Production of Liquid Fuel from Rice Husk and Sawdust

by

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A research report submitted in partial fulfillment of the requirements for the degree of Master of Science in Energy Science and Engineering

KHULNA UNIVERSITY OF ENGINEERING & TECHNOLOGY

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Declaration

This is to certify that the research work entitled "Production of Liquid Fuel from Rice Husk and Sawdust" has been carried out by Md. Kamrul Islam in the Department of Energy Science and Engineering, Khulna University of Engineering & Technology, Khulna, Bangladesh. The above research work or any part of this work has not been submitted anywhere for the award of any degree or diploma.

16.05.19
Signature of Supervisor

16.5.19
Signature of Candidate

DEDICATION

This project work is dedicated to my parents.

Approval

This is to certify that the research work submitted by Md. Kamrul Islam entitled "Production of Liquid Fuel from Rice Husk and Sawdust" has been approved by the board of examiners for the partial fulfillment of the degree of Master of Science in the Department of Energy Science and Engineering, Khulna University of Engineering and Technology, Khulna, Bangladesh in May 2019.

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Md. Kamrul Islam

Abstract

Energy is the key input to economic growth of a nation and there is a close relation between accessibility of energy and escalation in the quality of nation. As conventional energy sources are limited and will diminish in future after complete consumption, so it is the high time to deal with the renewable and non-conventional energy sources. The necessity of liquid fuel is not only in the transport sector rather it is required in industrial and power sector. Production of liquid fuel from ligno-cellulosic material is one of the sources of renewable energy. As Bangladesh is an agricultural country, rice husk and sawdust are the common sources among all sources of cellulosic material available in the country. In the present study, fermentation method is used to produce liquid fuel from rice husk and sawdust. For fermentation process simultaneous saccharification and fermentation (SSF) process being used. In the process, husk and sawdust was pretreated to neutralize it and then saccharification was carried out with cellulase enzyme with different proportion where temperature was maintained at around 37^{o}C for 84 hours. Fermentation reagents were prepared by adding yeast, peptone and dextrose for yeast inoculums. Saccharified slurry was clutched to fermentation in a mixture of reagents and fermentation medium. The process was carried out for one day in aerobic condition and then next three days in anaerobic condition at a temperature of 35^{o}C. After fermenting clear supernatant is obtained from centrifugation and then gas chromatography (GC) was performed on the product for estimation of ethanol. In the experimentation rice husk and enzyme mixture was added at the ratios of 2.5:1, 3:1 and 3.5:1 respectively. The yields of ethanol are respectively 9.55% (v/w), 8.73% (v/w) and 6.74% (v/w) from respective fermented liquid broth. Sawdust and enzyme ratio of 2.5:1 provide a very low about 1.23% (v/w) of ethanol. This shows that sawdust not prospective but rice husk is a prospective source of extraction of liquid fuel by fermentation.

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Nomenclature

SHF	Separate Hydrolysis Fermentation
SSF	Separate Hydrolysis and Fermentation
ATP	Adenosine Tri Phosphate
ADP	Adenosine Di Phosphate
NAD	Nicotinamide Adenine Dinucleotide
ADH	Alcohol Dehydrogenase
EtOH	Ethyl Alcohol
YPD	Yeast Peptone Dextrose
Expt.	Experiment
GC	Gas-Chromatographic
A-1	Rice Husk Sample in Experiment 1
A-2	Rice Husk Sample in Experiment 2
A-3	Rice Husk Sample in Experiment 3
S-1	Sawdust Sample in Experiment 4
S-2	Sawdust Sample in Experiment 5
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CHAPTER I

INTRODUCTION

1.1 General

Energy is the most important driver for the industrial development of a nation and there is a close relation between the accessibility of energy and the escalation in the development of a nation. Energy sources as conceived in the present world are divided into two major categories as renewable and non-renewable energy sources. Non-renewable or conventional energy sources do not form or replenish in a short period of time. Non-renewable energy sources come out of the ground as liquids, gases, and solids. The four major non-renewable energy sources are: Crude oil (Petroleum), Natural gas, Coal and Nuclear energy (Uranium). Crude oil is used to make liquid petroleum products such as gasoline, diesel and heating oil. Propane and other hydrocarbon gas liquids, such as butane and ethane, are found in natural gas and crude oil. Coal, crude oil, and natural gas are all considered as fossil fuels because they were formed from the buried remains of plants and animals that lived millions of years ago. Uranium ore, a solid, is mined and converted to a fuel used at nuclear power plants. Uranium is not a fossil fuel, but it may be classified as a non-renewable fuel. As the conventional sources (non-renewable) are limited and it will be exhausted after complete consumption of the reserve, so it is necessary to think about alternative fuel or new sources of energy. In that case renewable energy sources could be an immaculate preference to deals with [1]. Renewable energy is the energy that is generated from natural processes that are continuously replenished. The most abundantly available renewable energy is the solar energy which is basically the indirect source to other renewable energy. The other renewable energy includes various forms of biomass, wind, hydro, ocean-thermal, waves, tides and geothermal in special sense. This energy cannot be exhausted and is constantly renewed. Generally, it indicates energies that are non-traditional and have low environmental impact, doesn't pollute air enormously as non-renewable fuel does hence they are referred to as relatively clean energy.

For renewable energy sources rice husk and sawdust could be the choices for securing energy in an agriculture based country like Bangladesh. Bangladesh is an agricultural country and produces a vast amount of ligno-cellulosic biomass. These biomasses comprise of rice husk, rice straw, wheat straw, sugarcane baggase etc. Different crops like rice, wheat, sugarcane etc. are cultivated almost all over the country among them 74.85% of these cultivated land cultured rice. Among all the cultivated crops in Bangladesh rice straw hold the first rank position [2]. Rice husks are the largest mill-generated source of biomass available for energy use. As large quantities of rice husks are normally available at the rice mills, there are no additional efforts or costs involved in the collection of this biomass for use as an energy sources. Due to the availability of large quantities at any location, rice husks can be put to use for comparatively larger energy applications, like generation of steam for process heating applications, small scale heat exchanger operation etc. [3]. Chemical content of rice husk is 50% cellulose, 25-30% lignin, and 15-20% silica and in case of sawdust which is rich in ligno-cellulosic materials. These ligno-cellulosic biomasses can be a good source of starch which is the primary requirement of sugar production [4, 5]. Finally these sugars may be converted to ethanol by fermentation. Rice husk had been used as raw material of briquetting in Bangladesh. If it could be used to produce fermented oil then it may compensate the demand of transport-fuel. The production of ethanol from ligno-cellulosic waste comes under the second generation bio-fuel production. It is an alternative to the first generation biofuels which are produced directly from the food crops such as sugarcane, potatoes, corn etc. and emerges into food and fodder concerns [6, 7]. According to the Rice mills Owners Association of Bangladesh, there are about 100,000 rice mills and 90% of which are located in four cluster areas. These four cluster areas are Dinajpur (North Bengal), Sherpur (Near Bogura), Ishwardi (Near Kushtia) and Kaliakoir (Near Dhaka). Taking an average (lower-mid) capacity range of about 100-200 kW, there is a 50-100 MW power markets in these cluster areas [8]. Total rice production in Bangladesh is about 25.0 million tons/year [9]. Total amount of available husk, assuming 20% weight is converted into husk, equals 5 million tons/year. Therefore, total amount of available husk would be approximately 14 tons/day [10]. Saw mills are nested throughout the country hence sawdust exists in everywhere in Bangladesh. So, the option of choosing rice husk and sawdust to facilitate ethanol production would be a fair choice for their ease of availability and they are generally treated as waste.

Generally, biomass can be converted to convenient gaseous or liquid fuels by applying certain technologies like pyrolysis, gasification, anaerobic digestion or fermentation etc. These techniques may be broadly classified into thermo-chemical conversion and biochemical conversion. Thermo-chemical conversion is the process by which biomass is broken down into smaller molecules (both liquid and gaseous) at high temperature and pressure. Biochemical conversion is the process of converting biomass into convenient intermediate fuels carried out by the action of certain micro-organisms. The two most prominent biochemical conversion processes are anaerobic digestion and ethanol fermentation.

A number of studies have been conducted on ethanol production from lingo-cellulosic material. In 1995 Ingran and Doran [11] work on conversion of cellulosic materials to ethanol. In 2002 Sun and Cheng [12] gave the feedback of both separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) of ethanol production. Ayhan Demirbas (2003) stated Cellulosic materials can be used to produce bio-ethanol [13]. Ollosfon et al. (2008) avowed a review on bio-ethanol production using SSF methods of fermentation using wheat straw as lingo-cellulosic feedstock [14]. Yamada et al. (2011) studied on direct ethanol production from cellulosic materials using *Saccharomyces cerevisiae* with optimized cellulase expression from rice straw [15]. Shing et al. (2014) carried out experiments on ethanol production with enzymatic hydrolysis of microwave alkali pretreated rice husk for ethanol production by *Saccharomyces cerevisiae*, *Scheffersomyces stipitis* and their co-culture [16]. Sana et al. (2017) used SSF method for producing bio-ethanol from Pakistani ligno-cellulosic biomasses [17]. It has been observed that in Bangladesh there is ease of availability of biomass and most ethanol production methods from sugar containing materials hence it may be a blond preference to work on other biomasses containing cellulose and hemi-cellulose in order to produce ethanol.

Generally ethanol production processes are outlined as milling, sterilization, cooking, cooling, fermentation, distillation, dehydration, denaturing and fuel co-products [18]. In case of ligno-cellulosic material there are two types of fermentation processes: Separate Hydrolysis Fermentation (SHF) and Simultaneous Saccharification and Fermentation (SSF). SHF is the process of varying bioconversion conditions. First carried out it for hydrolysis in order to

produce monosaccharide sugar after that fermentation process will start. On the other hand, in SSF process ligno-cellulosic biomasses produce glucose and it rapidly converted to ethanol by the yeast. Both processes have been widely used for the production of ethanol. In contrast, ethanol production by SSF process can be simultaneously completed within a single step and provides a higher ethanol yield than SHF process [19, 20]. The choice of pretreatment methods plays an important role to increase the efficiency of enzymatic saccharification thereby making the whole process economically viable. Experimentally SSF method is more cost effective than SHF method [20, 21]. The main advantages of SSF over SHF are higher ethanol yields and less energetic consumption. The drawback of SSF is that the optimum conditions, especially the optimum temperature for the cellulases (enzyme) and the microorganism differ [22, 23]. At first ligno-cellulosic biomasses are pre-treated for saccharification comprised of removing dust and preparing for break down the cellulose into starch. During saccharification cellulase enzyme would have to be used. Then fermenting reagents would be prepared by using *Sacchromyces cervisae* in order to make yeast inoculums. These inoculums will convert the sugar materials (starch) into ethyl alcohol (ethanol) [24]. Wood consists of 90% of its masses as lignin and the rest is cellulose. Sawdust is that found in saw mills would be a potential source of starch generating biomass follow through ethanol production [25].

Typically ethanol is produced from a variety of sugar containing biomass by fermentation with yeast. In this case, generally sugarcane, molasses, sweet sorghum etc. are being used for fermentation. The focal attraction of sugar bearing materials for ethanol production lies in the fact that their carbohydrate content is already in fermentable form or in other word in simple sugar form such as glucose or fructose. Starches (such as cassava, corn, potatoes etc.) contain carbohydrate of larger molecular complexity, which have to be broken down to simple sugars by a saccharification process. On the other hand, carbohydrate in the cellulosic materials (rice husk, saw dust, agricultural residue etc.) have an even greater molecular complexity and need to be converted to fermentable sugars by enzyme or micro organisms, because yeast can act on simple sugars to produce ethanol. So, a number of works has been carried out to produce ethanol from sugar bearing materials in commercial scale but on the other hand due to complexity of conversion of cellulosic materials into sugar forms for production of ethanol from ligno-cellulosic material have done with relatively fewer numbers. In Bangladesh this been infrequent

to produce ethanol from ligno-cellulosic biomasses. Therefore, an attempt may be made to extract ethanol from biomass materials containing cellulose or ligno-cellulosic materials.

1.2 Objectives of the Research

The main objectives of the research are as follows:

i. To covert the ligno-cellulosic material (rice husk and sawdust in different proportion) into starch by cellulase enzyme.
ii. To convert the starch into ethanol by fermentation method using SSF process.
iii. To assess the properties of ethanol produced from rice husk and sawdust independently.
iv. To assess the properties of ethanol produced from mixtures of rice husk-sawdust.

CHAPTER II

THEORETICAL ASPECTS AND LITERATURE REVIEW

2.1 Renewable Energy Technologies

Renewable energy, often referred to as clean energy, comes from natural sources or processes that are constantly replenished. Such that solar, wind, tidal etc. are doing their regular task as usual vastly depends on their availability in nature. Renewable energy is habitually assumed as a new technology, exhausting nature's power has long been used for heating, transportation, lighting, and more. Various grains or grind flour in the wind mills are powered by the wind energy. The sun has provided during the daylong ray that is been captured and utilize to power generations. Besides this researchers are putting more effort on the potentials of utilizing appropriate technologies to recover energy and useful by products from non-biodegradable domestic and industrial solid wastes. Such materials include biomass residue, municipal solid wastes, medical wastes, industrial wastes, rubber and plastics etc. Biofuels are important for several reasons in fact biofuels are an encouraging alternative for liquid type transportation fuel that historically comes from petroleum. While other sources of renewable energy such as solar, wind, tidal energy are useful for electricity generation, used in domestic or industrial purposes; none of these sources are suitable for transportation fuel. Biofuels can overcome this problem as they are liquid can simply use in transportation sector. Regarding this renewable energy technologies have been discussed subsequently in the following sections.

2.1.1 Biofuel

Fuel that is derived from biomasses is referred to as biofuel. Biofuel often considered as a source of renewable energy, unlike fossil fuels such as petroleum, coal, and natural gas. There are two main types of biofuels known as ethanol and biodiesel [26]. The term biofuel is usually used to refer liquid fuels, such as ethanol and biodiesel that are used as replacements of transportation fuels like petroleum, diesel and jet fuel [26]. Biofuels are currently the only viable replacement

to hydrocarbon transportation fuels because it can be used in existing combustion engines, nominal changes to infrastructure are required for their employment [27]. This is the most prominent advantages as concern to the environmental impacts of fossil fuels that continues to rise; though biofuels are not as energy dense as conventional transportation fuels but it has an enormous prospect to be used in transportation sector. 1 gallon of biodiesel has 93% of the energy of 1 gallon of diesel and 1 gallon of ethanol has 73% of the energy of 1 gallon of gasoline [28].

2.1.2 Biofuel Production Processes

Biomass is the primary energy sources to produce biofuels. There are various adequate and specific technologies for biomass conversion processes. It can broadly be divided into 4 categories such as: 1) Physical 2) Agrochemical 3) Thermochemical and 4) Biochemical. They are shortly outlined in Table 2.1 [29]. The conversion of biomass is shown in Figure 2.1 as flow chart [30].

Table 2.1: Biomass Conversion Processes

Catagory	Process
Physical	➢ Briquetting ➢ Pelletisation
Agrochemical	➢ Fuel extraction (from freshly cut plants)
Thermochemical	➢ Combustion ➢ Carbonisation ➢ Pyrolysis ➢ Gasification ➢ Liquefaction
Biochemical	➢ Anaerobic digestion ➢ Biodiesel ➢ Ethanol fermentation

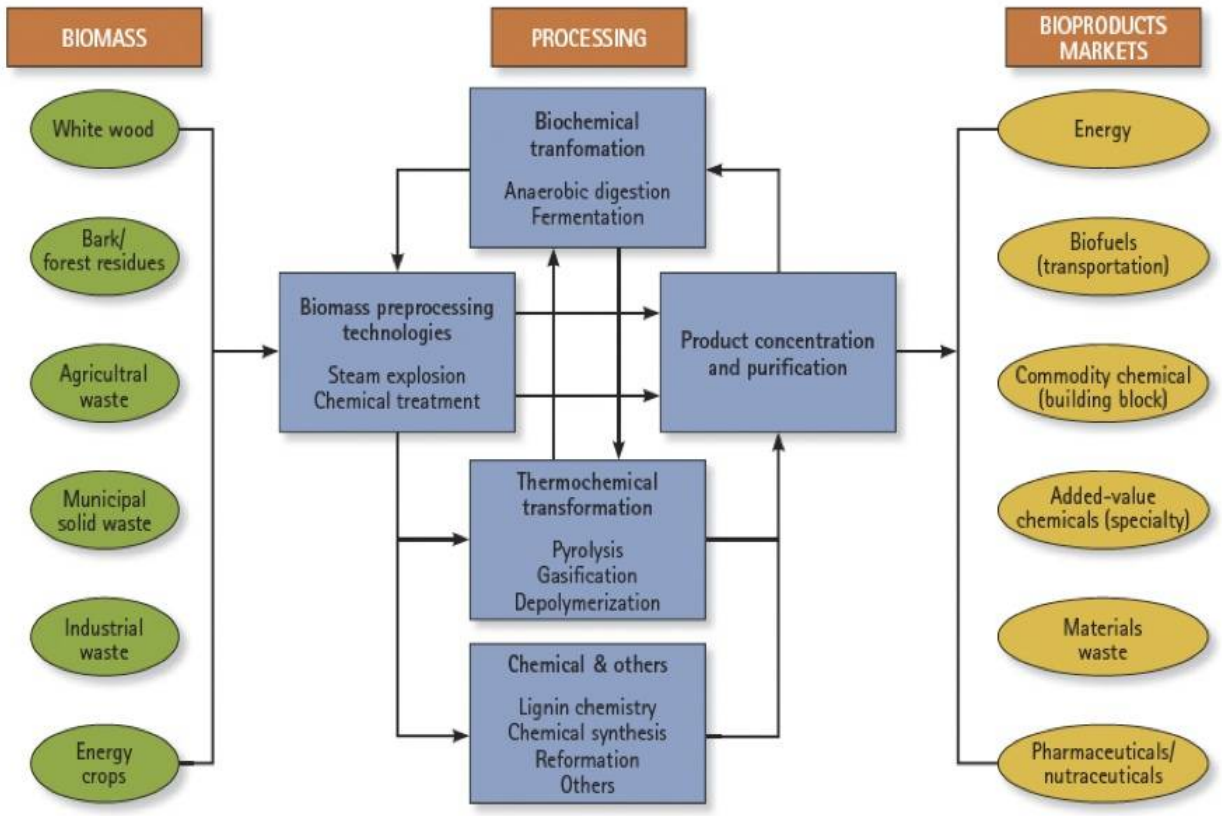

Figure 2.1: Flow Chart of Biomass Conversion Processes

Physical Conversion of Biomass

The simplest method of physical conversion of biomass through the compression of combustible material. Commonly classified into two processes briquetting and pelletisation. Briquetting is a well known technique. This is brought about by compression balling. Briquetting is carried out by compression under a die at high temperature for moisture removal and pressure. Pelletisation is a process in which wood wastes are compressed and extracted in the form of rods. Pelletising reduces the moisture content and increase the bulk density of the biomass [29]. The physical process of bioconversion can be illustrated in Figure 2.2 which shows a flow diagram of biomass briquette production [31].

