanut, palm, coconut, safflower, canola, sesame, cottonseed, etc. Once these fats or oils are filtered from their hydrocarbons and then combined with alcohol like methanol, biodiesel is brought to life from this chemical reaction. These raw materials can either be mixed with pure diesel to make various proportions, or used alone. Despite one's mixture preference, biodiesel will release a smaller number of its pollutants (carbon monoxide particulates and hydrocarbons) than conventional diesel. This is because biodiesel burns both cleaner and more efficiently. Even with regular diesel's reduced quantity of sulfur from the ULSD (ultra-low sulfur diesel) invention, biodiesel exceeds those levels because it is sulfur-free [24].

In some countries, biodiesel is less expensive than conventional diesel. Biodiesel is also safe to handle and transport because it is as biodegradable as sugar, 10 times less toxic than table salt, and has a high flash point of about 300 ^{o}F (148 ^{o}C) compared to petroleum diesel fuel, which has a flash point of 125 ^{o}F (52 ^{o}C) [24]. In the USA, more than 80% of commercial trucks and city buses run on diesel. The emerging US biodiesel market is estimated to have grown

200% from 2004 to 2005. "By the end of 2006, biodiesel production was estimated to increase fourfold [from 2004] to more than" 1 billion US gallons (3,800,000 m^3) [25].

2.5.3. GREEN DIESEL

Green diesel, also known as renewable diesel, is a form of diesel fuel which is derived from renewable feedstock rather than the fossil feedstock used in most diesel fuels. Green diesel feedstock can be sourced from a variety of oils including canola, algae, jatropha and salicornia in addition to tallow. Green diesel uses traditional fractional distillation to process the oils, not to be confused with biodiesel which is chemically quite different and processed using trans-esterification. “Green Diesel” as commonly known in Ireland should not be confused with dyed green diesel sold at a lower tax rate for agriculture purposes. Using the dye allows custom officers to determine if a person is using the cheaper dyed green diesel in higher taxed applications such as commercial haulage or cars [26].

2.5.4. BIOETHERS

Bioethers (also referred to as fuel ethers or oxygenated fuels) are cost-effective compounds that act as octane rating enhancers. They also enhance engine performance, whilst significantly reducing engine wear and toxic exhaust emissions. Greatly reducing the amount of ground-level ozone, they contribute to the quality of the air we breathe [27].

2.5.5. BIOGAS

Biogas is methane produced by the process of anaerobic digestion of organic material by anaerobes [27]. It can be produced either from biodegradable waste materials or by the use of

energy crops fed into anaerobic digesters to supplement gas yields. The solid byproduct, digestate, can be used as a biofuel or a fertilizer.

- Biogas can be recovered from mechanical biological treatment waste processing systems.

  Note: Landfill gas is a less clean form of biogas which is produced in landfills through naturally occurring anaerobic digestion. If it escapes into the atmosphere it is a potential greenhouse gas.
- Farmers can produce biogas from manure from their cows by using an anaerobic digester (AD) [28].

2.5.6 SYNGAS

Syngas, a mixture of carbon monoxide, hydrogen and other hydrocarbons is produced by partial combustion of biomass, that is, combustion with an amount of oxygen that is not sufficient to convert the biomass completely to carbon dioxide and water [29]. Before partial combustion the biomass is dried, and sometimes pyrolysed. The resulting gas mixture, syngas, is more efficient than direct combustion of the original biofuel; more of the energy contained in the fuel is extracted.

- Syngas may be burned directly in internal combustion engines, turbines or high-temperature fuel cells [28]. The wood gas generator is a wood-fueled gasification reactor that can be connected to an internal combustion engine.
- Syngas can be used to produce methanol, DME and hydrogen, or converted via the Fischer-Tropsch process to produce a diesel substitute, or a mixture of alcohols that can be blended into gasoline. Gasification normally relies on temperatures >700°C.

- Lower temperature gasification is desirable when co-producing biochar. However, this results in a Syngas polluted with tar.

2.5.7. SOLID BIOFUELS

Examples include wood, sawdust, grass trimmings, domestic refuse, charcoal, agricultural waste, non-food energy crops, and dried manure.

When raw biomass is already in a suitable form (such as firewood), it can burn directly in a stove or furnace to provide heat or raise steam. When raw biomass is in an inconvenient form (such as sawdust, wood chips, grass, urban waste wood, agricultural residues), the typical process is to densify the biomass. This process includes grinding the raw biomass to an appropriate particulate size (known as hogfuel), which depending on the densification type can be from 1 to 3 cm (1 in), which is then concentrated into a fuel product. The current types of processes are wood pellet, cube, or puck. The pellet process is most common in Europe and is typically a pure wood product. The other types of densification are larger in size compared to a pellet and are compatible with a broad range of input feedstocks. The resulting densified fuel is easier to transport and feed into thermal generation systems such as boilers. One of the advantages of solid biomass fuel is that it is often a by-product, residue or waste-product of other processes, such as farming, animal husbandry and forestry [30]. In theory this means there is no competition between fuel and food production, although this is not always the case [30].

A problem with the combustion of raw biomass is that it emits considerable amounts of pollutants such as particulates and PAHs (polycyclic aromatic hydrocarbons). Even modern pellet boilers generate much more pollutants than oil or natural gas boilers. Pellets made from agricultural residues are usually worse than wood pellets, producing much larger emissions of dioxins and chlorophenols [31].

A derivative of solid biofuel is biochar, which is produced by biomass pyrolysis. Biochar made from agricultural waste can substitute for wood charcoal. As wood stock becomes scarce this alternative is gaining ground. In eastern Democratic Republic of Congo, for example, biomass briquettes are being marketed as an alternative to charcoal in order to protect Virunga National Park from deforestation associated with charcoal production [32].

2.6. SECOND GENERATION BIOFUELS (ADVANCED BIOFUELS)

Second generation biofuels are biofuels produced from sustainable feedstock. Sustainability of a feedstock is defined among others by availability of the feedstock, impact on GHG emissions and impact on biodiversity and land use [7]. Many second generation biofuels are under development such as Cellulosic ethanol, Algae fuel [33], biohydrogen, biomethanol, DMF, BioDME, Fischer-Tropsch diesel, biohydrogen diesel, mixed alcohols and wood diesel. Cellulosic ethanol production uses non-food crops or inedible waste products and does not divert food away from the animal or human food chain. Lignocellulose is the "woody" structural material of plants. This feedstock is abundant and diverse, and in some cases (like citrus peels or sawdust) it is in itself a significant disposal problem.

Producing ethanol from cellulose is a difficult technical problem to solve. In nature, ruminant livestock (like cattle) eat grass and then use slow enzymatic digestive processes to break it into glucose (sugar). In cellulosic ethanol laboratories, various experimental processes are being developed to do the same thing, and then the sugars released can be fermented to make ethanol fuel. In 2009, scientists reported developing, using "synthetic biology", "15 new highly stable fungal enzyme catalysts that efficiently break down cellulose into sugars at high temperatures", adding to the 10 previously known [34]. The use of high temperatures, has been

identified as an important factor in improving the overall economic feasibility of the biofuel industry and the identification of enzymes that are stable and can operate efficiently at extreme temperatures is an area of active research [12]. In addition, research conducted at Delft University of Technology by Jack Pronk has shown that elephant yeast, when slightly modified can also create ethanol from non-edible ground sources (e.g. straw) [35].

Scientists working with the New Zealand company Lanzatech have developed a technology to use industrial waste gases, such as carbon monoxide from steel mills, as a feedstock for a microbial fermentation process to produce ethanol [36]. In October 2011, Virgin Atlantic announced it was joining with Lanzatech to commission a demonstration plant in Shanghai that would produce an aviation fuel from waste gases from steel production [37]. Scientists working in Minnesota have developed co-cultures of *Shewanella* and *Synechococcus* that produce long chain hydrocarbons directly from water, carbon dioxide, and sunlight [38]. The technology has received ARPA-E funding.

2.7. ISSUES WITH BIOFUEL PRODUCTION AND USE

There are various social, economic, environmental and technical issues with biofuel production and use. These include: the effect of moderating oil prices, the "food vs fuel" debate, poverty reduction potential, carbon emissions levels, sustainable biofuel production, deforestation and soil erosion, loss of biodiversity, impact on water resources, as well as energy balance and efficiency. The International Resource Panel, which provides independent scientific assessments and expert advice on a variety of resource-related themes, assessed the issues relating to biofuel use in its first report “Toward sustainable production and use of resources: Assessing Biofuels” [39]. In it, it outlined the wider and interrelated factors that need to be

considered when deciding on the relative merits of pursuing one biofuel over another. It concluded that, not all biofuels perform equally in terms of their impact on climate, energy security and ecosystems, and suggested that environmental and social impacts need to be assessed throughout the entire life-cycle.

Although there are many current issues with biofuel production and use, the development of new biofuel crops and second generation biofuels attempts to circumvent these issues. Many scientists and researchers are working to develop biofuel crops that require less land and use fewer resources, such as water, than current biofuel crops do. According to the journal "Renewable fuels from algae: An answer to debatable land based fuels" [40], algae is a source for biofuels that could utilize currently unprofitable land and waste water from different industries. Algae are able to grow in wastewater, which does not affect the land or freshwater needed to produce current food and fuel crops. Furthermore, algae are not part of the human food chain, and therefore, do not take away food resources from humans.

The effects of the biofuel industry on food are still being debated. According to a recent study entitled "Impact of biofuel production and other supply and demand factors on food price increases in 2008" [41], biofuel production was accountable for 3-30% of the increase in food prices in 2008. A recent study for the International Centre for Trade and Sustainable Development shows that market-driven expansion of ethanol in the US increased maize prices by 21 percent in 2009, in comparison with what prices would have been had ethanol production been frozen at 2004 levels [42]. This has prompted researchers to develop biofuel crops and technologies that will reduce the impact of the growing biofuel industry on food production and cost.

One step to overcoming these issues is developing biofuel crops best suited to each region of the world. If each region utilized a specific biofuel crop, the need to use fossil fuels to transport the fuel to other places for processing and consumption will be diminished. Furthermore, certain areas of the globe are unsuitable for producing crops that require large amounts of water and nutrient rich soil. Therefore, current biofuel crops, such as corn, are impractical in different environments and regions of the globe.

In 2012, the United States House Committee on Armed Services put language into the 2013 National Defense Authorization Act that would prevent the Pentagon from purchasing biofuels that offered improved performance for combat aircraft [43].

2.8. THIRD GENERATION BIOFUELS

Third-generation biofuels are produced from extracting oil of algae – sometimes referred to as "oilgae". Its production is supposed to be low cost and high-yielding – giving up to nearly 30 times the energy per unit area as can be realized from current, conventional 'first-generation' biofuel feedstocks.

2.8.1. ETHANOL BIOFUELS

As the primary source of biofuels in North America, many organizations are conducting research in the area of ethanol production. The National Corn-to-Ethanol Research Center (NCERC) is a research division of Southern Illinois University, Edwardsville, that is dedicated solely to ethanol-based biofuel research projects [44]. On the Federal level, the USDA conducts a large amount of research regarding ethanol production in the United States. Much of this research is targeted toward the effect of ethanol production on domestic food markets [3]. A

division of the U.S. Department of Energy, the National Renewable Energy Laboratory (NREL), has also conducted various ethanol research projects, mainly in the area of cellulosic ethanol [45].

2.8.2. ALGAL BIOFUELS

Algae-based biofuels have been hyped in the media as a potential panacea to our Crude Oil based Transportation problems. Algae could yield more than 2000 gallons of fuel per acre per year of production [46]. Algae-based fuels are being successfully tested by the U.S. Navy [47]. Algae-based plastics show potential to reduce waste and the cost per pound of algae plastic is expected to be cheaper than traditional plastic prices [48]. This oil-rich algae can then be extracted from the system and processed into biofuels, with the dried remainder further reprocessed to create ethanol.

The production of algae to harvest oil for biofuels has not yet been undertaken on a commercial scale. However, feasibility studies have been conducted to arrive at the above yield estimates. In addition to its projected high yield, alga-culture — unlike crop-based biofuels — does not entail a decrease in food production, since it requires neither farmland nor fresh water. Many companies are pursuing algae bio-reactors for various purposes, including the scaling up biofuels production to commercial levels [49]. Prof. Rodrigo E. Teixeira from the University of Alabama in Huntsville demonstrated the extraction of biofuels lipids from wet algae using a simple and economical reaction in ionic liquids [50].

2.8.3. JATROPHA

Several groups in various sectors are conducting research on Jatropha curcas, a poisonous shrub-like tree that produces seeds considered by many to be a viable source of biofuels feedstock oil [5]. Much of this research focuses on improving the overall per acre oil yield of Jatropha through advancements in genetics, soil science, and horticultural practices. SG Biofuels, a San Diego-based Jatropha developer, has used molecular breeding and biotechnology to produce elite hybrid seeds of Jatropha that show significant yield improvements over first generation varieties [8]. SG Biofuels also claims that additional benefits have arisen from such strains, including improved flowering synchronicity, higher resistance to pests and disease, and increased cold weather tolerance [51].

Plant Research International, a department of the Wageningen University and Research Centre in the Netherlands, maintains an ongoing Jatropha Evaluation Project (JEP) that examines the feasibility of large scale Jatropha cultivation through field and laboratory experiments [52]. The Center for Sustainable Energy Farming (CfSEF) is a Los Angeles-based non-profit research organization dedicated to Jatropha research in the areas of plant science, agronomy, and horticulture. Successful exploration of these disciplines is projected to increase Jatropha farm production yields by 200-300% in the next ten years [10].

2.8.4. GREENHOUSE GAS EMISSIONS

According to Britain's National Non-Food Crops Centre, total net savings from using first-generation biodiesel as a transport fuel range from 25-82% (depending on the feedstock used), compared to diesel derived from crude oil [53]. Nobel Laureate Paul Crutzen, however, finds that the emissions of nitrous oxide due to nitrate fertilizers is seriously underestimated, and

tips the balance such that most biofuels produce more greenhouse gases than the fossil fuels they replace. Producing lignocellulosic biofuels offers potentially greater greenhouse gas emissions savings than those obtained by first generation biofuels. Lignocellulosic biofuels are predicted by oil industry body CONCAWE [12] to reduce greenhouse gas emissions by around 90% when compared with fossil petroleum, in contrast first generation biofuels were found to offer savings of 20-70% [53].

Some scientists have expressed concerns about land-use change in response to greater demand for crops to use for biofuel and the subsequent carbon emissions [54]. The payback period, that is, the time it will take biofuels to pay back the carbon debt that they acquire due to land-use change, has been estimated to be between 100–1000 years, depending on the specific instance and location of land-use change. However, no-till practices combined with cover crop practices can reduce the payback period to 3 years for grassland conversion and 14 years for forest conversion [55]. Biofuels made from waste biomass or from biomass grown on abandoned agricultural lands incur little to no carbon debt [56].

2.9. BIOBUTANOL

Biobutanol is a four-carbon alcohol derived from the fermentation of biomass. When it is produced from petroleum-based feedstocks, it's commonly called butanol. Biobutanol is in the same family as other commonly known alcohols, namely single-carbon methanol and the more-well known two-carbon alcohol ethanol. The importance of the number of carbon atoms in any given molecule of alcohol is directly related to the energy content of that particular molecule. The more carbon atoms present, especially in a long carbon-to-carbon bond chains, the denser in energy the alcohol is. Breakthroughs in biobutanol processing methods, namely the discovery

and development of genetically modified microorganisms, has set the stage for biobutanol to surpass ethanol as a renewable fuel. Once considered usable only as an industrial solvent and chemical feedstock, biobutanol shows great promise as a motor fuel due to its favorable energy density of 0.81 Kg/L [23], compared to bioethanol of energy density of 0.785 Kg/L [23] and it returns better fuel economy and is considered a superior motor fuel. Bioethanol has a calorific value of 27 MJ/Kg or 21.2 MJ/L [23], while Biobutanol has a greater calorific value of 33.22 MJ/Kg and 26.9 MJ/L [23].

2.9.1. THE PROCESS OF BIOBUTANOL PRODUCTION

Butanol can be obtained using several chemical technologies. It is also possible to produce butanol in the process of fermentation by means of bacteria of the genus *Clostridium*. This process occurs under anaerobic conditions, and butanol as one of the products - called biobutanol [57].

Biobutanol is derived mainly from the fermentation of the sugars in organic feedstocks (biomass). Historically, up until about the mid-50s, biobutanol was fermented from simple sugars in a process that produced acetone and ethanol, in addition to the butanol component. The process is known as ABE (Acetone Butanol Ethanol) and has used unsophisticated (and not particularly hearty) microbes such as *Clostridium acetobutylicum.* The problem with this type of microbe is that it is poisoned by the very butanol it produces once the alcohol concentration rises above approximately 2 percent. This processing problem caused by the inherent weakness of generic-grade microbes, plus inexpensive and abundant (at the time) petroleum gave way to the simpler and cheaper distillation-from-petroleum method of refining butanol.

In recent years, with petroleum prices heading steadily upwards, and worldwide supplies getting tighter and tighter, scientists have revisited the fermentation of sugars for the manufacturing of biobutanol [40]. Great strides have been made by researchers in creating "designer microbes" that can tolerate higher concentrations of butanol without being killed off [40].

The ability to withstand harsh high concentration alcohol environments, plus the superior metabolism of these genetically enhanced bacteria has fortified them with the endurance necessary to degrade the tough cellulosic fibers of biomass feedstocks such as pulpy woods and switchgrass. The door has been kicked open and the reality of cost competitive; if not cheaper, renewable alcohol motor fuel is upon us.

Production of butanol by the anaerobic fermentation is one of the oldest industrial methods for obtaining this organic solvent [58]. In the early 20th century, interest in butanol resulted from an inadequate level of supply of natural rubber and increase of its market price. At that time, butanol was used as one of the raw materials for the production of butadiene, being a raw material for synthetic rubber production. Currently, butanol is considered as an alternative biofuel. Butanol is a colourless, flammable alcohol. It is used widely in industry, among others, applied as a solvent. It arouses particular interest due to the role it can play in the future as a biofuel. It is expected that production of biobutanol can reduce consumption of oil and natural gas by the automobile industry and reduce emissions of harmful gases into the atmosphere [57].

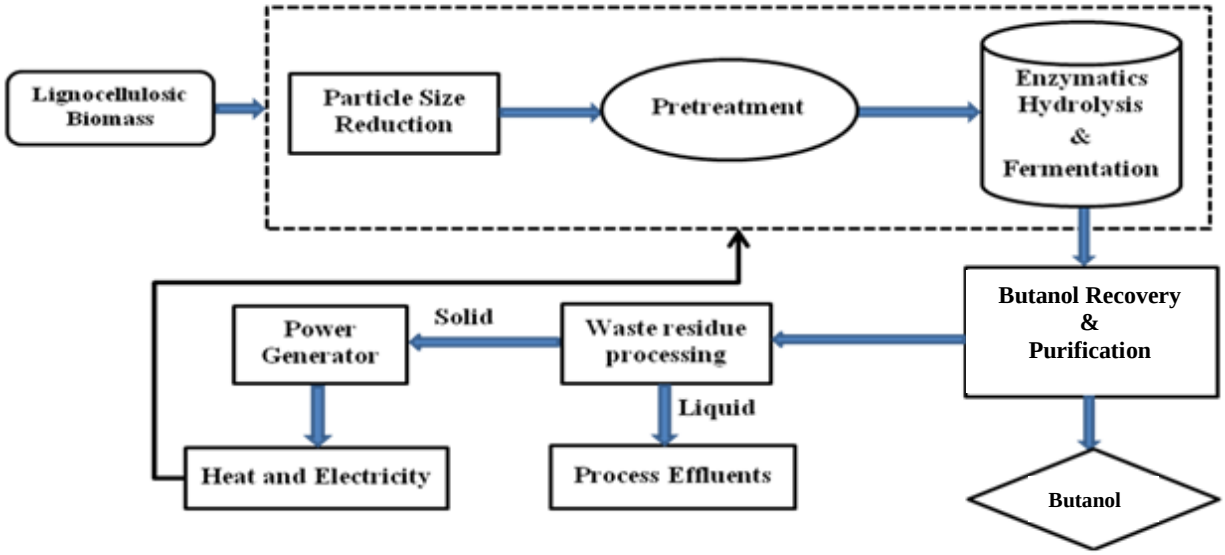


Figure 2.1.: Process flow of biological conversion of lignocellulosic biomass into butanol.

The petrochemical industry uses alcohols, mainly ethanol as a fuel additive, improving its quality. Research results show that the use of butanol for this purpose is much more useful than the application of ethanol. Butanol has a high calorific value, which is 29.2 MJ/dm^3 (melting point –89.5°C, boiling point 117.2 ^{o}C, flash point 36 ^{o}C, the self-ignition 340 ^{o}C) compared to ethanol which has a calorific value of 19.6 MJ/dm^3 (melting point –117.3 °C, boiling point 78.3 ^{o}C, flash point 13 ^{o}C, the self-ignition 366 ^{o}C) [58]. Furthermore, it also has a relatively low heat of vaporization, and is less corrosive than ethanol. All these features enhance its usefulness both as an additive to gasoline, as well as biofuels. Currently, butanol is used only as an additive to gasoline because there is no engine working exclusively on this alcohol. However, intensive research is carried out in this direction. Table 2.2 gives the basic properties of butanol as a fuel in comparison with the other liquid biofuels.

Table 2.2: Properties of butanol and other biofuels [58]

Fuel	Combustion energy [MJ/dm^3]	Evaporation heat [MJ/kg]	Air-fuel ratio	Heat of vaporization	RON Research Octane Number	MON Motor Octane Number
Petrol	32	0.36	14.6	0.36 MJ/kg	91 – 99	81 – 89
Butanol	29.2	0.43	11.1	0.43 MJ/kg	96	78
Ethanol	19.6	0.96	9.0	0.92 MJ/kg	130	96
Methanol	16	1.2	6.4	1.2 MJ/kg	136	104

2.9.2. BIOBUTANOL ADVANTAGES

So, with all of the chemistry and intense research notwithstanding, biobutanol have many advantages over the production of ethanol.

