PRODUCTION OF BIOETHANOL FROM WHEAT STRAW BY USING LOCALLY ISOLATED YEAST

BY; TEKALIGN MISGANA GEMESHA

A THESIS SUBMITTED TO

THE DEPARTMENT OF APPLIED CHEMISTRY

SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE MASTER'S OF SCIENCE IN APPLIED CHEMISTRY

OFFICE OF GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

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Approval of Board of Examiners

We, the undersigned, members of the Board of Examiners of the final open defense by Tekalign Misgana Gemesha have read and evaluated his thesis entitled ' **The production of bioethanol from Wheat straw by using locally isolated yeast'** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the MSc.

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Internal Examiner	Signature	Date
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External Examiner	Signature	Date

Declaration

I hereby declare that this MSc Thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Tekalign Misgana Gemesha

Signature:_____

This MSc Thesis has been submitted for examination with our approval as University advisors .

_____	_____	_____
Advisor Name	Signature:	Date of submission
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Co-advisor name	signature	Date of submission

Advisor's approval sheet

To: Applied Chemistry department

Subject: Thesis Submission

This is to certify that the thesis entitled ' The production of bio ethanol from wheat straw by using locally Isolated yeast ' submitted in partial fulfillment of the requirements for the degree of Master in Applied chemistry, the Graduate program of the department of Applied Natural science and has been carried out by Tekalign Misgana Id. No GSU/0508/06, under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence hereby he/she can submit the thesis to the department.

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Name of major Advisor	Signature	Date
_____	_____	_____
Name co-advisor	signature	Date

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ASTM	American Society for testing materials
C_2H_5OH	Ethanol
E10	10 percent ethanol 90 percent gasoline
E5	5 percent ethanol 95 percent gasoline
FAO	Food agriculture organization of United Nation
FTIR	Fourier transform infrared spectroscopy
FS	Fermentable sugar
HAB	Herbaceous and agricultural biomass
HMF	Hydroxyl methyl furfural
ICUMSA	International commission for uniform methods of sugar analysis
LCB	Lignocellulosic biomass
LCW	Lignocellulosic waste
TRS	Total reducing sugar
TS	Total solid
SCB	Sugar cane bagasse
SNNPR	Southern Nations, Nationalities, and Peoples region
TSS	Total sum of squares
USA	United States of America
VS	Volatile solid
YPD	Yeast extract peptone dextrose
WWB	Wood and woody biomass

ABSTRACT

Production of ethanol from utilized renewable resources such as crop residue can be used as an alternative energy sources .There for, the objective of this work was to use wheat straw sample collected from Wellega Zone, to evaluate bioethanol production potential of wheat straw by using locally isolated yeast. S. cereviciae prepared from teff flour was used for fermentation. Samples were air dried and ground to particle size less than 2.0 mm for proximate and chemical composition analysis. The ethanol production processes were carried out in four main stage such as pretreatment, hydrolysis, fermentation and distillations. For hydrolysis, the three factorial design 2×2×2 was applied to investigate the effect of the weight of the sample (50 and 70g), reaction time of (30 and 60min) and sulfuric acid of concentration (1% and 5%) with a solid to liquid ratio of 1:10 . The fermentation process was held at temperature of 30^{o}C and pH of 5, and treated using different acid concentrations and residence times. The results showed that maximum total sugar concentration 13.66% was obtained at a hydrolysis time of 60 minutes and acid concentration of 5%. In the process, it was observed that ethanol concentration increased with an increase in acid concentration, hydrolysis time and weight of the sample . Under these conditions, maximum bioethanol concentration production was 11% by volume, a maximum result as compared with literature data. Chemical characterization of the bio-ethanol produced as detected by FTIR confirmed the presence of O-H, C-O, -CH_2, and CH_3 functional groups in the ethanol produced, which indicate the presence of ethanol in the product. In conclusion, the results indicated that being available in plentiful amounts and non-edible material, wheat straw would be potential feedstock for bioethanol production in Ethiopia.

Key words; Lignocellulosic, wheat straw, bioethanol, process, Isolated yeast.

CHAPTER ONE INTRODUCTION

1.1 Background

In the 20th century, the world economy has been dominated by technologies that depend on fossil energy, such as petroleum, coal, or natural gas to produce fuels, chemicals, materials and power [1, 2]. The continued use of fossil fuels to meet the majority of the world's energy demand is threatened by increasing concentration of CO_2 in the atmosphere and concerns over global warming [3, 4]. The heightened awareness of the global warming issue has increased in the development of methods to mitigate greenhouse gases emission [5]. Reducing use of fossil fuels would considerably reduce the amount of CO_2 produced, as well as reduce the levels of pollutants [6]. As concern about global warming and dependence on fossil fuels grows, the search for renewable energy sources that reduce CO_2 emissions becomes a matter of widespread attention [7]. However, the utilization of these agricultural crops exclusively for energy production is heavily conflicting with food and feed production [8].Thus, the production of ethanol from lignocellulosic biomass of non-crop plants or non-edible parts of plants (second generation) approach is considered a sustainable solution for biofuel production.

Lignocelluloses wastes (LCW) refer to plant biomass wastes that are composed of cellulose, hemicellulose and lignin [9, 10]. They may be grouped into different categories such as wood residues (including sawdust and paper mill discards), grasses, waste paper, agricultural residues (including straw, Stover, peelings, cobs, stalks, nutshells, non-food seeds, bagasse, domestic wastes (lignocellulose garbage and sewage), food industry residues, municipal solid wastes and the like [11]. Currently, the second-generation bio-products such as bioethanol, biodiesel, bio-hydrogen and methane from lignocellulose biomass are increasingly been produced from wastes residues rather than from energy crops (such as jatropha, switchgrass, hybrid poplar and willow) they competes for land and water [12].

In terms of total production, wheat is the second most important grain crop in the world. A world annual wheat production in 2011 of 682 million tons and, in average, the harvesting of 1.3 kg of grain is accompanied by the production of 1 kg of straw; this gives an estimation of about 524 million tons of wheat straw in 2009 [13]. Therefore, wheat raw material is a rich source for the production of bioethanol [14].Ethiopia is the second largest wheat producing country in Africa next to South Africa. Bread wheat (Triticum aestivum) is the major variety of wheat grown in Ethiopia. The Oromia and Amhara regions produce 59% and 28% of the country's wheat, respectively, with an additional 10% coming from the Southern Nations, Nationalities, and Peoples region (SNNPR) and 3% from other regions [15].

The wheat straw produced has left on the field, plowed again into the soil, burned or even removed from the land depending on the decision made by the landowner. Farmers use crop residues for different purposes, e.g. for fuel as firewood and minor constructions, for roofing local houses, in the case of wheat, oat and barley straws. However, the major use is for livestock feed particularly for draught oxen during dry seasons. Animals feed on crop residues mainly in two ways. Alternatively, the residues are left in the threshing ground and consumed by animals together with the standing straws, which are left for aftermath grazing.

The conversion of cellulose to ethanol involves two processes: hydrolysis of cellulose present in wheat straw into fermentable reducing sugars and fermentation of the sugars to ethanol. The hydrolysis involves dilute H_2SO_4 acid, and the fermentation is carried out by the use of microorganisms like yeast or bacteria [16]. Due to the presence of lignin and hemicellulose, lignocellulosic biomass is not easily broken down into its components, which makes access of cellulose to acid difficult, for the extraction of sugars, an effective pretreatment is necessary for the liberation of polysaccharides from lignin and to access sugar components for hydrolysis step [17]. Thus, ethanol production is affected by using easily available substrates such as lignocelluloses and microorganisms, which could convert them to ethanol [18]. The yeast Saccharomyces cerevisiae is the primary organism that is used for ethanol production and efficiency of ethanol production is mainly dependent on the choice of yeast strain and continuous improvement using modern biotechnology tools [19].

The ability of yeast to produce ethanol depends on many factors such as strains, growth factors and optimum environmental conditions [20]. Isolated thermo tolerant yeast from fruits could promote higher yield of ethanol at higher temperature than commercial Saccharomyces cereviciae like baker yeast. Industrial cellulosic ethanol production is still a challenge due to high processing cost. One reason for the high cost is the high steam energy consumption in the di stillation of fermentation broth with low ethanol titer when lignocellulose materials are used as fe edstock [21]. Nevertheless, economic ethanol can produced from lignocellulosic substrates using S.cerevisiae [22]. Different pretreatment methods have evolved so far to increase the cellulose c ontent in the fermentation system to upgrade ethanol and hence it reduces the cost [23, 24].

The heart in ethanol production process is fermentation. Fermentation is carried out by a variety of microorganisms such as fungi, bacteria, and yeasts. S. cerevisiae is one of the widely studied and used yeasts at both industry and household levels. It tolerates a wide range of pH with acidic optimum which makes its fermentation less susceptible to infection than bacteria [25,26]. It also tolerates ethanol better than other ethanol producing microorganisms [27].Besides the five- and six-carbon sugars produced during hydrolysis of lignocellulosic biomass several inhibitors of ethanol fermentation are also generated.

These inhibitors pose hindrances such as inhibition of cell growth and sugar consumption during S. cerevisiae cultivation for ethanol production [28]. Different approaches have been used to solve the inhibitory effects of these chemicals in the production process [29, 30]. The choice of wheat straw to bioethanol conversion should be decided on the basis of overall economics (lowest cost), environmental (lowest pollutant) and energy (higher efficiency),i.e comprehensive process development and optimization are still required to make the process economically viable. An increasing petroleum price and negative impact of fossil fuels on the environment are encouraging the use of lignocellulosic materials to help meet energy needs [31]. Therefore, this study was intended to solve such type of problem and to convert this low valuable by product into highly valuable product.

1.2. Statement of the problem

Renewable energy plays an important role in the long-term energy supply security, diversification of energy mix, energy access, environmental security and sustainability. The challenges in utilization of renewable resources are a function of their costs compared to conventional generation ,their ability to be integrated into the grid ,which may require new controls to optimize variable output from multiple distributed sources and the availability of cost effective multiple hour storage .

Due to the tough crystalline structure, the enzymes currently available require several days to achieve good results. Either option is expensive. Currently the cost of enzymes is also too high. Acid is low cost, non-volatileness, easily available, and productive. However, bioethanol fermentation from lignocellulosic material can only be achieved by adequately pre-treating the lignocellulosic material. Lignocellulosic material contains cellulose, which is surrounded by a matrix of hemicellulose and lignin. Pre-treatment would remove the lignin and make the cellulose and hemicellulose accessible for conversion to sugars. Both cellulose and pentose converts in to fermentable sugar. However, the primary industrial yeast used in bioethanol production, Saccharomyces cerevisiae converts only hexose sugars such as glucose and is not able to co-ferment glucose and xylose. Thus, wheat straw can be used for the production of second-generation biofuel.