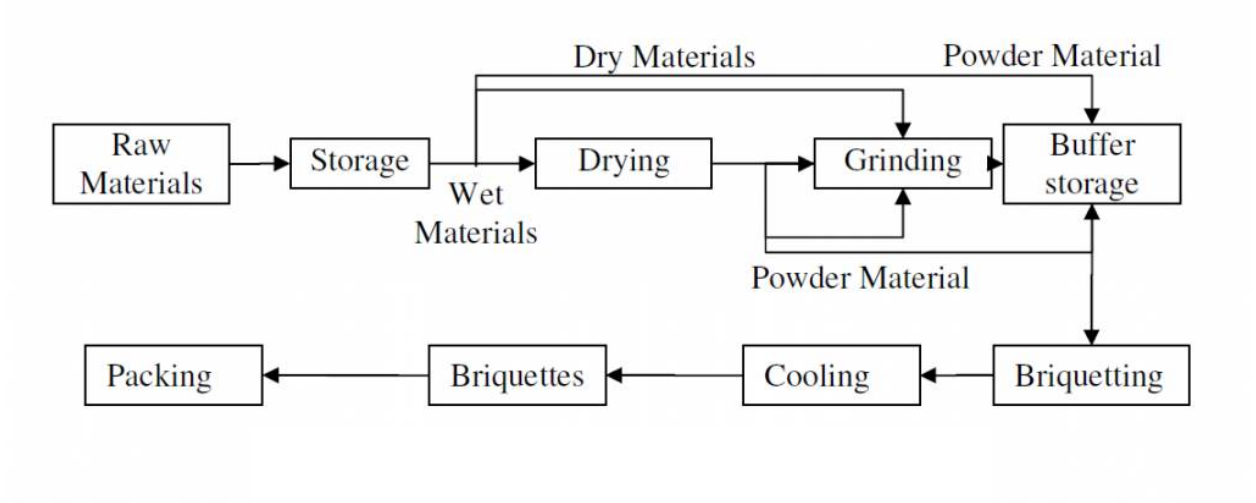

Figure 2.2: Flow Diagram of Biomass Briquette Production

Agrochemical Conversion of Biomass

Agrochemical fuel extraction describes the production of fuels from plants. The plant usually remains alive and unharmed. Generally liquid or solid fuels may be obtained directly from living or freshly cut plants. The oil of the plant itself can directly used as an energy source. The oils are essentially used for the production of food products and the manufacturing of paint, colors, soap and cosmetic articles [29].

Thermochemical Conversion of Biomass

Thermochemical processing is the use of heat to promote chemical transformations of biomass into energy and chemical products. It mainly includes the processes of Combustion, Carbonisation, Pyrolysis, Gasification and Liquefaction [32]. In Figure 2.3 shows a simple flow chart of thermochemical conversion processes of biomass [33]. The different thermochemical process are briefly described below:

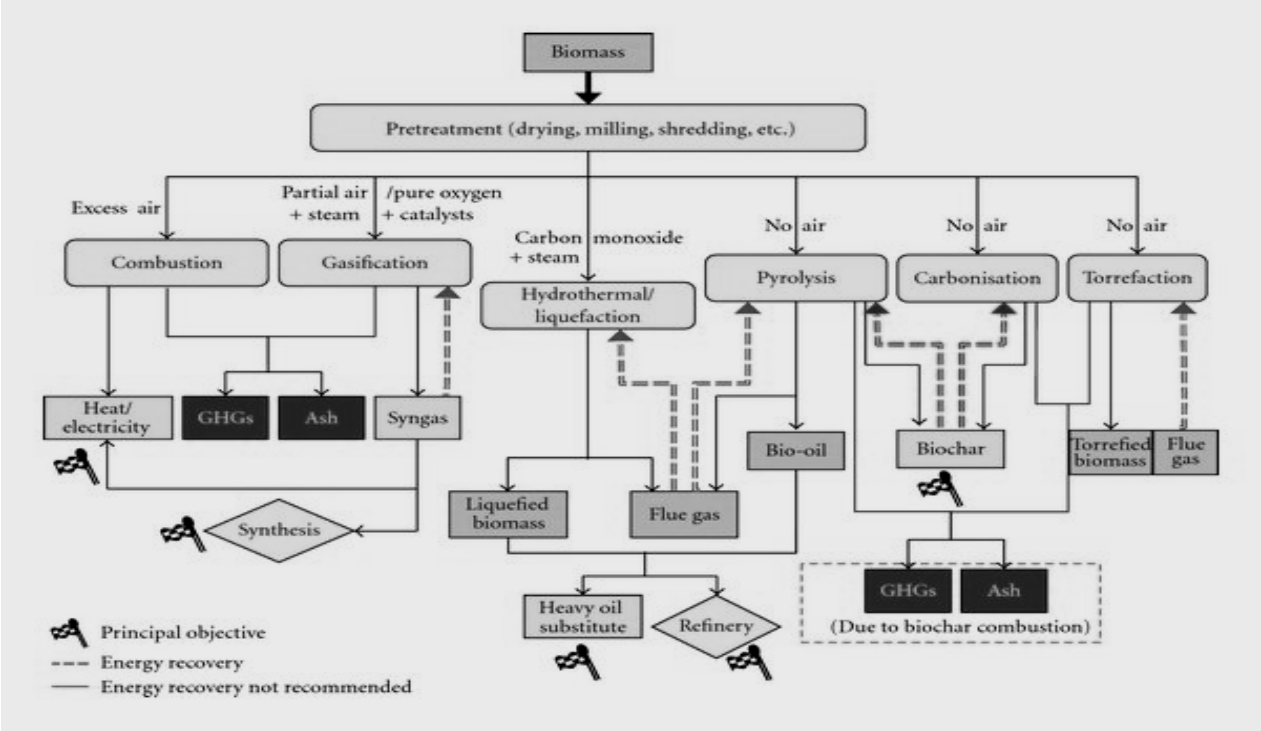

Figure 2.3: Flow Chart of Thermochemical Conversion Processes

1. Combustion: The oldest known and most widely used controllable energy source is known as biomass energy in the earth. Due to the rising cost of fossil fuel and the advance equipment development, the biomass energy is more practical. Conversion of biomass to heat is the commonly used technology around the world. The excess air helps to produce heat during combustion. The first stage of combustion involves the evolution of combustible vapors from the biomass, which burn as flames. The residual material, in the form of charcoal, is burnt in a forced air supply to give more heat. The hot air, hot water or steam is produced by combustion of gases or it can be used directly for drying purpose. The combustion efficiency depends primarily on good contact between oxygen in the air and the biomass fuel. The emission of CO_2, stem are the main product of biomass during combustion. Minimizations of these emissions of their possible effects are significant anxieties in the design of environmentally suitable biomass combustion methods [29, 32].

2. Carbonisation: In this process wood is heated slowly with a restricted air flow to form a high carbon product by removing volatile materials from it. The final product is known as the

charcoal. It is extensively used as a domestic fuel. Charcoal contains 20-25% volatiles and 75-80% fixed carbon on a dry basis [29]. The carbonisation process takes places in four main stages determined by temperature attained in each stage. In first stage referred as endothermic and involves the initial drying of the wood to be carbonized at a temperature up to 200^{o}C [29]. The second stage is known as pre-carbonisation stage, which includes to producing some pyroligneous liquids as well as small quantities of non condensable gases that are CO and CO_2 at a temperature range of 170-300^{o}C [29]. The third stage is exothermic and takes places in 250-300^{o}C. In this stage greater proportion of the light tars and charcoal is being produced. The fourth stage follows the temperature above 300^{o}C. In this stage the bulk of remaining volatile components of the charcoal are driven off, thus increasing the carbon content of charcoal [29]. Following the carbonisation, the charcoal product is allowed to cool, which may take a few hours to many days depending on the type of kiln used for the production.

3. Pyrolysis: Pyrolysis process can be used to produce liquid fuel. Generally, pyrolysis process converts liquid fuel by using biodegradable and non-biodegradable materials. Optimum conditions favors the high yield of liquid and bio-oil can be produced by rapid cooling of pyrolysis vapor [34]. Pyrolysis was performed the temperature between 400 and 650^{o}C without O_2 to decomposed organic materials. The solid non-volatile species is known as bio-char. A portion of the gas phase volatiles shrink into a black, viscous fluid termed bio-oil [35] that has a diversity of substitutes including pyrolysis oil, bio-crude oil, bio-fuel oil, wood liquid, wood oil, liquid smoke, wood distillates, pyroligneous tar, and pyroligneousacid [36]. Fast pyrolysis and slow pyrolysis was performed at the temperature range 400–600°C.It has been used for epochs to generate methanol and yields approximately equal extents of char, gas, and liquid [37, 38]. Relatively high liquid yield achieved through rapid heating rates of 10 to >1000°C/s in fast or flash pyrolysis. It is required short residence times of less than two seconds and rapid slaking of the vapors. Figure 2.4 represent a schematic arrangement of pyrolysis process [39].

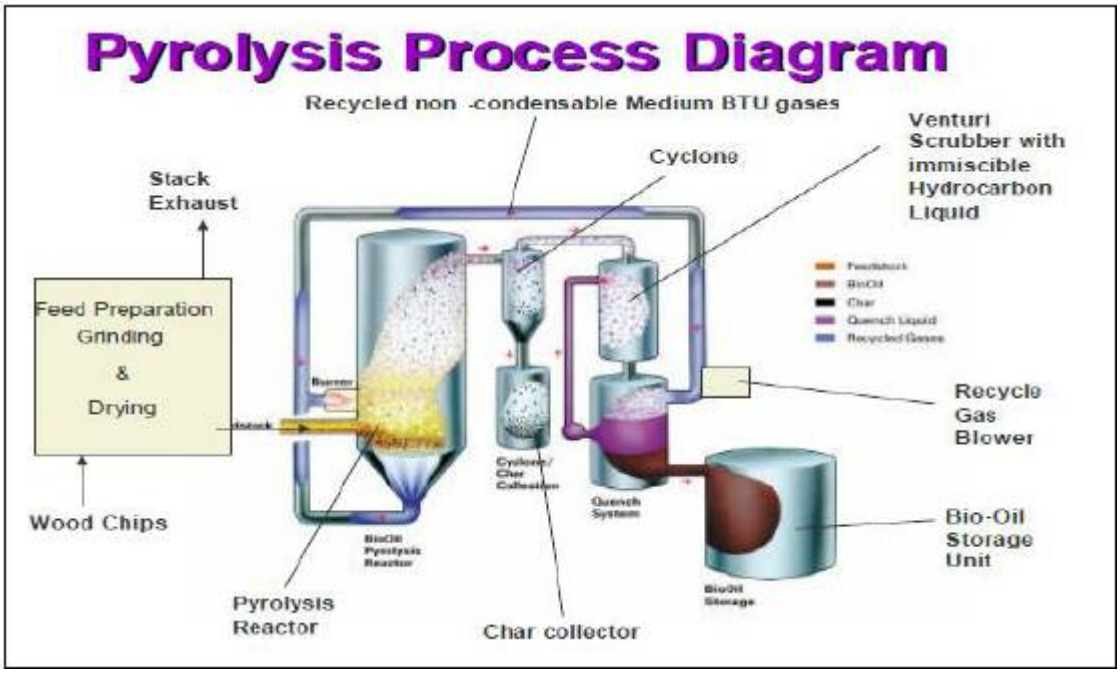

Figure 2.4: Schematic Arrangement of Pyrolysis Processs

4. Gasification: Gasification of biomass is thermal decomposition in the presence air. It is the conversion process of solid, carbonaceous fuels into combustible gas mixtures, known as producer gas. It is also referred to as wood gas, water gas and synthesis gas. This gas can burn directly in a furnace to generate process heat for electricity generation. It can also used in as fuel in internal combustion engines and gas turbines. Wood, municipal waste or other biomasses can partially oxidize at atmospheric pressure to develop a crude gas consisting primarily of H, CO and CO_2. This process is similar to production of gas by partial oxidation of natural gas or petroleum fractions. After purification, it can be subjected to the shift conversion of CO_2 and steam to obtain more CO and H to give a synthesis gas for methanol. When partial oxidation is carried out with air instead of oxygen the synthesis gas contains nitrogen. Removal of CO and CO_2 can give a mixture of hydrogen and nitrogen in the proper ratio to serve as ammonia for synthesis. The most widely applied reactors for gasification of biomass are countercurrent moving bed gasifier, cocurrent moving bed gasifier, cross-current moving bed gasifier and fluidized bed gasifier [40]. All the gasifiers have consisted several stages of gasification process (drying, pyrolysis, reduction and oxidation) with different temperature range. A typical schematic view of gasification process is shown in Figure 2.5 [41].

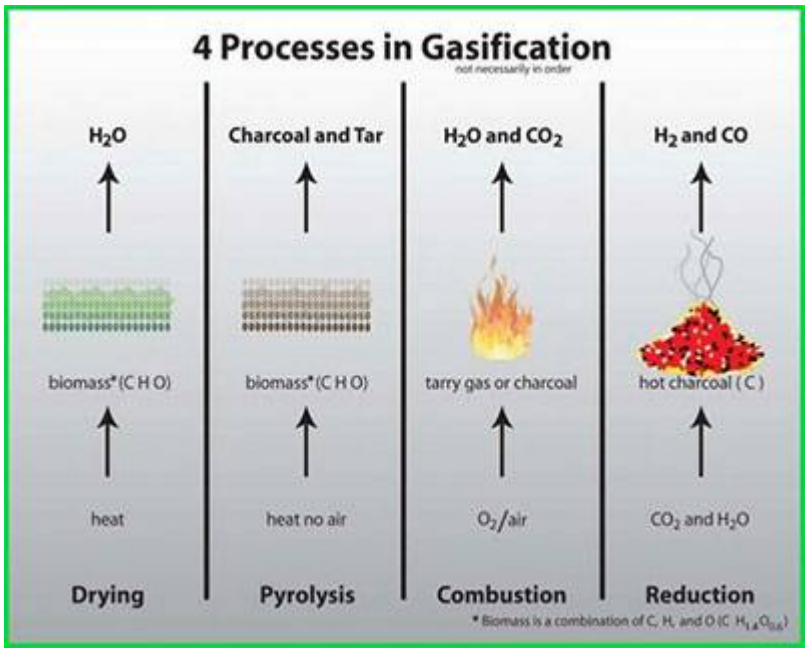

Figure 2.5: Schematic View of Gasification

5. Liquefaction: Liquefaction is a high pressure and temperature conversion process available to convert organic matter into oil. The reduction involves heating the raw material at 240-400^{o}C and high pressure (10.34 X 10^{6} to 27.58 X 10^{6} Pa) in the presence of CO, steam and a catalyst. About 318 liters of oil are produced per ton of organic inputs [54]. It can be achieved through two processes one is liquefaction through pyrolysis without any gasification medium (direct liquefaction) and other is liquefaction through methanol synthesis with gasification medium (indirect liquefaction or gasification + Fisher-Tropsch). Biomass can be converted into syngas through gasification and then into liquid hydrocarbons via Fisher-Tropsch, or into bio-oil/bio-crude through pyrolysis, hydrothermal conversion and solvolysis via liquefaction. Bio-oil and syngas can be further upgraded to liquid fuels such as methanol, gasoline, diesel fuel. On the other hand polyesters, polyurethane foams, phenolic adhesives can be obtained via solvolysis liquefaction. Valuable chemicals such as levulinic acid, hydroxymethylfurfuralthat formed by hydrolysis and dehydration of biomass can be further upgrade to liquid hydrocarbons [42]. A schematic view of direct liquefaction process of biomass conversion is illustrated in Figure 2.6 [43].