- Biobutanol has higher energy content than ethanol, so there is a much lower loss of fuel economy. With an energy content of about 105,000 BTUs/gallon (versus ethanol's approximate 84,000 BTUs/gallon), biobutanol is much closer to the energy content of gasoline (114,000 BTUs/gallon).
- Biobutanol can be easily blended with conventional gasoline at higher concentrations than ethanol for use in unmodified engines. Experiments have shown that biobutanol can run in an unmodified conventional engine at 100 percent, but to date, no manufacturers will warrant use of blends higher than 15 percent.
- Since it is less susceptible to separation in the presence of water (than ethanol), it can be distributed via conventional infrastructure (pipelines, blending facilities and storage tanks). There's no need for a separate distribution network.
- It is less corrosive than ethanol. Not only is biobutanol a higher-grade more energy dense fuel, it is also less explosive than ethanol.

- EPA test results show that biobutanol reduces emissions, namely hydrocarbons, carbon monoxide (CO) and oxides of nitrogen (NOx). Exact values depend upon the engine state of tune.

Furthermore, biobutanol as a motor fuel—with its long chain structure and preponderance of hydrogen atoms—could be used as a stepping-stone in bringing hydrogen fuel cell vehicles to the main stream. One of the biggest challenges facing hydrogen fuel cell vehicle development is the storage of on-board hydrogen for sustainable range and the lack of hydrogen infrastructure for fueling. The high hydrogen content of butanol would make it an ideal fuel for on-board reforming. Instead of burning the butanol, a reformer would extract the hydrogen to power the fuel cell.

2.9.3. BIOBUTANOL DISADVANTAGES

Currently, the only real disadvantage is there are many more ethanol refining facilities than biobutanol refineries. While ethanol refining facilities far outnumber those for biobutanol, the possibility of retrofitting ethanol plants to biobutanol is feasible. Also, if the refinements continue with genetically modified microorganisms, the feasibility of converting plants becomes greater and greater.

It’s clear that biobutanol is the superior choice over ethanol as a gasoline additive and perhaps eventual gasoline replacement. For the past 30 years or so, ethanol has had most of the technological and political support. It has seeded the market for renewable alcohol motor fuel. Biobutanol is now poised to pick up the mantle.

2.9.4. PROPERTIES OF BUTANOL AND SOME OTHER FUELS

Switching a gasoline engine over to butanol would in theory result in a fuel consumption penalty of about 10%, but butanol's effect on mileage is yet to be determined by a scientific study. While the energy density for any mixture of gasoline and butanol can be calculated, tests with other alcohol fuels have demonstrated that the effect on fuel economy is not proportional to the change in energy density [59].

2.9.5. OCTANE RATING

The octane rating of n-butanol is similar to that of gasoline but lower than that of ethanol and methanol. n-Butanol has a RON (Research Octane Number) of 96 and a MON (Motor Octane Number) of 78 (with a resulting "(R+M)/2 Pump Octane Number" of 87, as used in North America) while t-butanol has octane ratings of 105 RON and 89 MON [60]. t-Butanol is used as an additive in gasoline but cannot be used as a fuel in its pure form. This is because of its relatively high melting point of 25.5 °C. This causes it to gel and freeze near room temperature.

A fuel with a higher octane rating is less prone to knocking (extremely rapid and spontaneous combustion by compression) and the control system of any modern car engine can take advantage of this by adjusting the ignition timing. This will improve energy efficiency, leading to a better fuel economy than the comparisons of energy content different fuels indicate. By increasing the compression ratio, further gains in fuel economy, power and torque can be achieved. Conversely, a fuel with lower octane rating is more prone to knocking and will lower efficiency. Knocking can also cause engine damage [61]. The fuel-air charge is meant to be ignited by the spark plug only, and at a precise time in the piston's stroke cycle. Knock occurs when the peak of the combustion process no longer occurs at the optimum moment for the four-

stroke cycle. The shock wave creates the characteristic metallic "pinging" sound, and cylinder pressure increases dramatically. Effects of engine knocking range from inconsequential to completely destructive.

2.9.6. AIR-FUEL RATIO

Alcohol fuels, including butanol and ethanol, are partially oxidized and therefore need to run at richer mixtures than gasoline. Standard gasoline engines in cars can adjust the air-fuel ratio to accommodate variations in the fuel, but only within certain limits, depending on model. If the limit is exceeded by running the engine on pure butanol or a gasoline blend with a high percentage of butanol, the engine will run lean, something which can critically damage components. Compared to ethanol, butanol can be mixed in higher ratios with gasoline for use in existing cars without the need for retrofit, as the air-fuel ratio and energy content are closer to that of gasoline [62].

2.9.7. SPECIFIC ENERGY

Alcohol fuels have less energy per unit weight and unit volume than gasoline. To make it possible to compare the net energy released per cycle a measure called the fuels specific energy is sometimes used. It is defined as the energy released per air fuel ratio. The net energy released per cycle is higher for butanol than ethanol or methanol and about 10% higher than for gasoline [63].

2.9.8. VISCOSITY

The viscosity of alcohols increases with longer carbon chains. For this reason, butanol is used as an alternative to shorter alcohols when a more viscous solvent is desired. The kinematic viscosity of butanol is several times higher than that of gasoline and about as viscous as high quality diesel fuel [4].

Table 2.3.: Viscosity of butanol and other fuels

Substance	Kinematic Viscosity at 20^0C
Butanol	3.64 cSt
Ethanol	1.52 cSt
Methanol	0.64 cSt
Gasoline	0.4 - 0.8 cSt
Diesel	> 3 cSt
Water	1.0 cSt

2.9.9. HEAT OF VAPORIZATION

The fuel in an engine has to be vaporized before it will burn. Insufficient vaporization is a known problem with alcohol fuels during cold starts in cold weather. As the heat of vaporization of butanol is less than half of that of ethanol, an engine running on butanol should be easier to start in cold weather than one running on ethanol or methanol [62].

2.9.10. POSSIBLE BUTANOL FUEL MIXTURES

Standards for the blending of ethanol and methanol in gasoline exist in many countries, including the EU, the US and Brazil. Approximate equivalent butanol blends can be calculated from the relations between the stochiometric fuel-air ratio of butanol, ethanol and gasoline. Common ethanol fuel mixtures for fuel sold as gasoline currently ranges from 5% to 10%. The share of butanol can be 60% greater than the equivalent ethanol share, which gives a range from 8% to 16%. "Equivalent" in this case refers only to the vehicle's ability to adjust to the fuel.

Other properties such as energy density, viscosity and heat of vaporization will vary and may further limit the percentage of butanol that can be blended with gasoline [62]. Consumer acceptance may be limited due to the offensive smell of butanol [62]. Plans are underway to market a fuel that is 85% Ethanol and 15% Butanol (E85B), so existing E85 internal combustion engines can run on a 100% renewable fuel that could be made without using any fossil fuels. Since it is a longer hydrocarbon chain, butanol is be fairly non-polar. It is also more similar to gasoline than it is to ethanol. Butanol has been demonstrated to work in vehicles designed for use with gasoline without modification.

2.9.11. CURRENT USE OF BUTANOL IN VEHICLES

Currently no production vehicle is known to be approved by the manufacturer for use with 100% butanol. As of early 2009, only few vehicles are approved for even using E85 fuel (i.e. 85% ethanol + 15% gasoline) in the USA. However, in Brazil all vehicle manufacturers (Fiat, Ford, VW, GM, Toyota, Honda, Peugeot, Citroen and others) produce flex fuel vehicles that can run on 100% ethanol or any mix of ethanol and gasoline. These flex fuel cars represent 90% of the sales of personal vehicles in Brazil, in 2009. BP and Dupont, engaged in a joint venture to produce and promote butanol fuel, claim [64] that "biobutanol can be blended up to 10%v/v in European gasoline and 11.5%v/v in US gasoline" [64].

David Ramey drove from Blacklick, Ohio, to San Diego, California, using 100% butanol in an unmodified 1992 Buick Park Avenue [58]. In the 2009 Petit Le Mans race, the No. 16 Lola B09/86 - Mazda MZR-R, of Dyson Racing, ran on a mixture of biobutanol and ethanol developed by team technology partner BP.

2.10 BAMBOO

Bamboo is a taxonomic group of large woody grasses [11]. It grows naturally in subtropical and temperate zones on all over the world [65]. Bamboo species have high growth rates, the ability to grow in a wide range of climates, sequester carbon and have low ash content and alkali index [65, 66].

The chemical composition of Bamboo is similar to that of wood. The main constituents of bamboo culms are cellulose, hemi-cellulose and lignin, which amount to over 90% of the total mass. The minor constituents of bamboo are resins, tannins, waxes and inorganic salts. Compared with wood, however, bamboo has higher alkaline extractives, ash and silica contents (Tomalang et al. 1980; Chen et al., 1985).

Bamboo contains other organic composition in addition to cellulose and lignin. It contains about 2 – 6% starch, 2% de-oxidized saccharide, 2 – 4% fat, and 0.8-6% protein. The carbohydrate content of bamboo plays an important role in its durability and service life. Durability of bamboo against mold, fungal and bores attack is strongly associated with its chemical composition. Bamboo is known to be susceptible to fungal and insect attack. The natural durability of bamboo varies between 1 and 36 months depending on the species and climatic condition (Liese 1980). The presence of large amounts of starch makes bamboo highly susceptible to attack by staining fungi and powder-post beetles (Mathew and Nair 1988). Higher benzene – ethanol extractives of some bamboo species could be an advantage for decay resistance (Feng et al. 2002).

The ash content of bamboo is made up of inorganic minerals, primarily silica, calcium and potassium. Manganese and magnesium are two other common minerals. Silica content is the highest in the epidermis, with very little in the species can adversely affect the processing

machinery. The internode of solid bamboo has significantly higher ash, 1% NaOH, alcohol-toluene and hot water soluble than the nodes (Mabilangan et al. 2002).

Since the amount of each chemical composition of bamboo varies with age, height, and layers the chemical compositions of bamboo are correlated with its physical and mechanical properties. Such variation can lead to obvious physical and mechanical properties changes during the growth and maturation of bamboo.

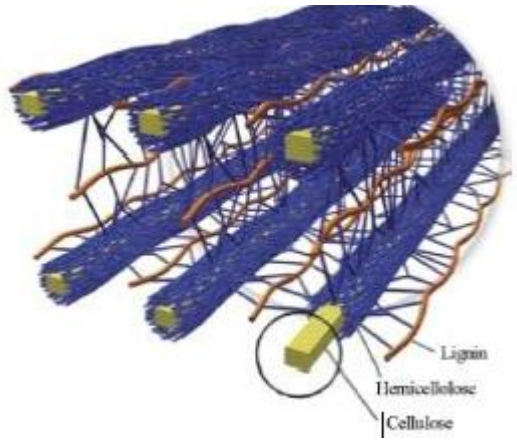


Figure 2.2. Typical Ligno-cellulose biomass

Ligno-cellulose: Ligno-cellulose is a loose compound of lignin and cellulose. Lignin is not a single chemical compound. The name represents a class of closely resembling chemical compounds.

Cellulose: Cellulose $(C_6H_{10}O_5)_n$ is a carbohydrate. It forms the primary structural component of green plants. For the plants, the primary cell wall is made of cellulose and the second cell wall is made of cellulose with a varying amount of lignin. Cellulose is also the most abundant form living terrestrial biomass in the world, which in combination with lignin and hemicellulose can be found in all the plants (Crawford, 1981). It is also the major constituent of paper and for the synthesis of the plastic celluloid.

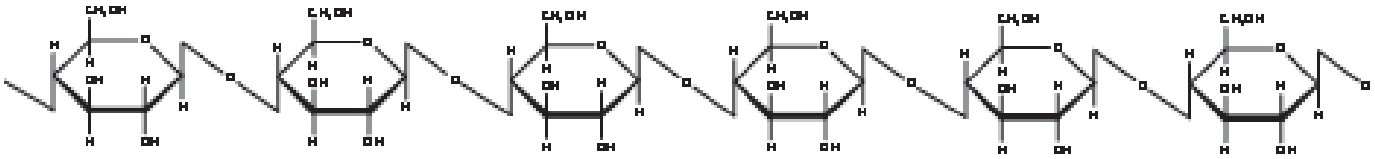


Figure2.3: Structure of Cellulose

Lignin: Lignin is an integral part of the cell walls of plants, especially in tracheids, xylem fibers and sclereids. It is the second most abundant organic compound on earth after cellulose. Lignin makes up about one-quarter to one-third of the dry mass of wood. The lignin fills the cell wall of the plant in the space among the cellulose, hemicellulose and pectin components. It confers mechanical strength to the cell walls and thus the whole plant. It is important in conducting water in culms. Since it is difficult to degrade, it helps to build a barrier to defend the plant against the invasion of pathogens and enhances the durability of the plant. The high lignified wood is durable and yields more energies than cellulose. However, it is a detrimental for paper making and, therefore, should be removed by pulping.

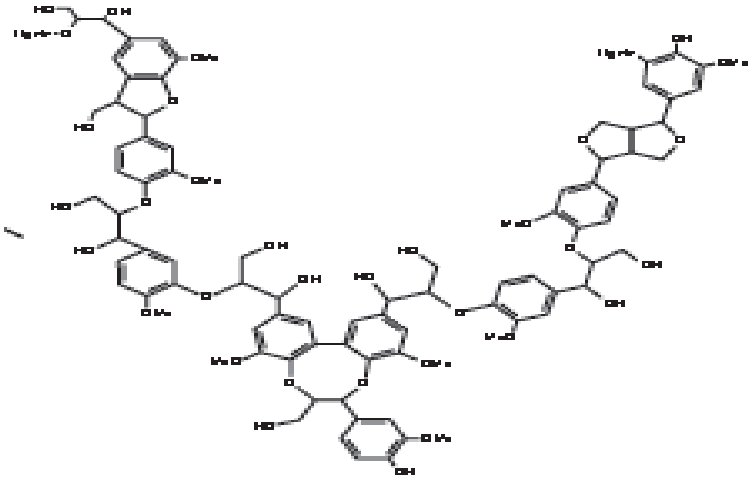


Figure 2.4: Structure of Lignin

Hemicellulose: Hemicellulose is similar to cellulose but is less complex. Hemicelluloses bind with pectin to cellulose to form a network of cross-linked fibers in plants. The hemicellulose in bamboo has its main component xylan between that of the hardwood and softwood.

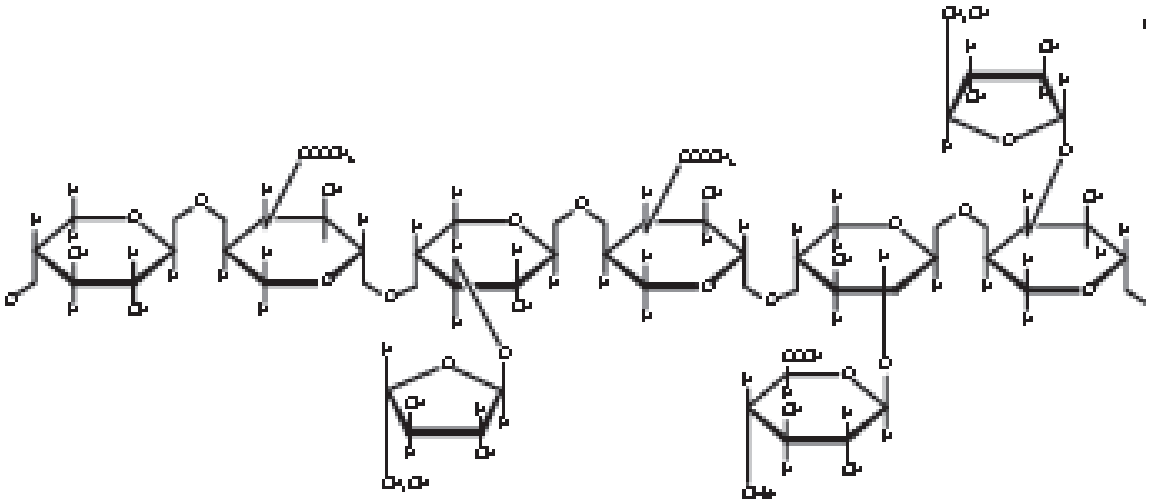


Figure 2.5: Structure of Hemicellulose

Table 2.4: Chemical Compositions of bamboo and softwood (Janssen, 1981).

	Cellulose (%)	Lignin (%)	Hemicellulose (%)
Bamboo	55	25	20
Softwood	50	25	25

Bamboo is truly amazing, it has over 1,000 uses that have been helping people, animals and the environment for hundreds of years.

`

2.11. PRE-TREATMENT

The pre-treatment of feedstock is needed to break down the lignocellulosic matrix and optimize the overall conversion of biomass to fuel. Pre-treatment methods are designed to remove the lignin and hemicellulose, reduce cellulose crystallininty and increase the porosity of the material to allow for better access of catalysts. Figure 2.1, presents an illustration for the effects of pretreatment. The ultimate goal is to improve the formation of sugars or availability to

subsequently form sugars by hydrolysis, avoid degradation and loss of carbohydrates, and avoid formation of byproducts that inhibit subsequent hydrolysis and fermentation processes [59].

Pretreatment techniques can be physical, chemical and biological [11]. Since each technology has inherent advantages and disadvantages, there is no "winning" method for pretreatment as of yet

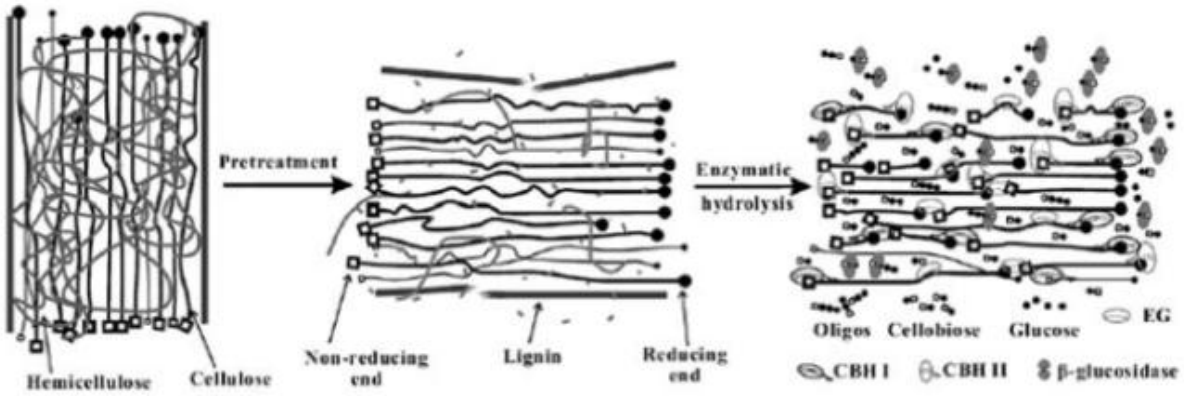


Figure 2.6. Dilute acid pre-treatment and enzymatic hydrolysis schematic from Wu et al. 2010.

Physical pretreatment approaches include: steam explosion; hot water; mechanical grinding or milling and high energy radiation. Mechanical comminution disrupts cellulose crystallininty and increases the surface area of the biomass. However, it does not remove lignin [60]. Seldom used exclusively, mechanical techniques are time and energy intensive.

Chemical pretreatment is perhaps the most widely studied group of technologies, with industrial applications of chemical pretreatment in paper production. Acid, alkaline, ammonia fiber explosion (AFEX), organosolv and ionic liquid pretreatment are major chemical pretreatment techniques. Of course, the effectiveness of each pretreatment depends on the substrate and treatment conditions.

Acid or base pretreatments can promote hydrolysis and improve the glucose yield by removing lignin and hemicellulose [67]. While concentrated acid treatments are effective, the recovery and neutralization of the acid, as well as the reactors necessary to handle the hazardous

and corrosive acid are expensive. Dilute acid treatments have been further investigated as feasible processes. Dilute sulfuric acid, dilute nitric acid, dilute phosphoric acid and dilute hydrochloric acid have been reported in literature [11].

Prior research by Pingali et al. found that dilute sulfuric acid pretreatment increased the cross-sectional radius of crystalline cellulose fibrils in switchgrass. They also found a removal of hemicellulose and formation of lignin aggregates [68]. Nitric acid reduces contaminant costs compared to sulfuric acid, but the higher cost of the acid counterbalances this alternative. Organosolv is a delignification process, and the organic solvents commonly used include methanol, ethanol, acetone, ethylene glycol, tetrahydrofurfuryl alcohol, glycerol and aqueous phenol [60]. Like concentrated acid treatments, the organic solvents are costly and must be recovered to reduce costs. Furthermore, the solvents can inhibit subsequent hydrolysis and fermentation.

Ionic liquids (ILs) can dissolve large amounts of cellulose at mild conditions and offer advantages over organosolv methods, particularly because the ILs are easily recovered in high purity. Ionic liquids include N-methylmorpholine-N-oxide monohydrate (NMMO), 1-nbutyl-3-methylimidazolim chloride (BMIMCl) and 1-allyl-3methylimdazolium chloride (AMIMCl). The interaction between the oxygen and hydrogen atoms of cellulose hydroxyl groups and the ionic liquids forms complexes that break the hydrogen bonds between molecular chains of cellulose. Consequently the cellulose is solubilized and recovered by precipitation in anti-solvents [67].

Biological pretreatment involves the used microorganisms, such as rot fungi and bacteria, to decompose the biomass, before enzyme hydrolysis. While brown and soft rot fungi break down cellulose, white rot fungi has been shown to degrade lignin by a co-oxidative process. The

greatest drawback of biological treatment is that the reaction is slow and requires monitoring of growth conditions.

In this study, dilute acid pretreatment using H_2SO_4 and NaOH were chosen due to the simple experimental setup, reasonable cost and proven efficacy for woody biomass samples.

2.11.1. SULFURIC ACID PRETREATMENT

Concentrated sulfuric acid has a very strong affinity for water. It is sometimes used as a drying agent and can be used to dehydrate (chemically remove water from) many compounds, e.g., carbohydrates. It reacts with the sugar sucrose, $C_{12}H_{22}O_{11}$, removing eleven molecules of water, H_2O, from each molecule of sucrose and leaving a brittle spongy black mass of carbon and diluted sulfuric acid. The acid reacts similarly with skin, cellulose, and other plant and animal matter.

Cellulosic raw materials are hydrolyzed by dilute sulfuric acid to remove hemicellulose, after which the solid residue is separated and is treated with concentrated sulfuric acid to dissolve cellulose contained therein. After blending and mixing of the residue in the concentrated sulfuric acid under mild reaction conditions, cellulose is re-precipitated by addition of water or an organic solvent such as methanol. The recovered cellulose can then be hydrolyzed by cellulose enzymes and/or dilute acids to provide a high yield of glucose. High level recovery and re-concentration of the sulfuric acid is also disclosed [6].