In addition to environment benefit, ethanol production from wheat straw can stimulate community based jobs and economic growth. Now a day, the production of wheat is increasing in Ethiopia and they generate a high amount of wheat straw. This by-product is mainly used as a low value cattle feed or simply deposited as a waste into landfill. Therefore, the Conversion of such waste into biofuel helps to reduce environmental pollution and energy problem of developing countries like Ethiopia. However, ethanol fermentation from wheat straw still needs to be studied in order to get high yields from the fermentation process. The conversion process of wheat straw to glucose should be studied to get maximum amount of bioethanol.

1.3 Objectives

1.3.1 General objective

The main objective of this study was to evaluate bioethanol production potential of wheat straw using locally prepared yeast.

1.3.2 Specific objectives

The specific objectives of this study were:

- To determine the amount of cellulose, hemicelluloses and lignin content and to analysis the moisture content, total solid (TS) and volatile solid (VS) contents of wheat straw.
- To determine the effects of weight, concentration of acid and time variation for efficient hydrolysis and fermentation of wheat straw using locally available yeast and H_2SO_4 catalyst.
- To determine the functional group and quantity of bioethanol produced from wheat straw
- To compere simple and double distillation process for the production of bioethanol from wheat straw.
- To camper the efficiency of commercial yeast and yeast extracted from teff flour in bioethanol production.

1.4 Significance of the study

- Revealed for researchers to see this very cheap and highly available by-product for other investigation.
- This study also highly contributes in the substitute fossil fuel by biofuel.
- Bioethanol production from wheat straw is considered as second-generation biofuel process since it has no direct conflict with human food.

  In general, this study urges the government to arrange stakeholders and different other concerning bodies to participate in bioethanol production. Furthermore, the result could strength the polities and strategies promoting green economy using biofuels, as alternative energy source.

CHAPTER 2 LETRATURE REVEUW

National energy security, energy sustainability and climate changes are the primary reasons to find alternative, renewable and reliable resources to fulfill energy demand. Currently, the world energy demand increases at an annual growth rate of 1.6% up to 45% by 2030. In addition, the consumption of fossil fuels produces different pollutants and causes environmental issues. These issues and depletion of fossil fuel resources have led a rapid expansion of renewable resources. At present, there are different renewable resources, namely wind, tide, hydropower and biomass. These renewable resources can satisfy energy demand in power sector, but transportation sector mainly depends on liquid fuels, which cannot produced from other renewable sources except biomass. The world energy outlook projected to meet 5% of world demand for transportation fuel by biofuels in 2030 [32].

Biofuels can be categorized into three major groups, namely first-generation, second generation and third-generation types. The main distinction among them is the type of feedstock used in the production process, their current and future availability. First generation biofuels are currently produced in large commercial quantities in many countries from agricultural crops such as sugarcane, maize, soybean and jatropha through well-established technologies such as hydrolysis, fermentation and trans-esterification. Bioethanol and biodiesel are the two most well-known examples of first-generation biofuels used in the transport sector and account for over 90% of global biofuel usage. While the properties of cellulosic ethanol are similar to those of first generation bioethanol, the relative abundance and wide range of lignocellulosic biomass used as feedstock, which is non-food, give them an advantage [33].

Bioethanol: a distilled colorless liquid fuel obtained from numerous potential feedstock varieties such as sugar beet, wheat, corn, cassava, fruits, bagasse, barley, molasses, skim milk (whey),potatoes, sorghum, switch grass and cellulose biomass such as wood, paper, straw and other cellulose wastes such as grasses, others includes municipal solid wastes. These various waste streams for ethanol production have their peculiar properties and generally differ.

2.1 The production of Bioethanol in Ethiopia

Ethanol production in Ethiopia linked with sugar factories. The total identified irrigable land for sugarcane plantation in the country is about 700, 000 hectares, estimated at a potential to produce one billion liters of ethanol [34]. At present, the main supply line in the domestic market dominated by two sugar factories (Fincha and Metehara) with the combination of their annual production capacity at around 11.1 million liters. In order to transform this potential into reality, the government developed a strategic plan in 2007 considering jatropha as a principal feedstock for biodiesel production and sugarcane as a principal feedstock for bioethanol production. Among other things, the strategy focused on esta blishing biofuel program, encouraging feedstock development, motivating customer demand, im proving environmental sustainability, awareness conception and promotion of biofuels, and rene wing energy policy to incorporate bioenergy in detail. As a continuation of this endeavor, there have been repeated efforts to initiate using bioethanol for domestic use and particularly, blending 5% of ethanol with gasoline in the year 2008 followed by 10% in the year 2011 and to increase the percentage in the years to come was the plan set out. With these, of course the plan includes expansion of sugar factories and building new ones though delayed during implementation [34].

2.2 Feed stocks for bioethanol production

Feed stocks typically grouped under the heading biomass and include agricultural residues, wood, municipal solid waste and dedicated energy crop [35]. Biomass has seen as an interesting energy source for several reasons, the main reason being the contribution provided by bioenergy to sustainable development [36]. Resources are often available locally, and conversion into secondary energy carriers is feasible without high capital investments [37]. Different feedstock types are available for the production of bioethanol as it can be derived from any biological raw materials that contain sugar, such as sugarcane, sugar beets and potatoes, or materials that can be converted into sugar from starch or cellulose, such as corn, wheat and other cereals [34]. Ethanol is also obtained from the agricultural products, products from food and biomass processing industries that are divided into three groups: raw sugar e.g. sugar beet and sugar cane, raw starch e.g. corn, rye, and triticale and lignocellulose materials such as grass, wood and straws [38].

2.2.1 Lignocellulosic based feedstock

Bioethanol can be produced from lignocellulosic biomass (LCB) such as crop residues for instance wheat straw, rice straw, corn cob, sugarcane bagasse (SCB), rice hulls, barley straw, sweet sorghum bagasse, and olive stones, hardwood, softwood, cellulose wastes, herbaceous biomass and municipal solid wastes [18] . These materials most often are underutilized and are sometimes disposed of in the environment without any treatment, leading to serious environmental pollution problems [39]. Lignocellulosic wastes (LCW) refer to plant biomass wastes are composed of cellulose, hemicellulose, and lignin. The lignocellulosic biomass, which represents the largest renewable reservoir of potentially fermentable carbohydrates on earth, is mostly wasted in the form of pre-harvest and post-harvest agricultural losses and wastes of food processing industries. Due to their abundance and renewability, there has been a great deal of interest in utilizing LCW for the production and recovery of many value added products [40].

Cellulose is an unbranched homopolysaccharide composed of β-D glucose units linked by (1, 4) glycosidic bonds. However, the basic building block of cellulose is a dimer of two glucose units known as cellobiose. Cellulose is the most abundant material on Earth, and it is the main constituent of plants. It is also present in bacteria, fungi, and algae even in animals. In nature, cellulose chains have a degree of polymerization (DP) of approximately 10,000 and 15,000 glucopyranose units in wood and native cotton celluloses, respectively [41]. This makes cellulose chemically stable, insoluble, and difficult to break down.

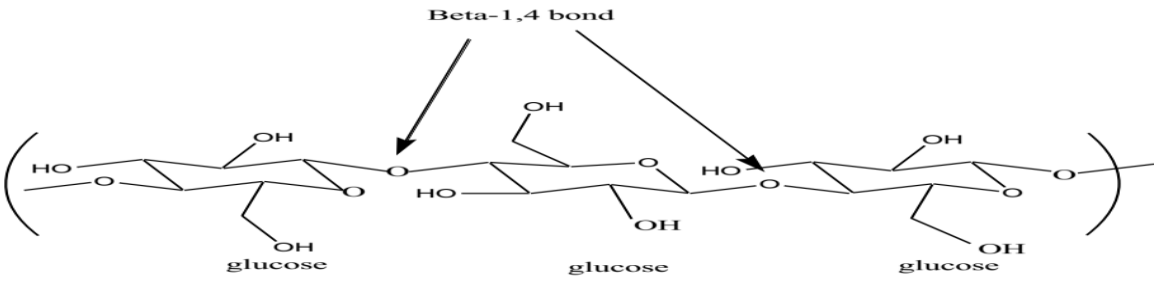


Figure 2.1: Molecular structure of cellulose : Ethanol production from cellulosic biomass by encapsulated saccharomyces cerevisiae [41]

Hemicellulose or polyose is a mixture of polymers comprising pentoses, hexoses hexuronic acids and deoxy-hexoses. Hemicelluloses differ from celluloses by composition of various sugar units and by much shorter and branched molecular chains. In contrast to cellulose which is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength. Therefore, dilute acid or base, as well as hemicellulase enzymes easily hydrolyze it [42].

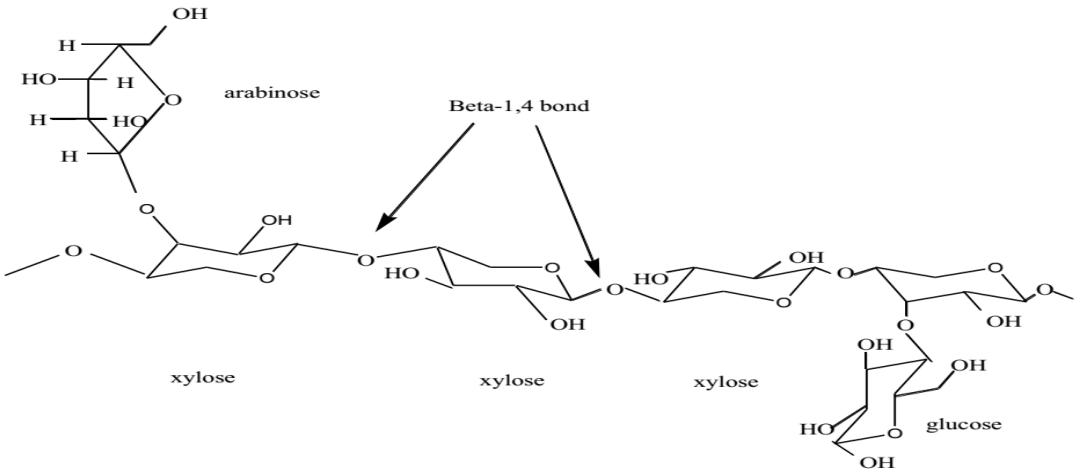


Figure2. 2; Molecular structure of hemicelluloses; characterization and computer simulation of corn stover /coal blends for gasification in a down draft gasifier [42]

Lignin is a complex, hydrophobic, cross-linked, three-dimensional aromatic polymer of phenylpropane building blocks. The mechanical strength properties of plants are mainly due to incorporation of lignin into their cell walls, whereby huge plants such as trees can remain upright. Lignin is one of the most complicated natural polymers with respect to its structure and heterogeneity, which make it extremely resistant to chemical and biological degradation [41].