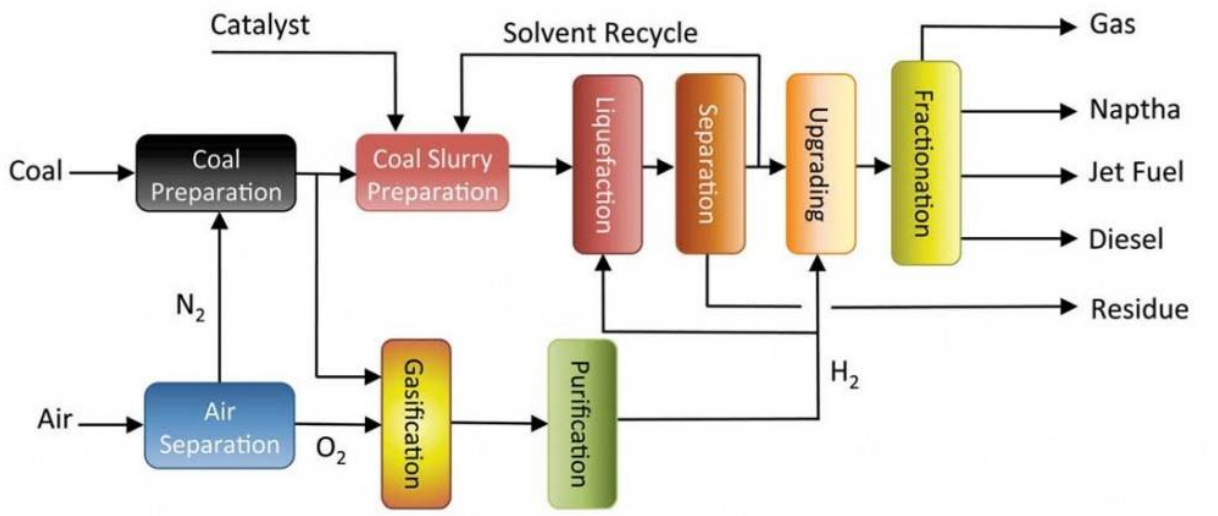

Figure 2.6: Direct Liquefaction Process of Biomass Conversion

Biochemical Process of Biomass Conversion

Biochemical conversion of biomass includes use of bacteria, microorganisms and enzymes to breakdown biomass into gaseous or liquid fuels. Liquid fuels, such as ethanol, methanol, biodiesel, Fischer-Tropsch diesel, and gaseous fuels, such as hydrogen and methane. The resource base for biofuel production is comprised of a comprehensive diversity of forestry based agricultural resources, industrial processing residue, municipal solid and urban wood residues. Globally, biofuels are most commonly used to power vehicles, domestic heating and for cooking.

1. Anaerobic Digestion: Anaerobic digestion is the decomposition of organic waste by bacteria in an oxygen free environment to gaseous fuel (mainly methane). This process breaks down the organic matter into simpler organic compounds. The final products are a mixture of methane, carbon dioxide and some trace gases known as biogas [29]. The process anaerobic digestion is known to exist for quite a long time. Biogas is also known as the swamp gas, sewer gas, fuel gas, marsh gas etc. In biogas production there need a digester which is sealed tank or container that control to achieve fermentation. The entire process takes place in three steps. At first step insoluble organic solids are converted to soluble compounds. In second step soluble compounds converted into short chain acids and alcohols. Finally products obtained in second steps are

converted to gases by different types of species of anaerobic bacteria. Figure 2.7 shows a typical view of anaerobic digestion process of biomass conversion [44].

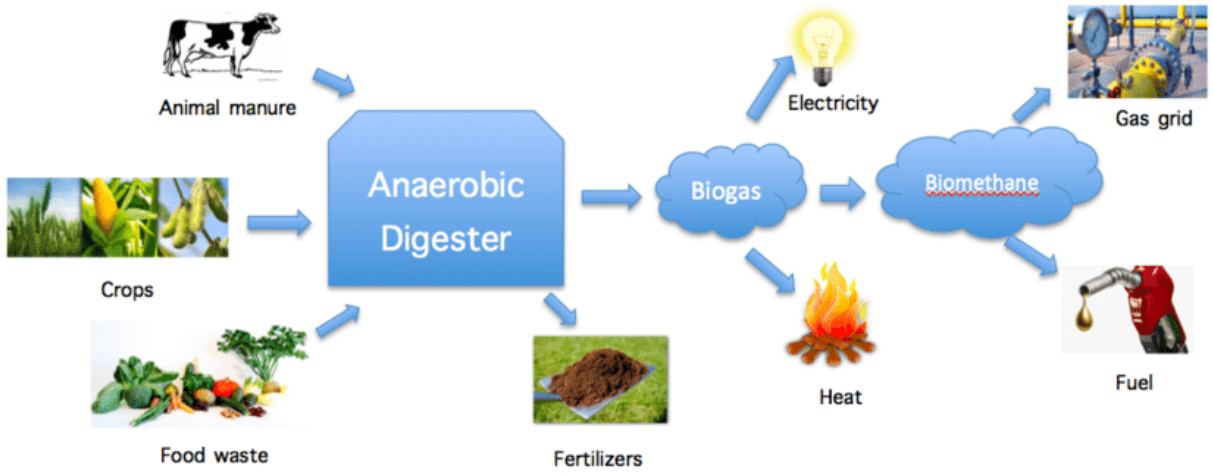

Figure 2.7: Anaerobic Digestion Process of Bioconversion

2. Biodiesel: Biodiesel production is the process of producing the biofuel, through the chemical reactions transesterification and esterification. This process involves short chain alcohols (typically methanol or ethanol) reacted with vegetable oils or animals fats. The alcohols used should be of low molecular weight ethanol being one of the most used one for its low cost for biodiesel production however greater conversions into biodiesel can be reached by using methanol. The biodiesel production can be proceed by acid or base catalyst however, most biodiesel produced by base catalyst. The acid catalyst has the high sensitivity with water. However, alkaline catalysis has the disadvantage of its soap production during the reaction [45]. As mentioned above biodiesel can be produced from straight vegetable oil, animal oil/fats, tallow and waste oils. The basic routes to biodiesel production are shortly described by firstly base catalyzed transesterification of the oil. Secondly direct acid catalyzed transesterification of the oil. Finally conversion of the oil to its fatty acids and then to biodiesel. In Figure 2.8 gives the schematic view of biodiesel production process [46]

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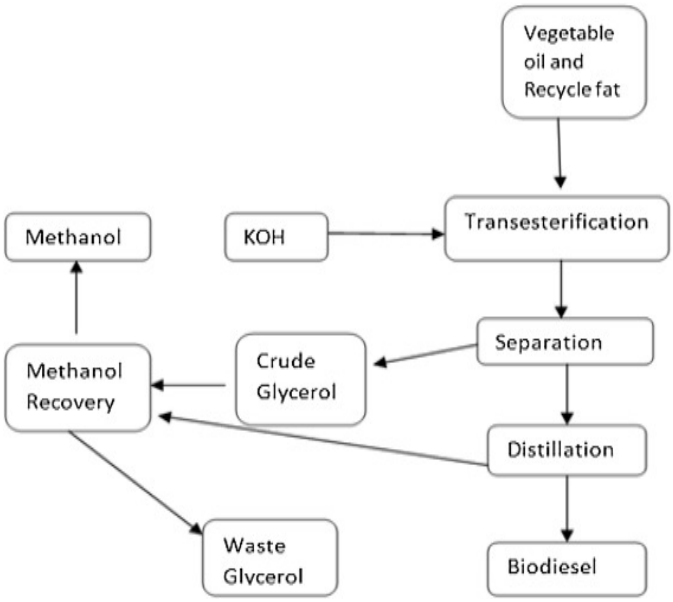

Figure 2.8: Schematic Diagram of Biodiesel Production Process

3. Ethanol Fermentation: Ethanol fermentation referred to as alcoholic fermentation which is a biological process that coverts sugar (such as glucose, fructose and sucrose) into cellular energy, producing ethanol and CO_2 as by products. Ethanol (C_2H_5OH) is a light alcohol and is a volatile, colorless, flammable liquid with a distinguishing odor. It is also known as ethyl alcohol and often abbreviated as Et-OH. The most common mode of production is the fermentation of sugar or starch from agricultural crops by yeasts or living microorganism. The procedure of ethanol manufacture depends on what kind of raw materials are being used. Ethanol production commonly carried out in the foremost three steps: (1) to obtain the solution encompassing fermentable sugars, (2) conversion of these sugars into ethanol by fermentation and (3) ethanol separation and purification, usually done by distillation–rectification–dehydration. The fermentation process can use any sugar-containing material to produce ethanol [47]. The fermentation reactions occur at temperatures between 25^{o}C and 30^{o}C and it last between 6 hr and 72 hr depending on the feedstock nature or fermentation process being used. The broth typically contains 8–14% of ethanol on a volume basis. The distillation step yields an azeotropic mixture made up of 95.5% alcohol and 4.5% water that is the ‘‘hydrous’’ or ‘‘hydrated’’ ethanol which is

then dehydrated to obtain an ‘‘anhydrous’’ ethanol containing up to 99.6% alcohol and 0.4% water[48].

The chemical equations below summarize the fermentation of sucrose ($C_{12}H_{22}O_{11}$) into ethanol (C_2H_5OH). Alcoholic fermentation converts one mole of glucose into two moles of ethanol and two moles of CO_2, producing two moles of ATP (Adenosine tri phosphate) in the process. The overall chemical formula for alcoholic fermentation is:

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

Sucrose is a dimer of glucose and fructose molecules. In the first step of alcoholic fermentation, the enzyme invertase cleaves the glycosidic linkage between the glucose and fructose molecules.

$$C_{12}H_{22}O_{11} + H_2O + \text{invertase} \rightarrow 2\ C_6H_{12}O_6$$

Next, each glucose molecule is broken down into two pyruvate molecules in a process known as glycolysis. Glycolysis is summarized by the equation:

$$C_6H_{12}O_6 + 2\ ADP + 2\ P_i + 2\ NAD^+ \rightarrow 2\ CH_3COCOO^- + 2\ ATP + 2\ NADH + 2\ H_2O + 2\ H^+$$

CH_3COCOO^- is pyruvate, and P_i is inorganic phosphate. Finally, pyruvate is converted to ethanol and CO_2 in two steps, regenerating oxidized NAD+ (Nicotinamide adenine dinucleotide) needed for glycolysis:

1^{st} step: $CH_3COCOO^- + H^+ \rightarrow CH_3CHO + CO_2$

2^{nd} step: $CH_3CHO + NADH^+ + H^+ \rightarrow C_2H_5OH + NAD^+$

The first step catalyzed by pyruvate decarboxylase and former reaction is catalyzed by alcohol dehydrogenase (ADH in baker's yeast). As shown by the reaction above, glycolysis causes the reduction of two molecules of NAD^+ to NADH. Two ADP molecules are also converted to two ATP and two water molecules via substrate-level phosphorylation [49, 50].

Bioethanol production from lingo-cellulosic wastes requires a number of stages, including the pretreatment of biomass with subsequent enzymatic hydrolysis followed by fermentation. During the fermentation process, sugars are converted into ethanol and carbon dioxide with the help of fermenting bacteria. A variety of microorganisms or enzymes are capable of fermenting sugars into bioethanol [51]. In case of ligno-cellulosic material there are two types of fermentation

processes: (a) Separate Hydrolysis and Fermentation (SHF) and (b) Simultaneous Saccharification and Fermentation (SSF). Both processes have been widely used for the production of ethanol. SHF is the process of varying bioconversion conditions. Firstly carried out it for hydrolysis in order to produce monosaccharide sugar after that fermentation process is begin. In other hand, SSF ligno-cellulosic biomasses produce glucose and it is rapidly converted to ethanol by the yeast [52, 53]. Yeast is eukaryotic organisms that are able to grow on different types of sugars while exhibiting high sugar and ethanol tolerance. There are various yeast being used for ethanol production such as *Saccharomyces cerevisiae, Scheffersomyces stipitis, Pachysolen tannophilus, Candida shehatae, Candida guillermondii*etc. Among them *Saccharomyces cerevisiae* is the most encouraging yeast strain intricate in the conversion of sugars into bioethanol. However, xylose, one of the major components of hemi-cellulose, cannot be converted into ethanol by most of the microbial strains used in industry. *Saccharomyces cerevisiae* has the ability to utilize both mono-meric sugars and sucrose, which make it an efficient microbe for use with a variety of substrates. Other benefits related to its application are resistance against high ethanol concentration, inhibitor resistance, and its ability to consume significant amounts of substrate under technologically suitable conditions [54, 55]. Figure 2.9 represents the basic formula of fermentation process [56].

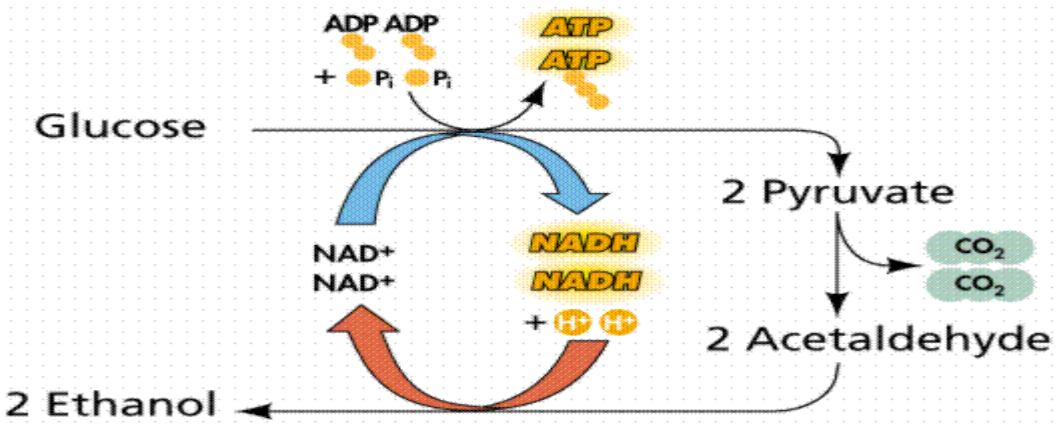

Figure 2.9: Basic Formula of Fermentation Process

(a) Separate Hydrolysis and Fermentation (SHF): The separate hydrolysis and fermentation (SHF) process is the oldest method used to produce bioethanol. In this process, externally produced enzyme blends are used to hydrolyze pretreated biomass to yield sugar monomers. The resulting enzymatic hydrolysate is used to produce biofuel by the action of fermenting bacteria. Both processes are performed separately because of the different temperature optima of hydrolytic enzymes (approximately 50°C) and fermentation (30-35°C) [57]. SHF can be run to allow each of the processes to take place at the optimal temperature. The dedicated hydrolysis in this process arrangement also allows for p^H to be adjusted following conversion to sugars in cases where there is a disparity between the p^H optima for the two processes. Moreover, separation of the hydrolysis and fermentation phases allows process tractability in the fermentation such as enabling batch and fed-batch processes [58]. The phases of this process are:

i) Pretreatment of lingo-cellulose using 2% NaOH and steam at 2 bars, for 30 minutes. After pretreatment, the liquid phase was reaped and neutralized with H_2SO_4 and in order to reach p^H 4.8 the pretreated substrate alternatively washed with distill water and H_2SO_4 [59].

ii) Enzymatic hydrolysis performed by specified or selected enzymes or microbes. The hydrolysis medium comprises of 1% yeast extract, 2% peptone, 7.5-10% biomass (dry weight), and citrate buffer of p^H 4.8. For the next stage of fermentation nutrients will added. At the end of this stage pretreated samples collected for estimate glucose or sucrose concentration [59].

iii) Fermentation is performed at 35^oC with designated yeasts in a water bath with shaker, for 2-3 days. For monitoring CO_2 and ethanol BlueSens sensors and gas counters are installed. Remained biomass is weighted at the end of the process. The complete process is carried out in 7–8 days [59].

Figure 2.10 shows the diagrammatic representations of SHF process [57].

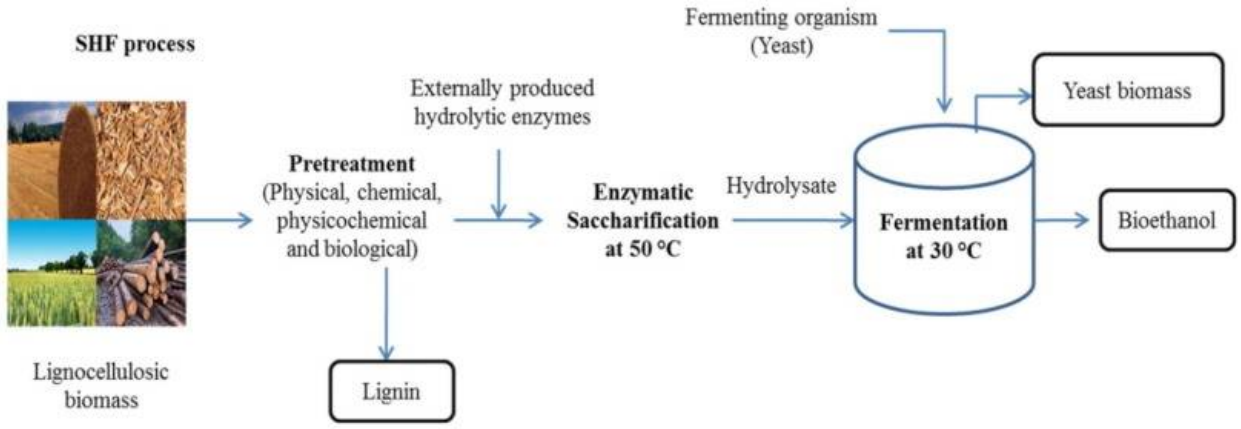

Figure 2.10: Diagrammatic Representation of SHF Process

(b) Simultaneous Saccharification and Fermentation (SSF): It is the option that is perform the enzymatic hydrolysis together with the fermentation, instead of consequent to the enzymatic hydrolysis is called simultaneous saccharification and fermentation (SSF). In SSF process ligno-cellulosic biomasses produce glucose and it rapidly converted to ethanol by the yeast. The difference between SSF process and SHF process is as follow: the enzymatic hydrolysis is carried out for a short period of 24 hours at 50^{o}C, tailed by inoculation. Afterwards the saccharification phase converts the biomass lignin or cellulosic material into sugar and then after fermentation processes are carried out simultaneously until the ethanol concentration is constant [59]. The major imbursements of performing the enzymatic hydrolysis together with the fermentation, instead of in a separate step after the hydrolysis, are the reduced final product reticence of the enzymatic hydrolysis and it minimizes the speculation costs. The main drawbacks, on the other hand, it needs to be finding out the optimum temperature and p^{H} for both enzymatic saccharification and fermentation. Fermenting microbes is not easily recycling. To overcome this situation primary requirement is to keep the temperature below 37^{o}C, whereas the low yeast concentration expedient to operate at a vast solid loading. Figure 2.11 gives the details of SSF process of biomass conversion [60].