The hydrolysis of cellulosic material using sulfuric acid, removes the hydrolyzed hemicellulose portion from the remainder of the cellulosic material residue. Blending and mixing residue with concentrated sulfuric acid under mild conditions to dissolve and partially hydrolyze

the cellulose portion of residue, while the lignin portion being substantially unaffected by the mild reaction conditions and remaining as a solid.

Hydrolyzing the cellulosic material with dilute sulfuric acid to remove the hemicellulose portion of the cellulosic material in the form of a liquid hydrolysate; separating solid residue including the cellulose and lignin portions of the cellulosic material from liquid hydrolysate.

2.11.2. SODIUM HYDROXIDE PRETREATMENT

Cellulose does not react directly with NaOH. What changes when you treat the wood with NaOH is the level of contamination with other sugars. The plant cell wall (the stuff wood is made of) is a mixture of many different substances, especially polysaccharides and lignin. All of these compounds are stable in NaOH. However, they can be extracted from the wood under different conditions. When you treat the wood with hydroxide, you extract many polysaccharides from the wood (called hemicelluloses) and get cellulose almost pure. When wood is treated with hydroxide 4H (care is needed, it is extremely corrosive and dangerous at such a high concentration), it extracts almost all the hemicellulose (xylans, xyloglucans, galactomannans, etc.) and get cellulose. This is not a chemical reaction, but a physical characteristic of these polysaccharides (solubility under these chaotropic conditions). Some fragmentation probably occurs (hydrolysis), but since cellulose is a very big molecule, it does not make much difference.

2.11.3. ENZYMATIC HYDROLYSIS

After pretreatment, hydrolysis (also referred to as saccharification) is used to convert the polysaccharides into sugars. The hydrolysis process is often carried out by enzymes isolated from bacteria [11] and fungi [11]. These depolymerize the cellulose into simple sugars. The main

groups of cellulases are (i) endoglucanase, (ii) exoglucanases and (iii) ß-glucosidases [70]. The endoglucanses attack regions of low crystallininty in the cellulose fibers to produce free chain ends. Then, the exoglucanase removes cellobiose units from the free chain ends, and finally the ß-glucosidase hydrolyzes the cellobiose to produce glucose [69]. A simplified schematic of cellulase activity is shown in Figure 2.6.

The three stages of hydrolysis are: the adsorption of enzyme to cellulose, biodegradation of cellulose to sugars and desorption of the cellulase. The cellulase activity, reaction conditions and substrate material all influence the rate and efficacy of enzymatic hydrolysis.

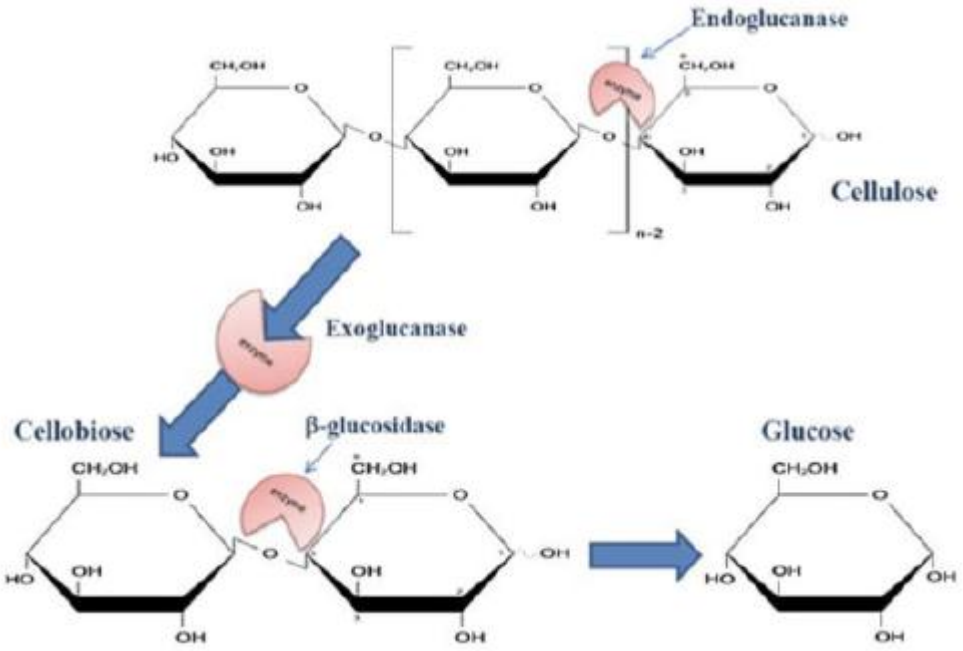


Figure 2.7. Illustration of cellulose degradation to glucose by endoglucanase, exoglucanase and β-glucosidase from Thirmal and Dahman18

In some cases, the addition of protein or additives that reduce the affinity between cellulase and lignin are used to increase the efficiency of the hydrolysis procedure [70]. Additional enzymes are required to breakdown the hemicellulose. These enzymes include xylanase, ß-xylosidase, glucuronidase, acetylesterase and glactomannanase and glucomannanse

[71]. In fully developed processes, enzymes can be recovered by membranes from the liquid supernatant and subsequently recycled.

2.11.4. FERMENTATION

Fermentation processes use a variety of bacteria and fungi to convert the sugars into alcohols of interest, namely ethanol and butanol. Recalling that the hydrolysis of cellulose and hemicellulose produces hexose (C6) and pentose (C5) sugars, it is desirable to find fermentation methods that utilizes both sugar sources. The most common fermentation yeast for ethanol production, *Saccharomyces cerevisiae* only utilizes C6 sugars, and sometimes, additional yeast strains will be added to utilize C5 sugars.

However, through genetically engineering microorganisms, researchers have designed new metabolic pathways to improve the C5 sugar processes [11].

2.11.5 CLOSTRIDIA

Clostridia are rod-shaped, spore forming, Gram positive bacteria with a natural acetone-butanol ethanol (ABE) fermentation process. Solventogenic clostridia can utilize a wide variety of substrates, including hexose, pentose and disaccharides, and convert them to desirable products. Strains of solventogenic clostridia include *C. acetobutylicum, C. beijerinckii, C. cellulolyticum, C. saccharoperbutylacetonicum, C. pasteurianum and C. isopropylicum* [72]. Clostridia have two phases to the production: acidogenesis and solventogenesis.

The pH of the medium plays an important role, as the formation of acids during the acidogenic phase decreases the pH, and once the pH reaches a critical point, solventogenesis begins. If the pH decreases below 4.5 before enough acids form, the solventogenic phase will be brief and have a lower yield [6]. The acidogenic phase generally occurs during the exponential

growth phase, with acetate, butyrate, hydrogen and carbon dioxide produced as major products. During the solventogenic phase, the acids are reassimilated into acetone, butanol and ethanol.

2.12. PRIOR WORK ON THE PRODUCTION OF ALTERNATIVE FUELS

There is ongoing research into finding more suitable biofuel crops and improving the oil yields of these crops. Using the current yields, vast amounts of land and fresh water would be needed to produce enough oil to completely replace fossil fuel usage. It would require twice the land area of the US to be devoted to soybean production, or two-thirds to be devoted to rapeseed production, to meet current US heating and transportation needs (Lane J, 2012).

Specially bred mustard varieties can produce reasonably high oil yields and are very useful in crop rotation with cereals, and have the added benefit that the meal leftover after the oil has been pressed out can act as an effective and biodegradable pesticide ("Mustard Hybrids for Low-Cost Biofuels and Organic Pesticides" Retrieved 2010-03-15.).

The NFESC, with Santa Barbara-based Biodiesel Industries is working to develop biofuels technologies for the US navy and military, one of the largest diesel fuel users in the world ("PORT HUENEME, Calif: U.S. Navy to Produce its Own Biofuels :: Future Energies: The future of energy". Future Energies Retrieved 2009-10-17).

A group of Spanish developers, working for a company called Ecofasa, announced a new biofuel made from trash. The fuel is created from general urban waste which is treated by bacteria to produce fatty acids, which can be used to make biofuels ("Newsvine - Ecofasa turns waste to biofuels using bacteria". Lele.newsvine.com. 2008-10-18 Retrieved 2009-10-17).

W. Leenakul and N. Tippayawong (2010) used biochemical conversion of lignocellulosic biomass to ethanol provides a sustainable energy production system, the bamboo (Dendrocalamus asper) was pretreated with dilute sulfuric acid prior to enzymatic hydrolysis

process to produce fermentable sugars. The amount of dry feedstock solid/liquid loading at 10% w/w was pretreated in an autoclave at different temperatures (120, 140°C) with different residence times (30, 60, 90 min) and different sulfuric acid concentrations (0.6, 0.9, 1.2% w/w). Maximum glucose and xylose yields were achieved at 140°C, 1.2% sulfuric acid concentration and 90 min. [74].

Nakorn Tippayawong and Nuttida Chanhom (2011) used Lignocellulosic biomass is an important alternative energy source to be utilized for ethanol production. In there work, bamboo (Dendrocalamus asper Backer) was used as biomass feedstock for conversion to fermentable sugars. Pretreatment was carried out with dilute sulfuric acid at concentrations between 0.4 – 1.6% w/w, and residence time between 45 – 135 min at a fixed temperature of 140^{o}C [75].

Although bamboo has been used to produce bio-ethanol, and only of recent when Amanda M. Rees [11], produced bio-butanol from bamboo using *Clostridium acetobutylicum*, this present study looks at the effect of temperature on the production of bio-butanol from bamboo, because bio-butanol was found to have a higher calorific value than that of bio-ethanol. However, there has been no prior efforts to develop and scale up the method that was used by Rees. This will be explored in this study using dilute H_2SO_4 acid for pre-treatment and varying the acid concentration, pre-treatment time and temperature.

REFERENCE

[1]. "AccessScience |Encyclopedia Article | Alcohol fuel".Accessscience.com. Retrieved 2008-11-06.

[3]. Energy – Consumption'!A1 "Consumption by fuel, 1965–2008" (XLS). Statistical Review of World Energy 2009, BP. 31 July 2006. Retrieved 24 October 2009.

[4]. State of the World 2009, Worldwatch Institute, 2009

[5]. Global Energy Review in 2011, Enerdata Publication

[6]. IEA Key energy statistics 2010 and IEA Key energy statistics 2009 oil page 11, gas p.13, hard coal (excluding brown coal) p. 15 and electricity p. 27

[7]. "Solar Radiation and Climate Experiment". National Aeronautics and Space Administration. Retrieved. 21 December, 2011.

[8]. Energy in Sweden 2010, Facts and figures Table 55 Regional energy use, 1990 and 2008 (kWh per capita)

[10]. http://www.businessdayonline.com/NG/index.php/economic-watch/30814-q3-2011-nigeria-records-218-mbd-in-crude-oil-production.

[11]. Amanda M. M. Rees: Bamboo to Butanol: Production of Lignocellulosic Butanol through Fermentation by Clostridia, Senior Thesis, Department of Chemical and Biochemical Engineering, Princeton University, Princeton, New Jersey, USA. 2012.

[12]. Wheeler, Jill (2008). Alternative Cars. ABDO. p. 21. ISBN 978-1-59928-803-1.

[13]. Thirmal, C.; Dahman, Y. Comparisons of existing pretreatment, saccharification and fermentation processes for butanol production from agricultural residues. *Canadian Journal of Chemical Engineering.* 2011, *9999,* 1-17.

[14]. Development and Demonstration of Hydrogen and Compressed Natural Gas (H/CNG) Blend Transit Buses, NREL, 2005, http://www.afdc.energy.gov/afdc/pdfs/38707.pdf

[15]. Kristin Brekke "Butanol, an energy alternative?" Ethanol Today, March 2007, Retrieved 2010-11-12**.**

[16]. Balmer, Robert T.. "14.15 Reversed Stirling Cycle Refrigeration". *Modern Engineering Thermodynamics*. Academic Press. ISBN 978-0-12-374996-3.

[17]. Raili Leino (22.10.2012). "Mullistava idea: Tulevaisuuden auto voi kulkea typpimoottorilla" (in Finnish). *Tekniikka&Talous*.

[18]. Towards sustainable production and use of resources: Assessing Biofuels, 2009, International Resource Panel, United Nations Environment Programme.

[19]. “Biofuels Make a Comeback Despite Tough Economy". Worldwatch Institute. 2011-08-31. Retrieved 2011-08-31.

[20]. Frauke Urban and Tom Mitchell 2011. Climate change, disasters and electricity generation. London: Overseas Development Institute and Institute of Development Studies.

[21]. Yirka, Bob (2011-09-05). "Pair claim they can make ammonia to fuel cars for just 20 cents per liter". Physorg.com. Retrieved 2011-09-12.

[22]. Patakova, P.; Linhova, M.; Rychtera, M.; Paulova, L.; Melzoch, K. Novel and neglected issues of acetone-butanol-ethanol (ABE fermentation by Clostridia: *Clostridum* metabolic diversity, tools for process mapping and continuous fermentation systems. *Biotechnology Advances.* 2012.

[23]. Jones, D.T., “Biobutanol”, Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand, Biotechnology. Vol. VI.

[24]. Duff, S.J.; Murray, W.D. Bioconversion of forest products industry waste cellulosics to fuel ethanol: a review. *Bioresource Technology.* **1996**, *55,* 1-33.

[25]. Lobovikov, M.; Paudel, S.; Piazza, M.; Ren, H.; Wu, J. World bamboo resources: A thematic study prepared in the framework of the Global Forest Resources Assessment 2005. *Food and Agriculture Organization of the United Nations.* 2007.

[26]. “With only 2/3 the energy of gasoline, ethanol costs more per mile". zFacts.com. 2007-04-27. Retrieved 2008-03-07.

[27]. Briggs, Michael (August 2004). "Widescale Biodiesel Production from Algae". Archived from the original on March 24, 2006. Retrieved 2007-01-02 publisher = UNH Biodiesel Group (University of New Hampshire).

[28]. Demirbas, A. (2009). "Political, economic and environmental impacts of biofuels: A review". *Applied Energy* 86: S108–S117. DOI:10.1016/j.apenergy.2009.04.036. edit

[29]. Evans, G. "Liquid Transport Biofuels - Technology Status Report", *National Non-Food Crops Centre*, 2008-04-14. Retrieved on 2009-05-11.

[30]. “Biofuel Production". *European Biofuels Technology Platform*. Retrieved 17 May 2011.

[31]. “IEA says biofuels can displace 27% of transportation fuels by 2050 Washington". *Platts*. 20 April, 2011.

[32]. Pingali, S.; Urban, V.; Heller, W.; McGaughey, J.; O'Neill, H.; Foston, M.; Myles, D.; Ragauskas, A.; Evans, B. Breakdown of Cell Wall Nanostucture in Dilute Acid Pretreated Biomass. *Biomacromolecules*. 2010, *11*, 2329-2335.

[33]. UNEP.org-Properties of oxygenates (PDF).

[34]. "BIOGAS: No bull, manure can power your farm." Farmers Guardian (September 25, 2009): 12. General OneFile. Gale.

[35]. Lane, Jim. "US warplanes can fly faster, carry additional weapons load using advanced fuels and biofuels." *Biofuels Digest*, 21 May 2012.

[36]. "Threat to Great Apes Highlighted at Virunga Meeting". America.gov. Retrieved 2010-07-14.

[37]. Wu, X.; McLaren, J.; Madl, R.; Wang, D. Biofuels from Lignocellulosic Biomass. *Sustainable Biotechnology.* **2010,** 19-41.

[38]. Fisher, Lawrence M. April 24th 2007. "Carbon gas is explored as a source of ethanol" *New York Times*.

[39]. Bailey, J.E. and Ollis, D.F., Biochemical Engineering Fundamentals, 2nd Ed., p163-172, McGraw-Hill, 1986.

[40]. Durre, Peter, "Biobutanol: An Attractive Biofuel." Biotechnology, Journal 2. 12 (2007): 1525-1534.

[41]. Huang, He, Hui Lui, and Yi-Ru Gan. "Genetic Modification of Critical Enzymes and Involved Genes in Butanol Biosynthesis from Biomass." *Biotechnology Advances* 471.1 (2010).

[42]. Zheng, Y.; Zhongli, P.; Zhang, R. Overview of biomass pretreatment for cellulosic ethanol production. *Int. J. Agricu. & Biol Eng.* 2009, *2*, 51-68.

[43]. National Renewable Energy Laboratory (2007-03-02). "Research Advantages: Cellulosic Ethanol". National Renewable Energy Laboratory. Retrieved 2012-04-02.

[44]. "Jack Pronk's elephant yeast". Tnw.tudelft.nl. Retrieved 2010-07-14.

[45]. R. E. Teixeira (2012). "Energy-efficient extraction of fuel and chemical feedstocks from algae". *Green Chemistry* 14 (2): 419-427. DOI:10.1039/C2GC16225C.

[46]. ScienceDirect.com - Biomass and Bioenergy - Impact of biofuel production and other supply and demand factors on food price increases in 2008.

[47]. B.N. Divakara, H.D. Upadhyaya, S.P. Wani, C.L. Laxmipathi Gowda (2010). "Biology and genetic improvement of Jatropha curcas L.: A review". *Applied Energy* 87 (3): 732-742. DOI:10.1016/j.apenergy.2009.07.013.

[48]. REN21 (2011). "Renewables 2011: Global Status Report". pp. 13–14.

[49]. Coughlan, M.P.; Ljungdahl, L.G. Comparative biochemistry of fungal and bacterial cellulolytic enzyme system. *Biochemistry and Genetics of Cellulose Degradation*; Aubert, J.; Benguin, P.; Millet, J. Ed. 1988.

[50]. Searchinger, Timothy; Ralph Heimlich, R.A. Houghton, Fengxia Dong, Amani Elobeid, Jacinto Fabiosa, Simla Tokgoz, Dermot Hayes, Tun-Hsiang Yu (2011 [last update]). "Use of U.S. Croplands for Biofuels Increases Greenhouse Gases Through Emissions from Land-Use Change". sciencemag.org. DOI:10.1126/science.1151861. Retrieved November 8, 2011.

[51]. http://www.oilgae.com/energy/sou/ae/re/be/alc/but/but.html

[52]. National Non-Food Crops Centre. "GHG Benefits from Use of Vegetable Oils for Electricity, Heat, Transport and Industrial Purposes, NNFCC 10-016", Retrieved on 2011-06-27.

[53]. "Valcent Products Inc. Develops "Clean Green" Vertical Bio-Reactor". Valcent Products. Retrieved 2008-07-09.

[54]. DICTIONARY OF AUTOMOTIVE TERMS - "En" (scroll way down to "engine knock")". Retrieved 2008-03-21.

[55]. Ethanol Research (2012-04-02). "National Corn-to-Ethanol Research Center (NCERC)". Ethanol Research. Retrieved 2012-04-02.

[56]. Cedric Briens, Jan Piskorz and Franco Berruti, "Biomass Valorization for Fuel and Chemicals Production - A Review," 2008. International Journal of Chemical Reactor Engineering, 6, R2.

[57]. "Council Directive 85/536/EEC of 5 December 1985 on crude-oil savings through the use of substitute fuel components in petrol". Eur-lex.europa.eu. Retrieved 2010-07-14.

[58]. http://www.license.umn.edu/Products/Co-cultured-Synechococcus-and-Shewanella Produce-Hydrocarbons-without-Cellulosic-Feedstock__20100084.aspx".

[59]. Sun, Y.; Cheng, J. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*. **2002**, *83*, 1-11.

[60]. Yeoman CJ, Han Y, Dodd D, Schroeder CM, Mackie RI, Cant IK. (2010) Yeoman CJ, Han Y, Dodd D, Schroeder CM, Mackie RI, Cann IK (2010). "Thermostable enzymes as biocatalysts in the biofuel industry". *Adv. Appl. Microbiol.* 70: 1–55. DOI:10.1016/S0065-2164(10)70001-0. PMID 20359453.. Advances in Applied Microbiology 70: 1 – 55.

[61]. THE FUTURIST, Will Thurmond. July–August 2007.

[62]. The Impact of US Biofuel Policies on Agricultural Price Levels and Volatility, By Bruce A. Babcock, Center for Agricultural and Rural Development, Iowa State University, for ICTSD, Issue Paper No. 35. June 2011.

[63]. "Virgin unveils 'ground-breaking' jet fuel". *Travel Weekly.co.uk*. 11 October 2011. Retrieved 14, October, 2011.

[64]. American Coalition for Ethanol (2008-06-02). "Responses to Questions from Senator Bingaman". American Coalition for Ethanol. Retrieved 2012-04-02.

[65]. Sergeeva, Y. E.; Galanina, L. A.; Andrianova, D. A.; Feofilova, E. P. (2008). "Lipids of filamentous fungi as a material for producing biodiesel fuel". *Applied Biochemistry and Microbiology* 44 (5): 523. DOI:10.1134/S0003683808050128. edit

[66]. Is Algae Based Biofuel a Great Green Investment Opportunity". Green World Investor. 2010-04 -06. Archived from the original on 17 June 2010. Retrieved 2010-07-11.

[67]. Navy demonstrates alternative fuel in riverine vessel". Marine Log. 2010-10-22. Retrieved 2010 - 07-11.

[68]. Biofuels Magazine (2011-04-11). "Energy Farming Methods Mature, Improve". Biofuels Magazine. Retrieved 2012-03-08.

[69]. Evans, Jon (14 January 2008). "Biofuels aim higher". *Biofuels, Bioproducts and Biorefining (BioFPR)*. Retrieved 2008-12-03.

[70]. Sheehan, John; et al. (July 1998). "A Look Back at the U. S. Department of Energy's Aquatic Species Program: Biofuels from Algae". National Renewable Energy Laboratory. Retrieved 16 June 2012.

[71]. "Customs seize illegal fuel". BBC News. 2004-12-09. Retrieved 2010-07-25.

[72]. Laurent, L. Production of biobutanol from white grape pomace by Clostridium saccharobutylicum using submerged fermentation. M.S. Thesis, Auckland University of Technology. 2010.

[73]. Hydrogen/Natural Gas (HCNG) Fuel Blends". Office of Energy Efficiency and Renewable Energy (EERE). 2009-10-07. Retrieved 2010-07-11.

[74]. Leenakul, W.; Tippayawong, N. Dilute Acid Pretreatment of Bamboo for Fermentable Sugar Production. Journal of Sustainable Energy & Environment 1 (2010) 117-120.