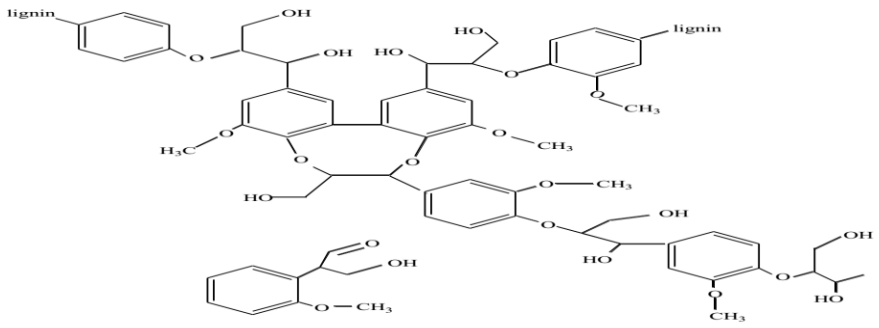


Figure 2. 3: Molecular structure of lignin; Biomass gasification using bubbling fluidized - bed gasified: investigation of the effect of different catalyst on tar reduction [43]

Table 2.1 The contents of cellulose, hemicellulose, and lignin in common agricultural residues and wastes; Hydrolysis of lignocellulosic materials for ethanol production [44]

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40 – 55	24 – 40	18 – 25
Softwood stems	45 – 50	5 – 35	25 – 35
Nut shells	25 – 30	25 – 30	30 – 40
Grasses	25 –40	35 – 50	10 – 30
Corn cobs	45	35	15
Paper	85 – 99	0	0 – 15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15 – 20	80 – 85	0
Cotton seed hair	80 – 95	5 – 20	0
News paper	40-55	25_40	18_30
waste paper from chemical pulps	60-70	10-20	5-10
Primary wastewater solids	8-15	Na	Na
Solid cattle manure	1.6 – 4.7	1.4 – 3.3	2.7 – 5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.5	12
Swine waste	6	28	Na

Extractives: are woody compounds that are soluble in neutral organic solvents or water. The extractives usually represent a minor fraction (between 1-5%) of lignocellulosic materials. They contain a large number of both lipophilic and hydrophilic constituents. The extractives can be classified in four groups: (a) terpenoids and steroids, (b) fats and waxes, (c) phenolics constituents and, (d) inorganic components [45, 46].

2.3 Ethanol production in global

Today, bio-ethanol is the most dominant bio-fuel and its global production showed an upward trend over the last 25 years with a sharp increase from 2000. As of 2005, worldwide production capacity for bio-ethanol fuel was about 45 billion liters per year, with approximately 15% annual growth between 2000 and 2005. This value increased to 49 billion liters in 2006, when the Americans produced 75% of the total world ethanol output, followed by Asia/Pacific and Europe/Africa with respective values of 15 and 10% [41].

The industrial alcohol market showed a rather modest rate of growth similar to the increase in Gross Domestic Product in many countries. The market for beverage alcohol in most developed countries is stagnating, due to increased health awareness. In 2009, production of fuel ethanol reached an estimated 76 billion liters, an increase of 10 percent over 2008. The United States and Brazil accounted for 88 percent of global ethanol production in 2009. Most of the increased production occurred in the United States [41].After a significant downturn in the U.S. fuel ethanol market in 2008, U.S. production rose 16 percent to about 41 billion liters in 2009, accounting for approximately 54 percent of global ethanol production. According to one estimate, U.S. ethanol (which is mostly corn based) displaced more than 360 million barrels of imported oil for gasoline production.

The highest sugar prices in years, combined with adverse weather conditions in a major producing region, resulted in a drop in Brazil‟s ethanol production from 27.1 billion liters in 2008 to 26.3 billion liters in 2009. All ethanol produced in Brazil is from sugar cane. All fueling stations in Brazil sell both pure ethanol and gasohol, a 25 percent ethanol/75 percent gasoline blend. Flex-fuel cars, which can use pure ethanol, gasoline, or any blend of the two, provide the flexibility to choose fuel based on price at the pump. They have been widely embraced by drivers and represent more than 95 % of all new cars sold in Brazil.

In recent years, significant global trade in fuel ethanol has emerged, with Brazil being the leading exporter. However, Brazilian ethanol export declined by almost 31 percent in 2009. International demands declined in great part because of the global economic crisis [41].

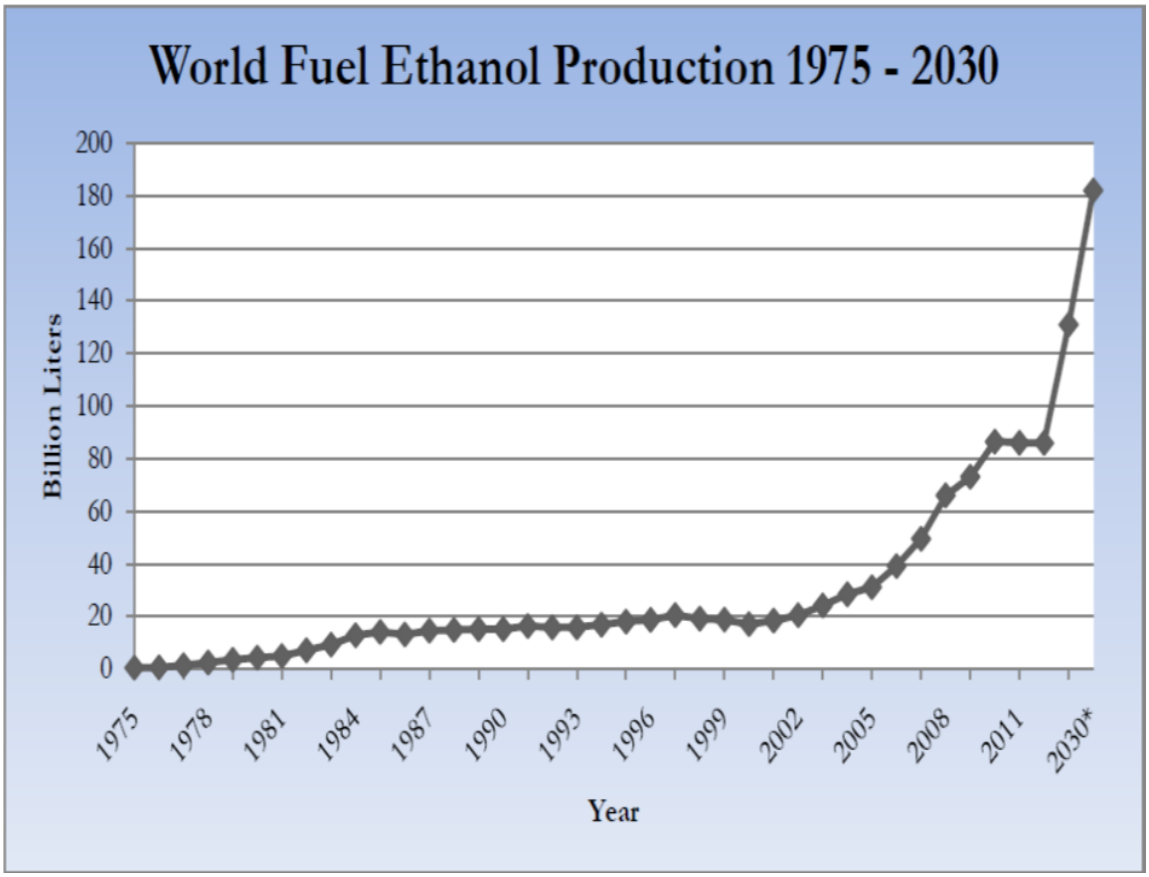


Figure2.4 World fuel ethanol production trend; Effect of corn grain variety on the bioethanol production efficiency [38]

2.4 Biomass Characterization

Biomass is biological material derived from living, or recently living organisms. It includes plants, leftovers from agricultural materials and forestry processes, as well as organic industrial, animal and human wastes [47]. Biomass has highly variable properties, especially with respect to moisture, structural components and inorganic constituents. This is directly related to the conditions of biomass growth, i.e., the properties of the soil that are associated with the location site, weather conditions and, especially, with use of chemicals (pesticides) and fertilizers [48].

The structural organic components are the hemicellulose, cellulose and lignin, from where the designation of lignocellulosic biomass was originated, and additionally extractives that may comprise organic and inorganic matter in their composition. There are different groups of biomass which can be classified in (1) wood and woody biomass (WWB), (2) herbaceous and agricultural biomass (HAB) including straws (HAS) and residues (HAR), (3) aquatic biomass, (4) animal and human biomass wastes, (5) contaminated biomass and industrial biomass wastes (semi-biomass) and (6) biomass mixtures (blends from the previous varieties).

Table 2.2. Chemical characteristics and structural components composition of various biomass groups and sub-groups [49]

Biomass	WBB	mean	HAB	mean	HAS	Mean
Proximate analysis wt%.ar)						
Moisture	4.7-62.9	19.3	4.7-62.9	19.3	7.4-16.8	10.2
Volatile matter	30.4-79.7	62.9	41.5-76.6	66.0	58-73.9	66.7
Fixed carbon	6.5-24.1	15.1	9.1-35.3	16.9	12.5-17.8	15.3
Ash	0.1-8.4	2.7	0,1-8.4	2,7	4.3-18.6	7.8
Structural component(wt,%.daf)						
Cellulose	12.4-65.5	39.5	23.7-87.5	46.1	18-54.8	45.4
Hemicellulose	6.7-65.6	34.5	12.3-54.5	30.2	18-39	31
Lignin	10.2-44.5	26	0.0-54.3	23.7	14.9-21.7	23.1
Extractive	1-9.9	3.1	1.2-86.8	13.7	3.8-21.7	13.6

Where; wood and woody biomass (WWB), herbaceous and agricultural biomass (HAB) including straws (HAS) and residues (HAR),

2.5 Overview of Ethanol production Process

Ethanol can be produced in two different ways. Either chemically, by hydration of ethylene, which is derived from crude oil or natural gas, or by fermentation of sugar containing feeds, starchy feed materials or lignocellulosic materials. About 5% - 10% of the ethanol produced in the world is a petroleum product.

The two primary ways of producing fuel ethanol from cellulosic feedstock are: Biochemical conversion process and thermo chemical conversion process [50].

2.6 Biochemical Conversion

Biological conversion consists of exposing biomass to certain microorganisms. The secondary fuels produced are the result of metabolic activity of the microorganisms. Production of ethanol through fermentation through anaerobic digestion is the most common biological conversion processes. Ethanol fermentation from carbohydrates is probably one of the oldest processes known to man. Today, it is widely regarded as an important potential alternative source of liquid fuels for the transport sector [51]. Typical lignocellulose-to-ethanol processes consist of at least four steps. These are pretreatment to enhance biomass digestibility, hydrolysis of cellulose to sugar monomers, fermentation of sugars to ethanol, and recovery of ethanol by distillation/evaporation from process stream [52].

2.6.1 Pretreatment

Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic chemical composition and structure so that hydrolysis of carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields [1,53]. The effect of pretreatment of lignocellulosic materials has been recognized for a long time. The purpose of the pretreatment is to remove lignin, reduce cellulose crystalline and increase the porosity of the materials.

Pretreatment must meet the following requirements: Improve the formation of sugars or the ability to subsequently form sugars by acidic or enzymatic hydrolysis; avoid the degradation or loss of carbohydrate; avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes. Physical, chemical, physico-chemical, and biological processes have been used for pretreatment of lignocellulosic materials [1, 44].