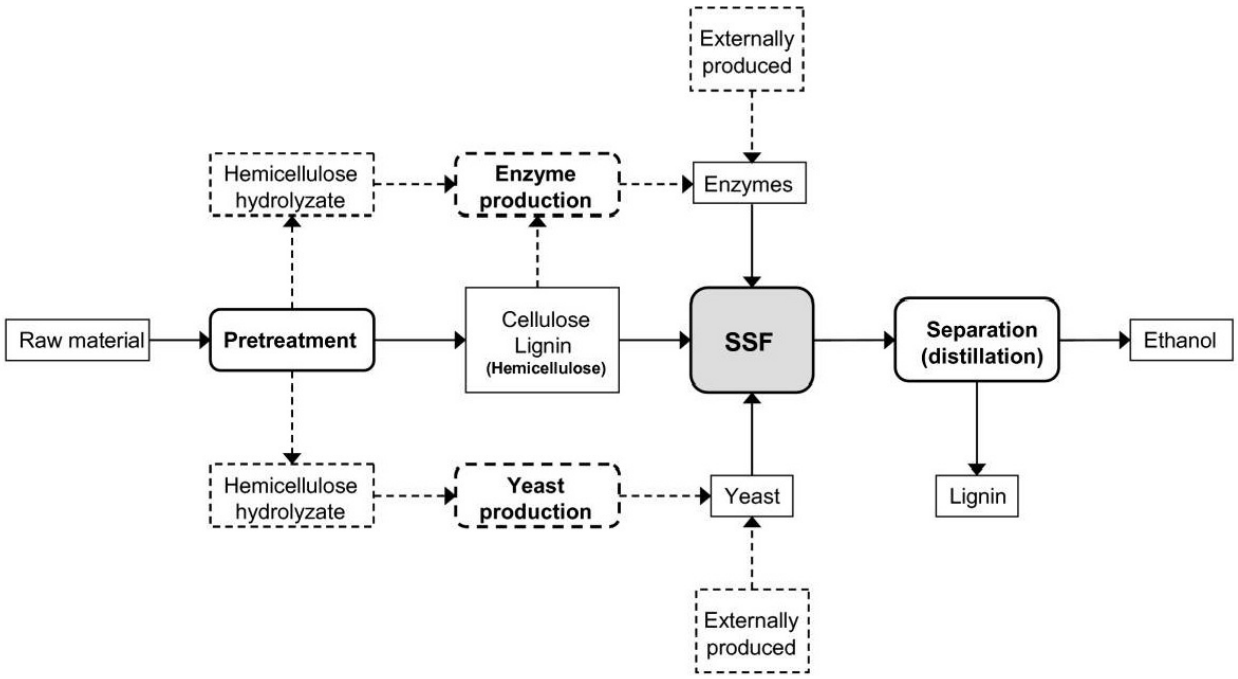

Figure 2.11: Schematic Representation of SSF Process

So, in short it generally state that hydrolysis and fermentation step is conducted simultaneously after pretreatment process for producing bioethanol. SHF was the conventional method that hydrolysis was carried out in the primary steps and then fermentation process has to perform. This process first allowed producing monosaccharide sugar in hydrolysis stage, so for fermentation sugar is being ready. The whole method, each process would get optimum condition, *Saccharomyces cereviceae*at 32°C, and enzyme at 50°C [61, 62]. In SSF, saccharification and fermentation situated in a single reactor, enzyme or microbes and yeast put together, so glucose or sucrose is rapidly converted into ethanol [63].

Ethanol

Ethanol is a chemical compound, a simple alcohol with the chemical formula C_2H_6O. Its formula can be also written as CH_3-CH_2-OH or C_2H_5-OH (an ethyl group linked to a hydroxyl group) and is often abbreviate as EtOH. Ethanol is a volatile, flammable, colorless liquid with a slight distinctive odor [64]. Ethanol is generally produced by fermentation of sugars by yeasts or via petrochemical processes. About 5% of the ethanol produced in the world in 2003 was actually a petroleum product. It is synthesis of catalytic hydration of ethylene with the aid of

H_2SO_4 as the catalyst. It can also be obtained via ethylene or acetylene, from calcium carbide, coal, oil gas, and other sources. Petroleum derived ethanol is almost identical to bioethanol chemically and only differ by the aid of radiocarbon dating [65]. Bioethanol is typically attained from the conversion of carbon-based feedstock. Agricultural feed stocks are well-thought-out as renewable because they get energy from the sun and using photosynthesis reaction that provide all kind of minerals needed for plant growth (such as nitrogen and phosphorus).Ethanol can be derived from a variety of feed stocks such as sugarcane, bagasse, miscanthus, sugar beet, sorghum, grain, switch grass, barley, hemp, kneaf, potatoes, sweet potatoes, cassava, sunflower, fruit, molasses, corn, stover, wheat straw, cotton, other biomass, as well as many types of cellulose waste and harvesting [66]. For ethanol production first process uses microbes or enzymes and yeast fermentation to convert the herb cellulose into ethanol while the second process uses pyrolysis to convert the whole plant to either a liquid fuel or a syngas. D Glucose and other sugars in the feed stocks are converted into ethanol and carbon dioxide during fermentation.

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

Pure ethanol is a flammable, colorless liquid with a boiling point of 78.5°C. Its low melting point of -114.5°C allows it to be used in antifreeze products. It has a pleasant odor reminiscent of whiskey. Its density is 789 g/l. its flash point 13^oC and having a molecular weight 46.07 g/mol [67].

Significance of Ethanol as a Fuel:

Ethanol has a number of uses in different sectors but in energy point of view it has a significant uses. It is used as a fuel. It burns in air to give carbon dioxide and water and can be used in combination with petrol.

$$C_2H_5\text{-}OH + 3O_2 \rightarrow 2CO_2 + 3H_2O$$

Mixture of petrol and ethanol is known as gasohol with 10-20% ethanol. The wide use of ethanol is as an engine fuel and fuel additives. It has been used as rocket fuel and is currently in light weight rocket-powered aircraft. Unleaded gasoline blended with ethanol are being used in automobile. There are two efficient blends of ethanol and gasoline generally used. The most

common blend is 10% ethanol and 90% gasoline. This mixture will power cars and requires no changes to existing internal combustion engine. The other combination called E85 is comprised of 85% ethanol and 15% unleaded gasoline. E85 is being used widely in flex-fuel vehicles. More than 97% of U.S. gasoline contains ethanol, typically in a mixture called E10, made up of 10% ethanol and 90% gasoline, to oxygenate the fuel and reduce air pollution. Ethanol has a higher octane number than gasoline, providing quality blending properties. Minimum octane number rations avert engine knocking and maintain drivability. Although the 10%-90% ethanol mixture has been in use for several years, the high percentage E85 mixture cannot to be used without modifications to existing internal combustion engines. Commercial fuel cells regulate on transformed natural gas, hydrogen or methanol. Due to its wide range of availability, low cost, high purity and low toxicity ethanol is a smart substitute to other engine fuel. There are a wide range of fuel cell perceptions that have been trialed including direct-ethanol fuel cells, auto-thermal reforming systems and thermally integrated systems. The mainstream of work is being conducted at a research level even though there are a number of organizations at the beginning of commercialization of ethanol fuel cells [66].

2.2 Literature Review

Number of studies have been reported regarding the biofuel (bioethanol) production. Ingran and Doran (1995) reported about the recombinant of the strains of microorganisms to produce ethanol from hemi-cellulosic material [11]. Sun and Cheng (2002) review a paper titled "Hydrolysis of lingo-cellulosic materials for ethanol production" and reported Separate Hydrolysis and Fermentation and Simultaneous Saccharification and Fermentation both processes have been widely used for the production of ethanol [12]. Iranmahboob et al. (2002) optimized acid hydrolysis regarding the production of ethanol. They produce ethanol from wood chips [68]. Ayhan Demirbas (2003) stated that cellulosic materials can be used to produce bioethanol. He concluded that the cellulose portion is hydrolyzed by acids or enzymes into glucose/sugar that is fermented to bioethanol [13]. Gasper et al. (2007) used corn fiber as raw material for producing ethanol. They uses cellulytic enzyme for breakdown of hemicelluloses of the corn fiber [69]. Farid Talbenia (2008) reported in his doctoral thesis entitled "Ethanol production from cellulosic biomass by encapsulated *Saccharomyces cerevisiae*" about

encapsulated cell system to produce bioethanol [70]. Ollosfon et al. (2008) avowed a review on bioethanol production using SSF methods of fermentation. They used wheat straw as a lingo-cellulosic feedstock [14]. Pejin et al. (2009) used SSF process for producing ethanol. They used wheat straw as raw material in their investigation [71]. Yanasae et al. (2010) reported their research work demonstrating direct ethanol fermentation from amorphous cellulose using cellulase expressing yeast [72]. Yamada et al. (2011) studied on the direct ethanol production from cellulosic materials using a diploid strain of *Saccharomyces cerevisiae* with optimized cellulase expression in their study they used their previously developed method to optimize cellulase expression levels in yeast, they constructed a diploid *Saccharomyces cerevisiae* strain optimized for expression of cellulolytic enzymes, and attempted to improve the cellulose-degradation activity and enable direct ethanol production from rice straw, one of the most abundant sources of ligno-cellulosic biomass [15]. Vazirzadeh et al. (2012) reported on bioethanol production by using white onion as feedstock and yeast as fermented material [73]. Kumari and Pramanik (2013) investigated on ethanol production using *Ipomoea carnea*as a raw feedstock. They used hybrid yeast to produce bioethanol from this biomass [74]. Reza Robati (2013) used same strategic approach as Vazirzadeh et al. (2012) to produce ethanol but he took green onion as feedstock [75]. In 2014, Irfan et al. stated in their article about the extraction of ethanol from agricultural wastes using *Saccharomyces cerevisia* they used three biomasses sugarcane bagasse, rice straw and wheat straw as raw material [76]. Shing et al. (2014) worked out on ethanol production by enzymatic hydrolysis of microwave alkali pretreated rice husk by *Saccharomyces cerevisiae*, *Scheffersomyces stipitis* and their co-culture. In this case, rice husk was used as raw material and enzymatic hydrolysis was carried out by yeast [16]. Karagoz and Ozkan (2014) work out in the same ethanol production by *Saccharomyces cerevisiae*, *Scheffersomyces stipitis* and their co-culture but they used wheat straw as feedstock [77]. Zabed et al. (2016) reported on bioethanol production from lingo-cellulosic material. Their research highlighted on an overview on the diversity of biomass, technological approaches and microbial contribution to the conversion of ligno-cellulosic biomass into ethanol [78]. Sana et al. (2017) conducted experiments using SSF method for producing bioethanol which was stated their article and they use Pakistani ligno-cellulosic biomasses as raw material [17]. Azhar et al. (2017) suggested that yeast is the best starin applied in bioethanol production. They showed both acidic and enzymatic hydrolysis method for sugar formation [79]. Roozeboom et al. (2018) reported on

production in long term biomass and potential ethanol yields of annual and perennial biofuel crops. They reported sweet sorghum produced substantially more ethanol than all other crops in their investigation [80]. Cacua et al. (2018) reported on their research on production of bioethanol from pretreated rice husk hydrolyzed with acid cellulase at pilot scale [81]. Agarwal et al. (2019) studied on bioethanol production from an agro-waste (de-oiled rice bran) by using *Saccharomyces cerevisiae* as yeast strain [82]. So, investigation on research outline shows that in Bangladesh production of bioethanol from ligno-cellulosic biomass is incomprehensible. Also, the characteristics of biomass may vary from region to region depending on climate and weather. Therefore, attempts may be made to extract bioethanol from ligno-cellulosic material available in Bangladesh.

CHAPTER III

METHODOLOGY AND EXPERIMENTATION

3.1 Major Outlines

As mentioned before, when the hydrolysis and fermentation are performed in a single unit, it is known as simultaneous saccharification and fermentation (SSF). In this process, the enzyme (cellulase and microorganisms) is added to the saccharification process and glucose is immediately consumed by the fermenting microorganism. Thus, the inhibition effect caused by sugars over the cellulases is neutralized. At first ligno-cellulosic biomasses are pre-treated for saccharification comprised of removing dust and preparing for breakdown of the cellulose into starch. During saccharification cellulase enzyme are used. Then fermenting reagents would be prepared by using *Saccharomyces cerevisiae* in order to make yeast inoculums. These inoculums will convert the sugar materials (starch) into ethyl alcohol (ethanol).

3.1.1 Sample Preparation

The feedstock is first kept at room temperature for chemical pre-treatment. The ligno-cellulosic substrates will be soaked into 2.5% (w/v) NaOH for 60 minutes at a solid liquid ratio of 1:10. After soaking, the substrates will be subjected to sterilization at 121°C for approximately 90 minutes. Then the sterilized substrates will be washed with distilled water in hot condition. The pretreated neutralized substrates will be oven-dried at 110^{o}C and stored in airtight envelops.

3.1.2 Saccharification and Fermentation

For enzymatic saccharification, the cellulase enzyme along with respected buffer added with the pretreated substrate at ratios of 1:2.5, 1:3.5 and 1:30 respectively and kept it in a shaking incubator at 37^{o} C for 84 hours. In this case p^{H} is maintained at 4.8 to 5.0.

Yeast, Peptone, and Dextrose (YPD) medium will be prepared by adding 1gm glucose, 2 gm peptone, and 1gm yeast extract in 100 ml distilled water. *Saccharomyces cerevisiae* will be added to the YPD medium to make the yeast inoculums. The inoculums culture will be added to facilitate the fermentation process during ethanol production. Fermentation medium will be prepared by 0.375 gm Yeast, 0.02 gm $CaCl_2$, 0.2 gm $(NH_4)_2SO_4$, 0.0625 gm $MgSO_4$ and 0.09375 gm KH_2PO_4 relevant to respective buffer and poured into a conical flask along with 62.5 ml distilled water and stirred well. For the fermentation process, saccharified slurry will be poured into a jar fermenter along with the fermentation medium and yeast inoculums. The solid liquid ratio of the substrate will be maintained at 5% (w/v) in each case. The SSF will carry out under aerobic conditions for 24 hours and shifted to anaerobic conditions for the next 72 hours with incubation at 35°C to complete the reaction.

3.2 Experiment

The detailed description of the experimental procedures for the ethanol production is presented in the following sections. Each sample is carried out for three successive experiments for general observation.

3.2.1 Sample Preparation (Experiment 1 to 12):

For the first three (No. 1 to 3) experiments 150 gm rice husk and 1500 ml distilled water is needed. 2.5 % (w/v) NaOH solution prepared by 37.5 gm NaOH added to 1500 ml distilled water. Then 150 gm rice husk is added to this solution and soaked it for 60 min. After soaking pretreated substrate was sterilized at 121^oC for 90 min. The sterilized samples were washed several times with distilled water until neutrality (p^H was found 6.93). Then the sample is filtered and oven dried it at 110^0 C for 8 hours.

For the second three experiments (No. 4 to 6) 150 gm sawdust was used in replace of rice husk and similarly the samples were prepared as described early. It was subjected to neutrality and p^H obtained was 6.94.

For the third and fourth sets of experiments (No. 7 to 12) sample was prepared as described in first three experiments. In all cases subjected to neutrality p^H was obtained 6.87. Figure 3.1 shows pretreated sample drying and the solution were sterilization at 121^{o}C in an incubator.

Figure 3.1: Sample Oven Drying and Sterilization at 121^{o}C.

3.2.2 Saccharification

Saccharification process was carried out in order to convert the ligno-cellulosic materials to simple sugar form. Detailed procedures of all experiments are given in following sections.

Experiment 1 to 6:

At first 0.05 M sodium citrate buffer was prepared by adding 3.675 gm sodium citrate with 250 ml distilled water. Then 12.5 gm prepared sample along with 5 gm cellulase enzyme (sample: enzyme ratio 2.5: 1) is added with the buffer solution for enzymatic saccharification. P^H of these solutions was obtained as listed in the Table 3.1. Finally the solutions were kept in a shaking

incubator for 84 hours with a temperature of 37^{o}C. Figure 3.2 shows that in saccharification process, it is to be noted that cellulase enzyme was added in order to breakdown the cellulose material.

Table 3.1: p^{H} Variation in First Six Experiments during Saccharification Steps

Experiment No.	p^{H}
Expt. 1 (rice husk)	4.85
Expt. 2 (rice husk)	4.85
Expt. 3 (rice husk)	4.86
Expt. 4 (sawdust)	4.93
Expt. 5 (sawdust)	4.90
Expt. 6 (sawdust)	4.86

Figure 3.2: Cellulase Enzyme Addition to Pretreated Sawdust Sample during Saccharification Process.