[75]. Tippayawong, N.; Chanhom N. Conversion of Bamboo to Sugars by Dilute Acid and Enzymatic Hydrolysis. International Journal Of Renewable Energy Research, IJRER: Vol. 1, No. 4, pp. 240-244, 2011

CHAPTER THREE

MATERIALS AND METHODS

3.1. MATERIALS

The Bamboo (*Bambusa vulgaris*) sample was obtained from A.K. Merchants & Co. Opposite Games Village, Along Airport Road, Abuja. Two commercially available enzymes were ordered through Sigma Aldrich: a cellulase cocktail from the fungi *Trichoderma reesei* (Celluclast 1.5 L) and ß-glucosidase from *Aspergillus niger* (Novozym 188). All chemicals were reagent grade ordered from Sigma (St. Lousi, MO, USA) and Fisher (Fair Lawn, NJ, USA).

Figure 3.1: Bamboo Stick

Figure 3.2: Bamboo Sample

3.2. RAW MATERIAL COMPOSITION

X-Ray Diffraction technique was used to determine the different compounds that were found in bamboo sample, after breaking down to powder.

X-ray diffraction is based on the Bragg's law of diffraction, expressed as:

$$n\lambda = 2dsin\theta$$

Where λ is the wavelength of the incident radiation (Cu radiation), d is the spacing between the (hkl) planes, n is an integer, and φ is the angle between the incident beam and the scattering planes. The XRD is used to obtain information on the crystalline structure of the waxes obtained. The diffractograms of the waxes at room temperature are required in order to analyse the crystallinity of the sample.

Thin film X-ray diffraction analysis of wax was carried out using X'Pert PRO MPD diffractometer (PANanalytical, Almelo). The PAnalytical MPD Pro XRD system was switched on alongside the chiller to maintain the desire temperature while avoiding overheating. The XRD system was then operated from the computer using the X'Pert data collector software. The sample was prepared by depositing the wax on thin film, placed in the sample holder and pressed down to prevent a shift in the peak by making sure the surface was at the zero line. The XRD data were acquired using copper K-Alpha1 [Å] radiation from a rotating anode generator operated at 40kV and 30mA in the range of $2\theta = 20.0297^{\circ} - 65.3387^{\circ}$. The sample was then set in the XRD using the Gonio scan. The scan was started using the PSD scanning mode. The scanning step was 0.066^{o} while the scan step time was 26.67sec. The scan type was continuous with a divergent slit size of 0.2^{o}, chosen for high intensity.

Figure 3.3: X-Ray Diffraction Machine

3.3. DILUTE ACID PRE-TREATMENT

The bamboo was ground to fine particles using a mortar and sieved to select particles of 250-500μm. Dilute sulfuric acid (0.5 and 1.0% v/v) solutions were prepared, and acid solutions were added to 300mg samples of bamboo in 20mL glass vials. Then, the samples were heated and stirred at various temperatures (25, 110, 120, 150 and 200°C) using a hotplate and an oven for temperatures above 110^{o}C. The temperature was monitored using a thermometer. The samples were heated for (2 and 4 hours). The surface morphologies of the sample were imaged with an optical microscope after the pre-treatment. Samples of the solution were collected for subsequent sugar analysis. After heating, the samples were removed and allowed to cool to room temperature. The remaining solid was filtered and the insoluble solids were washed with deionized water until the pH of the wash was above 5.

Figure 3.4: Pounding of bamboo

Figure 3.5: Grinding of Bamboo

Figure 3.6: Dilute Acid Pre-treatment

Figure 3.7: Filtrate of Pre-treatment

3.4. OPTICAL MICROSCOPY ANALYSIS

All the samples (before and after the pre-treatment stage) were allowed to dry. They were all ground using 320 grit paper to the finial polishing stage of 600. The surface morphology of the samples was then studied under a digital microscope.

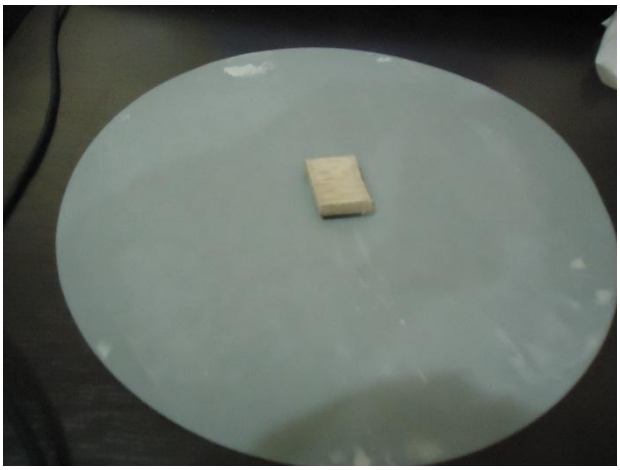


Figure 3.8: Polishing of Bamboo Sample

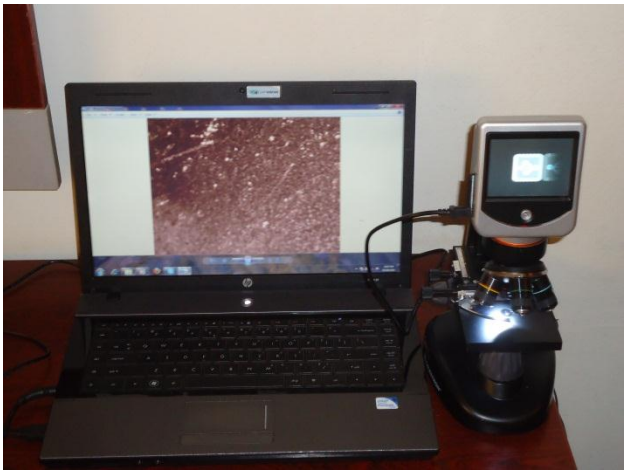


Figure 3.9: Digital Microscope (Celestron Model #44345)

3.5. MOISTURE ABSORPTION

The samples were weighed before the dilute acid pre-treatment and were left to dry for 24 hours after the acid pre-treatment. The samples were then weighed again using a digital weighing balance, in order to determine the weight loss of the bamboo sample after dilute acid pre-treatment.

Figure 3.10: Digital Weighing Balance

3.6. ENZYMATIC HYDROLYSIS

The washed and filtered biomass solids from the pre-treatment stage were transferred to conical flasks. A 10mL of citrate buffer (pH 4.8) was, then added, along with 1.2 mL of *cellulase* from (*Trichoderma reesei*) and 0.3 mL of ß-glucosidase from (*Aspergillus niger*). Samples were then placed in a New Brunswick Scientific incubator shaker at 37 °C for 72 hours on a rotary shaker. This was operated at a speed of 121 rpm. Additionally, 1 mL samples were collected for analysis after 72 hours. A sample of un-pretreated biomass with enzymes was used as a control.

Figure 3.11: Enzymatic Hydrolysis Process

Figure 3.12: New Brunswick Scientific incubator shaker

3.7. SUGAR ASSAY

A qualitative test using Benedict's reagent to test for the presents of reducing sugars. 1 mL of standard glucose, sucrose, the filtrate from the pre-treated bamboo and the filtrate from the enzymatic hydrolysis were put into different test tubes and few drops of the Benedict's solution were added.

DNS (3,5-dinitrosalicylic acid) reagent was prepared at Sheda Science and Technology Complex, (SHESTCO), Abuja, Nigeria. This was done by mixing 45 gms of sodium potassium

tartrate dissolved in 75 mL of water and 1.5 gm of DNS was dissolved in 30mL of 2M/Liter NaOH.

Stock standard sugar (glucose) was prepared by dissolving 250 mg of glucose in was and water was added until the volume reached 100 mL and 10mL from the stock solution was taken and water was added until the volume reached 100 mL.

Seven clean, dry test tubes were taken and solutions of the standard sugar solution (glucose), ranging from 0 to 3 mL were measured into different test tubes. The volume was made up to 3 mL with distilled water in all of the test tubes. 1 mL of DNS reagent was added to all the test tubes and were covered with cotton wool and kept in a boiling water bath for 10 minutes.

3 mL of the filtrate (from both the pre-treatment and the enzymatic hydrolysis) were also measured into test tubes before adding 1 mL of DNS reagent. They were covered with cotton wool and kept in a boiling water bath for 10 minutes.

The test tubes were then allowed cooled to room-temperature, because the absorbance is sensitive to temperature and the extinction at 540 nm against a blank sample, using UV-Visible Spectrophotometer was used to measure the absorbance of the samples.

Figure 3.13: Standard Sugar and Pre-treated Samples with DNS reagent

Figure 3.14: UV-Visible Spectrophotometer.

3.8. REMOVAL OF INHIBITORS

Removal of Inhibitors was carried by detoxification of hydrolysate using $Ca(OH)_2$ according to methods reported by Ezeji et al [76]. The pH of the hydrolysate was adjusted to 10.1 using $Ca(OH)_2$ and then Na_2SO_3 was added at 1 mg/mL. Next, the mixture was incubated in water bath for 1h at 45°C, and then the sample was centrifuged at 5000 rpm for 10 min. After centrifuging, the precipitate was discarded. Finally, the supernatant pH was adjusted to 6.8 using HCl and sterilized through a filter before fermentation.

Figure 3.15: Sample being centrifuged

3.9. FERMENTATION PROCESS

Strains of *Clostridium acetobutylicum* ATCC 824 were obtained from the American Type Culture Collection, (ATCC, Manasai, KS). The hydrolysate pH was adjusted to 6.5 using sodium hydroxide solution. Two samples were prepared: an untreated sample of hydrolysate and a sample with sugar and growth media supplements, which is the ATCC Medium: 2107 Modified Reinforced Clostridial Broth. The supplements included tryptose (1g), beef extract (1g), yeast extract (0.3g), dextrose (0.5g), sodium chloride (0.5g), soluble starch (0.1g), L-Cysteine (0.05g), sodium acetate (0.3g) were all dissolved in 100mL of distilled water.

After the pH was adjusted and media was added, the samples were sterilized through a filter paper and then placed in an anaerobic chamber overnight. Once the hydrolysate samples adjusted to the anoxic conditions, the samples were inoculated with 10% (v/v) of bacteria (C. acetobutylicum) culture growing on minimal media. A SHEL Lab Bactron Anaerobic Environmental Chamber (Cornelius, OR) was used to carry out the fermentation experiments.

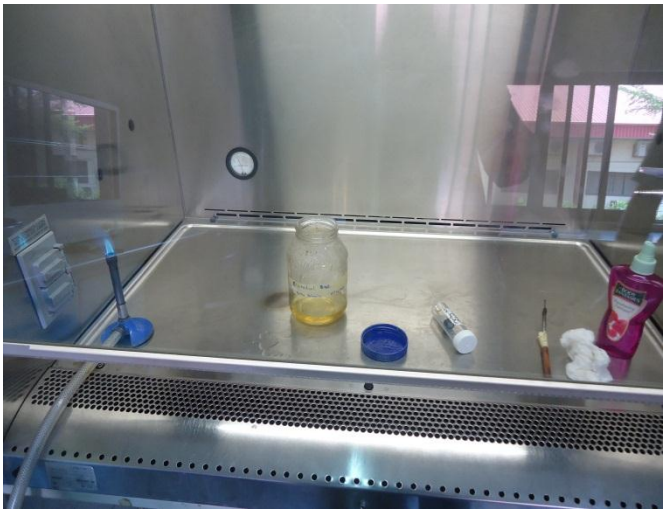


Figure 3.16: Purifier Biosafety Cabinet

Figure 3.17: Anaerobic Chamber

REFERENCE

[76]. Ezeji, T.; Qureshi, N.; Blaschek, H. Butanol production from agricultural residues: impact of degradation products on Clostridium beijerinckii growth and butanol fermentation. Biotechnology and Bioengineering. 2007, 97, 1460- 1469.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. MATERIAL COMPOSITION

Table 4.1: Material Composition of un-pretreated bamboo sample

S/No.	Material Composition	Chemical Formula	Percentage Composition (%)
1	D-Sorbitol (glucitol)	$C_6H_{14}O_6$	12.34
2	n-Docosane	$C_{22}H_{46}$	10.47
3	Lithium Myristate	$LiC_{14}H_{27}O_2$	17.86
4	n-Nonacosane	$C_{29}H_{60}$	47.66
5	Sexiphenyl	$C_{36}H_{26}$	11.67

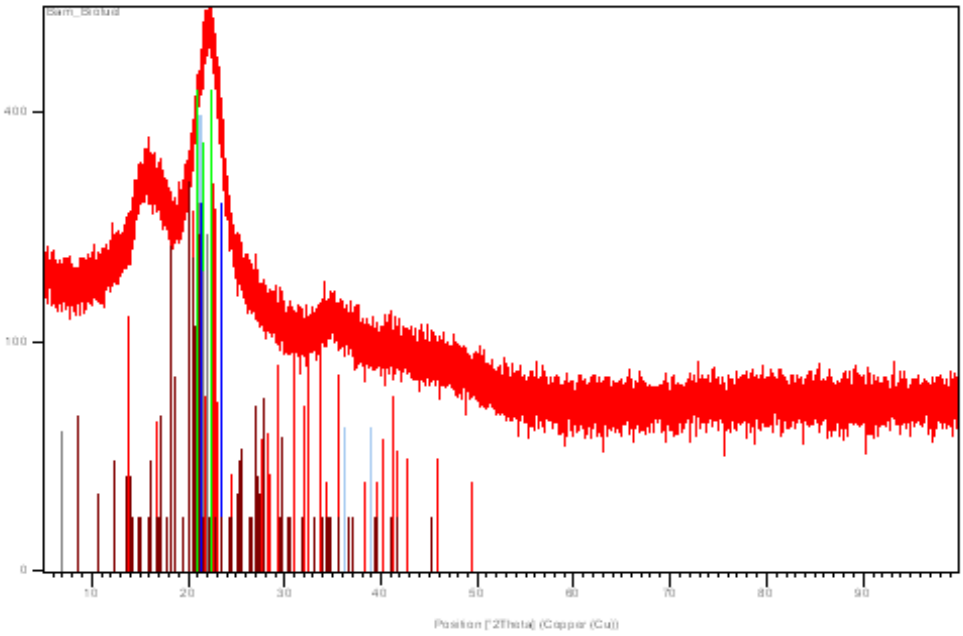


Figure 4.1: XRD of Un-pretreated bamboo sample

Table 4.1 presents the material composition and chemical formulae of different compounds obtained from the un-pretreated bamboo sample using X-Ray Diffraction technique. The results obtained show different hydrocarbons, elements such as Si and N, and compounds such as carbowax and starch. Most of the compounds are hydrocarbons that contain cellulose in them. This makes it possible to obtain reducing sugars, such as glucose, after dilute acid pre-

treatment and enzymatic hydrolysis. These are necessary for the production of butanol by fermentation. For example D-Sorbitol (glucitol) is a sugar alcohol.

4.2. EFFECTS OF TEMPERATURE ON DILUTE ACID PRE-TREATMENT

During dilute acid pre-treatment, the hemicellulose was hydrolyzed to release solubilized sugars in the pre-hydrolysate, while the cellulose and lignin remained mostly in the solid biomass. Using the glucose assays, the sugars in the pre-hydrolysate liquor were quantified to compare the effects of residence time, temperature and acid concentration during pre-treatment. Table 4.2 summarizes the sugar concentration results of glucose equivalents.

From the results obtained in Table 4.2, as the temperature and acid concentration increases, so does the amount of sugar solubilized during pre-treatment. Both higher temperatures and acid concentration were needed to remove the maximum amount of hemicellulose, and the highest glucose yield (91.80 mg/g). These were found at conditions of 1% (v/v) H_2SO_4 at 200^oC.

Table 4.2: Pre-hydrolysate sugar measurements after dilute H_2SO_4 acid pre-treatment of bamboo

Tempt. (^oC)	Concn. % (v/v)	Time (hr)	Yield (mg/g)
25	0.5	2	6.20
		4	6.70
	1.0	2	20.50
		4	5.50
110	0.5	2	12.90
		4	17.00
	1.0	2	22.30
		4	19.20
120	0.5	2	25.10
		4	22.60
	1.0	2	27.20
		4	27.90
150	0.5	2	28.70
		4	49.70
	1.0	2	29.50
		4	37.30
200	0.5	2	42.00
		4	56.10
	1.0	2	47.70
		4	91.80

Unpre-treated sample	1.0	4	4.10
Pre-treated with water	1.0	4	5.00
Sugar Cane	1.0	4	140.00

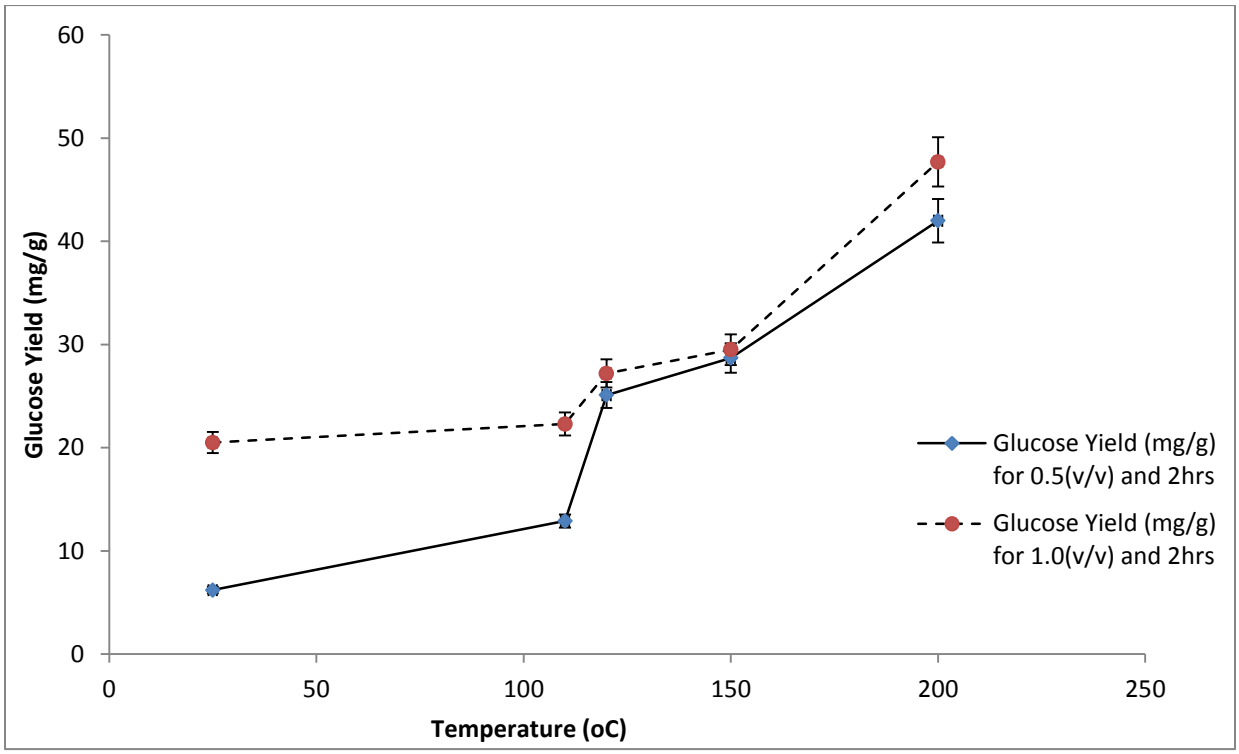


Figure 4.2: Plot of Glucose Yield (mg/g) against Temperature (^{o}C) for 2hours from dilute acid pre-treatement.

Figure 4.2 shows the relationships between the pre-treatment temperature and the amount of glucose released from the bamboo. For the bamboo samples that were subjected to dilute acid pre-treatment at room temperature, the glucose yields were the lowest. However, as the acid concentration increases, the glucose yields also increase from 6.2 mg/g at 0.5% (v/v) for 2 hours to 20.5 mg/g at 1.0% (v/v) for 2 hours. In the case of the sample pre-treated at 110^{o}C, the glucose yields were higher compared to those at room temperature. The glucose yield increased from 12.9 mg/g at 0.5% (v/v) for 2 hours to 22.3 mg/g at 1.0% (v/v) for 2 hours and for samples pre-treated at 120^{o}C. Furthermore, the glucose yields increased from 25.1 mg/g at 0.5% (v/v) for 2

hours to 27.2 mg/g at 0.5% (v/v) for 2 hours, as the acid concentration increased. For samples pre-treated at 150^{o}C, the glucose increased from 28.7 mg/g at 0.5% (v/v) for 2 hours to 29.5 mg/g at 1.0% (v/v) for 2 hours. Finally, for 200^{o}C, the glucose yield increased from 42 mg/g at 0.5% (v/v) for 2 hours to 47.7 mg/g at 1.0% (v/v) for 2 hours.

The increase in glucose yields were as result of the combined effect of the increase in temperature and acid concentration, which were able to solubilize more glucose.

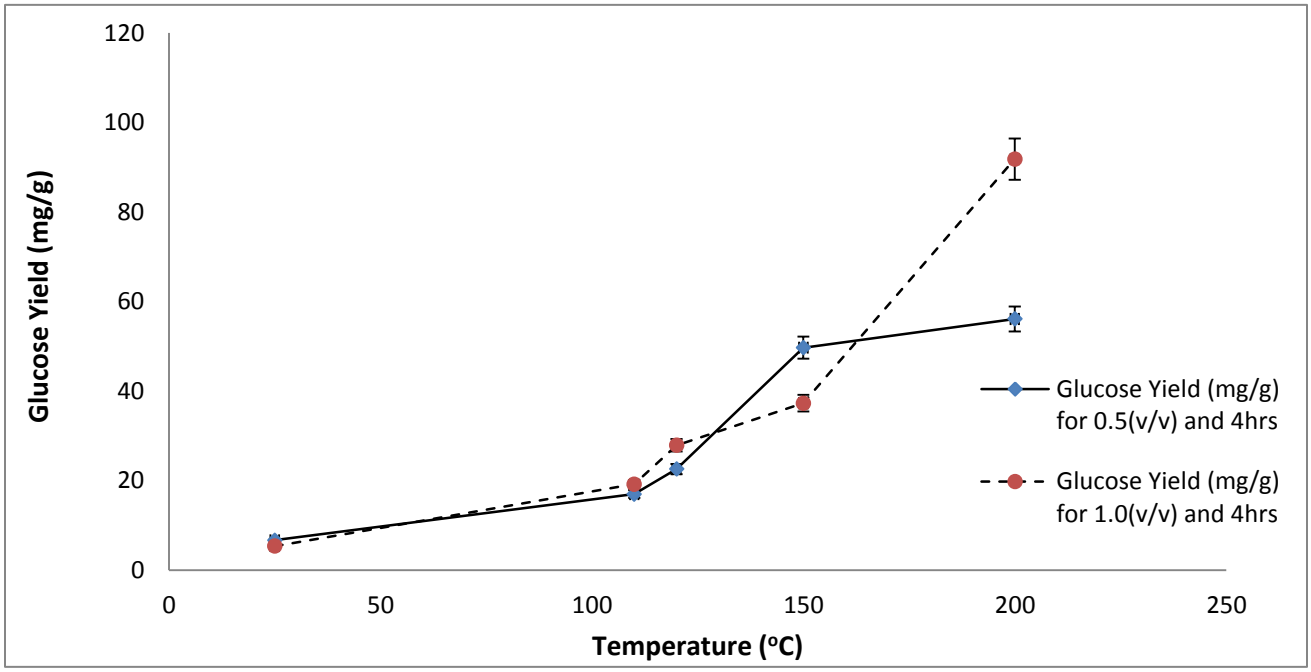


Figure 4.3: Plot of Glucose Yield (mg/g) against Temperature (^{o}C) for 4hours from dilute acid pre-treatement.