2.6.2 Hydrolysis

Hydrolysis is the unit operation that depolymerizes the polysaccharide chains of cellulose and hemicellulose into fermentable oligosaccharides and/or monosaccharides [54]. After the pretreatment process, there are two types of processes to hydrolyze the feedstocks for fermentation into ethanol, most commonly used are acid (dilute and concentrated) and enzymatic hydrolysis. In addition, there are some other hydrolysis methods in which no chemicals or enzymes are applied. For instance, lignocellulose may be hydrolyzed by gamma-ray or electron-beam irradiation, or microwave irradiation. However, those processes are commercially unimportant.

Hydrolysis of cellulosic materials includes the processing steps that convert the carbohydrate polymers e.g. cellulose and hemicellulose into monomeric sugars. Cleavage of these polymers can be catalyzed enzymatically by cellulases or chemically by acids such as sulfuric acid [17]. The factors that have been identified to affect the hydrolysis of cellulosic biomass include porosity or accessible surface area, cellulose fiber crystallinity, and the content of lignin and hemicellulose [41]. Both enzymatic and chemical hydrolyses require a pretreatment to increase the susceptibility of cellulosic materials [55].

2.6.2.1 Acid hydrolysis

Dilute Acid Hydrolysis. The dilute acid process is conducted under high temperature and pressure, and has a reaction time in the range of seconds or minutes, which facilitates continuous processing. The combination of acid and high temperature and pressure dictate special reactor materials, which can make the reactor expensive. The first reaction converts the cellulosic materials to sugar and the second reaction converts the sugars to other chemicals.

Unfortunately, the conditions that cause the first reaction to occur also are the right conditions for the second to occur [55]. The principle of acid hydrolysis is to apply temperature and pressure in order to soften lignocellulosic providing better penetration of the acid, and then degrade carbohydrate part of wood into monosaccharides. During treatment various products are formed: monosaccharides (xylose, arabinose, mannose etc.), while lignin and part of cellulose remain as solid residue. Research works on the dilute acid hydrolysis of different lignocellulosic materials have defined optimal process conditions: temperature 80-200^{0}C, sulfuric acid concentration 0.25–8 wt%, and reaction time 10-2000 min. Sulfuric acid is a commonly used acid due to low cost, nonvolatileness and affordable corrosion strength [56]. Another study carried reported that acid concentration in the dilute-acid hydrolysis process is in the range of 2-5% [57]. Acid hydrolysis typically uses dilute (4 wt. %) sulfuric acid at high temperatures (120-200^{0}C) for up to 2 hrs to break the cellulose chains into glucose.

The challenges associated with this method include cost and production of compounds inhibitory to fermentation organisms. Some of the costs of using acid can be mitigated through acid recovery and by-product recovery. Gypsum is produced in large quantities during neutralization of the hydrolyzate with lime at the end of the process, which could be used to make building supplies like wallboard. Additionally, the remaining lignin can be burned for process heat [54].

Despite all of the benefits of sulfuric acid hydrolysis, some limitations take place including high corrosion rates and expensive construction materials. In addition, liquors have to be neutralized prior to fermentation of sugars, thus gypsum is formed. The large amounts of gypsum negatively influence the downstream process, and results in a low-value byproduct stream. Thus, the treatment entails considerable expenses, which limits wide commercial implementation in comparison with other possible methods of hydrolysis [58].

According to the Salve on dilute hydrolysis, higher sugar concentration liberated from acid hydrolysate compared to enzyme hydrolysate . Another study on acid hydrolysis reported that maximum glucose recover obtained was 49.51% [59]. The biggest advantage of dilute acid processes is their fast rate of reaction, which facilitates continuous processing. Since 5-carbon sugars degrade more rapidly than 6-carbon sugars, one-way to decrease sugar degradation is to have a two-stage process.

The first stage is conducted under mild conditions to recover the 5-carbon sugars while the second stage is conducted under harsher conditions to recover the 6-carbon sugars [55].

2.6.3 Fermentation

Lignocellulose is often hydrolyzed by acid treatment. The hydrolysate obtained is then used for bioethanol fermentation by microorganisms such as yeast. The fermentation of ethanol is the biological process that converts fermentable sugars such as glucose and xylose to cellular energy with microorganisms which produce waste by-products ethanol and carbon dioxide anaerobically by the metabolic pathways of sugar. S. cerevisiae is yeast that is most widely used in the production of ethanol from hexoses but cannot utilise pentoses. P. stipitis converts both hexoses and pentoses into ethanol at relatively high conversion [60]. In general, the conversion of lignocellulosic material to sugar and then ethanol is governed by (equation 2.1) below:

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6 + \text{yeast} \rightarrow 2nC_2H_5OH + 2nCO_2 \quad (2.1)$$

From the above equation the first step is hydrolysis and the second step is fermentation. According to the reactions, the theoretical maximum yield is 0.51 kg bioethanol and 0.49 kg carbon dioxide per kg of xylose and glucose. The overall reaction of this fermentation of hexose sugar (glucose) by yeast has been expressed by Gay-Lussac which forms the basis of calculating fermentation efficiency as:

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2 \quad (2.2)$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (2.3)$$

Saccharomyces cerevisiae is the most favored organism for ethanol production from hexoses. P. stipitis and Candidashehatae are capable of fermenting both hexose (glucose) and pentose (xylose) sugars to ethanol [61]. Xylose-fermenting microorganisms are found among bacteria, yeast and filamentous fungi. Today, xylose fermenting bacteria include both native and genetically engineered organisms, and many have characteristics useful for simultaneous saccharification and fermentation [17]. One of the most effective bioethanol producing yeasts, S. cerevisiae, has several advantages owing to its high bioethanol production from hexoses, high tolerance to bioethanol and other inhibitory compounds in the acid hydrolysates of lignocellulosic biomass.

However, because wild-type strains of this yeast cannot utilize pentoses, such as xylose and arabinose bioethanol production from a lignocellulose hydrolysate is inadequate. For xylose-using S.cerevisiae, high bioethanol yields from xylose also require metabolic engineering strategies to enhance the xylose [17]. The optimal pH range for Saccharomyces cerevisiae varies between 4.0 and 6.0 depending upon the fermentation medium. The pH affects the efficiency of ethanol fermentation by influencing the activity of plasma proteins and intracellular enzymes. If enzymes are deactivated by pH < 4.0 the yeast will not be able to grow and produce ethanol efficiently observed an increase in ethanol production as well as fermentation efficiency with an increase in pH from 4.0 to 5.0 and found the optimum pH for Saccharomyces cerevisiae species around pH 4.5 [62] .

Another study carried by showed that Saccharomyces cerevisiae worked well between a pH range of 4.0-4.5 and yielded slightly better results at pH 4.0 with fermentation of Kalecik Karası and Narince types of grapes[62] . One of the most successful microorganisms for bioethanol production is Saccharomyces cerevisiae. Although the wild-type strain has high bioethanol productivity and very tolerant to high ethanol concentrations and inhibitory compounds, it is unable to ferment pentoses (hemicelluloses). According to study reported that 0.45 g ethanol/g glucose [63]. Pichia stipitis, Candida shek8hatae and Pachysolan tannophilus are promising microbes that are capable of fermenting both hexoses and pentoses. However, S. cerevisiae is still the most commercialized and dominated strains for bioethanol production [64].

Microorganisms for bioethanol fermentation can best be described in terms of their performance parameters and other requirements such as compatibility with existing products, processes and equipment. The performance parameters of fermentation are temperature range, pH range, alcohol tolerance, growth rate, productivity, osmotic tolerance, specificity, yield, genetic stability, and inhibitor tolerance [65]. All the recombinant strains are mesophilic organisms and function best between 25℃, and 33^0C.

2.6.3.1 Yeast

Yeasts are ascomycetous or basidiomycetous fungi that reproduce vegetatively by budding or fission, and that form sexual states which are not enclosed in a fruiting body. The yeast species are all characterized by a common features have-respect to morphological and physiological traits. This type of description, in which physiological characters are important, distinguishes yeast taxonomy from other fungal taxonomy [66]. Identification of yeast genera can often be achieved by morphological tests supplemented with a few physiological tests. With regard to the latter, sole carbon and nitrogen source assimilation by yeasts may be determined by auxanography which nowadays can be conveniently carried out using commercially available kits: Analytical Profile Index (API) strips (BioMérieux, France) or the automated/computerized BCCM/Allev 2.00 system [67].

Sugar assimilation and fermentation tests are commonly accomplished using glucose, galactose, maltose, sucrose, lactose, raffinose, trehalose and xylose.With regard to fermentation of these sugars has argued that the anaerobic liberation of CO_2 into durham tubes is not very accurate for detecting slowly fermenting yeast species [68]. Ethanol production assays are deemed more appropriate determinants for yeast characterization [69]. Yeasts are used in many industrial processes, such as the production of alcoholic beverages, biomass and various metabolic products. The last category includes enzymes, vitamins, capsular polysaccharides, carotenoids, polyhydric alcohols, lipids, glycolipids, citric acid, ethanol, carbon dioxide and compounds synthesized by the introduction of recombinants DNA into yeasts. Some of these products were produced commercially while others are potentially valuable in biotechnology [70].

2.6.4 Separation

2.6.4.1 Distillation

Distillation is the method used to separate two liquid based on their different boiling points. However, to achieve high purification, several distillations are required. This is because all materials have intermolecular interactions with each other, and two materials will co-distill during distillation [71]. The ethanol is then extracted from this solution by fractional distillation. Although the boiling point of ethanol, 78.3℃, is significantly lower than the boiling point of water, 100^0C, these materials cannot be separated completely by distillation.

In a distillation, the most volatile material (i.e. the material that has the lowest boiling point) is the first material to distill from the distillation flask, and this material is the azeotrope of 95% ethanol which has the lowest boiling point. If an efficient fractionating column is used, 95% alcohol could be obtained first and then a small intermediate fraction of lower concentration, and then water. But no matter how efficient the fractionating column used, 95% alcohol cannot be further concentrated by distillation because the vapor has exactly the same composition as the liquid; towards distillation, then, 95% alcohol behaves exactly like a pure compound [50].

2.7 Optimization of bioethanol production from wheat straw and other biomasses

Integration of first and second-generation bioethanol production process is considered to be an intermediate step towards full scale lignocellulosic bioethanol production. Recognizing that in sugarcane based industries bagasse is already used in electricity generation, a simulation and optimization approach was used to optimize plant operation [72]. Only two decision variables were considered: the fraction of feedstock entering the boiler, and the fraction of the bagasse surplus (not burned) used for second generation bioethanol production, being the rest sold. The process was simulated using the software EMSO, and a particle swan optimization algorithm handled the optimization variables. The authors showed that the production of 2G bioethanol increases thermal demands in at least 25%, which narrows the decision feasible range on how much bagasse, can be diverted to production of second generation fuel. Furthermore, electric power surplus is diminished in at least 31%.