Experiment 7 to 12:

For the experiments 7 to 9 first prepared 0.05 M sodium citrate buffer by adding 3.675 gm sodium citrate with 350 ml distilled water. Then 17.5 gm prepared sample (rice husk) along with 5 gm cellulase enzyme (sample: enzyme ratio 3.5: 1) added with the buffer solution for

enzymatic saccharification. Next for the experiments 10 to 12, .05 M sodium citrate buffer by adding 3.675 gm sodium citrate with 300 ml distilled water. Then 15 gm prepared sample (rice husk) along with 5 gm cellulase enzyme (sample: enzyme ratio 3: 1) added with the buffer solution for enzymatic saccharification. P^H of these solutions was obtained as listed in the Table 3.2. In final step the solutions were kept in a shaking incubator for 84 hours with a temperature of 37^{o}C. Figure 3.3 shows that sample places to shaking incubator at 37^{o}C.

3.2.3 Fermentation:

At first the fermentation reagents were prepared by adding 2 gm glucose, 2 gm peptone and 1gm yeast extract in 100 ml distilled water for culture of *Saccharomyces cerevisiae*. The solution kept in an incubator at 37^{o}C for 24 hours. The p^H of this solution was 5.25 for all experiments. Figure 3.4 represent the solution preparation steps for yeast inoculumns.

Table 3.2: p^H Variation in Last Six Experiments during Saccharification Steps

Experiment No. with husk-enzyme ratio	p^H
Expt. 7 (husk : enzyme=3.5:1)	4.95
Expt. 8 (husk : enzyme=3.5:1)	4.90
Expt. 9 (husk : enzyme=3.5:1)	4.89
Expt. 10 (husk: enzyme=3:1)	4.93
Expt. 11 (husk: enzyme=3:1)	4.92
Expt. 12 (husk: enzyme=3:1)	4.95

Figure 3.3: Samples Placed in Shaking Incubator to Complete the Saccharification Process

Figure 3.4: Solution Preparation for Yeast Inoculums.

Then the fermentation medium was made by adding 0.375 gm yeast extract, 0.02 gm calcium chloride, 0.02 gm ammonium sulfate, 0.0625 gm magnesium sulfate, 0.09375 gm mono-potassium phosphate with 62.5 ml distilled water. Then the fermentation reagents were added with the liquid slurry at a ratio of 1:25. Details of the quantity of the fermentation reagents and p^H obtained for the final solutions are listed in Table 3.3.Saccharified slurry was prepared by mixing with above solution in a conical flask and stirred well. The flask was placed in a dark place in aerobic condition for 24 hours and then placed in an incubator with anaerobic condition at 35^{o}C for 72 hours. Figure 3.5 exhibits the fermentation process in aerobic condition and later in anaerobic condition.

Table 3.3: Details of Fermentation Reagents and p^H Obtained in Fermentation Process

Experiment No.	Sample	Sample Enzyme Ratio	Fermentation Reagents (ml)	p^H
Experiment 1	Rice husk	2.5 : 1	12.5	6.30
Experiment 2				6.29
Experiment 3				6.30
Experiment 4	Sawdust	2.5 : 1	12.5	6.28
Experiment 5				6.24
Experiment 6				6.18
Experiment 7	Rice husk	3.5 : 1	16.5	6.20
Experiment 8				6.21
Experiment 9				6.24
Experiment 10	Rice husk	3.0 : 1	14.5	6.20
Experiment 11				6.21
Experiment 12				6.24

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Figure 3.5: Fermentation Process in Aerobic Condition and Later in Anaerobic Condition

3.3 Sample Collection:

After fermentation a clear supernatants was obtained from centrifugation and the sample was kept in an appended tube for ethanol estimation. After the gas chromatographic test ethanol was estimated. For collecting the clear supernatants fermented broth of each experiment, they were collected first in a 1.5 ml appended tubes and centrifugation was done with 1200 rpm about 7 minutes. Finally after centrifugation clear experimented liquid samples were collected in another appended tube. After that gas-chromatographic (GC) tests were carried out for estimating the fermented bioethanol. Figure 3.6 displays the centrifugation process of the experimented sample broth.

Figure 3.6: Centrifugation of the Experimented Sample Broth

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Results:

Gas-chromatographic (GC) test of all the samples were done in Quality Control Department of Active Fine Chemicals Ltd., Dhaka. After GC test of the fermented sample following results were obtained for each sample of the experiment. Each sample was first run with a standard sample of different retention time and match up to this sample with experimented sample for final estimation of ethanol. Tailing factor and the peak area were recorded with different retention time. Experiment 1 to 12 where biomass samples were mixed with cellulase enzyme with different ratios is denoted as different names in order to simplification are listed in Table 4.1

Table 4.1: Details of Sample Identification of Various Feedstocks

Experiment No. and Biomass	Biomass Enzyme Ratio	Sample ID
Expt. 1 (Rice Husk)	2.5 : 1	A-1
Expt. 2 (Rice Husk)		A-2
Expt. 3 (Rice Husk)		A-3
Expt. 4 (Sawdust)	2.5 : 1	S-1
Expt. 5 (Sawdust)		S-2
Expt. 6 (Sawdust)		S-3
Expt. 7 (Rice Husk)	3.5 : 1	H-1
Expt. 8 (Rice Husk)		H-2
Expt. 9 (Rice Husk)		H-3
Expt. 10 (Rice Husk)	3.0 : 1	B-1
Expt. 11 (Rice Husk)		B-2
Expt. 12 (Rice Husk)		B-3

For the first set of experiments (Expt. No. 1-3), the gas chromatographer used was Shimadzu-GC-2010 Plus model, whose column thickness was 0.15 μm, inner diameter 0.53 mm and length 30 m (0.15x0.53x30) for ethanol estimation. For rest of the experiments (Expt. No. 4-12) Shimadzu GC-2010 model gas chromatographer was used with column thickness 0.15 μm, inner diameter 0.53 mm and length 15 m (0.15x0.53x15) for ethanol estimation. The oven temperature of the gas chromatographer was maintained 120^{o}C during the experiments. The variation of the retention time of standard sample could be due to the different column dimensions used in this study. The results show that the ethanol estimated in all samples have a peak approximately at same retention time as standard samples. This indicates the purity of the ethanol samples according to standard sample which are almost identical. The tailing factor varied from 1.87 to 3.81 represents the coefficient of peak symmetry. It could be associated with the quality of a separation of peak to identify the quantity of ethanol. It was to be noted that the results obtained with saw dust sample was not very satisfactory, that is why experiments with mixing of saw dust with rice husk was not conducted; rather effect of enzyme concentration on rice husk fermentation was investigated. Standard of the sample was run three times in this experiment. It might be mentioned that double run of the sample was performed showing the similar result identified from GC peaks. Thus the subsequent run of the sample was performed in a single time in other experiments. The retention time of the standards match with the ethanol sample for those experiments.

4.1.1 Results of Experiments 1 to 3:

The results of the experiments 1 to 3 are present in Figure 4.1, 4.2, 4.3 and 4.4. Details of the results are tabulated in Table 4.2, 4.3, 4.4 and 4.5.

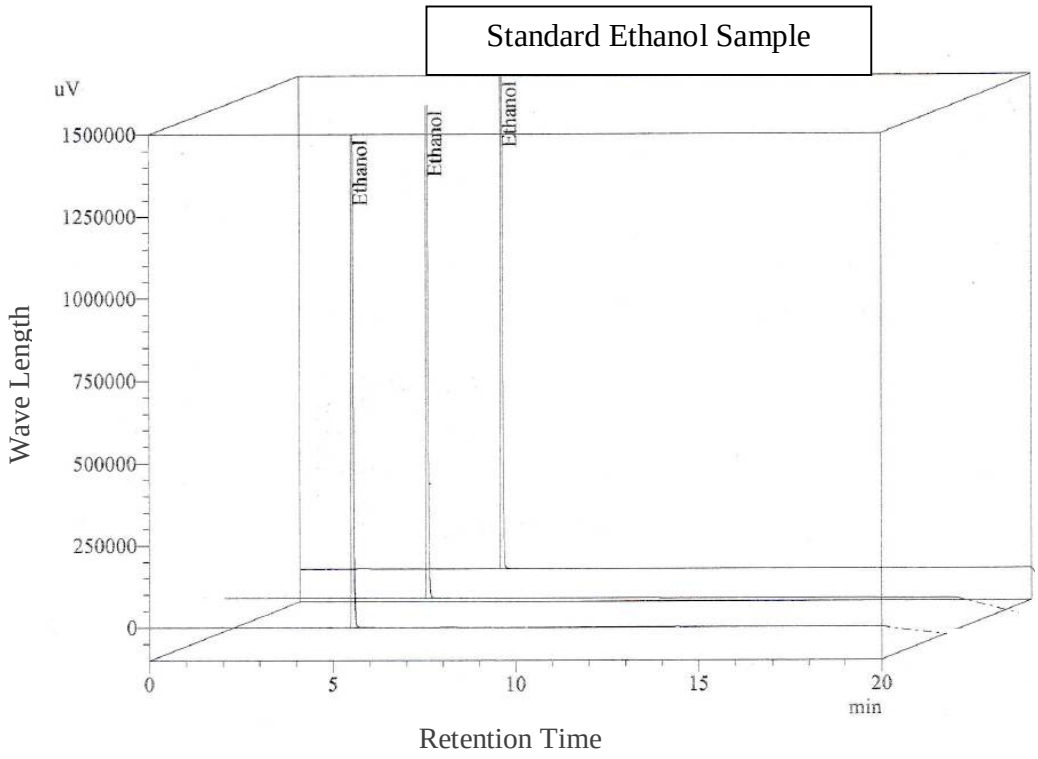

Figure 4.1: GC Peak of a Standard Ethanol Sample

Table 4.2: GC Test Result for a Standard Ethanol Sample

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol Standard	Standard	5.53	1.51	9727703	100.00
Ethanol Standard	Standard	5.52	1.50	9614855	100.00
Ethanol Standard	Standard	5.52	1.51	9679873	100.00
Average	-	5.52	1.51	9674144	100.00
% Relative Standard Deviation	-	0.1	0.5	0.6	0.0

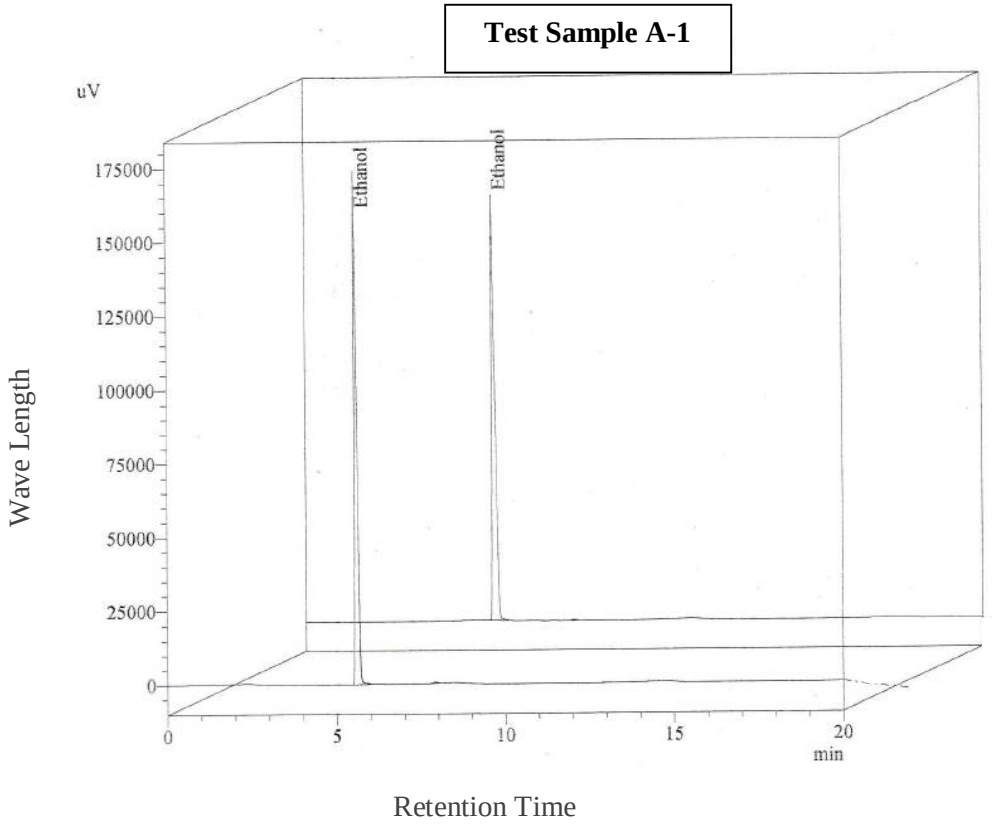

Figure 4.2: GC Peak of 1st Sample A-1 (Experiment 1)

Table 4.3: GC Test Result for 1st Sample A-1 (Experiment 1)

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol	Sample A-1	5.56	2.23	936214	99.64
Ethanol	Sample A-1	5.56	1.90	928846	99.62
Average	-	5.56	2.07	932530	99.63
% Relative Standard Deviation	-	0.0	11.3	0.6	0.0

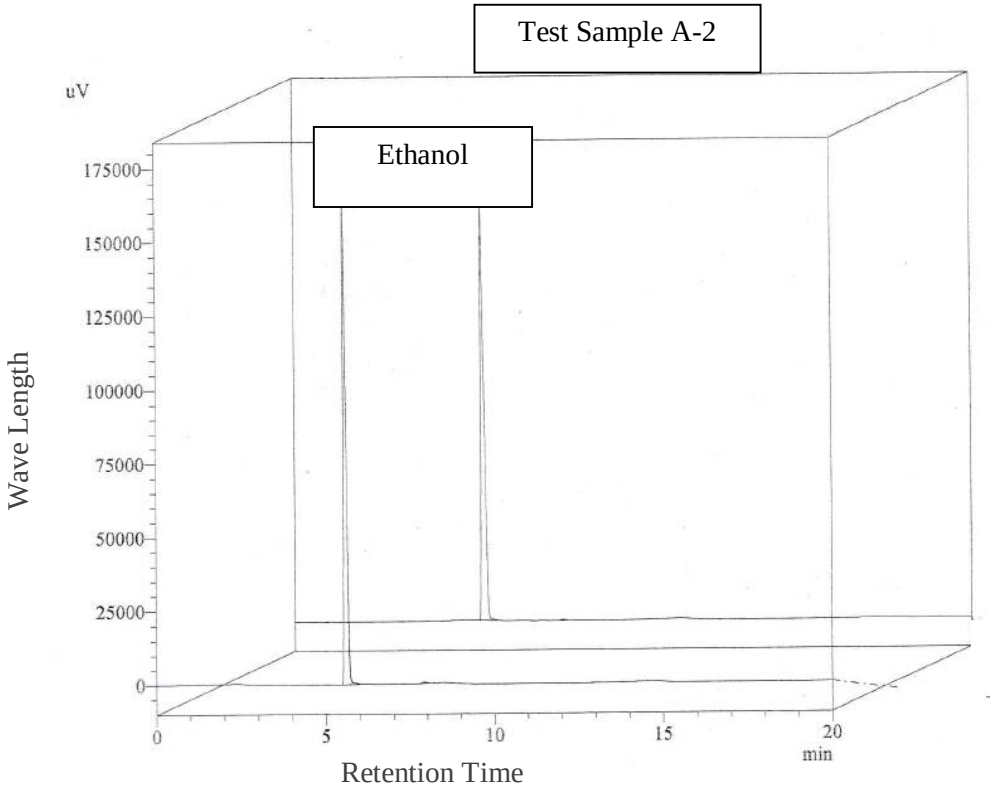

Figure 4.3: GC Peak of 2nd Sample A-2 (Experiment 2)

Table 4.4: GC Test Result for 2nd Sample A-2 (Experiment 2)

Title	Sample ID	Retention Time	Tailing Factor	Area (mm^2)	% of Area
Ethanol	Sample A-2	5.57	1.77	928221	99.55
Ethanol	Sample A-2	5.56	1.80	917625	99.53
Average	-	5.56	1.79	922923	99.54
% Relative Standard Deviation	-	0.0	1.19	0.8	0.0

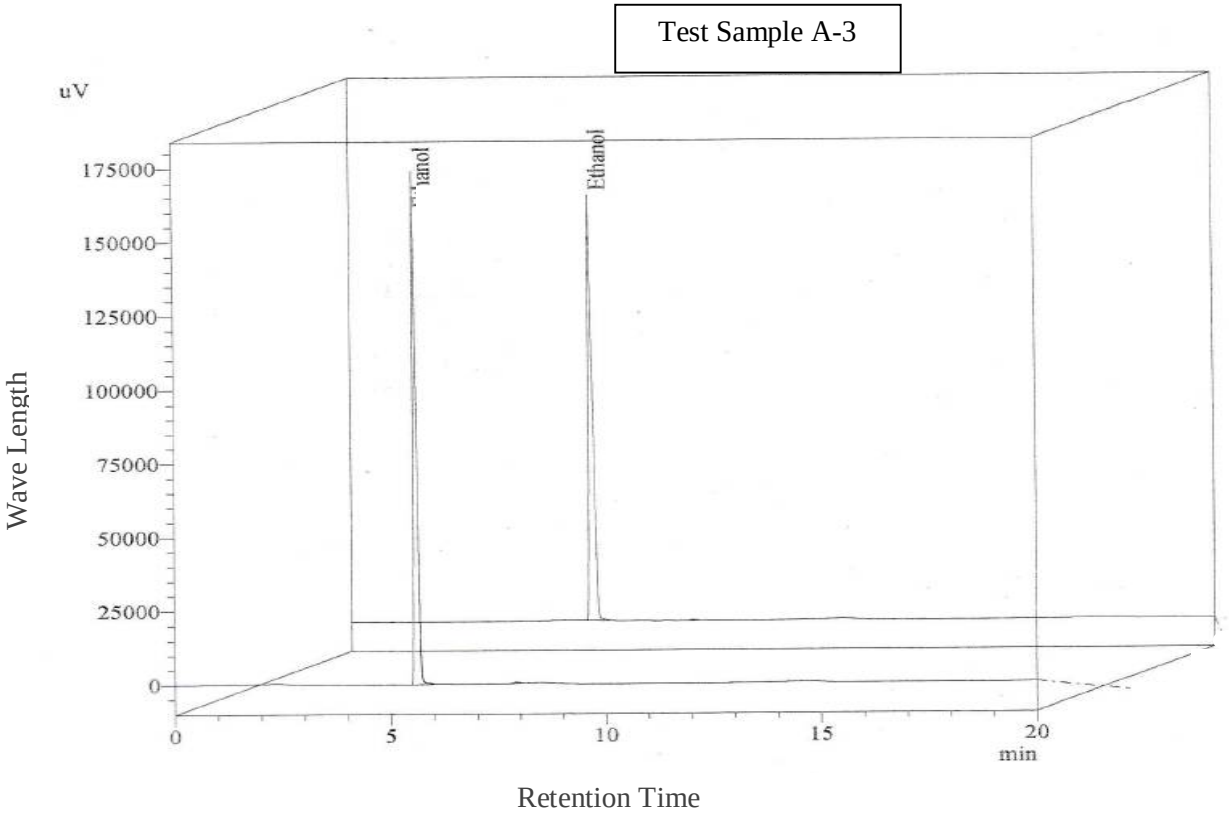

Figure 4.4: GC Peak of 3rd Sample A-3 (Experiment 3)

Table 4.5: GC Test Result for 3rd Sample A-3 (Experiment 3)

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol	Sample A-3	5.54	1.93	922625	99.48
Ethanol	Sample A-3	5.54	1.71	908367	99.44
Average	-	5.54	1.82	915496	99.46
% Relative Standard Deviation	-	0.0	8.54	1.1	0.0

4.1.2 Results of Experiments 4 to 6:

The results of the experiments 4 to 6 are present in Figure 4.5, 4.6, 4.7 and 4.8. Details of the results are tabulated in Table 4.6, 4.7, 4.8 and 4.9.