Figure 4.3 shows the relationships between the pre-treatment temperature and the amount of glucose released from the bamboo after 4 hours. Among the samples, that were not heated, the glucose yields were the lowest. However, the glucose yields were a little higher than those pre-treated for 2 hours, the glucose yields decreased from 6.7 mg/g at 0.5% (v/v) for 4 hours to 5.4 mg/g at 1.0% (v/v) for 4 hours. For samples pre-treated at 110^{o}C, the glucose yields increased from 17.0 mg/g at 0.5% (v/v) for 4 hours, to 19.2 mg/g at 1.0% (v/v) for 4 hours. Also, for

samples pre-treated at 120^{o}C, the glucose yields increased from 22.6 mg/g at 0.5% (v/v), for 4 hours to 27.9 mg/g at 1.0% (v/v) for 4 hours, as the acid concentration increase. For samples pre-treated at 150^{o}C the glucose yields decreases from 49.7 mg/g at 0.5% (v/v) for 4 hours to 37.3 mg/g at 1.0% (v/v) for 4 hours. Finally, for samples pre-treated at 200^{o}C, the glucose yields increases from 56.1 mg/g at 0.5% (v/v) for 2 hours, to 91.8 mg/g at 1.0% (v/v) for 4 hours.

The increase in glucose yields is as result of the combine effect of increase in temperature, increase in acid concentration and residence time. This is shown in Figures 4.2 and 4.3.

Furthermore, a concern that arises in pre-treatment and hydrolysis techniques is that sugars can degrade to form undesired products. However, the results from this study showed that, as the residence time increased from 2.0 to 4.0 hours, the amount of glucose generally increased. In contrast, a decrease in sugar concentrations (over long time intervals) would have indicated the presence of side reactions that convert or degrade the sugars into alternate products (i.e. acids). A notable increase of solubilized glucose was observed in the 1% (v/v) 200°C sample. However, this was partially attributed to the evaporation of water during the heating procedure. Still, this increase in glucose might indicate that the residence time could be extended even further to liberate the maximum amount of sugars from the hemicellulose.

The initially low glucose levels correspond with the release of glucose present in the hemicellulose. This is more easily hydrolyzed due to the short branches and short lateral chains. After longer residence times, the prehydrolysate liquor collected from the samples heated at 200°C continued to have increasing glucose yields. This suggests that, after the hemicellulose had been hydrolyzed, the cellulose began to break down into glucose as well.

4.3. EFFECTS OF DILUTE ACID PRETREATMENT ON BAMBOO MICROSTRUCTURE

The following optical micrographs were observed for the pretreated samples in dilute H_2SO_4 acid with different temperature, concentration and time.

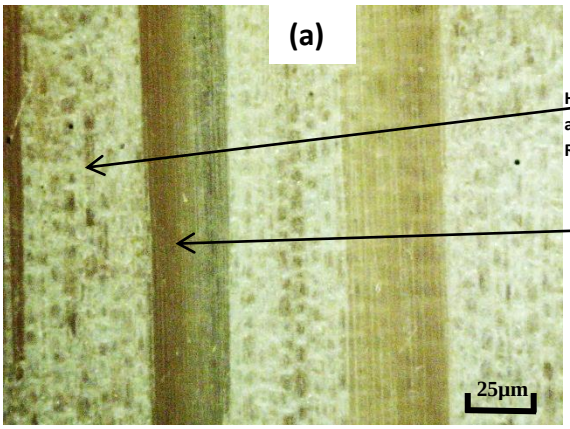


Figure 4.4a: Micrograph of un-pretreated bamboo. x400

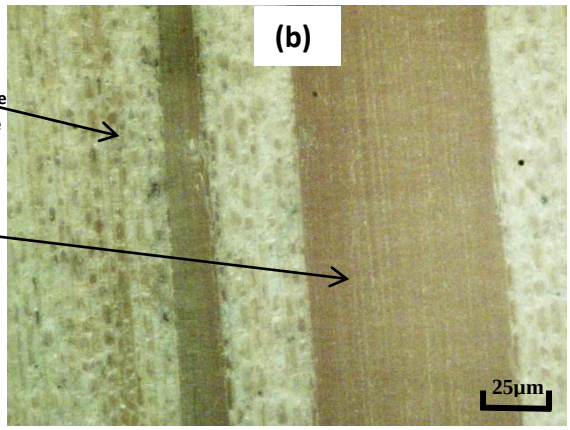


Figure 4.4b: Micrograph of pretreated bamboo with 0.5%(v/v) H_2SO_4 for 2hours at 25°C. x400

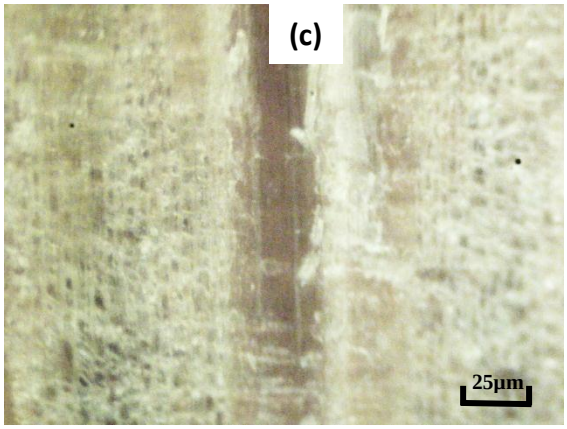


Figure 4.4c: Micrograph of pretreated bamboo with 0.5%(v/v) H_2SO_4 for 2hours at 110°C. x400

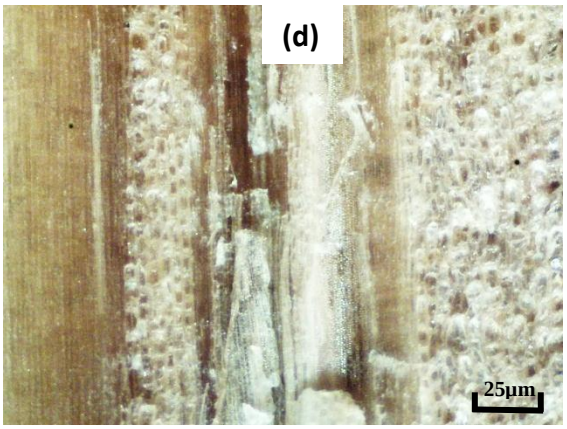


Figure 4.4d: Micrograph of pretreated bamboo with 0.5%(v/v) H_2SO_4 for 2hours at 120°C. x400

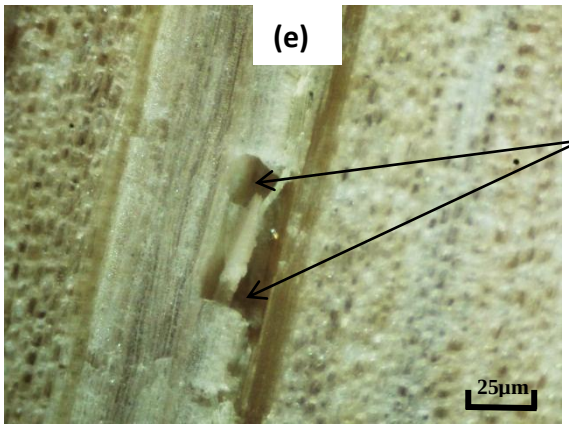


Figure 4.4e: Micrograph of pretreated bamboo with 0.5%(v/v) H_2SO_4 for 2hours at 150°C. x400

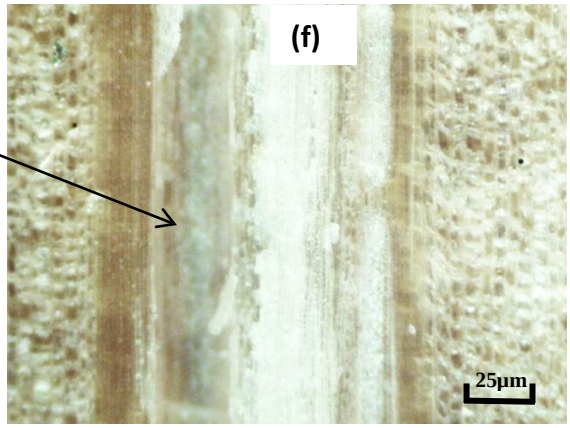


Figure 4.4f: Micrograph of pretreated bamboo with 0.5%(v/v) H_2SO_4 for 2hours at 200°C. x400

To have a better understanding of the effects of dilute sulfuric acid treatment on bamboo particles, optical microscopy images of Samples were obtained. Prior work by Pingali et. al. (2010) used small angle neutron scattering to examine the effects of dilute acid pretreatment on cell wall nanostructure. Their findings suggested that pretreatment times of 1 hour or longer at 160°C degrade the cellulose network and alter cell wall surfaces at the micrometer scale. Amanda Rees used Environmental SEM, (ESEM) to study the structure of bamboo after dilute acid pretreatment. She also investigated the relationship between the sugar yields and the morphological changes in cell wall structure. Since sugar concentrations in prehydrolysate corresponds to hydrolyzed hemicellulose, then it can be assumed that as the amount of solubilized glucose increases, so does the sample porosity. The micrographs in Figure 4.4a-f depict the structure of the untreated bamboo samples and the samples that were treated with water and 1.0% v/v dilute sulfuric acid at 200°C. The untreated sample contains amorphous regions, with larger particles and solid surfaces, while the sample that was heated in water has defined porous regions, where the cell walls were visible. The cell walls comprise lignin, which gives the cells structural integrity [11]. Pretreatments, using only water and heat, were able to remove the easily accessible hemicellulose and cellulose, but did not impact the lignin [11].

Figure 4.4a shows the surface morphology of un-pretreated bamboo. This reveals the hemicellulose and cellulose region surrounded by lignin, while Figure 4.4b shows the structure of the bamboo after pre-treatment with dilute sulphuric acid for 2 hours. The structure is similar to that of Figure 4.4a. Figures 4.4c, d and e, shows a reduction in the crystalline structure of the pre-treated bamboo at 110 ^{o}C, 120 ^{o}C and 150 ^{o}C, respectively, for 2 hours and 0.5% (v/v). As the temperature increases, the reduction in crystallinity increases. Here, the hydrolysis of hemicellulose begins occurs. This corresponds to the amount of sugar that is been dissolved in

the filtrate. Figure 4.4f shows higher reduction of crystallinity and a considerable amount of porosity and higher amount of sugar compared to the prior ones, this indicates that the cellulose began to break down into glucose. This corresponds to the high glucose yield of 42 mg/g that was produced at 200^{o}C and 0.5% (v/v) concentration for 2 hours.

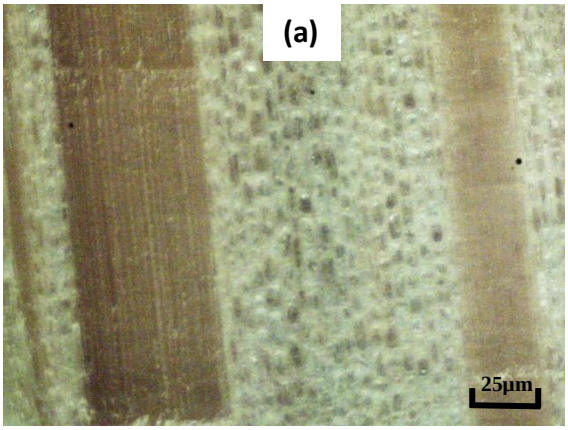


Figure 4.5a: Micrograph of pretreated bamboo with 0.5%(v/v) H_2SO_4 for 4hours at 25°C. x400

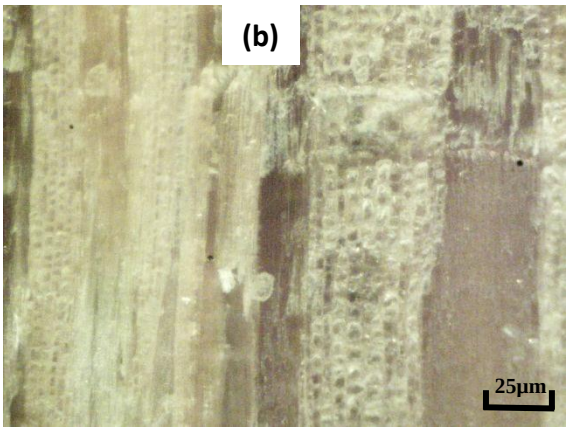


Figure 4.5b: Micrograph of pretreated bamboo with 0.5%(v/v) H_2SO_4 for 4hours at 110°C. x400

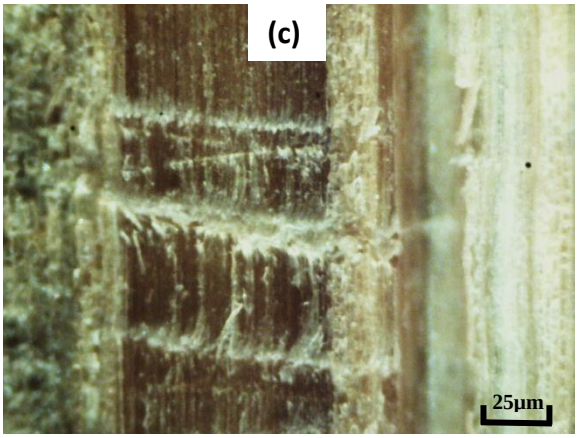


Figure 4.5c: Micrograph of pretreated bamboo with 0.5%(v/v) H_2SO_4 for 4hours at 120°C. x400

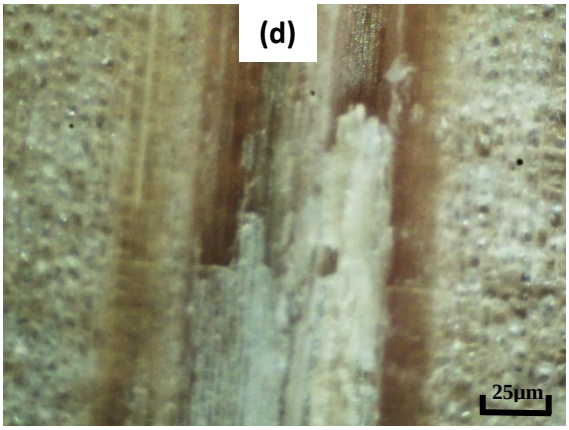


Figure 4.5d: Micrograph of pretreated bamboo with 0.5%(v/v) H_2SO_4 for 4hours at 150°C. x400

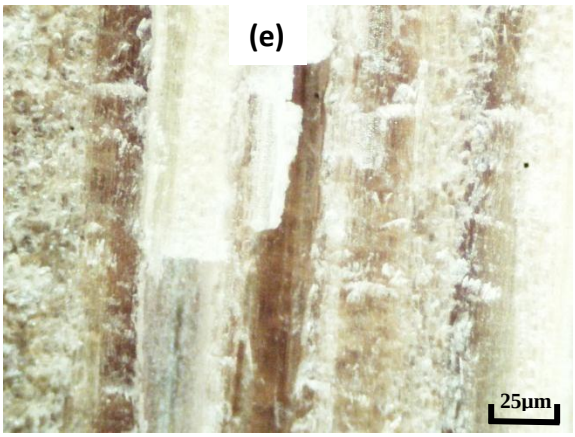


Figure 4.5e: Micrograph of pretreated bamboo with 0.5%(v/v) H_2SO_4 for 4hours at 200°C. x400

Figure 4.5a shows little difference in the crystallinity compared to the un-pretreated bamboo after pre-treatment at room temperature for 4 hours and a concentration of 0.5% (v/v). However, Figures 4.5b and c shows clear evidence of disorder in the crystalline structure of the pre-treated bamboo. This indicates the hydrolysis of hemicellulose in the bamboo sample. Figures 4.5d and e exhibit greater disorder in the crystallinity and some levels of porosity. This is consistent with the higher level of glucose yield of 56.1 mg/g in the filtrate. This corresponds to the amount of hemicellulose that was hydrolyzed and part of cellulose that were broken down into glucose. This was as a result of an increase in temperature and residence time from 2 to 4 hours.

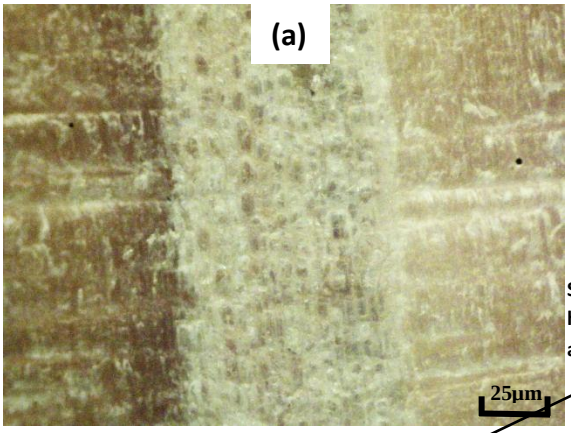


Figure 4.6a: Micrograph of pretreated bamboo with 1.0%(v/v) H_2SO_4 for 2hours at 25°C. x400

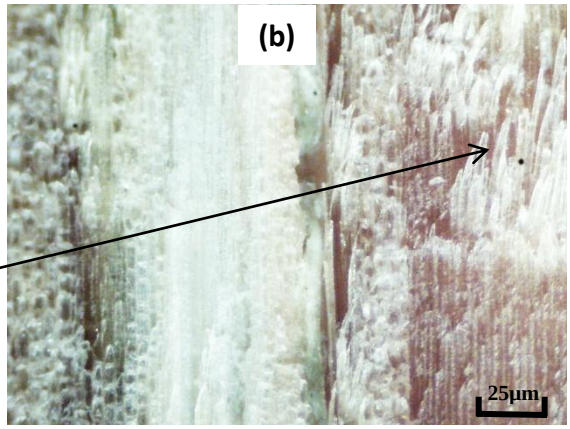


Figure 4.6b: Micrograph of pretreated bamboo with 1.0%(v/v) H_2SO_4 for 2hours at 110°C. x400

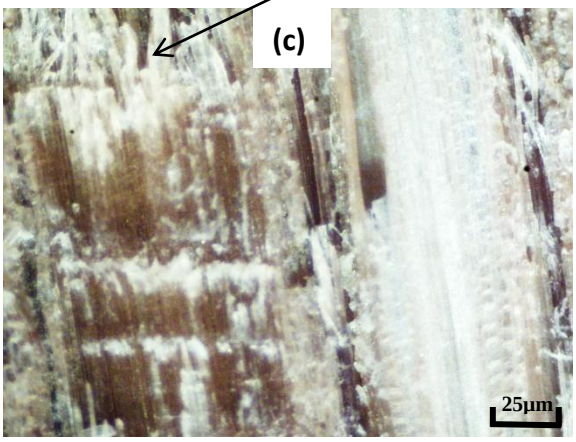


Figure 4.6c: Micrograph of pretreated bamboo with 1.0%(v/v) H_2SO_4 for 2hours at 120°C. x400

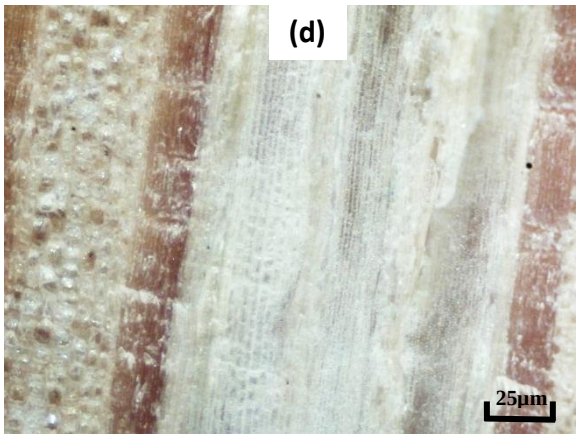


Figure 4.6d: Micrograph of pretreated bamboo with 1.0%(v/v) H_2SO_4 for 2hours at 150°C. x400

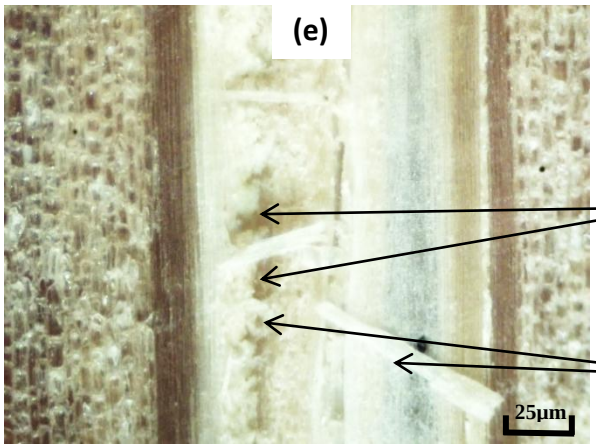


Figure 4.6e: Micrograph of pretreated bamboo with 1.0%(v/v) H_2SO_4 for 2hours at 200°C. x400

Figure 4.6a, shows difference in the crystallinity compared to the un-pretreated bamboo after pre-treatment at room temperature for 2 hours and 1.0% (v/v), this change is as a result of hydrolysis of hemicellulose due to the short branches and short lateral chains. Figure 4.6b and c, shows the stretching of the hemicellulose and cellulose which is as a result of an increase in temperature and acid concentration, the branch structure begins to loosening up to from longer chains and therefore allowing more hemicellulose to be hydrolyzed to provide more glucose yields of 22.3 mg/g at 110^{o}C and 1.0% (v/v) for 2 hours and 27.2 mg/g 120^{o}C and 1.0% (v/v) for 2 hours compared to that at room temperature. Figure 4.6d, shows removal of large amount of hemicellulose and part of cellulose begin broken down to glucose, which can be compared to the sugar assay test results, which shows high amount of glucose yield of 29.5 mg/g 150^{o}C and 1.0% (v/v) for 2 hours. Figure 4.3e, shows removal of hemicellulose and break down of cellulose to hemicellulose and also shows a disruption of the lignin structure, thereby given access to more hemicellulose and cellulose region. This gives reason for a higher glucose yield of 47.7 mg/g 200^{o}C and 1.0% (v/v) for 2 hours, here the combination of temperature and residence time increases the glucose which is released from the pre-treated bamboo sample.