On the other hand, a flow sheet for the production of bioethanol from switch grass via biochemical route was proposed by optimizing a superstructure embedding a number of alternatives [73]. Two technologies were considered in the pre-treatment stage: dilute acid and ammonia fiber explosion. Cellulose to bioethanol conversion was achieved by using SHF and a number of distillation and dehydration alternatives were considered . The optimal flow sheet consists of dilute acid hydrolysis and molecular sieves. For straw samples, temperatures around 120^0C-175^0C were optimal for dilute acid pretreatment. For woody samples, temperatures around 175^0C were also optimal, though not as drastically as for straw. In the case of both groups of biomass, temperature was the most influential factor in ethanol yield. Woody samples gave the highest ethanol yields at 10% biomass solid loading, while wheat straw was optimal at 5% and barley straw at 10%.

Wheat straw and soft wood gave the highest ethanol yields with 30 minutes, while barley straw and hard wood gave the highest yields with 45 minutes of treatment.In the case of both groups of biomass, temperature was the most influential factor in ethanol yield. Straw samples gave the highest yields at a $Ca(OH)_2$ loading of 0.05 g/g dry biomass, and woody samples varied, at 0.05 g/g dry biomass for hard wood and 0.1 g/g dry biomass for soft wood. For reaction time, woody materials gave the highest yields with 1 hour, whereas straws varied slightly with wheat straw at 1 hour and barley straw at 1-2 hours.

Soaking in aqueous ammonia: for both straw and woody samples 9% biomass solid loading were optimal in terms of ethanol yield. Both straw samples gave the highest ethanol yields with 15% ammonia (wt %), while woody samples differed slightly, with optimal for hard wood at 19% ammonia and soft wood at 15%. Optimal reaction time for wheat straw was 36 hours while barley straw required 39 hours. Both woody samples had the highest ethanol yields at 36 hours. Comparison of three optimized pretreatment processes against feedstocks. I n the table below, of the three pretreatment processes tested (dilute acid, lime and soaking in aqueous ammonia (SAA)), SAA pretreatment was found to yield the highest amounts of ethanol for wheat straw, barley straw and hard wood at 149.0, 147.1 and 75.4 g/g dry biomass, respectively. Soft wood samples reached its highest ethanol yield (29.1 g/g dry biomass) when subjected to lime pretreatment.

However, based on the reaction time of each pretreatment technology, the ethanol yield rates (mg/g dry biomass/hour) of samples pretreated by dilute acid were much higher than those by alkaline. Thus, in this project, it could be concluded that the dilute acid pretreatment was recommended because of its comparatively high efficiency. Certainly a more detailed cost estimate on energy consumption, equipment investment and maintenance, etc. should also be conducted as a comprehensive evaluation on each pretreatment technology[74] .

Table 2.3 Evaluation of Pretreatment technologies for Converting Washington biomass to bioethanol [74]

Feedstock	Dilute Acid Pretreatment			Lime Pretreatment			SAA Pretreatment		
	Ethanol (mg/g dry biomass)	Reaction time (min)	Ethanol yield rate (mg/g dry biomass/h)	Ethanol (mg/g dry biomass)	Reaction time (hour)	Ethanol yield rate (mg/g dry biomass/h)	Ethanol (mg/g dry biomass)	Reaction time (hour)	Ethanol yield rate (mg/g dry biomass/h)
Wheat Straw	147.8	30	295.6	131.5	1	131.5	149.0	36	4.1
Barley Straw	141.5	30	283.0	128.1	2	64.0	147.1	39	3.8
Hard Wood	42.4	45	56.5	66.0	1	66.0	75.4	36	2.1
Soft Wood	22.4	45	29.9	29.1	1	29.1	25.6	36	0.7
Biomass Mixture	90.2	30	180.4	85.6	1	85.6	100.6	36	2.8

CHAPTER 3 MATERIALS AND METHODS

3.1. Chemicals and equipments

3.1.1 Chemicals and reagents

All the chemicals and reagents used in this study were an analytical grades and includes;

Sodium hydroxide (NaOH, min. assay 98% BDH Chemicals Ltd pool England cellulose), sulphuric acid (H_2SO_4, (98%, England)), Dextrose sugar, yeast extract, urea, $MgSO_4.7H_2O$, Yeast (Saccharomyces cerevisiae), (manufactured in furnace by S.I.Lesaffre with the strain safinstant). Fehling reagents grade one and two, HCl (GR grade E-Merck ,India) ,HNO_3 ,acetone, benzene, distilled water, methyl blue, $K_2Cr_2O_7$,chloramphenicol,ethanol (97%) ,acetone ,yeast of Tef flour, YPDA.

3.1.2 Equipments

Digital balances (model-Sartorius with 0.01mg sensitivity, and model EP214C), Sieves (mesh size of 2.0mm Sortmks 3332 , PFEUFFR Germany) , vacuum filter (model BN 3 STAATLICH, Berlin),pHmeter (pHmeter3310 ,JENWAY) , shaker (EXCELLAE24R) , condenser, Filterpaper,funnel,Incubator Model D.91126 schwabach FRG ,made in Germanny), scissor, tongue, stirrer Autoclave(model LXB75 made in China) , Oven (model 105, Industrial estate , Ambala cantt made in India),Laminar air flow work station (model N201330E made in USA), Furnace, heating mantle (Labmaster, isopad,type LMUL/ER/1L with 220/240 volts), Vessels, Soxhlet, tirmple, piterdish, flasks, measuringcylinder, alcoholmetere, burret, dropper, testtube, crucible, electrical grinder (ZAIBA super blender), distillation setup, water bath , reflux, thermometer, ,Fourier transform infrared spectroscopy (prinks Elmer spectrum, 65 FTlR).

3.2 Sample collection and preparation

The sample of Wheat straw was randomly collected from Guduru wereda,Horo Guduru Zone of Ethiopia farmers farm lands.The collected sample was brought to the laboratory of applied chemistry department of Adama Science and Technology University, Ethiopia.

In the laboratory, the wheat straw sample was cut by scissor into pieces of about 3-5 cm length for drying and grinding. The sample was first sun dried, then dried in oven at 105^{o}C for 2 hours to obtain an easily crushable material. After drying, the sample was grounded with electrical grinder with a maximum ground particle size of less than 2 mm.

3.3 Characterization of Wheat straw

3.3.1 Proximate analysis

The proximate analysis gives information about total solid (TS), moisture content (MC), volatile matter content (VM), the fixed carbon content (FC) and the ash content of the sample [75].

I. Total solid (TS)

It was determined according to TSS D 2540-99 standard method. To analysis the total solid of wheat straw, 40g of sample was dried at 105^{o}c for 4hrs by using oven drying until its weight was constant.

$$\text{TS (\%)} = \frac{\text{Weight of the sample befor oven drying}}{\text{Weight the sample after oven drying}} \text{X100 Or TS (\%)} = \frac{Final\ weight}{Intial\ weight}\text{X100} \qquad (3.1)$$

II. Moisture Content

A moisture content of wheat straw was determined according to ASTM2867-99. 40g of wheat straw was weighed in clean preheated moisture crucible of known weight by using sensitive balance. The sample and crucible were kept in an oven 105^{o}c for an hour. The crucible was covered and transferred to desiccators, and weighed after reaching room temperature. The crucible was heated in the oven for another two hours and was re-weighed. This was repeated until constant weight was obtained. The loss of weight was calculated as percent of weight and expressed as moisture content.

$$\text{Moisture content (\%)} = \frac{W1 - W2}{w1} \times 100 \qquad (3.2)$$

Where: W1= Initial weight W2= weight after drying

III. Volatile Matter Content

The percentage of volatile matter of wheat straw sample was determined by the standard method (ASTM D5832-98) .2 grams of the sample was measured on the balance and placed in a crucible. And then , both the sample and the crucible weighed (W_1).It was then heated up to 950^{o}c for exactly 7 minutes in a furnace .After heating , the sample was removed , cooled in desiccators and then weighed (W_2). The volatile matter content was determined by the following formula ;

$$\text{Volatile content (\%)} = \frac{Loss\ in\ weight\ due\ to\ volatile\ matter}{Weight\ of\ sample\ taken\ )} \times 100 \quad \text{Or}$$

$$\text{Volatile content (\%)} = \frac{W1 - W2}{w1 - W3} \times 100 \qquad (3.3)$$

Where: W1= Original weight of the sample + weight of crucible

W2 = Weight of crucible +ash sample

W3=Weight of empty crucible

IV. Ash Content

Determination of ash content in a sample was determined according to standard procedure (ASTM D2866-94) of heating in the furnace at 550^{o}C temperature for 2hrs.A crucible was weighed empty, and then samples were put in it. 25.5g of sample and the crucible were placed and heated in a muffle furnace for 2 hours at 550^{o}C .The crucible was removed from furnace and placed in a desiccators to cool, then was reweighed.

$$\text{Ash content (\%)} = \frac{(W1\_W2}{W1} \text{ X } 100 \qquad (3.4)$$

Where: W1= Original weight of the sample

W2 = Weight of sample after cooling

V. Fixed Carbon Content

This is the residue left after the moisture, volatile and ash is given up. It is deduced by subtracting from 100, the percentage of moisture, volatile matter and ash content. The fixed carbon content (FC) is given as;

$$FC = 100 - (\%\text{moisture} + \%\text{volatile matter} + \%\text{ ash}) \qquad (3.5)$$

3.3.2 Chemical composition of Wheat straw

I. Determination of extractives:

Oven dried samples of wheat straw was placed into trimble which is plugged with small amount of cotton and placed in a soxhlet extraction tube. An exhaustive ethanol extraction was complete in 6 hours using the Soxhlet method [76].

$$\text{Extractive (\%)} = \frac{W1-W2}{W1} \text{X}100 \qquad (3.6)$$

Where: W1 = oven dry sample W2 = extracted residue

II. Determination of cellulose content

2g of extractive free sample of wheat straw was transferred into flask that contains 150 mL of alcoholic nitric acid solution and boiled for 3hours under reflux. The alcoholic nitric acid solution consisted of mixing one volume of 68% (w/w) solution of nitric acid with four volumes of 97% purity alcohol. After each cycle, the solution was removed for a fresh volume. At the end the cellulose was washed by distilled water to 7 pH and weighed after drying at 105^{o}C.

$$\text{Cellulose (\%)} = \frac{W1-W2}{W1} \text{ x } 100 \quad (3.7)$$

Where: W1 = oven dry sample W2 = extracted residue

III. Determination of lignin:

2g of extractive free sample was placed in flask and 72% sulfuric acid was added. The flask was kept in water bath at 30^{o}C, during dispersion of the material for 1hr.After that distilled water was added and placed in autoclave for 121^{o}C to 1hr.

Next, the insoluble material (lignin) was filtered by vacuum filtration. The lignin was washed until became acid free (with hot water) and after dried the solid sample was weighted [77].

$$\text{Lignin (\%)} = \frac{W1-W2}{w1} \text{ x } 100 \quad (3.8)$$

Where: w_1 = oven dry sample w_2 = extracted residue

IV. Determination of hemicellulose content

The hemicellulose content (WH) was calculated by difference, assuming that extractives, cellulose and lignin and are the only components of the entire biomass [78]

100 = WC + WH +WE + WL

$$WH = 100 - (WC + WE + WL) \quad (3.9)$$

Where: WC, WH, WE, WL are cellulose content, hemicellulose content, extractive, and lignin content respectively.