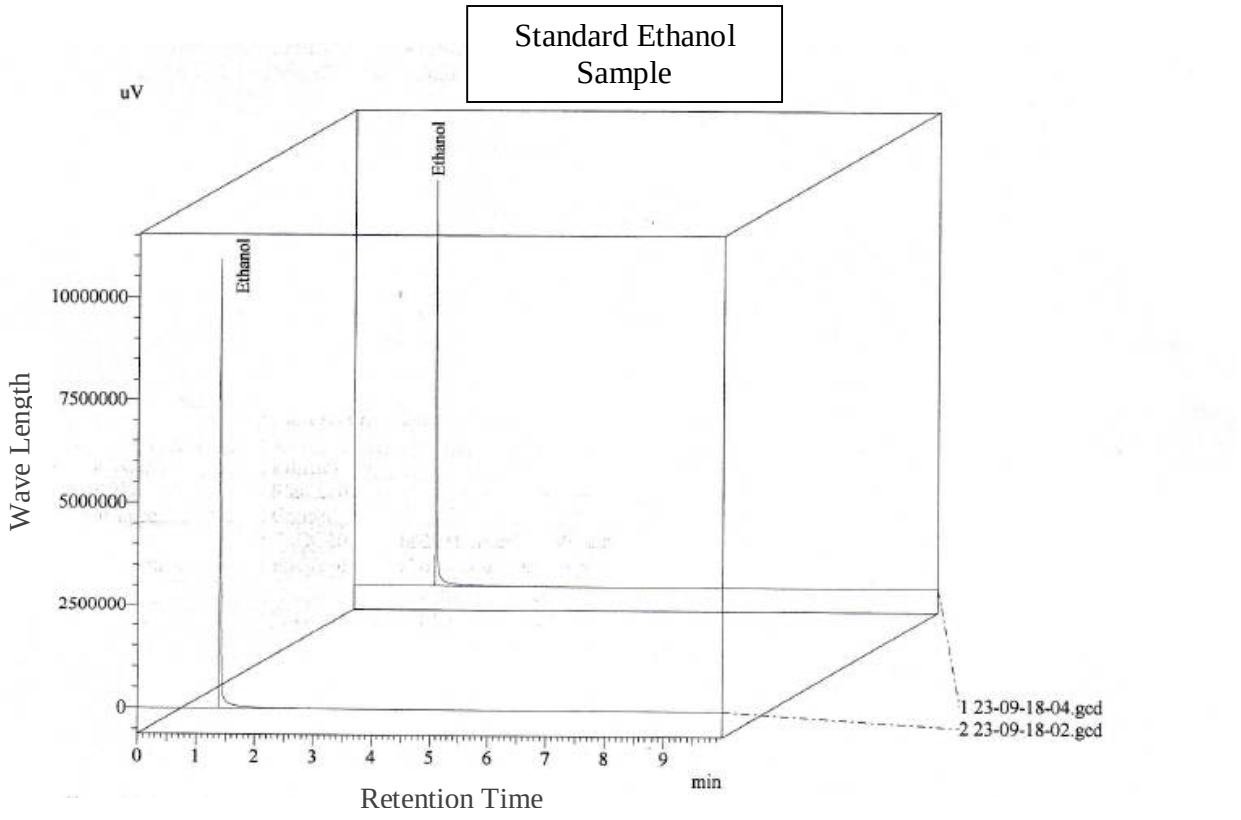

Figure 4.5: GC Peak of Standard Ethanol Sample

Table 4.6: GC Test Result for Standard Ethanol Sample

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol Standard	Standard	1.40	1.85	18924117	100.00
Ethanol Standard	Standard	1.40	1.89	19634638	100.00
Average	-	1.40	1.87	192793378	100.00
% Relative Standard Deviation	-	0.0	1.51	2.61	0.0

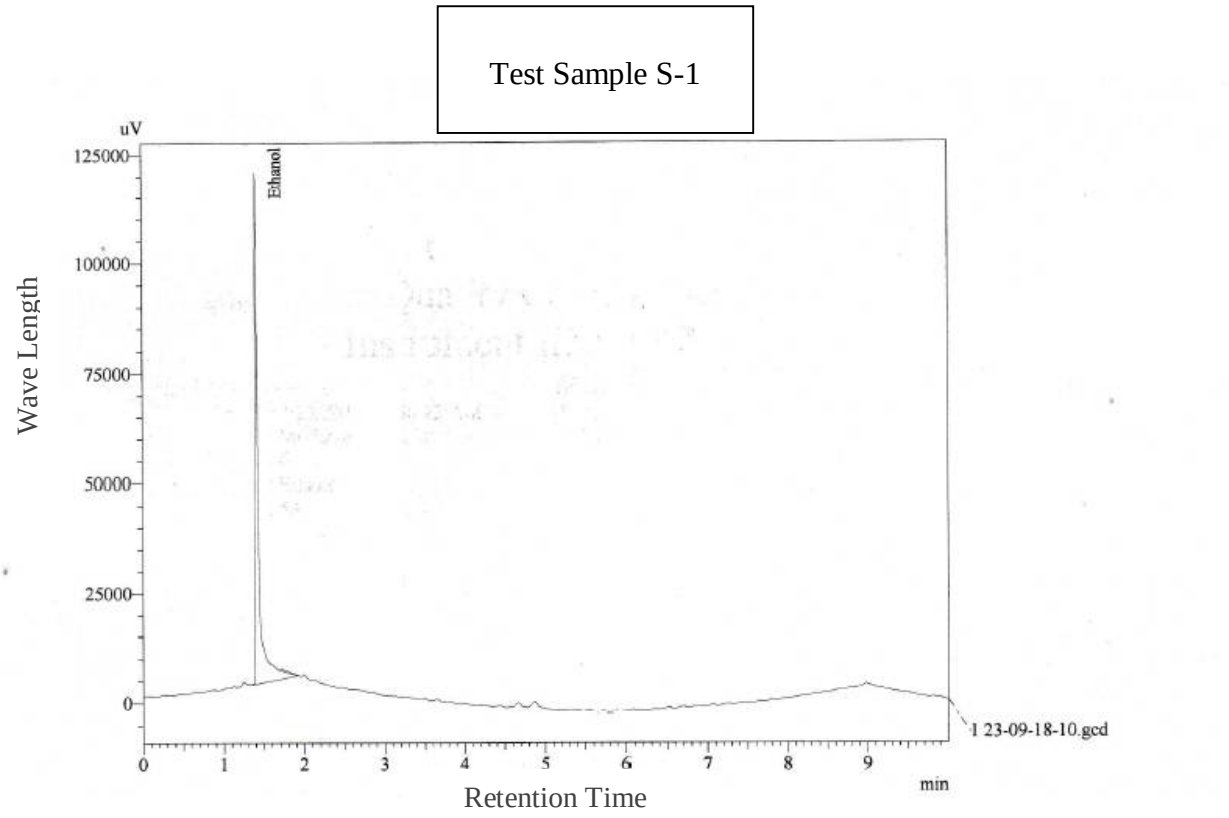

Figure 4.6: GC Peak of 4th Sample S-1 (Experiment 4)

Table4.7: GC Test Result for 4th Sample S-1 (Experiment 4)

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol	Sample S-1	1.41	3.62	3074580	99.9
Average	-	1.41	3.62	3074580	99.9
% Relative Standard Deviation	-	0.0	0.0	0.0	0.0

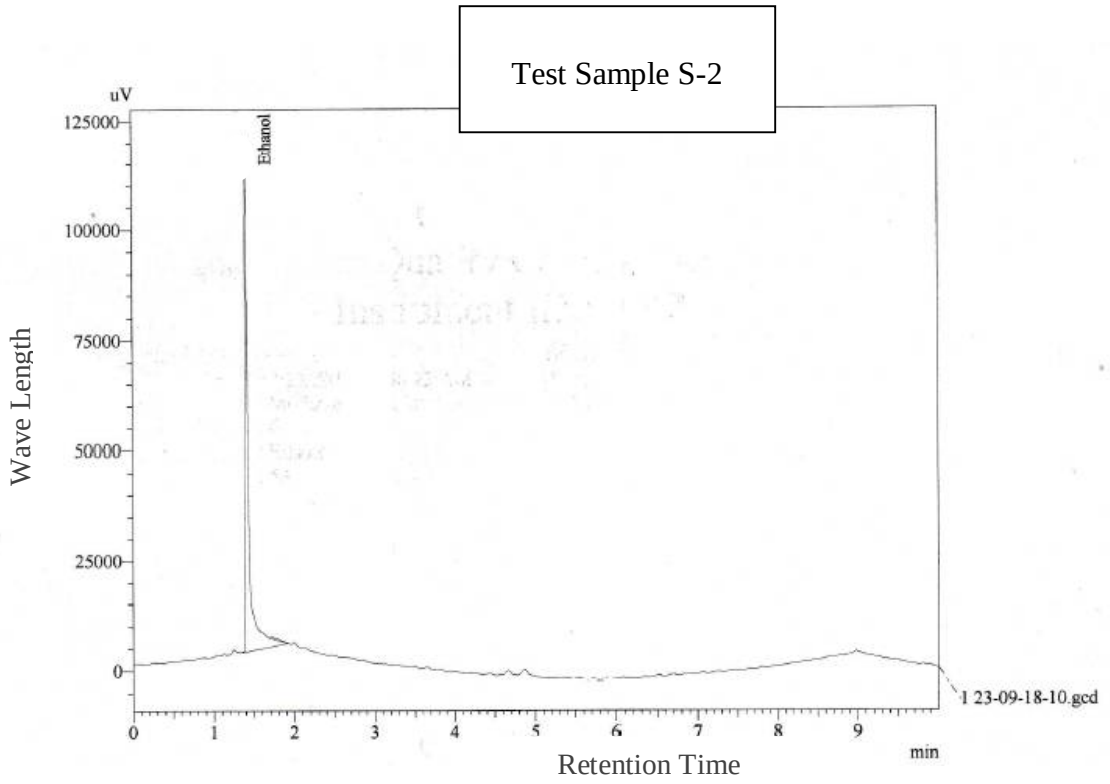

Figure 4.7: GC Peak of 5th Sample S-2 (Experiment 5)

Table 4.8: GC Test Result for 5th Sample S-2 (Experiment 5)

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol	Sample S-2	1.53	3.40	2377431	99.9
Average	-	1.53	3.40	2377431	99.9
% Relative Standard Deviation	-	0.0	0.0	0.0	0.0

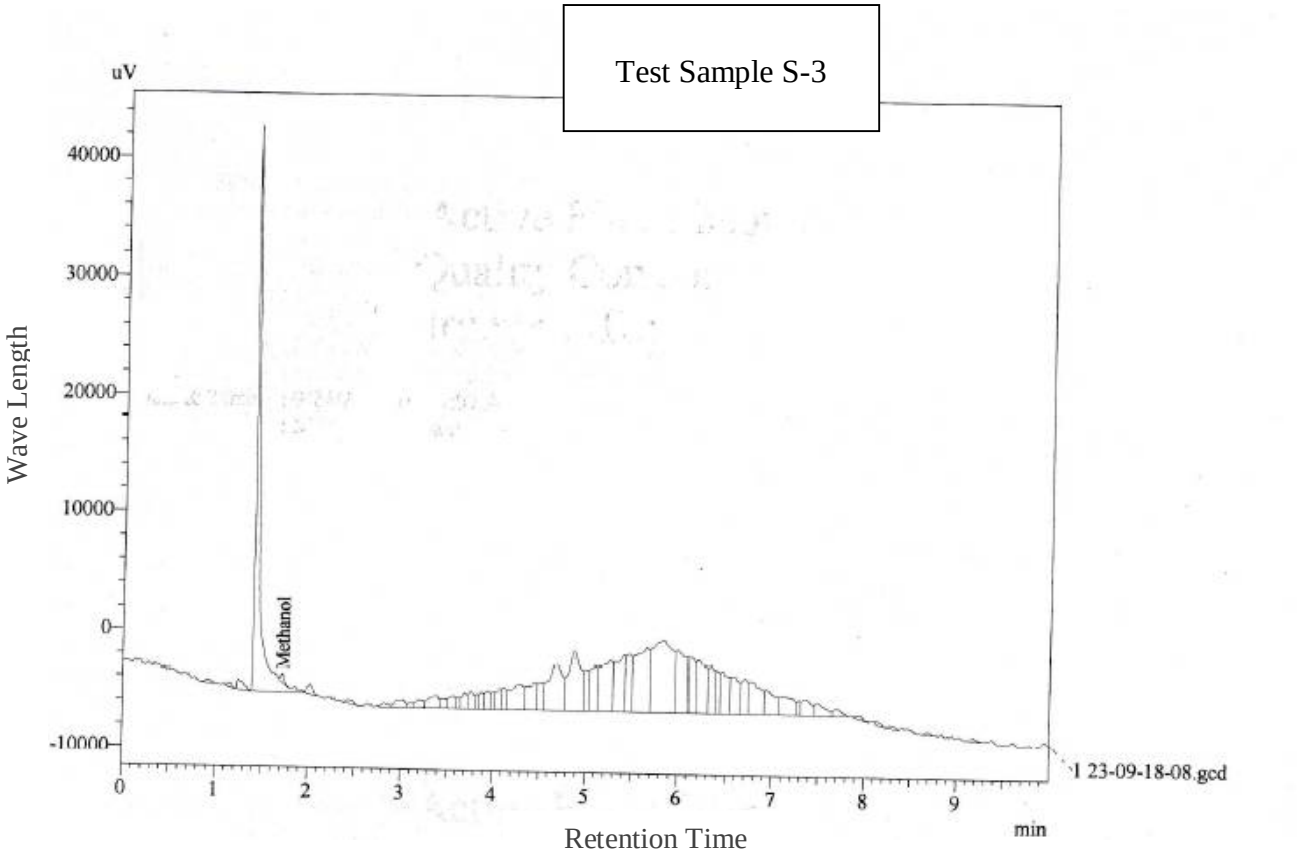

Figure 4.8: GC Peak of 6th Sample S-3 (Experiment 6)

Table 4.9: GC Test Result for 6th Sample S-3 (Experiment 6)

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol	Sample S-3	1.72	0.0	1659950	2.0
Average	-	1.72	0.0	1659950	2.0
% Relative Standard Deviation	-	0.0	0.0	0.0	0.0

4.1.3 Results of Experiments 7 to 9:

The results of the experiments 7 to 9 are presented in Figure 4.9, 4.10, 4.11 and 4.12. Details of the results are tabulated in Table 4.10, 4.11, 4.12 and 4.13.