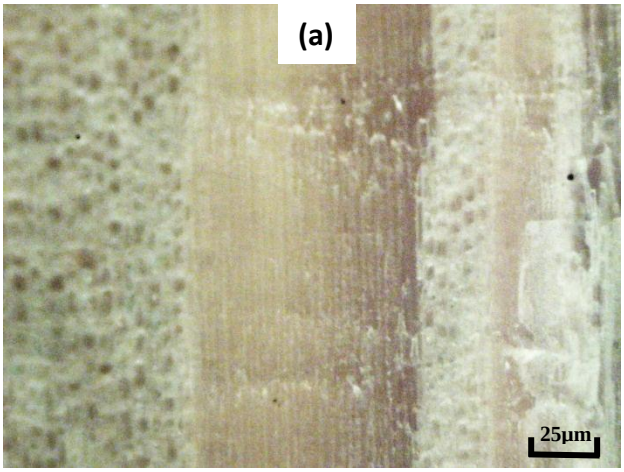


Figure 4.7a: Micrograph of pretreated bamboo with 1.0%(v/v) H_2SO_4 for 4hours at 25°C. x400

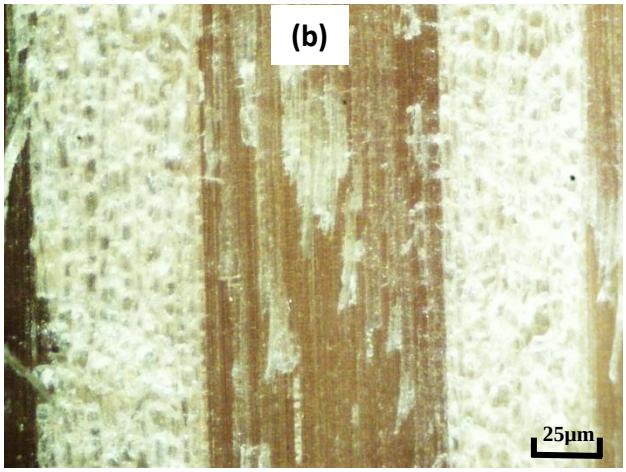


Figure 4.7b: Micrograph of pretreated bamboo with 1.0%(v/v) H_2SO_4 for 4hours at 110°C. x400

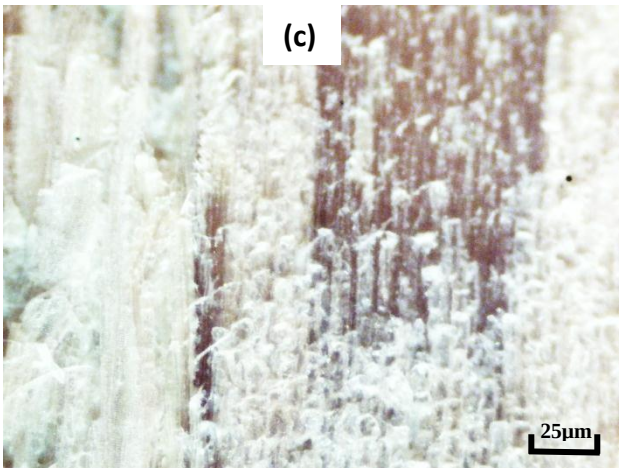


Figure 4.7c: Micrograph of pretreated bamboo with 1.0%(v/v) H_2SO_4 for 4hours at 120°C. x400

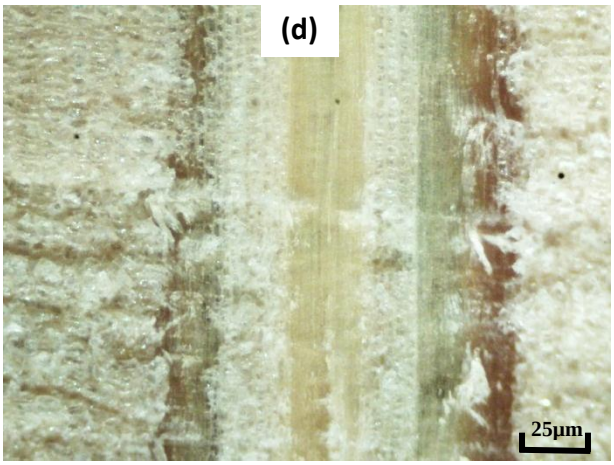


Figure 4.7d: Micrograph of pretreated bamboo with 1.0%(v/v) H_2SO_4 for 4hours at 150°C. x400

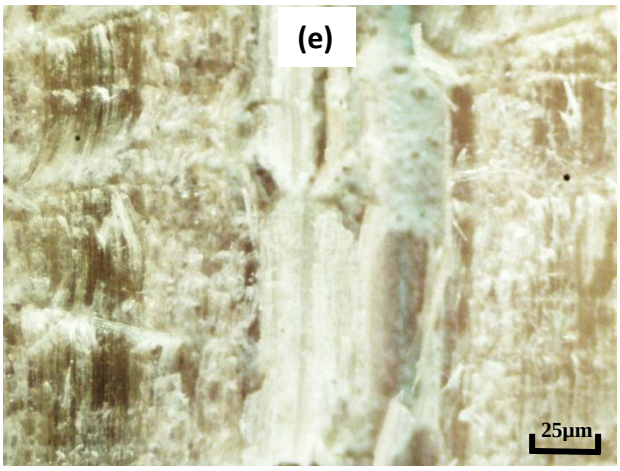


Figure 4.7e: Micrograph of pretreated bamboo with 1.0%(v/v) H_2SO_4 for 4hours at 200°C. x400

Figure 4.7a, shows a considerable dissolution of hemicellulose. Figure 4.7b shows that larger portions of the hemicellulose have been hydrolyzed which gives a glucose yield of 19.2 mg/g at 110^{o}C and 1.0% (v/v) for 4 hours. In Figure 4.7c, stretching of hemicellulose and a partial break down of cellulose to glucose throughout the regions and there is a considerable amount of pores, which increases the active sites necessary for enzymatic hydrolysis and has a glucose yield of 27.9 mg/g at 120^{o}C and 1.0% (v/v) for 4 hours. Figure 4.7d shows that a large amount of hemicellulose hydrolyzed and cellulose been broken down during the pre-treatment to form glucose of 37.3 mg/g at 150^{o}C and 1.0% (v/v) for 4 hours. Figure 4.7e shows vigorous removal of hemicellulose and the breakdown of cellulose and significant disruption of lignin resulting in higher level of porosity and a maximum glucose yield of 91.8 mg/g at 200^{o}C and 1.0% (v/v) for 4 hours, this is as a result of an increase in temperature, acid concentration and residence time. This results shows that at 200^{o}C and 1.0% (v/v) and 4 hours pre-treatment, maximum sugar can be obtained which is necessary for the fermentation and it makes it possible to have enough active sites to which the enzymes can attach themselves to during enzymatic hydrolysis.

4.4. EFFECTS OF TEMPERATURE ON MOISTURE ABSORPTION

Table 4.3: Moisture Absorption of Sample during Dilute Acid Pre-treatment

Tempt. (°C)	Concn. % (v/v)	Time (hr)	Final weight (g)	Finial weight (g)	Change in mass (g)	Moisture Absorption (%)
	0.5	2	2.37	2.40	0.03	1.27
25		4	2.03	2.09	0.06	2.96
	1.0	2	2.31	2.37	0.06	2.60
		4	2.29	2.32	0.03	1.31
	0.5	2	2.34	2.36	0.02	0.85
110		4	2.56	2.64	0.08	3.13
	1.0	2	2.46	2.79	0.33	13.41
		4	2.24	2.57	0.33	14.73
	0.5	2	2.86	2.91	0.05	1.75
120		4	2.37	2.65	0.28	11.81
	1.0	2	2.28	2.54	0.26	11.40
		4	2.84	3.44	0.60	21.13
	0.5	2	2.08	2.17	0.09	4.33
150		4	2.09	2.66	0.57	27.27
	1.0	2	2.90	3.41	0.51	17.59
		4	3.07	4.08	1.01	32.90
	0.5	2	2.46	2.84	0.38	15.45
200		4	2.77	3.62	0.85	30.69
	1.0	2	2.46	3.05	0.59	23.98
		4	2.38	3.52	1.14	47.90

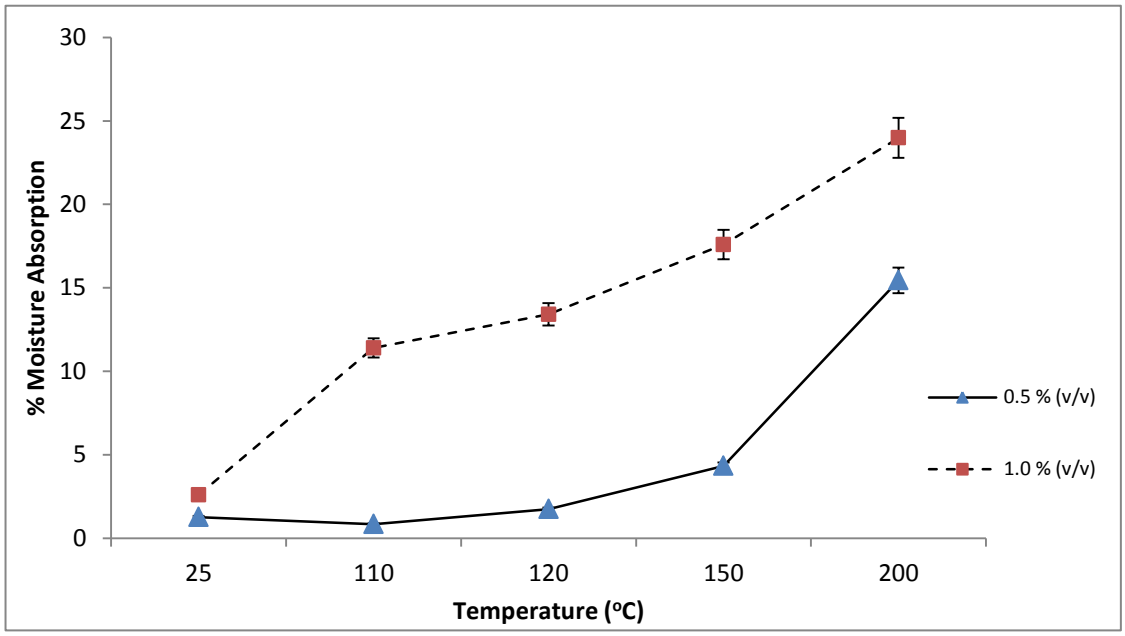


Figure 4.8: Plot of % Moisture Absorption against Temperature for 2hrs at different acid concentration.

Figure 4.8 shows the % moisture aborption of the pre-treated bamboo sample for 2hours, with concentration of 0.5 % (v/v) and 1.0 % (v/v) 2M dilute sulfuric acid at different temperatures of 25 ^{o}C, 110 ^{o}C, 120 ^{o}C, 150 ^{o}C and 200 ^{o}C. At room temperature the % moisture aborption was 1.27%, which was found to decrease to 0.85% at 110 ^{o}C, and then increase continuiously to 1.75% at 120 ^{o}C, 4.33% at 150 ^{o}C and 15.45% at 200 ^{o}C for 0.5 % (v/v). While for 1.0% (v/v), there was a large increase from 2.60% at 110 ^{o}C to 11.40% at 120 ^{o}C, and to 13.41% at 150 ^{o}C, which increased to 17.59% at 150 ^{o}C and finally increased to 23.98% at 200^{o}C. This increase in the moisture aborption can be attributed to the hydrolysis of the bamboo in which dilute H_2SO_4 acid reacts with cellulose to produce $C_6H_{10}(SO_3)O_5$; which involves the splitting of a bond and the addition of the hydrogen cation and the hydroxide anion from the water.

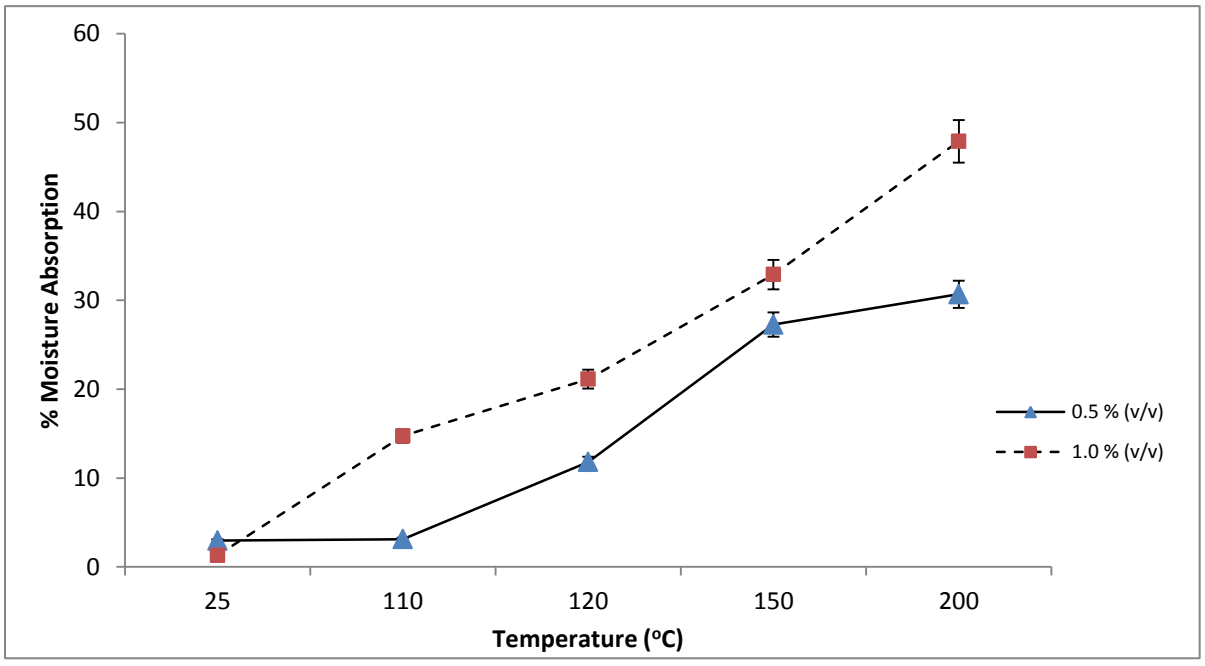


Figure 4.9: Plot of % Moisture Absorption against Temperature for 4hrs at different acid concentration.

Figure 4.9 show the % moisture aborption of the pre-treated bamboo sample for 4hours, with concentration of 0.5 % (v/v) and 1.0 % (v/v) 2M dilute sulfuric acid at different temperatures of 25 ^{o}C, 110 ^{o}C, 120 ^{o}C, 150 ^{o}C and 200 ^{o}C. At room temperature the % moisture aborption was 2.96%, which was found to increase continuously to 3.13% at 110 ^{o}C, 11.81% at 120 ^{o}C, 27.27% at 150 ^{o}C and 30.69% at 200 ^{o}C for 0.5 % (v/v). While for 1.0% (v/v), there was large increase from 1.31% at 110 ^{o}C to 14.73% at 120 ^{o}C, then increase to 21.13% at 150 ^{o}C, which increased to 32.90% at 150 ^{o}C and finally increased to 47.90% at 200^{o}C. This results shows increase in % moisture absortion, also it has been observed that the maximum moisture aborption for figure 4.9 was greater than that of figure 4.8, this was due to the residence time of 4 hours in which the pre-treatment took place, which allow a longer time for hydrolysis of hemicellulose and cellulose, which also allows a longer time for the reaction and more splitting of bond and the addition of the hydrogen cation and the hydroxide anion from the water.

4.5. EFFECTS OF TEMPERATURE ON ENZYMATIC HYDROLYSIS

During hydrolysis, cellulases are used to digest the biomass remaining after pretreatment. In addition to Celluclast, (Novozymes) which contains cellulases from T. reesei, ß-glucosidase from A. niger was also added to help hydrolyze the cellobiose, which is an inhibitor of cellulase activity. Table 4.4. contains a summary of sugars present in hydrolysate samples.

Based on the pretreatment results showing that higher temperatures and acid concentrations lead to greater solubilized glucose, it was also expected that the glucose concentrations after enzymatic hydrolysis would follow similar trends. Indeed, the maximum glucose yield 153.1 mg/g was found for the samples pretreated at 200°C. Figure 4.12 and 4.13 shows the graphs of glucose yields measured after 72 h of enzymatic hydrolysis.

In some cases, the hydrolaysates of the pretreated bamboo had lower quantities of glucose than expected. This could be simply be because of the presence of an inhibitor generated at higher pretreatment temperature. It has been suggested in prior work by Donohoe et al. (2011) that at higher temperatures, the lignin can extrude from within the cell walls and form droplets on the surface of the material, which reduces the accessibility of the cellulose or the hydrolyzed cellulose gives rise to oligomers or that the glucose formed is subsequently degraded (Saeman, J.F., 1945).

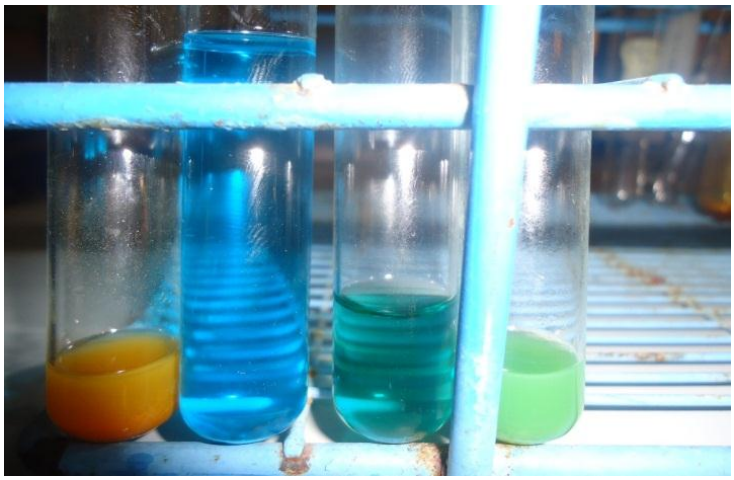


Figure 4.10: Qualitative test using Benedict's reagent to test for the presence of reducing sugars

Figure 4.10 shows the qualitative test using Benedict's reagent to test for the presents of reducing sugars, which showed a colour change to confirm the presence of glucose and sucrose in the filtrate from the pre-treated bamboo and the enzymatic hydrolysis. The solution which turned from deep blue to green ppt confirms the presence of sucrose and the second solution which turned from deep blue to yellow ppt to confirm the presence of glucose.

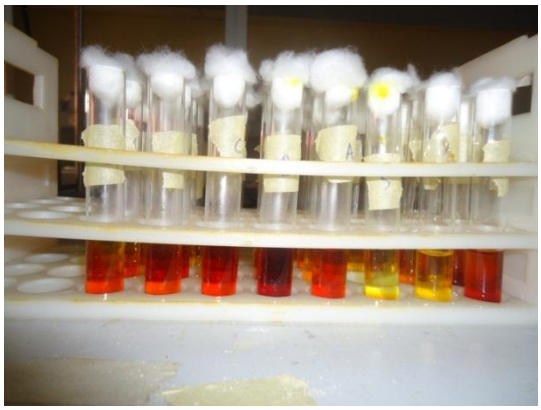


Figure 4.11: Quantitative test using DNS reagent to test for the presence of reducing sugars

Figure 4.11 shows a colour change from yellow to orange, the samples with thickers colour change indicate the presence of higher concentration of reducing sugars and this can be seen in the difference in pre-treatment temperature. The samples with the highest sugar concentration wer detected using the UV-Visible Photospectrometer were darker than the others.

Table 4.4: Sugars measured in hydrolysate after enzymatic hydrolysis

Tempt. (^{o}C)		Concn.% (v/v)	Time (hr)	Yield (mg/g)
		0.5	2	20.90
25			4	54.80
		1.0	2	31.90
			4	66.40
		0.5	2	18.20
110			4	70.10
		1.0	2	41.00
			4	91.30
		0.5	2	39.00
120			4	79.60
		1.0	2	71.30
			4	102.50
		0.5	2	41.10
150			4	83.00
		1.0	2	78.60
			4	107.70
		0.5	2	56.20
200			4	92.50
		1.0	2	96.70
			4	153.00
Unpre-treated sample		1.0	4	9.20
Pre-treated with water		1.0	4	3.30
Sugar Cane	1.0	4	176.30	

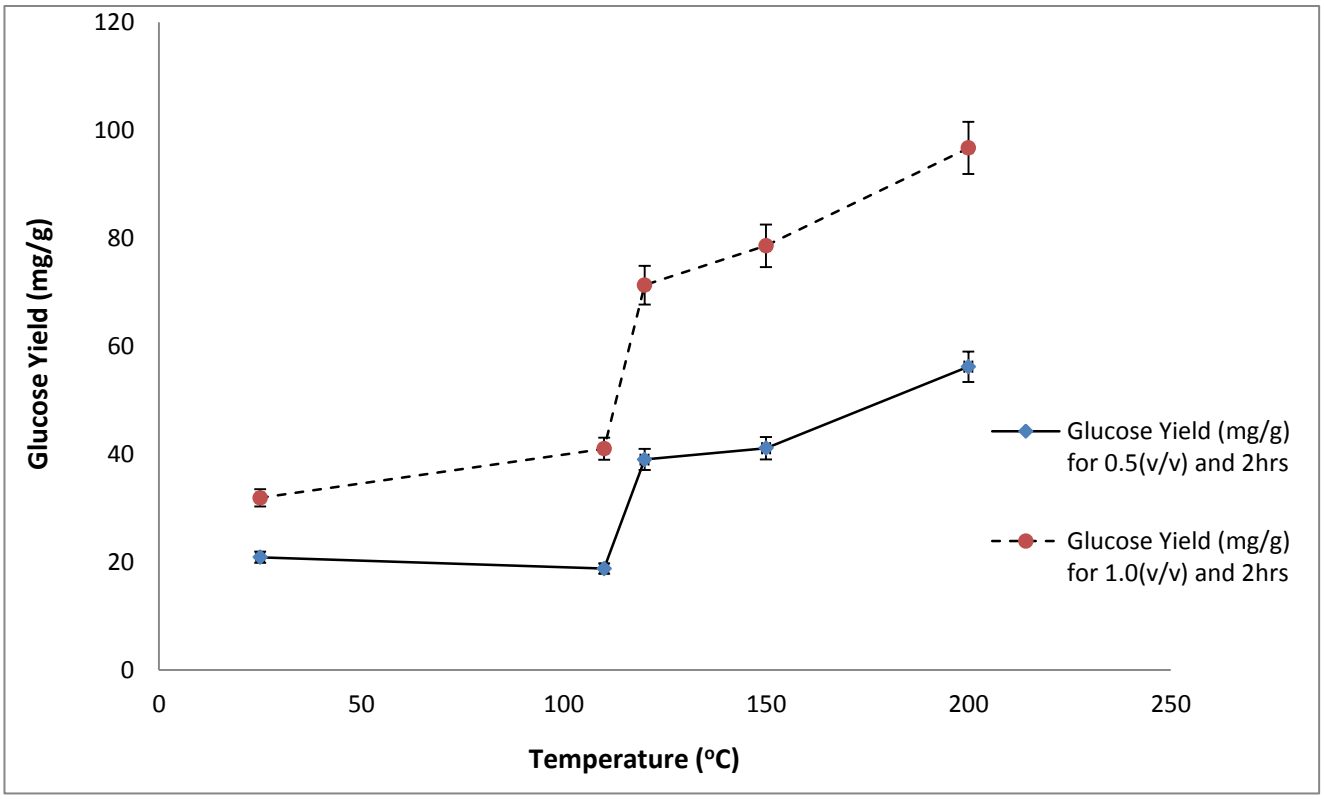


Figure 4.12: Plot of Glucose Yield (mg/g) against Temperature (^{o}C) for 2hrs after Enzymatic Hydrolysis.