3.4 Bio ethanol production process

In this study the effect of parameters such as time, temperature, concentration of acid, weight of sample loaded for hydrolysis process and production efficiency or performance of yeast for fermentation process, etc were used .

3.4.1 Sample Pretreatment

A. Pretreatment.First distilled water was prepared and then 50 g and 70g of the sample was soaked in a distilled water of 500 mL and 700mL in conical flasks for 24 hours. Then, the lignocellulosic biomass was rapidly heated at 121°C by high-pressure steam without addition of any chemicals in autoclave. The biomass/steam mixture was held for 15 minutes to promote hemicellulose hydrolysis and terminated by an explosive decompression.

After finishing the given pretreatment time and temperature the sample in autoclave was allowed to cool and the soluble portion was separated from the non-soluble portion. The non-soluble portion was hydrolyzed in the next steps and the soluble solution was placed in another conical flask.

3.4.2 Dilute Acid Hydrolysis

This process was conducted under high temperature and pressure, and has a reaction time in the range of seconds or minutes, which facilitates continuous processing. The three-parameter and two-level (2^3 = 8) factorial design was applied to hydrolysis step of the experimentation. Factorial design represents, for hydrolysis experiments there are thee variables that were changed in two conditions or levels (maximum and minimum) in which the changes of the variables were studied. For each one the hydrolysis parameters (acid concentration, hydrolysis time and weight of hydrolyzed sample), there are four low and four high levels in the design .1% (v/v) and 5%(v/v) diluted sulfuric acid was added to the non-soluble component obtained from pretreatment steps in the order of experimental design. The straw was then hydrolyzed in the reactor at a temperature of 121^{o}C and at a time of 30 and 60 min .The values of each parameters were prepared according to Table 3.1.

Table 3.1. Numeric values of parameters in hydrolysis process

Treatments number	Acid concentration (% by volume)	Hydrolysis time(min)	Weight of the sample(g)
1	1	30	50
2	5	30	50
3	1	60	50
4	5	60	50
5	1	30	70
6	5	30	70
7	1	60	70
8	5	60	70

After hydrolysis, the solid part was separated from the liquid in the hydrolyzate by filtration . After the solid part was separated, it was again washed by distilled water. The filtered hydrolyzate was neutralized with 10 M NaOH until the pH became in a range of 4.9-5.2. Then the soluble component mixed with the previously filter solution from the pretreatment step for the next procedure.

Determination of sugar content

The amount of sugar in the hydrolyzed samples was determined by Fehling method [59]. Fifty mL of each hydrolyzed sample solution was dissolved in 10 mL of distilled water and 2 mL of concentrated 1MHCl was added and boiled. The obtained sample was neutralized with 1NaOH and the solution was made up to a volume of 300 ml and taken into the burette.

The 5 mL of Fehling A and 5 mL of Fehling B were taken and mixed with 90 mL of distilled water in 250 mL Erlenmeyer flask and two drop of Methylene blue indicator was added. The solution in the flask was titrated until disappearance of blue color and the volume at which brick red color observed were recorded. For each sample, the sugar content was calculated by using the formula given below [79].

Sugar content (%) = $\frac{300mlX\ f}{V}$ X 100…… (3.10)

Where: f- Fehling factor (0.051); v- volume used in the titration (titrate value) (mL).

3.4.3 Fermentation process

3.4.3.1 Procedures for isolation and growth of yeast from teff flour

-20g of teff flour was dissolved in 500 mL of distilled water in high land bottle and allowed for 72 hours for the development of yeast.

-YPDA was prepared from the mixture of 10g yeast extract agar, 20g peptone , 20g glucose,0'1g/L chloramphenicol and 20g bacto agar by dissolving into 80mL of distilled water by string .Then distilled water was added to the solution until the final volume was 1L. -The media was sterilized in autoclave at 120^{o}c for 15 minutes and stored refrigerator at 4^{o}c.

The prepared yeast from teff flour samples by using serial dilution method followed by plating aliquots of appropriate dilution of samples on yeast extract peptone dextrose agar (YPDA). One ml of the sample was transferred to nine ml of sterile distilled water to be successively diluted to 10^{-1} up to 10^{-6}. Aliquots of 0.1 ml from final dilutions (10^{-3} and 10^{-4}) were spread onto YPDA [80]. The YPDA medium contains g/L of yeast extract 10, peptone 20, dextrose (glucose) 20, and agar 20. It supplemented with 0.1 mg/mL chloramphenicol to inhibit bacterial growth [81]. The plates were incubated at 37^{o}C for 72 hrs. Morphologically distinguished colonies were then selected under a dissection microscope. Yeast isolates were purified by sub culturing on YPD medium by streaking. The Pure culture was kept on YPD slant agar and stored at 4℃ for further study. According Apiradee the morphology of cells of ethanol, sugar and thermo tolerant yeasts and their appearance on solid medium, on YPD agar and YPD liquid medium was examined, after incubating at 37^{o}C for 3 days [13]. The cultural features recorded were texture, color and surface of colonies. One of the most effective bioethanol producing yeasts, S. cerevisiae, has several advantages owing to its high bioethanol production from hexoses, high tolerance to bioethanol and other inhibitory compounds in the acid hydro lysates of lignocellulose biomass. However, because of wild-type strains of this yeast cannot utilize pentoses, such as xylose and arabinose bioethanol production from a lignocellulose hydrolysate is inadequate.

Microorganisms: S. cereviciae isolated from teff flour by using the above procedures was collected from a piterdish and applied for the next experiment.

Procedures in liquid media Preparation:

First, 100 mL media containing 10 g Sugar (Dextrose), 0.2 g yeast extract, 1.0g urea and 1.0g $MgSO_4.7H_2O$ was prepared. The 100 mL media was sterilized in autoclave. And 2 g of yeast, Saccharomyces cerevisiae was added in a 250 mL conical flask and then properly covered with aluminum foil. The conical flasks was placed in incubator for 24 hours at a temperature of 30^oC and 200 rpm.

Adjustment of pH: Before addition of any microorganism to the diluted hydrolyzed sample, pH of these samples had to be adjusted. Otherwise, the microorganism will die in hyper acidic or basic state. A pH of around 5.0 - 5.5 was maintained. The hydrolyzed samples were primarily checked for pH using a digital pH meter. The pH meter was adjusted to 5.0 - 5.5. When the pH went below 5.0 - 5.5, sodium hydroxide solution was added drop wise to the flask with constant stirring until the pH reaches to a range of 5.0 - 5.5. When the pH went beyond 5.0 - 5.5, concentrated sulfuric acid was added drop wise to maintain the pH in the range.

Procedures of the Experiment: The yeast, Saccharomyces Cerevisiae culture was added with the proportion of 1:10 to the hydrolyzed sample. The vessel was lidded with a piece of ginned cotton covered with aluminum foil. Fermentation was let take place. After 72 hours of fermentation, the sample was taken out and distilled i.e. incubation time, yeast concentration (yeast proportion) and fermentation temperature was set to be at 72 hours, 10% and 30°C respectively.

3.4.4 Double Distillation process

A distillation process were used to separate the bioethanol from water in the liquid mixture. All distillation experiments were carried out at a temperature of 85°C and a distillation time of 3 hours by distillation setup. The distillate collected by simple distillation was redistilled to optimize the concentration of bio-ethanol for all samples.

3.5 Quantification and Characterization and of bioethanol produced from wheat straw

The ethanol concentration of the samples collected every 3 hours interval by simple distillation and double distillation of fermented solution was measured by alcoholmeter. An alcoholmeter is a hydrometer, which is used for determining the alcoholic strength of liquids. The functional groups of wheat straw bioethanol were determined by using prinks Elmer spectrum 65 FTlR with the help of lR correlation charts in Addis Ababa University, 4kilo campus.

Procedures for sample preparation for FTIR

From high ethanol contents (11%) of sample, 0.5mL of liquid solution was taken to Addis Abeba University. The lR spectrum was reported by % transmittance.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Characterization of Wheat straw

4.1.1 Proximate analysis

Table 4.1: The results of proximate analyses of the wheat straw sample

Physical composition	Weight percentage (%wt. dry basis)
Total solid (%)	93.649
Moisture content (%)	6.375
Volatile matter (%)	72.33
Ash content (%)	12.6
Fixed carbon content (%)	8.649

According to Cicilia Sambusitti, the total solid of wheat straw was 94±4 % [81]. S.V.Vassilev, D .Baxter and T.J Morgan reported that the moisture content of wheat straw was 7.4-16.8, volatile content of 58-73.9%, ash content of 4.3-18.6% '[49]. Mosier reported that, fixed carbon content of wheat straw was 7-9.9% [17]. The results from these studies are in a comparable range with literature values as reported by the mentioned researchers. The moisture content is a measure of the amount of water in the wheat straw. Moisture content analysis used for the determination of proportionality of solid to liquid ratio in the pretreatment and hydrolysis method with increasing moisture content it affects the product quality. The analysis of total moisture is used to determine other properties such as volatile matter, ash content and fixed carbon. The sample of wheat straw with higher moisture content needs more heat for moisture vaporization. Ash is a measure of inorganic impurities in the wheat straw. Finally, fixed carbon (FC) it is the carbon found in the material, which is left after volatile materials are driven off, this is used for the determination of carbon in the wheat straw [44].

4.1.2 Chemical composition analysis of Wheat straw

Table 4.2: The results of chemical composition of Wheat straw sample

Chemical composition	Weight percentage (w/w %)
Extractive	5
Cellulose	30
Hemicellulose	50
Lignin	15

Sun.Y and Cheng.J.reported that , the chemical composition of wheat straw was, 30% cellulose, 50% hemicellulose,lignin 15% and 1-5% extractive [1,44] .The results from this study were in a comparable range with literature values as reported by the above researchers. In this study, wheat straw contained high contents of the total cellulose of approximately 30% cellulose and 50% hemicellulose. The low level of lignin present in the sample of wheat straw appeared more advantageous than the other types, for example, 18-25% in hard wood steam, 25-35% in Soft wood steam , 30-40% in Nuts shells, and 10-35% in grasses . The lower the lignin content the easer hydrolysis condition, and decrease formation of toxic chemicals such as, aromatic, polyaromatic, phenolic and aldehydic (inabitory compounds) [5].