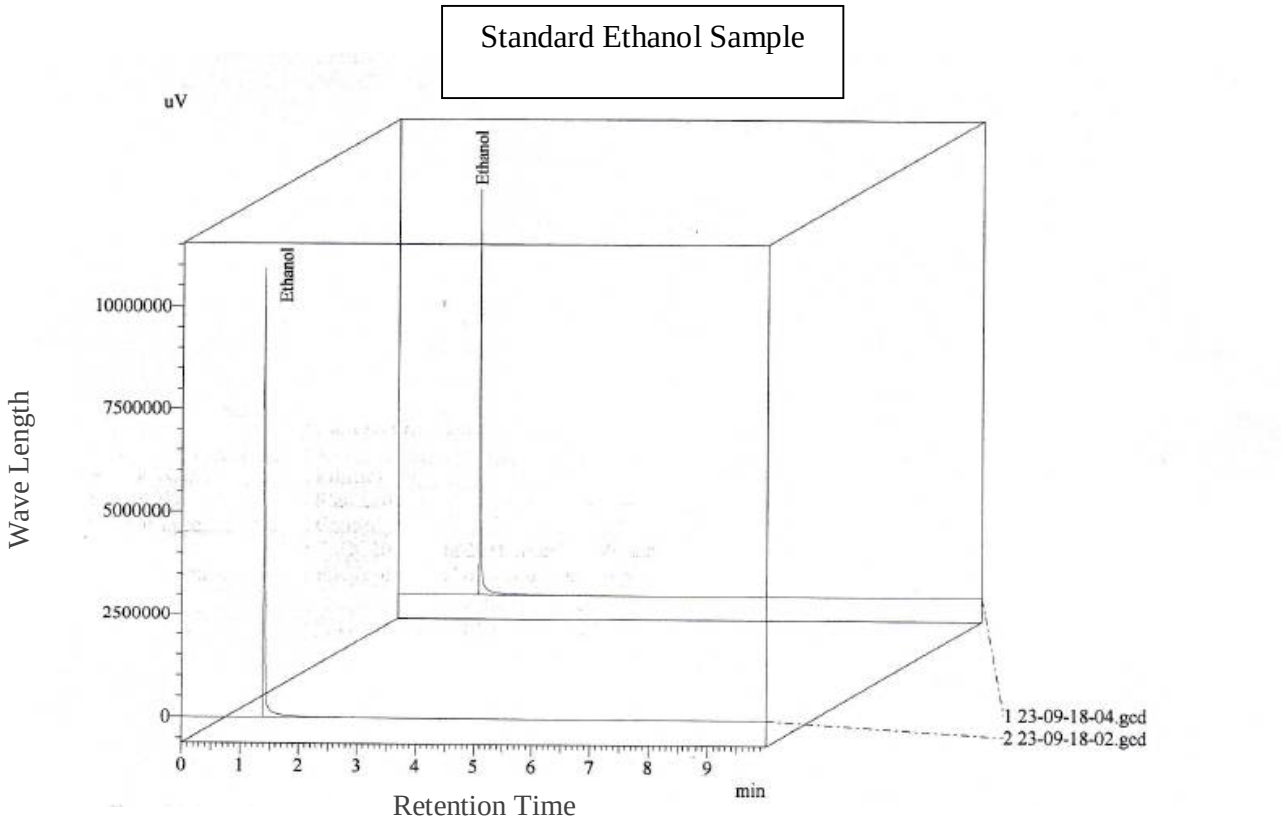

Figure 4.9: GC Peak of Standard Ethanol Sample

Table 4.10: GC Test Result for Standard Ethanol Sample

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol Standard	Standard	1.40	1.88	18924117	100.00
Ethanol Standard	Standard	1.40	1.90	19634638	100.00
Average	-	1.40	1.89	192793378	100.00
% Relative Standard Deviation	-	0.0	0.74	2.61	0.0

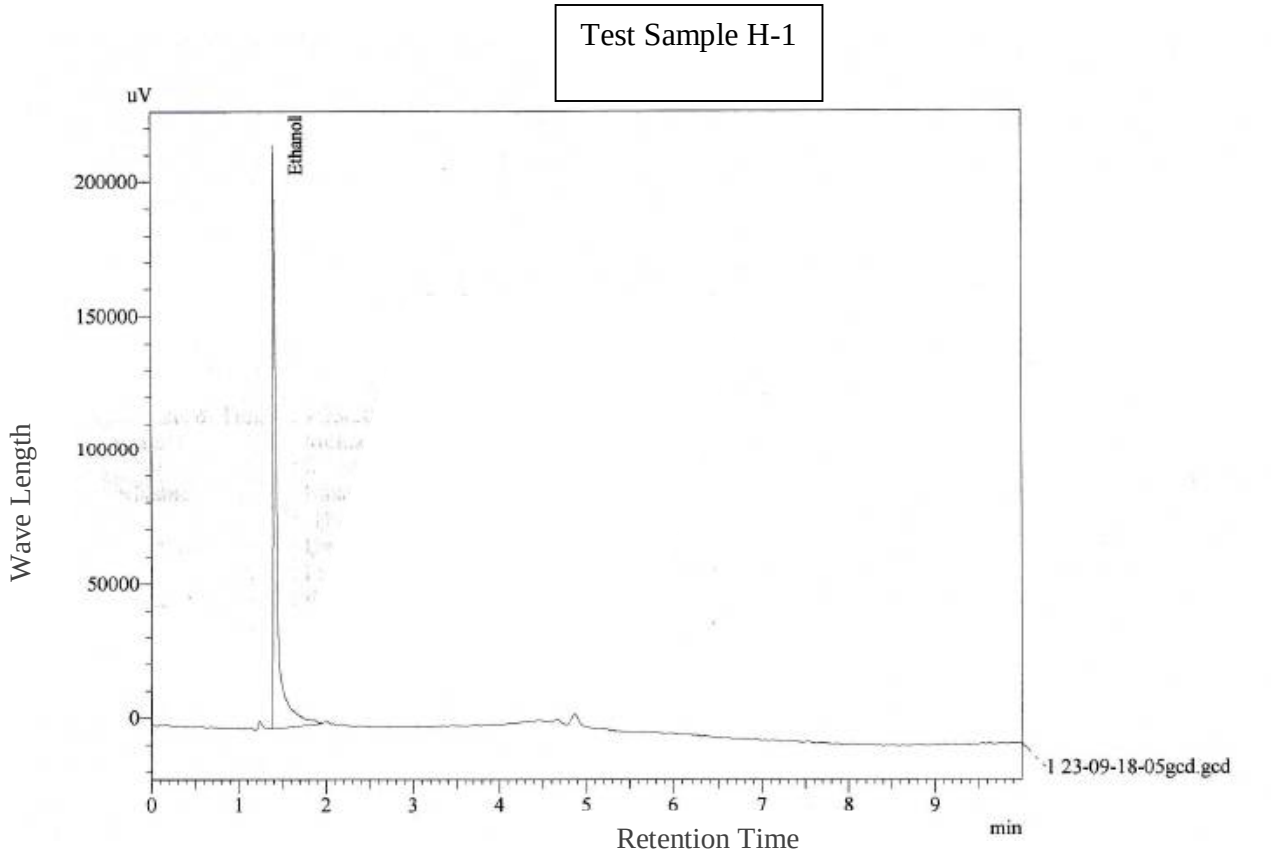

Figure 4.10: GC Peak of 7th Sample H-1 (Experiment 7)

Table 4.11: GC Test Result for 7th Sample H-1 (Experiment 7)

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol	Sample H-1	1.41	3.82	13129229	100
Average	-	1.41	3.82	13129229	100
% Relative Standard Deviation	-	0.0	0.0	0.6	0.0

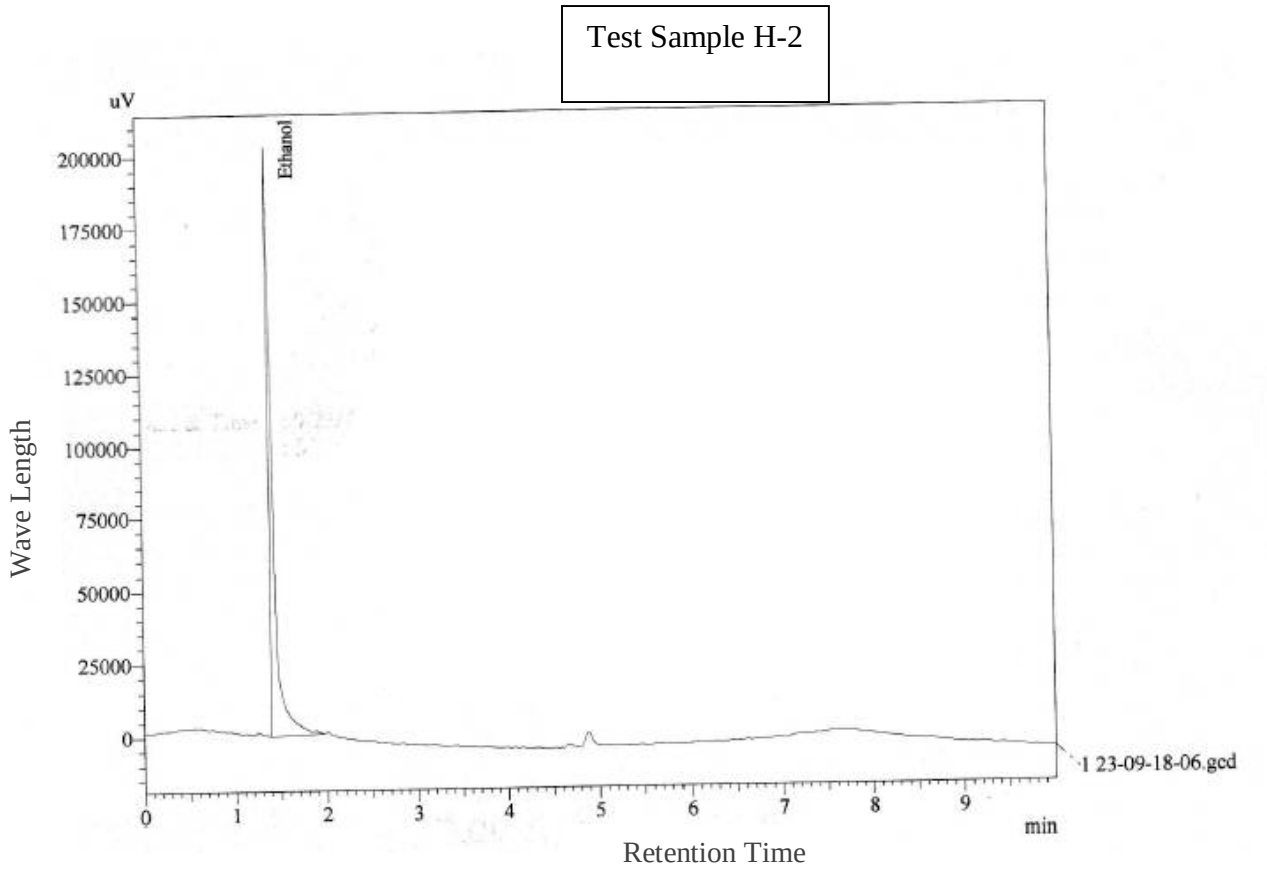

Figure 4.11: GC Peak of 8th Sample H-2 (Experiment 8)

Table 4.12: GC Test Result for 8th Sample H-2 (Experiment 8)

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol	Sample H-2	1.41	3.81	12897879	100
Average	-	1.41	3.81	12897879	100
% Relative Standard Deviation	-	0.0	0.0	0.6	0.0

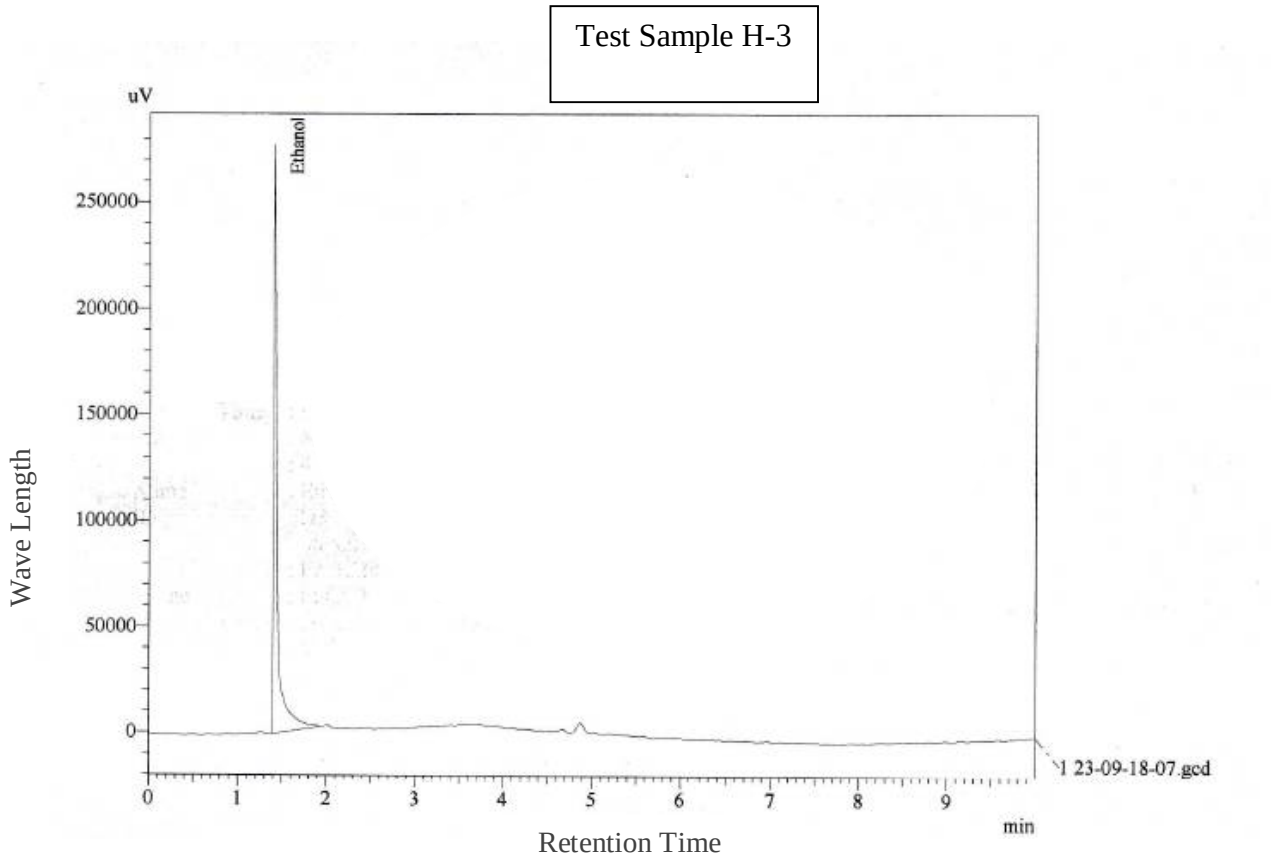

Figure 4.12: GC Peak of 9^{tn} Sample H-3 (Experiment 9)

Table 4.13: GC Test Result for 9th Sample H-3 (Experiment 9)

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol	Sample H-3	1.41	3.72	12974994	100
Average	-	1.41	3.72	12974994	100
% Relative Standard Deviation	-	0.0	0.0	0.6	0.0

4.1.4 Results of Experiments 10 to 12:

The results of the experiments 10 to 12 are presented in figure 4.13, 4.14, 4.15 and 4.16. Details of the results are tabulated in Table 4.14, 4.15, 4.16 and 4.17.

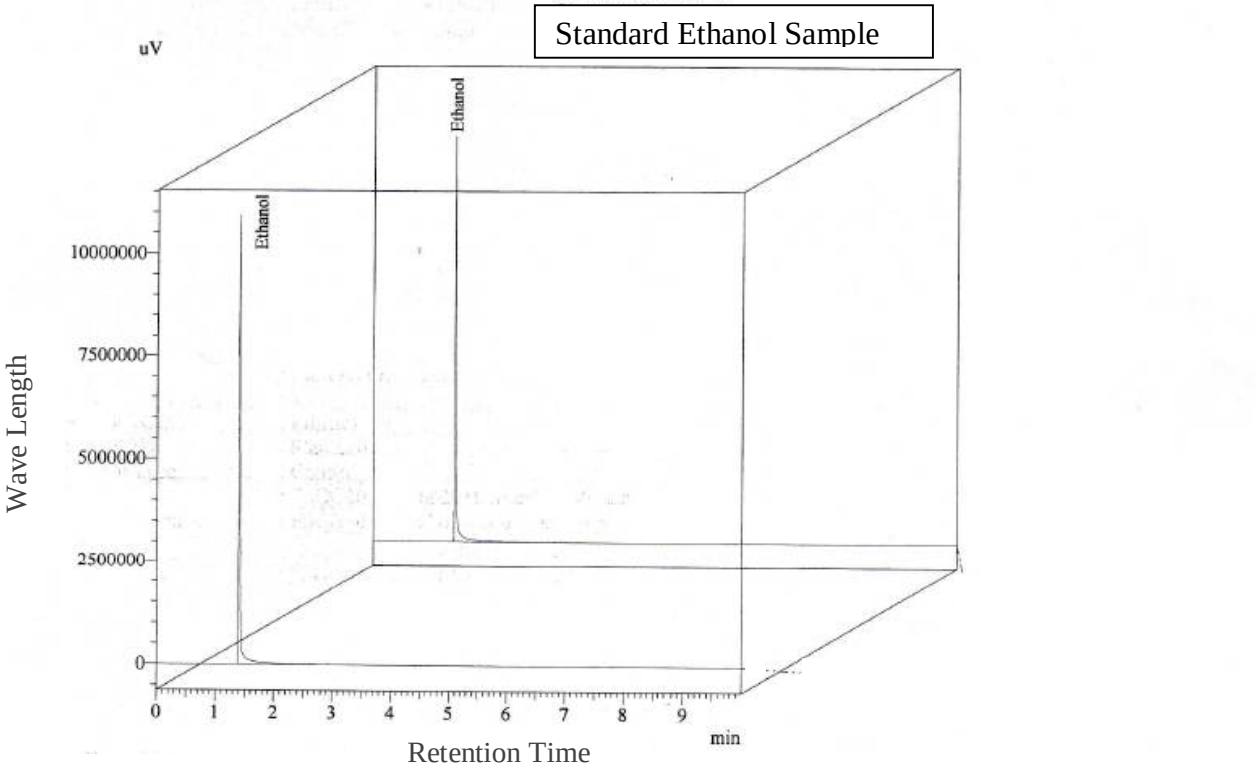

Figure 4.13: GC Peak of a Standard Ethanol Sample

Table 4.14: GC Test Result for Standard Ethanol Sample

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol Standard	Standard	1.40	1.88	18824117	100.00
Ethanol Standard	Standard	1.40	1.90	18634638	100.00
Average	-	1.40	1.89	18729378	100.00
% Relative Standard Deviation	-	0.0	0.74	0.72	0.0