Figure 4.12 shows the relationship between glucose formed during enzymatic hydrolysis and temperature after pretreatment of 0.5% and 1.0% (v/v) for 2hrs. From the graph above it shows that as the pretreatment temperature increase the glucose yields also increase.

The glucose yield from the graph shows a decrease in glucose yield from 20.9 mg/g at 25^{o}C to 18.8 mg/g at 110^{o}C and 0.5% (v/v), this may be due to the presence of an inhibitor generated at 110^{o}C and 0.5% (v/v), at this temperature lignin can extrude from within the cell walls and form droplets on the surface of the material, which reduces the accessibility of the cellulose, which in turn will reduce the glucose yield produced. But at 120^{o}C and 0.5% (v/v) there was a sharp increase to 39.0 mg/g of glucose yield, the glucose yield increases slightly to 41.1 mg/g at 150^{o}C and 0.5% (v/v) and finally increases to 56.2 mg/g at 200^{o}C and 0.5% (v/v).

The increase in glucose yields at higher temperature from 120^{o}C, may be as a result of the enzymes to access cellulose and hemicellulose after lignin has been broken down.

The glucose yield from the graph shows an increase in glucose yield from 31.9 mg/g at 25^{o}C and 1.0% (v/v) to 41.0 mg/g at 110^{o}C and 1.0% (v/v), at 120^{o}C and 1.0% (v/v) there was a sharp increase to 71.3 mg/g of glucose yield, the glucose yield increases slightly to 78.6 mg/g at 150^{o}C and 1.0% (v/v) and finally increases to 96.7 mg/g at 200^{o}C and 1.0% (v/v). The increase in glucose yields at higher temperature may be as a result of the enzymes to access cellulose and hemicellulose after lignin has been broken down.

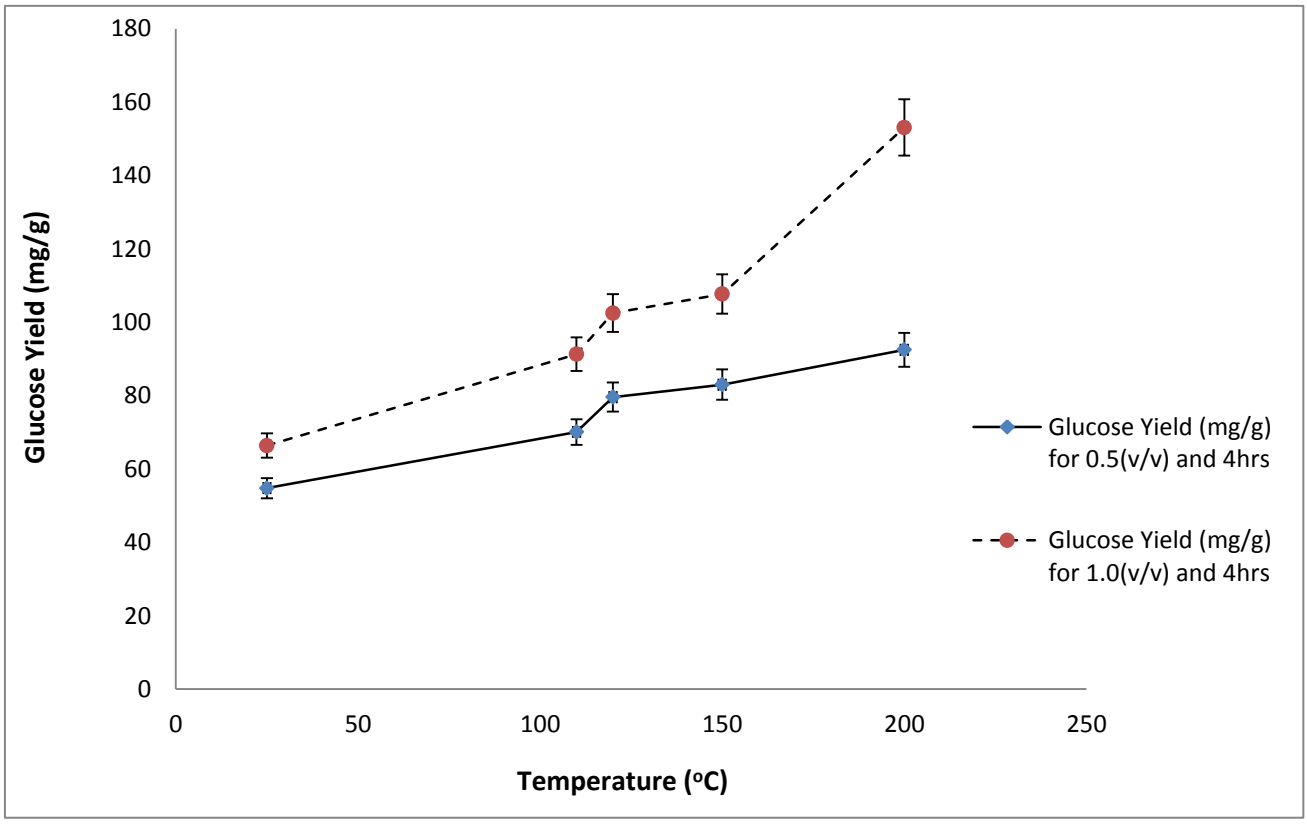


Figure 4.13: Plot of Glucose Yield (mg/g) against Temperature (^{o}C) for 4hrs after Enzymatic Hydrolysis.

Figure 4.13 shows the relationship between glucose formed during enzymatic hydrolysis and temperature after pretreatment of 0.5% and 1.0% (v/v) for 4hrs. From the graph above it shows that as the pretreatment temperature increase the glucose yields also increase.

The glucose yield from the graph shows an increase in glucose yield from 54.8 mg/g at 25^{o}C to 70.1 mg/g at 110^{o}C and 0.5% (v/v), at 110^{o}C and 0.5% (v/v), at 120^{o}C and 0.5% (v/v) there was an increase to 79.6 mg/g of glucose yield, the glucose yield increases to 83 mg/g at 150^{o}C and 0.5% (v/v) and finally increases to 92.5 mg/g at 200^{o}C and 0.5% (v/v). The increase in glucose yields there may be as a result of both the pretreatment temperature and the residence time, which provides the enzymes with more surface area of cellulose and hemicellulose after lignin has been broken down.

The glucose yield from the graph shows an increase in glucose yield from 66.4 mg/g at 25^{o}C and 1.0% (v/v) to 91.3 mg/g at 110^{o}C and 1.0% (v/v), at 120^{o}C and 1.0% (v/v) there was an increase to 102.5 mg/g of glucose yield, the glucose yield increases slightly to 107.7 mg/g at 150^{o}C and 1.0% (v/v) and finally increases to 153.1 mg/g at 200^{o}C and 1.0% (v/v). The maximum glucose yield was found to be 153.1 mg/g at 200^{o}C, this is as a result of the combination of pretreatment temperature, residence time and acid concentration, which totally degrade lignin and allows the enzymes to have access to more cellulose and hemicellulose after lignin has been broken down. From the surface morphology below figure 4.15e, it shows how the lignin has been broken down and removal to make the enzymes have more cellulose and hemicellulose surface to attach themselves to. From figure 4.15d, it shows a clear link of how much cellulose and hemicellulose has been hydrolyzed by enzymatic hydrolysis.

Table 4.5: Total Sugars released from milled bamboo samples after dilute acid pre-treatment and enzymatic hydrolysis

Tempt. (^{o}C)	Concn.% (v/v)	Time (hr)	Yield (mg/g)
25	0.5	2	27.10
		4	61.50
	1.0	2	52.40
		4	71.80
110	0.5	2	31.10
		4	87.10
	1.0	2	63.30
		4	110.50
120	0.5	2	64.10
		4	102.20
	1.0	2	98.50
		4	130.40
150	0.5	2	69.80
		4	132.70
	1.0	2	108.10
		4	145.00
200	0.5	2	98.20
		4	148.60
	1.0	2	144.40
		4	244.80
Unpre-treated sample	1.0	4	13.3
Pre-treated with water	1.0	4	8.30
Sugar Cane	1.0	4	316.30

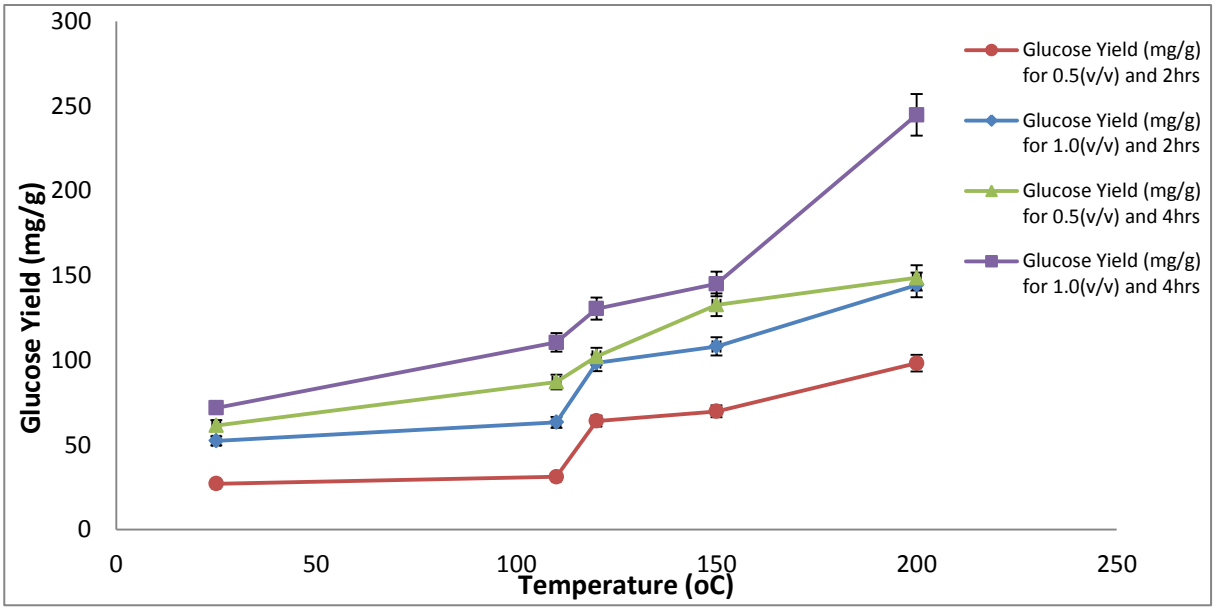


Figure 4.14: Plot of total glucose (mg/g) released from milled bamboo samples after dilute acid pre-treatment and enzymatic hydrolysis against temperature (^{o}C) for 2 hours and 4 hours.

Figure 4.14 shows a plot of total glucose released after dilute acid pre-treatment and enzymatic hydrolysis against temperature (^{o}C), when the acid concentration was varied between 0.5% and 1.0% (v/v) for 2 hours and 4 hours. From the plot, it is clear that pre-treatment temperature, dilute acid concentration and residence time increase the total amount of glucose released from the milled bamboo sample after pretreatment and enzymatic hydrolysis. This can be related to the surface morphology of the bamboo sample images, because the images shows that as the pre-treatment temperature, dilute acid concentration and residence time increases more hemicellulose and cellulose is being removed, thereby creating pores in the bamboo sample. This allows more enzymes to be able to attach themselves to the available active sites and thereby increase the enzymatic hydrolysis, which in turn produces more total sugars, compared to the lower pretreatment temperatures. It can be seen from the plot that at 25 ^{o}C and 0.5% (v/v) for 2 hours, the total sugars produced was 27.1 mg/g, which has the lowest amount of sugars compared to 200 ^{o}C and 1.0% (v/v) for 4 hours, the total sugars produced was 244.8 mg/g.

4.6. EFFECTS OF ENZYMATIC HYDROLYSIS ON BAMBOO MICROSTRUCTURE

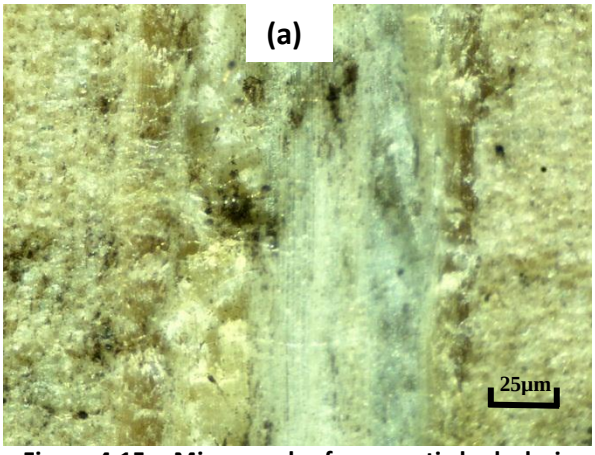


Figure 4.15a: Micrograph of enzymatic hydrolysis on pretreated bamboo with 0.5%(v/v) H_2SO_4 for 4hours at 25°C. x400

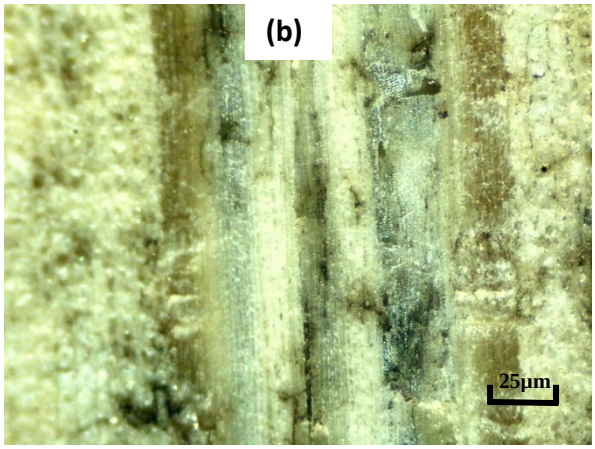


Figure 4.15b: Micrograph of enzymatic hydrolysis on pretreated bamboo with 1.0%(v/v) H_2SO_4 for 4hours at 25°C. x400

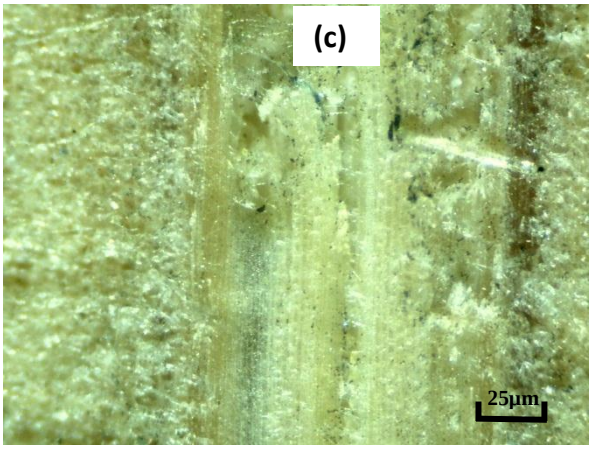


Figure 4.15c: Micrograph of enzymatic hydrolysis on pretreated bamboo with 0.5%(v/v) H_2SO_4 for 4hours at 200°C. x400

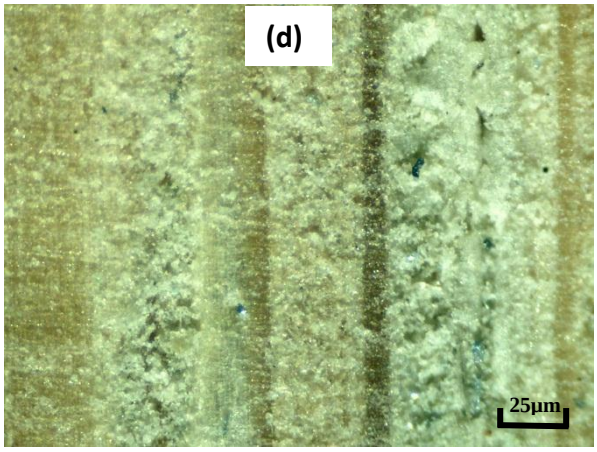


Figure 4.15d: Micrograph of enzymatic hydrolysis on pretreated bamboo with 1.0%(v/v) H_2SO_4 for 4hours at 200°C. x400

Figure 4.15a shows the microstructure of bamboo sample that was enzymatically hydrolyzed after it was pretreated with 0.5% (v/v) H_2SO_4 for 4 hours. Here the hemicellulose and cellulose region were reduced and the porosity increases, this is due to the hydrolysis of the hemicellulose and cellulose into glucose and the chemical degradation of the lignin portion. Figure 4.15b shows the microstructure of bamboo sample that was enzymatically hydrolyzed after it was pretreated with 1.0% (v/v) H_2SO_4 for 4 hours, which shows that the more the pores,

the lower the fraction of hemicellulose and cellulose. This difference may be due to the increase in acid concentration from 0.5 to 1.0%(v/v). It can be seen from Figure 4.15c, which was pretreated for 4 hours, 0.5% (v/v) and 200^{o}C, here more hemicellulose and cellulose region was hydrolyzed and the lignin was broken down and removed from the interface of the bamboo yet.

Figure 4.15d, which was obtained for, 1.0% (v/v) and 200^{o}C, here almost all the hemicellulose and cellulose were hydrolyzed from the substrate of the bamboo sample. The lignin portion was broken down and removed from the substrate of the bamboo, and this may also serve as inhibitor and reduce the total amount of glucose produce, during pretreatment and enzymatic hydrolysis.

The objective of pretreatment is to improve the digestibility of the bamboo and increase the accessibility of the cellulose fibers, and pretreatment with 1% dilute sulfuric acid showed more loose fibers and rough surfaces after hydrolysis, this suggests that the pretreatment with dilute sulfuric acid effectively increased the enzyme accessibility, because when pores are created they give room for more active sites for the enzymes to attach themselves to, thereby increasing the glucose yield during enzymatic hydrolysis.

4.7. FERMENTATION AND INHIBITOR REMOVAL

After adding $Ca(OH)_2$ and Na_2SO_3 and centrifuging, a white precipitate was formed, which was filtered off using a filter paper and a clear solution was produced, the white precipitate that were filtered off were the inhibitors which may be produced at high temperature as a result of the degradation of lignin in the sample.

The ATCC Medium: 2107 Modified Reinforced Clostridial Broth. The supplements included tryptose (1g), beef extract (1g), yeast extract (0.3g), dextrose (0.5g), sodium chloride (0.5g), soluble starch (0.1g), L-Cysteine (0.05g), sodium acetate (0.3g) were all dissolved in

100mL of distilled water, did not yield any bacterial growth on the plate or the culture medium or in the sugar samples after 24 hours. The prevention of growth may be due to the presence of inhibitors.

The addition of another standard minimal media supplements which includes aspartate as a buffering agent (2g/L), ammonia chloride for a nitrogen source (50mL/L), iron sulfate (20mL/L), biotene for vitamins (1.6mL/L) and PABA (20mg/mL), it was put in an anaerobic chamber for three days. After three days, it was notice that the bacterial had grown and the sugars solution form a couldly solution and when shaked it forms a foaming precipitate above the liquid solution.

4.8. BUTANOL MEASUREMENT

Raman spectroscopy was used to test mixtures of butanol and water (1, 2, 5, 10% v/v), and relative peak intensities between 1420 and 1450 cm^{-1} were used to create a calibration curve shown below. Primary alcohols have association bands near 1420 cm^{-1} corresponding to OH deformation, which disappear upon dilution.

Since these peak intensities correspond to the concentration of butanol in the mixture, the unknown concentration of butanol produced by clostridia was determined using the linear equation:

$$y = 0.3834x + 1.1055$$

where y is the intensity of the peak of interest relative to the 1% butanol peak and x is the concentration of butanol in the mixture in % v/v.