4.2 The total sugar content of Wheat straw

Table 4.3. The total sugar content of wheat straw in different conditions of hydrolysates

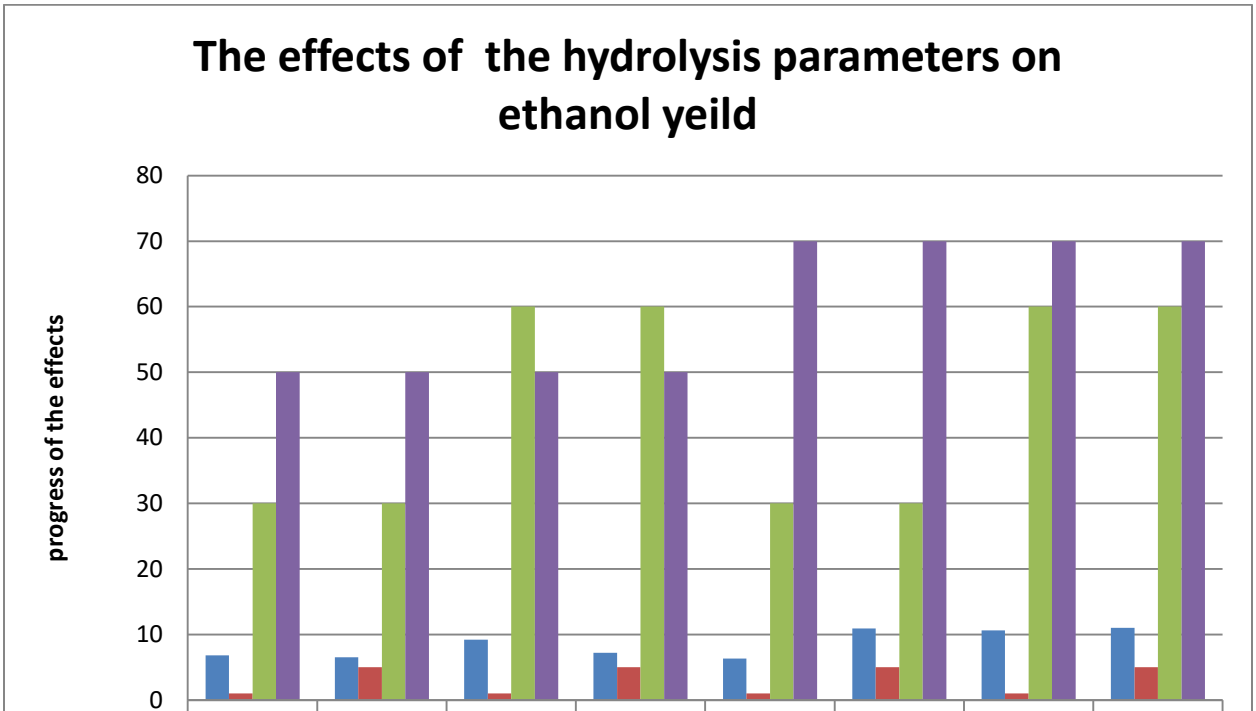
Sample type	Weight of the sample(g)	Concentration of $H_2SO_{4\,(\%)}$	Time of hydrolysis(min)	Sugar content (%)
A	50	1	30	12.5
B	50	5	30	12.9
C	50	1	60	12.64
D	50	5	60	12.86
E	70	1	30	13.30
F	70	5	30	13.45
G	70	1	60	13.43
H	70	5	60	13.66

Table 4.3 shows that as the weight of the sample, concentration of sulphuric acid used for hydrolysis and time of hydrolysis were increased, the sugar contents of the samples also increased. Because, when large amount of sample powder were hydrolyzed with high acid concentration for longer time of hydrolysis, hemicellulose and cellulose components of the straw was decomposed to sugar or glucose. The increasing of sugar content in acid treated samples with increasing of acid concentration may be due to decreasing of the degradation of monomeric sugars (xylose, glucose) to furfural and HMF or may be it could attributed from the conversion of glucose to levulinic and formic acid, which leads to decrease in glucose yield. These substances are toxic substances for yeast and can inhibit the yeast growth [82]. Based on this, the maximum yield of sugar concentration (13.66%) was maximum sugar content produced for 5% of acid concentration and hydrolysis time of 60 min. The observed result is coincide with the previous study [83, 84].

4.5 Bioethanol production from wheat straw

4.5.1 Hydrolysis process

The effects of the operating conditions on the ethanol yield were investigated and the optimal values were determined in this study.



	1	2	3	4	5	6	7	8
ethanol.c	6.8	6.5	9.2	7.2	6.3	10.9	10.6	11
Acid .c	1	5	1	5	1	5	1	5
Time	30	30	60	60	30	30	60	60
weight	50	50	50	50	70	70	70	70

Figure 4.1 The effects of acid hydrolysis parameters on the yields of bioethanol

Regarding acid hydrolysis of wheat straw, Figure 4.1 showed that, as the weight of the sample was increased , the yields of ethanol was also increased. This is because of increasing the sugar contents of the hydrolysate that leads the high yields of ethanol. In another hand at lower amounts of weight of the sample (50g), as the concentration of acid used for hydrolysis was increased the yields of ethanol was decreased, this is may be due to the formation of inhibitory compounds that affects the fermentation capacity of yeasts.

But with larger amounts of weight of the sample(70g) ,as the concentration of acid used for fermentation was increased, the yields of ethanol was also increased due to the degradation of cellulose and hemicellulose by large amounts of acid concentration. Generally, acid has positive effects on the yields of ethanol. With smaller weight of the sample (50g), increasing time of hydrolysis (30 to 60 min) with increasing the concentration of acid used for hydrolysis, decreases the yields of ethanol. In addition, with large weight of the sample (70g), increasing the time of hydrolysis increases with increasing the concentration of acid used for hydrolysis used, the yields of ethanol (6.3%-11.0 %). The concentration of acid used for hydrolysis and time of hydrolysis has inter dependent effects on the yields of ethanol. **7**0g of weight of the sample, 5% of acid concentration and 60minutes of hydrolysis were the conditions at which a maximum ethanol concentration (11%) was produced.

4.5.2 Fermentation process for the production of bioethanol from bioethanol

Fermentation process is another process that can be optimized in the production of optimum bioethanol yield. There are different parameters that are optimized to optimize the yield of bio-ethanol. Temperature, time, weight of sample and productivity of yeast (ethanol production ability of yeast) are the common parameters of fermentation process optimization. But for this study productivity of yeast was used as optimization criteria making other parameters to be constant .In this case 30^0c fermentation temperature, 72hrs time of fermentation, 25g of sample weight and 5% of H_2SO_4 were used constantly .The productivity efficiency of isolated yeast prepared from teff flour and the productivity efficiency of commercial yeast were used to optimize the yield of bioethanol . S.cerevisiae ferments sugars of liginocellulosic biomass than other micro organizems. Isolated strains fermented concentrated sugarcane syrups as well as high sucrose solution in synthetic medium with conversion efficiency of 89-92% [85]. According to Gaur, K work, S.cerevisiae strains has an ability to produce 5.8-11.6% bioethanol from biomasses [86].

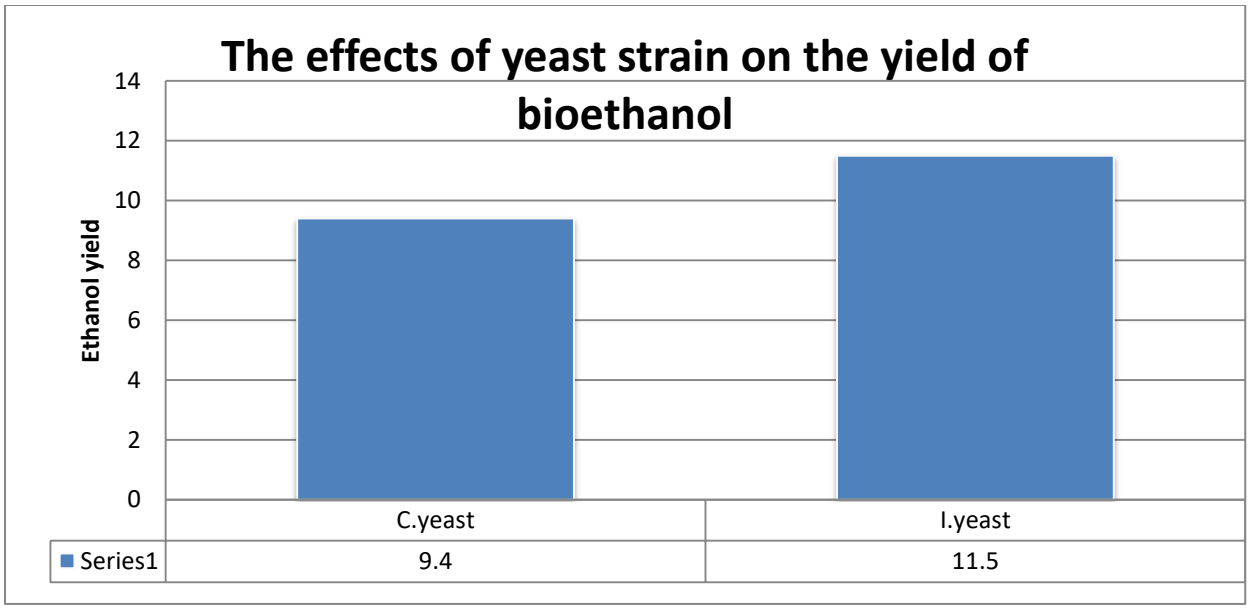


Figure 4.2.The effects of Commercial yeast and Isolated yeast on bio-ethanol yield

According to Figure 4.2 Isolated yeast yields 11.5%v/v of bio-ethanol and commercial yeast one produces 9.4% v/v of ethanol. There for 11.5%v/v of bio-ethanol was the optimum ethanol produced by isolated yeas from teff flour. Isolated yeast from teff flour processes more ethanol concentration than commercial yeast. In contrast to this study, Ballesteros obtained that 4.8% bioethanol by volume from wheat straw by using commercial yeast[87]. This implies that, the fermenting efficiency of locally isolated yeast was greater than the fermenting efficiency of commercial yeast. There for S.cerevisiae isolated from Teff flour was used for the production of bioethanol from wheat straw for fermentation process of all samples used in this research.

4.5.3 Double Distillation process for optimization of bioethanol yields

To obtain the maximum yields of bio-ethanol concentration, double distillation process was used. In this study to get maximum bio-ethanol concentration, simple distillation process and double distillation process were used to increase the yield. Temperature (85^0C) and time (3hrs) of distillation of wheat straw hydrolysate were constant in both simple and double distillation process.