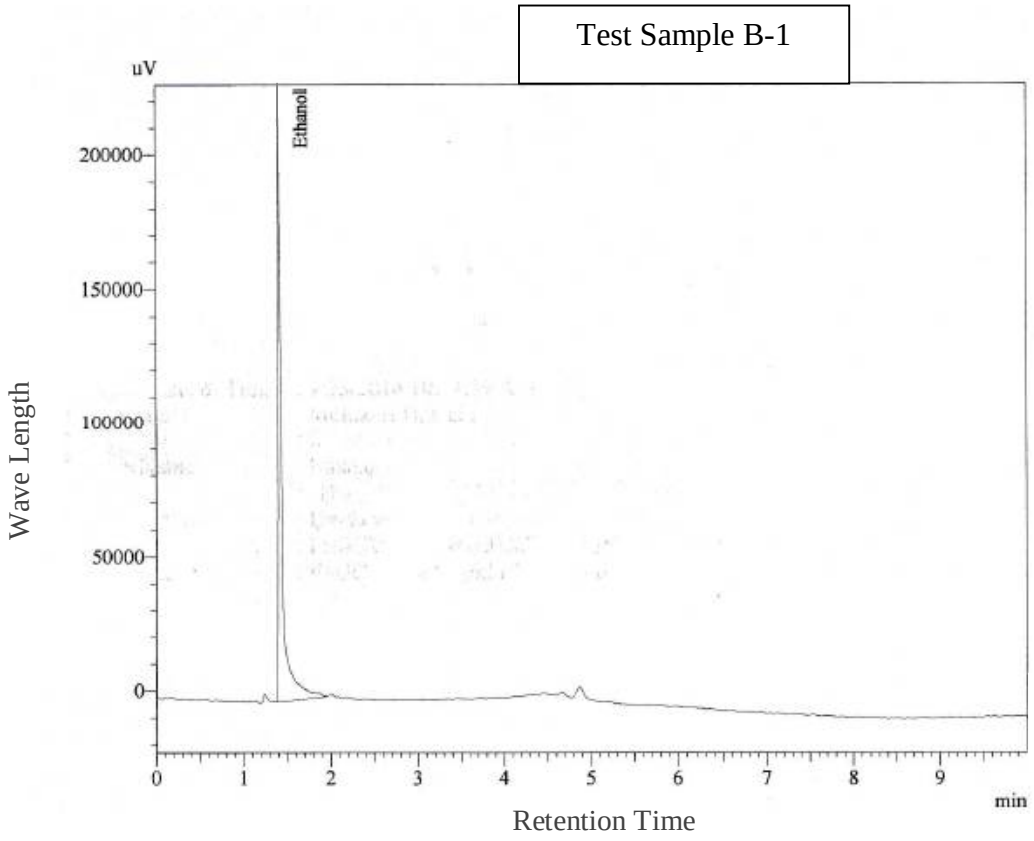

Figure 4.14: GC Peak of 10th Sample B-1 (Experiment 10)

Table 4.15: GC Test Result for 10th Sample B-1 (Experiment 10)

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol	Sample B-1	1.43	3.80	1638821	100
Average	-	1.43	3.80	1638821	100
% Relative Standard Deviation	-	0.0	0.0	0.0	0.0

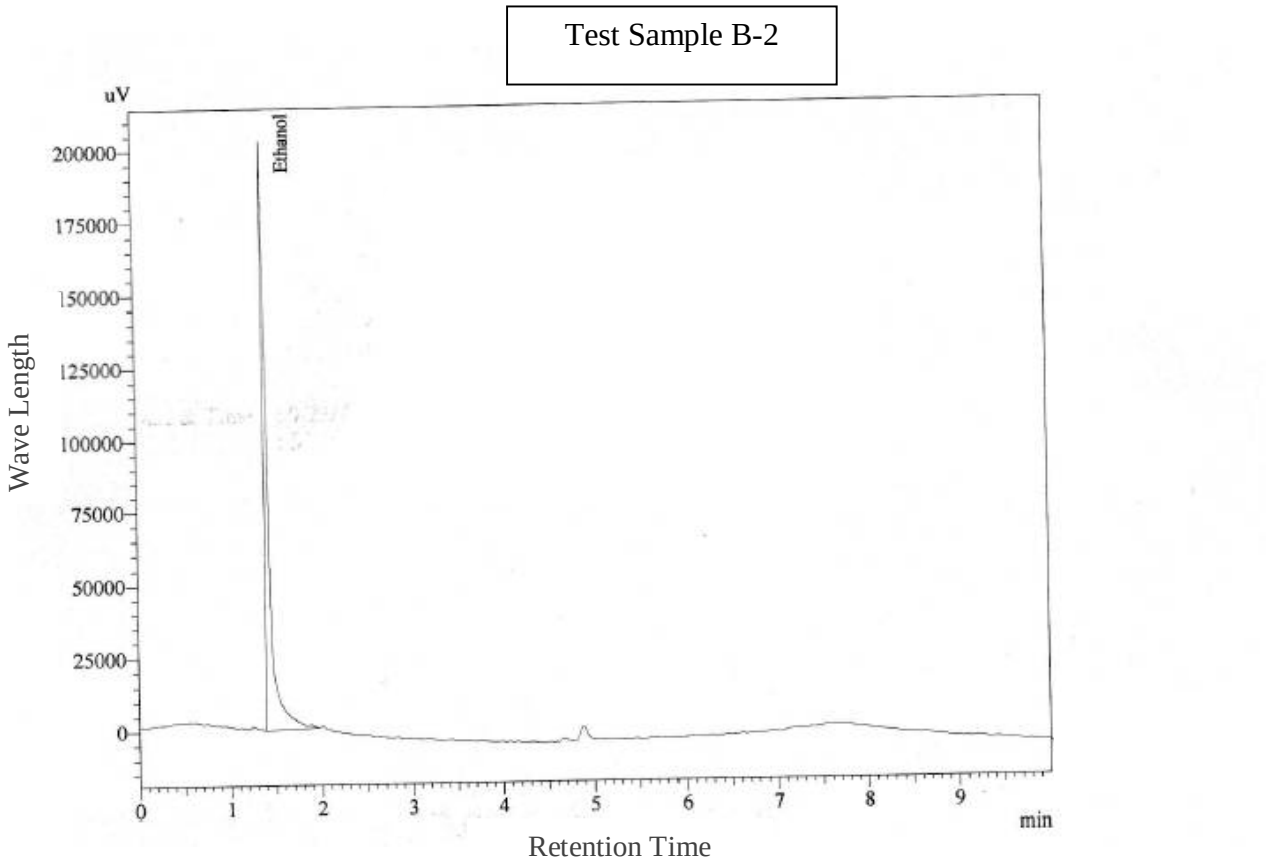

Figure 4.15: GC Peak of 11th Sample B-2 (Experiment 11)

Table 4.16: GC Test Result for 11th Sample B-2 (Experiment 11)

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol	Sample B-2	1.43	3.81	1642566	100
Average	-	1.43	3.81	1642566	100
% Relative Standard Deviation	-	0.0	0.0	0.0	0.0

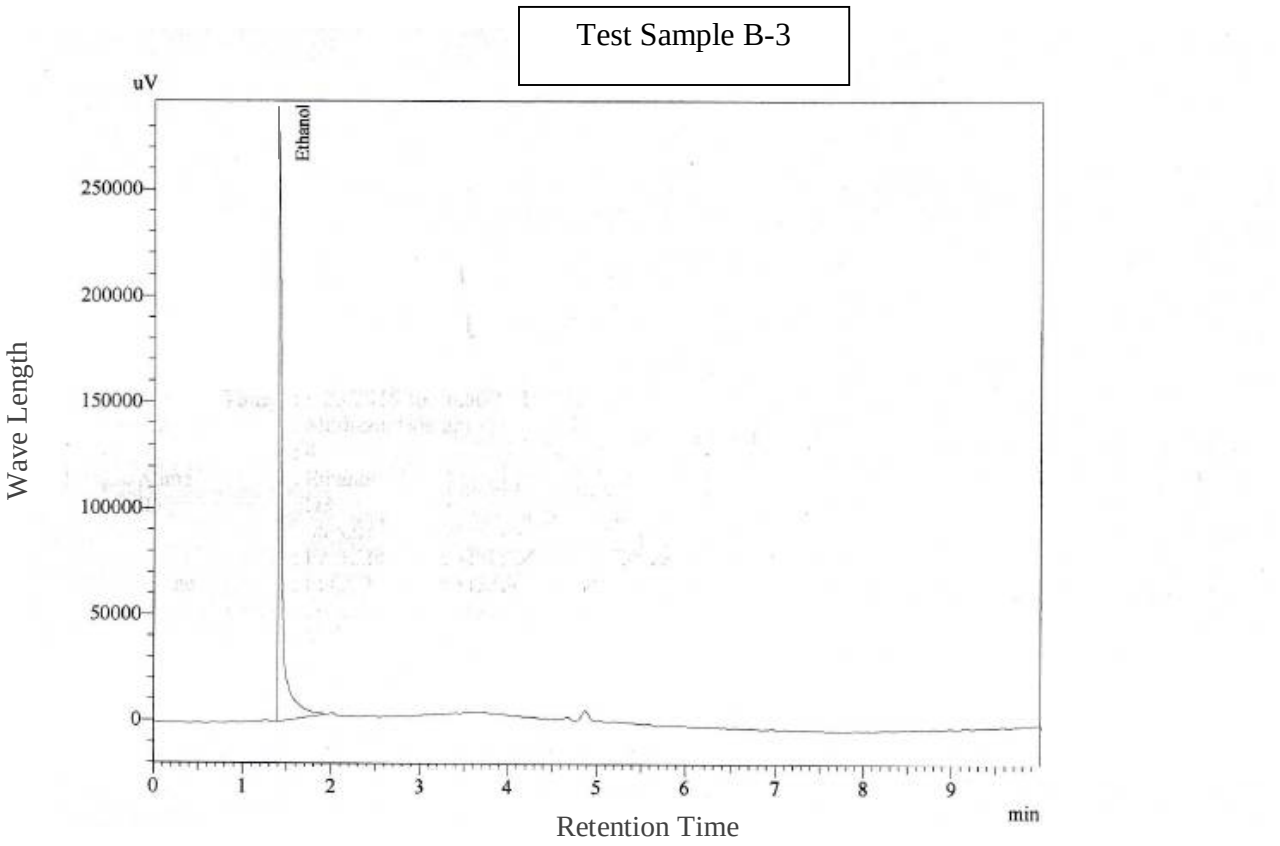

Figure 4.16: GC Peak of 12th Sample B-3 (Experiment 12)

Table 4.17: GC Test Result for 12th Sample B-3 (Experiment 12)

Title	Sample ID	Retention Time (min)	Tailing Factor	Area (mm^2)	% of Area
Ethanol	Sample B-3	1.41	3.77	1625711	100
Average	-	1.41	3.77	1625711	100
% Relative Standard Deviation	-	0.0	0.0	0.0	0.0

4.2 Summary of the Results

The estimated ethanol is calculated as:-

$$\text{Estimated ethanol} = \frac{\text{Average area of the respective sample}}{\text{Average area of the standard sample of ethanol}} \text{ X } 100\%$$

Here both samples are taken down to same amount. For the 1st sample A-1 it is calculated as = (932530/9674144) X 100% = 9.64%. Similarly, the values for other samples are calculated and outputs are listed in the tabulated form in the Table 4.18.

Table 4.18: Summary of the Results (Experiment 1-12)

Sample : Enzyme Ratio	Sample ID	% of ethanol (v/w)	Average % of Ethanol (v/w)
Rice husk : Enzyme= 2.50 : 1.0	A-1	9.64	9.55
	A-2	9.54	
	A-3	9.46	
Saw dust : Enzyme = 2.50 : 1.0	S-1	1.59	1.23
	S-2	1.23	
	S-3	0.86	
Rice husk : Enzyme= 3.50 : 1.0	H-1	6.81	6.74
	H-2	6.69	
	H-3	6.73	
Rice husk : Enzyme= 3.0 : 1.0	B-1	8.75	8.73
	B-2	8.77	
	B-3	8.68	

4.3 Discussions

Figure 4.1 represents the GC result of a standard ethanol sample and Figure 4.2, 4.3 and 4.4 represent the results of three test samples which denotes as A-1, A-2 and A-3 respectively. Retention time varies to 5.54 min to 5.56 min and tailing factor varies between 1.71 to 2.23. Relative Standard deviation (%RSD) in the area varies 0.6%, 0.8% and 1.1% respectively in three samples. Results show that the purity of ethanol gained from the fermented broths is

approximately identical as standard ethanol sample and tailing factor indicates peaks symmetry. For the 1st three samples where the pretreated rice husk mixed with the cellulase enzyme with a ratio of 2.5:1 shows the percentage of the ethanol as 9.64% (v/w), 9. 54% (v/w) and 9.46% (v/w) respectively. Hence, the percentage of average ethanol yield is about 9.55 % (v/w).

Figure 4.5 represents the GC result of a standard ethanol sample and Figure 4.6, 4.7, and 4.8 show the results of the three test sample of S-1, S-2 and S-3, where the sample originates from the heterogeneous sawdust and mixed with cellulase enzyme (with a ratio 2.5:1). Here the retention time is varies between 1.41 to 1.72 mins and tailing factor varies between 0 to 3.62. The estimated ethanol percentage is 1.59% (v/w), 1.23% (v/w) and 0.86% (v/w) respectively. Hence, the average percentage of ethanol is 1.23% (v/w). Nevertheless samples are varied due to its heterogeneous characters.

Again Figure 4.9 represents the GC result of a standard ethanol sample and Figure 4.10, 4.11, and 4.12 shows the results of three test sample of H-1, H-2 and H-3, where the sample originates from the rice husk with the cellulase enzyme ratio of 3.5:1. Here, the retention time for all cases is 1.41 mins with relative standard deviation 0 and tailing factor varies between 3.72 to 3.82. The estimated ethanol percentages are 6.81% (v/w), 6.69% (v/w) and 6.73% (v/w) respectively. Hence, the average percentage of ethanol is 6.74% (v/w).

Finally Figure 4.13 represents the GC result of a standard ethanol sample and Figure 4.14, 4.15, and 4.16 show the three test sample of B-1, B-2 and B-3, where the samples originates the rice husk with the cellulase enzyme (ratio 3:1). Here the retention time varies from 1.41 mins to 1.43 mins which indicates a small variations and tailing factor varies between 3.77 to 3.81. The estimated ethanol percentages are 8.75% (v/w), 8.77% (v/w) and 8.68% (v/w) respectively. Hence, the average percentage of ethanol is 8.73% (v/w).

From the above experiments, it can be envisioned that the higher amount of ethanol was obtained from rice husk sample compared to the sawdust sample. No experiment was further performed using sawdust sample due to lower yield of ethanol and high enzyme cost. The ethanol yield with the enzyme and rice husk ratio of 1:2.5, 1:30 and 1:3.5 was obtained as 9.55 % (v/w), 8.73%

(v/w) and 6.74% (v/w) respectively. This showed that ethanol concentration decreased with decreasing the concentration of enzyme.

Sana et al. (2017) studied on ethanol production from Pakistani lingo-cellulosic material by cellulase enzyme in saccharification process. It was reported around 13.72% (v/w) of ethanol produce from rice hulls substrates. Cacua et al. (2018) reported on their research on production of bioethanol from pretreated rice husk which is hydrolyzed with acid cellulase at pilot scale, they found about 10.3% (v/w) ethanol. In recent year Agarwal et al. (2019) evaluated the bioethanol production from an agro-waste (de-oiled rice bran) by *Saccharomyces cerevisiae* and reported maximum 9.68% (v/w) ethanol. Thus, ethanol obtained from rice husk in this method could be more beneficial using enzyme and sample ratio of 2.5:1. Further study should be concentrated to optimize enzyme-husk ratio in the saccharification steps of bioethanol fermentation production process to get better yield.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The data presented in this research signify the outcome of the liquid fuel (ethanol) from a ligno-cellulosic (rice husk and sawdust) material. Generally different liquid fuels are extracted from ligno-cellulosic material via different chemical processes such as transesterification, pyrolysis etc. Most of those liquid fuels are vegetable oils. So, the fermentation method of ethanol production could be one of the promising procedures. Total rice production in Bangladesh is quite sufficient that produce ample amount of rice husk as by product. In this research there were approximately 9.55% (v/w), 8.73% (v/w) and 6.74% (v/w) ethanol production from respective fermented liquid broth from the ligno-cellulosic material where the ratios of cellulase enzyme and raw material were 1:2.5, 1:30 and 1:3.5 respectively. While from the heterogeneous sawdust bioethanol obtained was about 1.23% (v/w) at an enzyme and raw material ratio of 1:2.5 which is very low. So, the option of choosing rice husk to facilitate ethanol production would be a fair choice for their ease of availability where they are generally treated as waste.

5.2 Recommendations

The recommendations for the further research are given below:

1) Specific species of rice husk may be used to produce ethanol for identifying immaculate choice for better ethanol yield production.
2) Homogeneous mixture of sawdust may be used for ethanol production.
3) Other species of yeast such that *Scheffersomyces stipitis*, *Pachysolen tannophilus* etc. may be used for contrast the outcome of ethanol production.

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