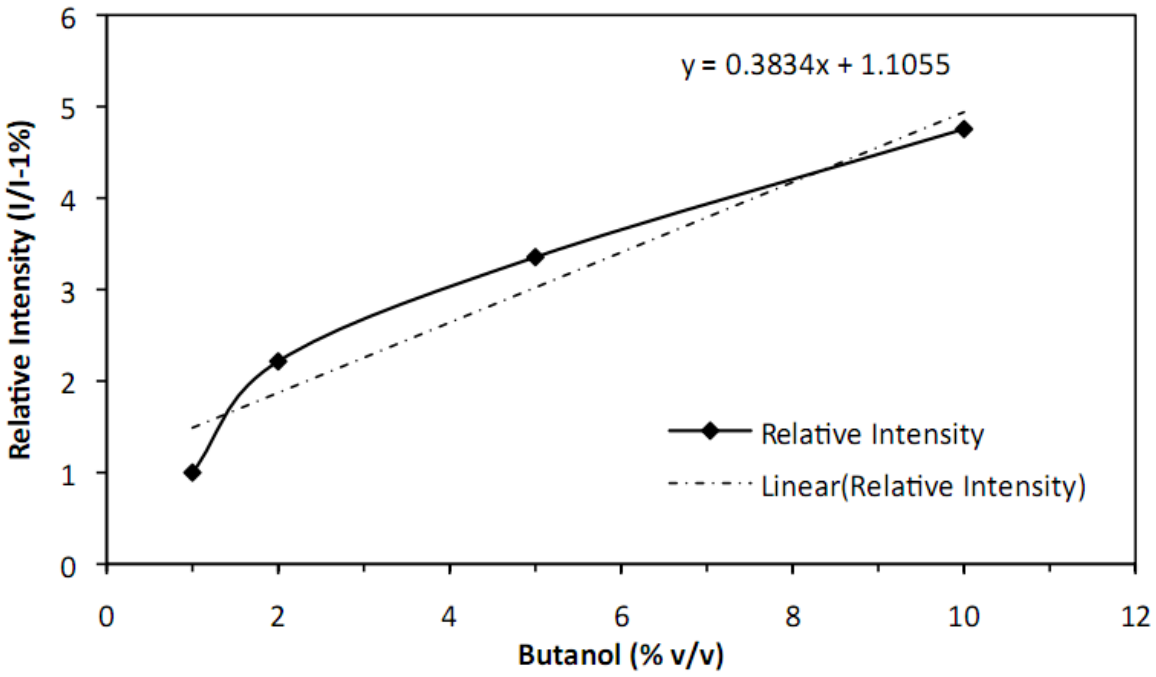


Figure 4.16: Calibration curve of Raman peak intensities for butanol mixtures in water

Results found that 10.4mg/mL of butanol (72.8mg, 1.3%) was produced in sample treated at 1% H_2SO_4 for 110°C. While no previous studies of butanol production from bamboo hydrolysates have been found, this is yield is greater than the reported yield of ethanol from bamboo (0.834 g/L) by Mutreja et al. [77].

REFERENCE

[77]. Mutreja, R.; Das, D.; Goyal, D.; Goyal, A. Bioconversion of agricultural waste to ethanol by SSF using recombinant cellulase from Clostridium thermocellum. Enzyme Research. 2011.

CHAPTER FIVE

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

5.1 CONCLUSIONS

The results presented in this study demonstrate the feasibility of butanol production from bamboo-derived sugars and the results shows an effect of temperature on the production of sugars. While there is still considerable work to be done to further optimize the system, this research contributes to the understanding of the effects of pretreatment on bamboo in terms of solubilized sugar production and morphological changes observed in the bamboo microstructure. Furthermore, glucose yields after dilute sulfuric acid pretreatment showed significant improvement, and following enzymatic hydrolysis, maximum glucose solubilized from bamboo was 244.8 mg/g.

Since bamboo is a renewable cellulosic feedstock that grows in a wide range of climates and does not divert from the food streams, it is a promising alternative to current fuel feedstocks. Additionally, butanol offers numerous advantages over ethanol as a fuel (i.e. greater energy density and less corrosive to existing infrastructure). In 2011, the global market for biofuels reached a record size of $83 billion dollars. This has been attributed in part to the rising cost of feedstock commodities, such as sugar and vegetable oil. Future work investigating the use of bamboo for sugar and fuel production has immense potential to impact biofuel production, with positive environmental and social implications as well.

5.2 SUGGESTIONS FOR FUTURE WORK

The results of this study suggest the need for further experimental work which includes:

1. The experiment should be performed at temperatures above 200^{o}C to determine if higher sugar yield can be obtained.

2. HPLC should be used to determine the concentration of sugar since it is faster and more accurate.

3. Improvement in the fermentation process by using genetically engineered strains of clostridia that produce more butanol (i.e. C. Beijerinckii), greater solvent tolerance, and the ability to utilize cellulose directly.

REFERENCE

[78]. "ButylFuel, LLC Main Page". Butanol.com. 2005-08-15. Retrieved 2010-07-14.

[79]. Scott SA, Davey MP, Dennis JS, Horst I, Howe CJ, Lea-Smith DJ, Smith AG. 2010. Biodiesel from algae: challenges and prospects. Current Opinion in Biotechnology. 21(3):277-86.

[80]. Redman, G., The Andersons Centre. "Assessment of on-farm AD in the UK", *National Non-Food Crops Centre*, 2008-06-09. Retrieved on 2009-05-11.

[81]. Renewable fuels from algae: An answer to debatable land based fuels (Nithya Srinath) Academia.edu

[82]. "Can algae-based plastics reduce our plastic footprint?" Smart Planet. 2009-10-07. Retrieved 2010-04-05.

[83]. Biofuels Digest (2011-05-16). "Jatropha blooms again: SG Biofuels secures 250K acres for hybrids". Biofuels Digest. Retrieved 2012-03-08.

[84]. SG Biofuels (2012-03-08). "Jmax Hybrid Seeds". SG Biofuels. Retrieved 2012-03-08.

[85]. Plant Research International (2012-03-08). "JATROPT (Jatropha curcas): Applied and technical research into plant properties". Plant Research International. Retrieved 2012-03-08.

[86]. Kim, Hyungtae; Seungdo Kim, Bruce E. Dale (2009). "Biofuels, Land Use Change, and Greenhouse Gas Emissions: Some Unexplored Variables". pubs.acs.org. DOI:10.1021/es802681k. Retrieved November 8, 2011.

[87]. Fargione, Joseph; Jason Hill, David Tilman, Stephen Polasky, Peter Hawthorne (2008). "Land Clearing and the Biofuel Carbon Debt". sciencemag.org. DOI:10.1126/science.1152747. Retrieved November 12, 2011.

[88]. Atsumi, Shota; Hanai, Taizo; Liao, James C. (2008), "Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels", *Nature* **451** (7174): 86–89, DOI:10.1038/nature06450, PMID 18172501

[89]. J.L. Smith; J.P. Workman (December 20, 2007). "Alcohol for Motor Fuels". Colorado State University. Retrieved 2008-01-29.

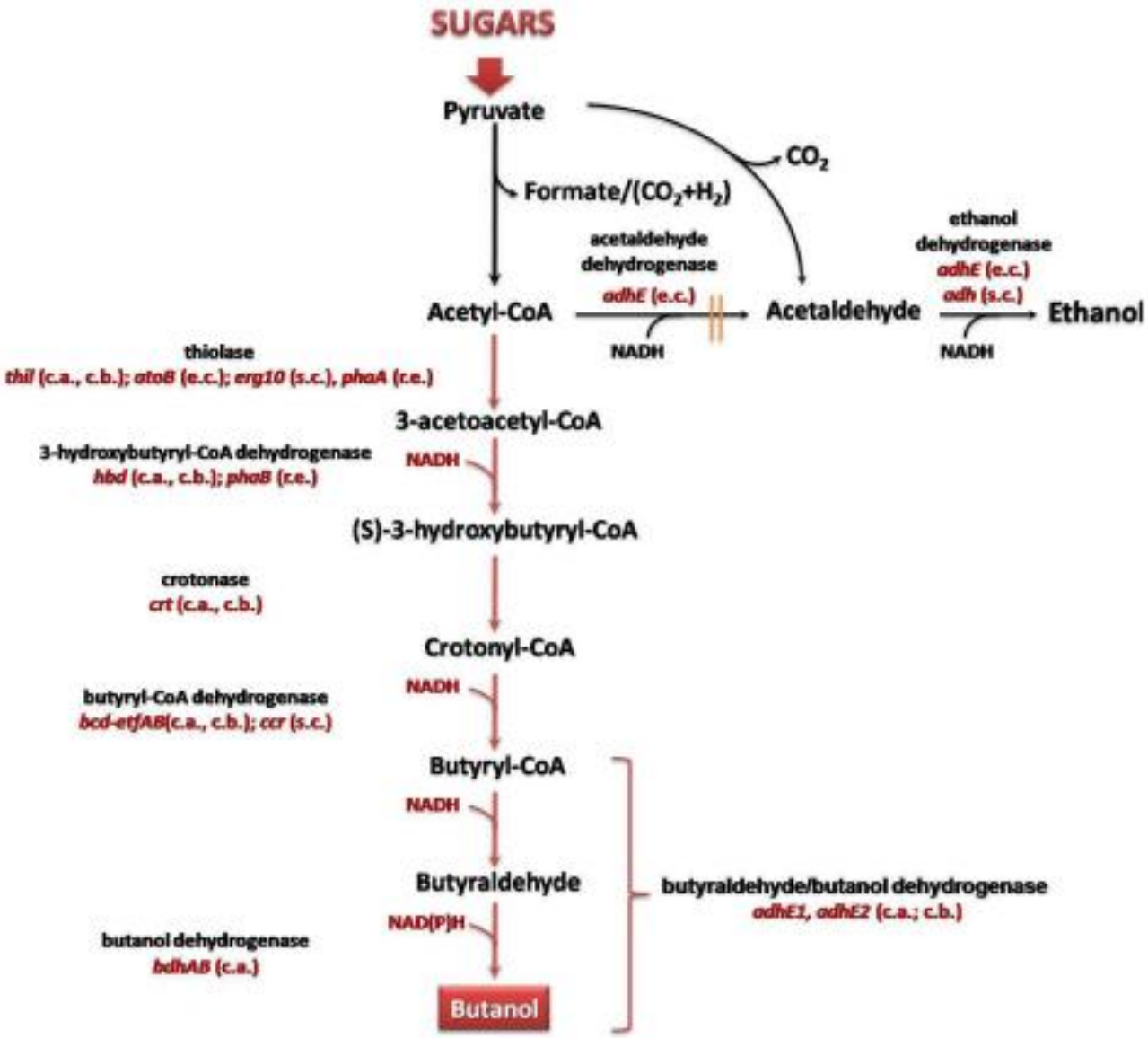
[90]. Scurlock, JMO.; Dayton, DC; Hames B. Bamboo: An overlooked biomass resource? *Biomass and Bioenergy*. Dept. of Energy, Oak Ridge. 2000.

[91]. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y.; Holtzapple, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology* 2005, *96,* 673-686.

[92]. Strobel, G.; Knighton, B.; Kluck, K.; Ren, Y.; Livinghouse, T.; Griffin, M.; Spakowicz, D.; Sears, J. (2008). "The production of myco-diesel hydrocarbons and their derivatives by the endophytic fungus Gliocladium roseum (NRRL 50072)". *Microbiology (Reading, England)* 154 (Pt 11): 3319-3328. DOI:10.1099/mic.0.2008/022186-0. PMID 18957585. edit.

[93]. EurekAlert. (2009). 15 new highly stable fungal enzyme catalysts that efficiently break down cellulose into sugars at high temperatures.

Appendix I



Metabolic pathway of Clostridia for ethanol and butanol production from Dellomonaco et al. Microbial Cell Factories 2010.

Appendix II

Table I: Glucose standard and Dinitrosalicylic acid (DNS) assay measurement

Test Tube No.	Standard Glucose (ml)	H_2O (ml)	DNS reagent (ml)	Absorbance (540nm)
Blank	0.0	3.0	1.0	0.000
1	0.2	2.8	1.0	0.141
2	0.4	2.6	1.0	0.274
3	0.6	2.4	1.0	0.410
4	0.8	2.2	1.0	0.552
5	1.0	2.0	1.0	0.677
6	1.5	1.5	1.0	1.048
7	2.0	1.0	1.0	1.380
8	2.5	0.5	1.0	1.692
9	3.0	0.0	1.0	1.982

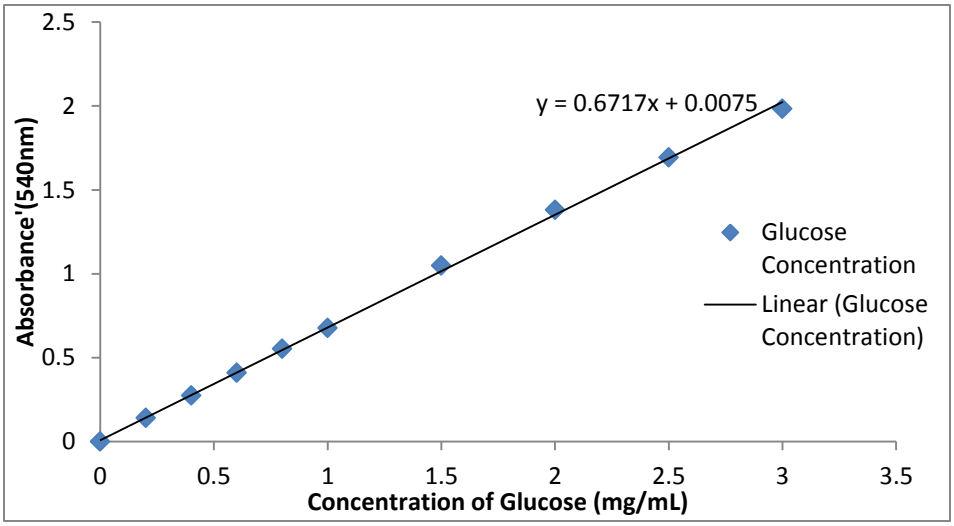


Figure I: Glucose standard curve and equation fit line for Dinitrosalicylic acid (DNS) assay

Calculation for sugar concentration of samples pretreated at 200°C:

$$C = \frac{X_{absorbance} - 0.0075}{0.6717} \qquad (1)$$

where C is concentration of glucose (mg/mL), X is absorbance of sample.

To calculate the yield in mg/g, the concentration found in Equation (1) was used in equation (2):

$$Yield = \frac{C \times V}{S} \qquad (2)$$

where V is the volume of the prehydrolysate (mL), S is the size of the bamboo substrate (mg).

Appendix III

Table II: Absorbance, Concentration and Yield of Pre-hydrolysate sugar measurements after dilute H_2SO_4

Tempt. (^oC)	Concn. % (v/v)	Time (hr)	Absorbance (540nm)	Concentration (mL)	Yield (mg/g)
	0.5	2	0.049	0.062	6.20
25		4	0.053	0.067	6.70
	1.0	2	0.145	0.205	20.50
		4	0.044	0.054	5.50
	0.5	2	0.094	0.129	12.90
110		4	0.122	0.170	17.00
	1.0	2	0.157	0.223	22.30
		4	0.136	0.192	19.20
	0.5	2	0.176	0.251	25.10
120		4	0.159	0.226	22.60
	1.0	2	0.190	0.272	27.20
		4	0.194	0.279	27.90
	0.5	2	0.200	0.287	28.70
150		4	0.341	0.497	49.70
	1.0	2	0.206	0.295	29.50
		4	0.258	0.373	37.30
	0.5	2	0.290	0.420	42.00
200		4	0.384	0.561	56.10
	1.0	2	0.328	0.477	47.70
		4	0.624	0.918	91.80
Unpre-treated sample	1.0	4	0.035	0.041	4.10
Pre-treated with water	1.0	4	0.041	0.050	5.00
Sugar Cane	1.0	4	0.948	1.400	140.00

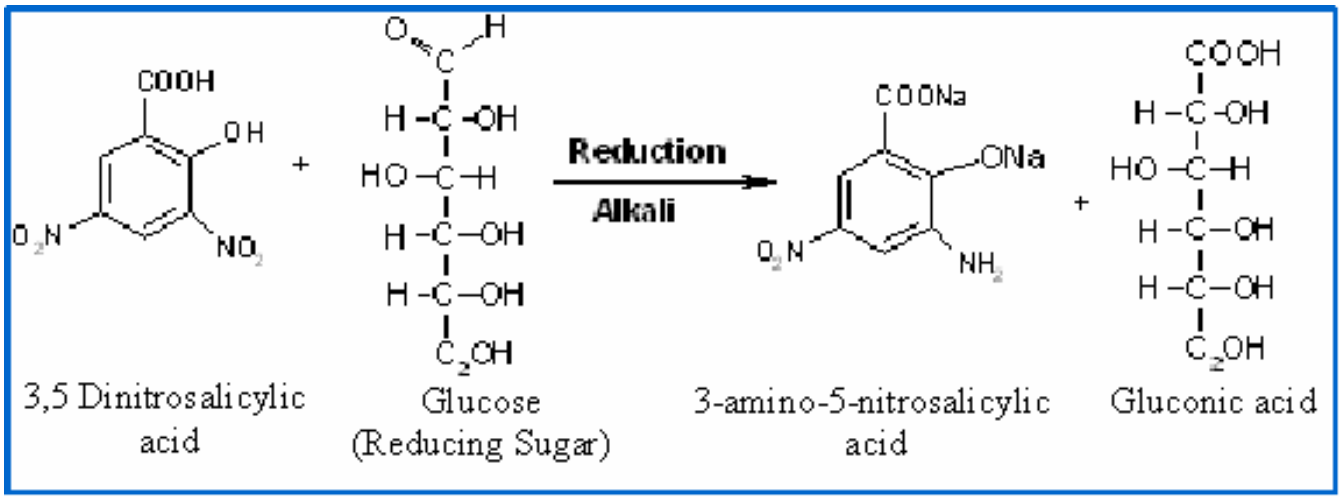
Appendix IV

Table III: Absorbance, Concentration and Yield of hydrolysate sugar measurements after Enzymatic Hydrolysis

Tempt. (^{o}C)	Concn. % (v/v)	Time (hr)	Absorbance (540nm)	Concentration (mL)	Yield (mg/g)
	0.5	2	0.148	0.209	20.90
25		4	0.376	0.548	54.80
	1.0	2	0.222	0.319	31.90
		4	0.454	0.664	66.40
	0.5	2	0.130	0.182	18.20
110		4	0.478	0.701	70.10
	1.0	2	0.283	0.410	41.00
		4	0.621	0.913	91.30
	0.5	2	0.269	0.390	39.00
120		4	0.542	0.796	79.60
	1.0	2	0.486	0.713	71.30
		4	0.696	1.025	102.50
	0.5	2	0.284	0.411	41.10
150		4	0.565	0.830	83.00
	1.0	2	0.535	0.786	78.60
		4	0.731	1.077	107.70
	0.5	2	0.385	0.562	56.20
200		4	0.629	0.925	92.50
	1.0	2	0.657	0.967	96.70
		4	1.035	1.530	153.00
Unpre-treated sample	1.0	4	0.069	0.092	9.20
Pre-treated with water	1.0	4	0.030	0.033	3.30
Sugar Cane	1.0	4	1.192	1.763	176.30

For total Sugar, the yield of Pre-hydrolysate sugar measured after dilute H_2SO_4 is added to yield of hydrolysate sugar measurements after Enzymatic Hydrolysis.

Appendix V



3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino-5-nitrosalicylic acid under alkaline conditions.

Equation for Sulfuric Hydrolysis of Cellulose

$C_6H_{10}O_5 + 3H_2SO_4 \longrightarrow C_6H_{10}(SO_4)_3O_2 + 3H_2O$

Equation for Conversion of Cellulose to Glucose

$(C_6H_{10}O_5)_n + nH_2O \longrightarrow n(C_6H_{12}O_6)$

Equation for Benedicts reagent for reducing sugars

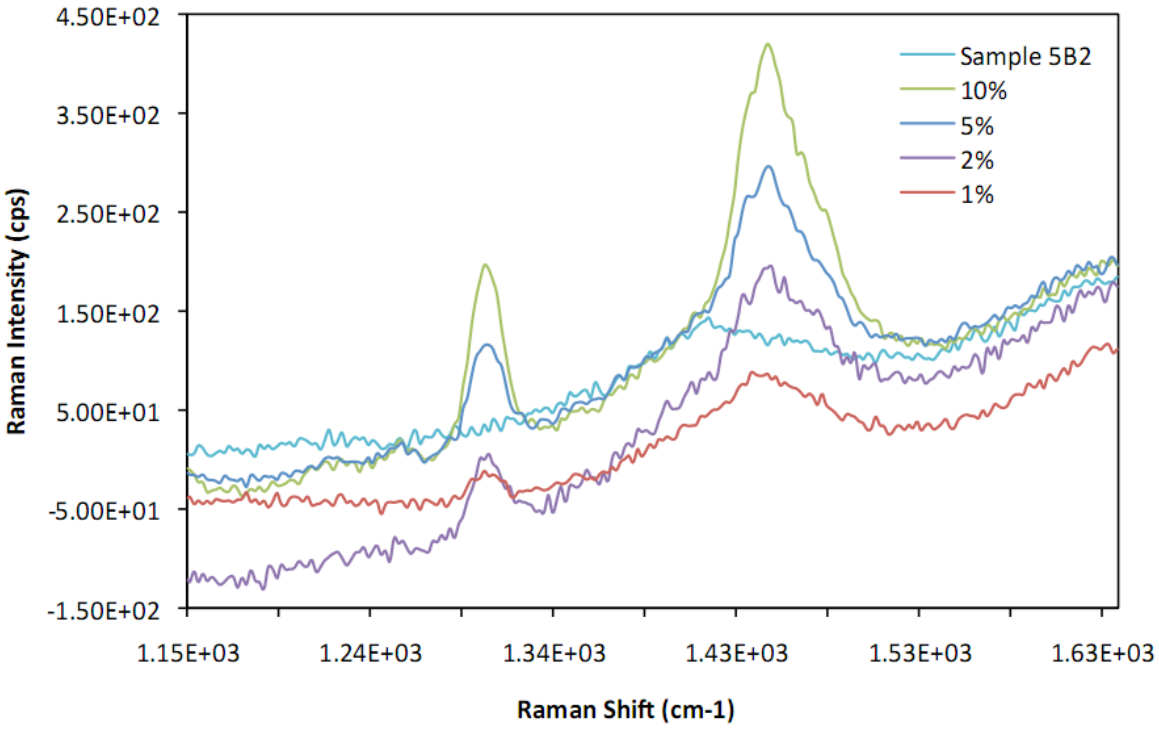
$C_6H_{12}O_6 + 2CuSO_4.5H_2O \longrightarrow C_6H_{12}O_7 + Cu_2O + 2H_2SO_4 + 8H_2O$

Glucose has a –CHO group, when benedicts reagent reacts will glucose, this –CHO group gets oxidized to –COOH group.

Equation for fermentation of Glucose

$C_6H_{12}O_6 \longrightarrow C_4H_9OH + H_2O + 2CO_2$

Appendix VI



Sample 5B2 Raman Spectroscopy Data: Comparison of peak intensities at 1420 C 1450 cmC1 for butanol Mixtures in water (1, 2, 5, 10% v/v), which was used to determine the unknown concentration of