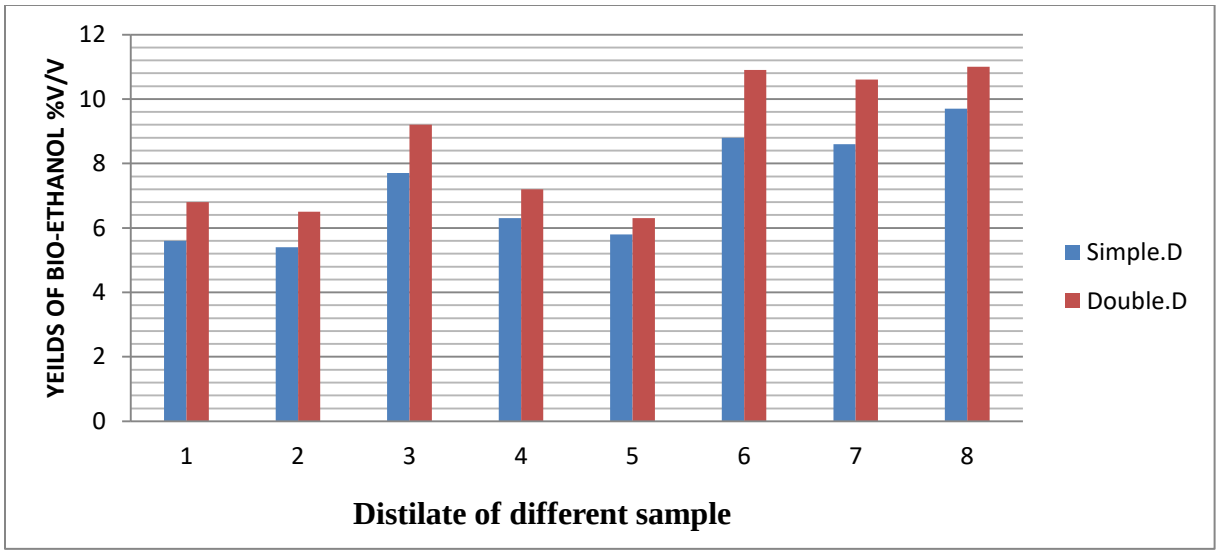


Figure 4.3 The effects of distillation type on the yields of bio-ethanol

From Figure4.3 as the concentration of bioethanol produced by simple distillation was increased from 5.4%v/v to 9.7%v/v, the concentration of bio-ethanol produced by double distillation from wheat straw also increased from 6.5%v/v to 11%v/v , this is because as the produced distillate by simple distillation was redistilled by double distillation process the amount of water contents of the distillate was decreased, and decreasing the amounts of water in the distillate increasing the concentration of bio-ethanol of the distillate. And the ethanol contents of each sample after double distillation were greater than that of simple distillation .

4.6 Fourier Transform Infrared spectroscopy (FTIR) for bioethanol Characterization

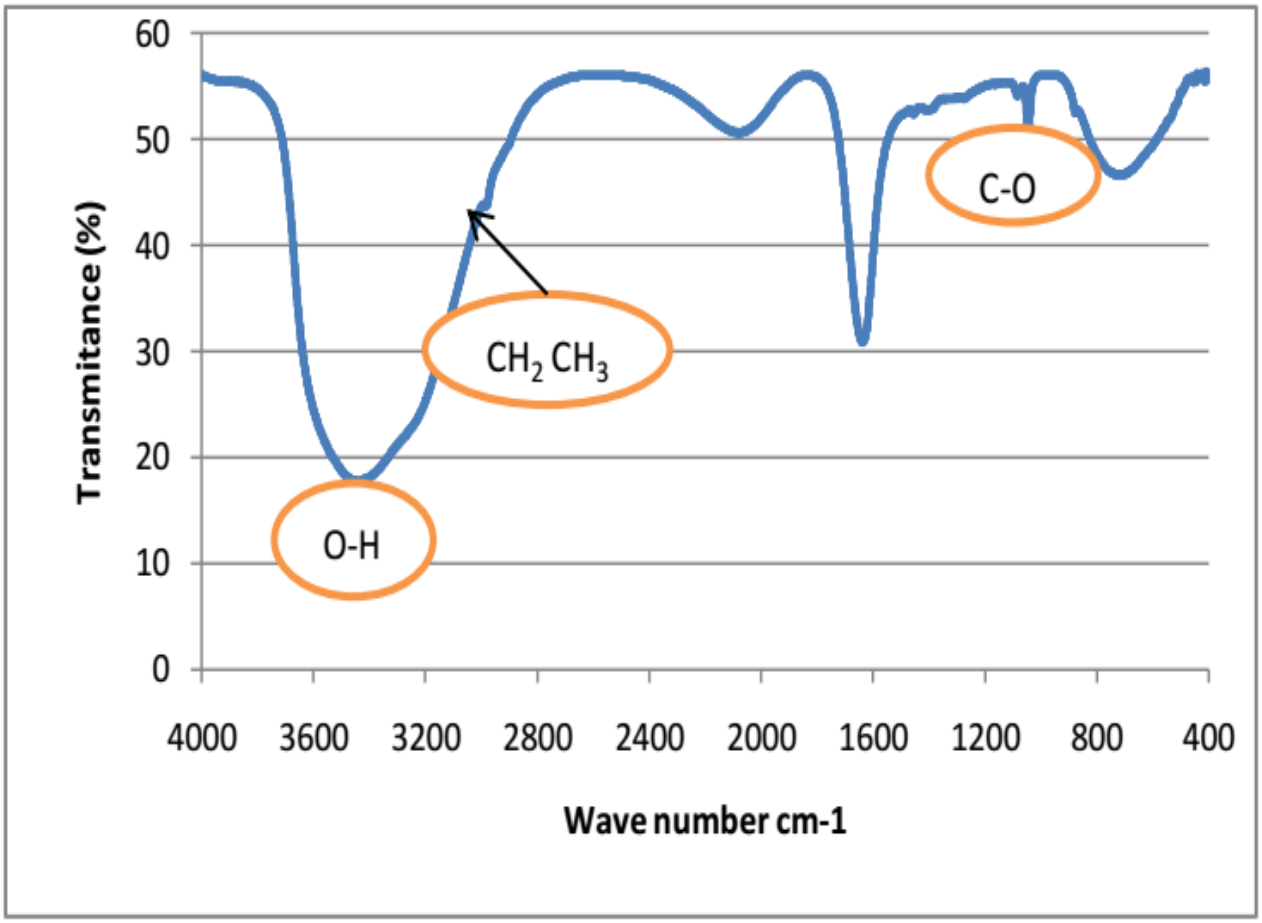


Figure 4.4. FTIR result 11%v/v of bio- ethanol yield at acid concentration of 5%, temperature of 210^{o}c, and for a hydrolysis of 60 minutes

Alcohols have characteristic IR absorptions associated with the O-H, C-O and the C-H stretching vibrations. When run as a liquid film the region 3500-3200 cm^{-1} with a very intense and broad band indicated the O-H stretch of alcohols, while the region 1260-1050 cm^{-1} confirms the C–O stretch. The bands at around 2880 and 2930 cm^{-1} were assigned as the symmetric stretching modes of the –CH_2 and–CH_3 groups, respectively [88]. This assures that the product obtained from wheat straw was ethanol due to the confirmation of these regions as shown in Figure 4.4.

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The study examines the potential of wheat straw for bio- ethanol production using locally prepared Yeast s.cerevesiae, isolated from teff flour for fermentation. From the result obtained, it can be concluded that the yield of ethanol decreases at very low hydrolysis acid concentration, weight of the sample and very low hydrolysis time. This indicated that determination of optimal conditions required to provide the maximum yield of fermentable sugars is the major factors in the treatment of lignocellulose. Hence, the optimum combinations of the three factors chosen for optimum ethanol yield were (70 g sample) 5% v/v H_2SO_4 (hydrolysis acid concentration) and 60 minutes (hydrolysis time).

The optimization study showed that the highest bio-ethanol concentration 11% v/v was observed by the use of isolated yeast than commercial yeast under the optimum conditions with 5%v/v of dilute H_2SO_4, 60 min hydrolysis time, at 72hrs of fermentation time and by using double distillation method. Production of bioethanol from wheat straw by using locally isolated yeast from teff flour was th e new discovery of this research.

Finally it can be concluded that wheat straw can be biomass source on a local basis for the production of bioethanol and may be included in sustainable energy development program of the country.

5.2 Recommendation

Further researches have to be carried out to increase the yield of bioethanol from wheat straw by use other microorganisms, which are capable of converting 5- and 6- carbon sugar into ethanol. Collaborate research and pilot-scale production should be encouraged by involving different stake holders so as to determine the feasibility of bio-ethanol production from wheat straw. Producing ethanol from renewable resources is becoming an important issue for the whole world. Therefore, the work needs to continue for scaling up of ethanol production from wheat straw. Further optimization of the Pretreatment, Fermentation and Distillation processes are recommended to maximize the yield of ethanol from wheat straw.

Alternative extraction methods of ethanol such as enzymatic extraction need to be done in order to investigate the variation that could arise on the quality and quantity of the ethanol yield because of using different extraction methods. Further investigation should be done to analyze the potential of bio-ethanol production from wheat straw using combination of yeasts, because xylose can't be converted by Saccharomyces cerevisae. An economic feasibility analysis of the overall conversion process is necessary for the purpose of commercialization. Finally, it is recommended that further researches should be done on bioethanol production from wheat straw.

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APPENDIX

Appendix A soxhlet extraction unit set up

Appendix B Reflux set up

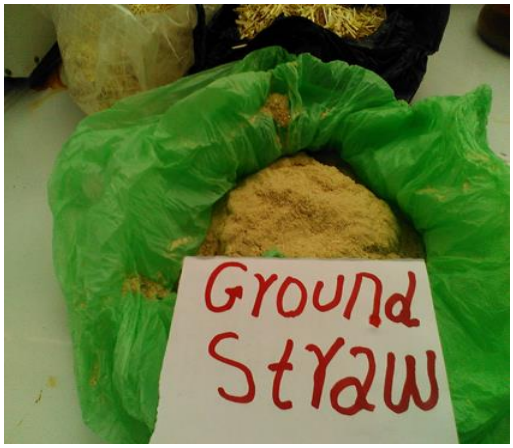
Appendix C Electric Muffle Furnace

Appendix D. Distillation set up

Appendix E.Autoclave

Appendix F.2 Teffe flour

Appendix F. Different Samples



Appendix F.1 cut wheat straw

Appendix F.2 ground wheat straw

Appendix F.3 caltured media

Appendix F.4 socked & hydrolysed sample

Appendix G. Ethanol yields of wheat straw under different hydrolysis conditions

Types of samples	Weight of the sample in(g)	Acid concentration (%)	Time(min)	Ethanol yield(%v)	
				BY simple distillation	BY double distillation
A	50	1	30	5.6	6.8
B	50	5	30	5.4	6.5
C	50	1	60	7.7	9.2
D	50	5	60	6.3	7.2
E	70	1	30	5.8	6.3
F	70	5	30	8.8	10.9
G	70	1	60	8.6	10.6
H	70	5	60	9.7	11

Appendix H Sample product which has been tested by ECAE

S/code	Characteristics tested result		Specification test method
	Relative density	Ethanol content % by volume	
I.Yeast	0.9823	11.5	ES318;2012 ES 804;2012
C.yeast	0.9872	9.4	ES 318;2012 ES 804;2012
A	0.9904	6.8	ES 318;2012 ES 804;2012
B	0.9907	6.5	ES 318;2012 ES 804;2012
C	0.9862	9.2	ES 318;2012 ES 804;2012
D	0.9895	7.2	ES 318;2012 ES 804;2012
E	0.9887	6.3	ES 318;2012 ES 804;2012
F	0.9847	10.9	ES 318;2012 ES 804;2012
G	0.9862	10.6	ES 318;2012 ES 804;2012
H	0.9852	11.0	ES 318;2012 ES 804;2012