WOLKITE UNIVERESTY

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DEPARTMENT OF CHEMICAL ENGINEERING

PROCESS STREAM

TITLE: OPTIMIZATION OF ACID HYDROLYSIS IN ETHANOL PRODUCTION FROM PROSOPIS JULIFLORA

A Final Year Thesis Project Proposal To Be Submitted to Wolkite University, College of Engineering and Technology Department of Chemical Engineering of Chemical Engineering In Partial Fulfillment For The Requirement For The Degree Of Bachelor Of Science In Chemical Engineering (Processes Engineering Stream

BY:

	ID/NO
1. DEFAR HASSEN	254/08
2. MINTESNOT GETU	624/08
3. MUKTAR ABAS	658/08
4. TSEGA DEREGA	902/08
5. WASIHUN MEGESTU	915/08

20/04/2013 E.C

DECLARATION

We hereby declare that the thesis is based on our original work except for flotation and citations which have been duly acknowledge. We also declare that the paper previously or currently has not been submitted for any other department at Wolkite University.

Prepared by:

Name:ID	No:	Signatures:
1. DEFAR HASSEN	254/08	…….
2. MINTESNOT GETU	624/08	……
3. MUKTAR ABAS	658/08	…….
4. TSEGA DEREGA	902/08	…….
5. WASIHUN MEGESTU	915/08	…….

This thesis has been submitted for examination with my approval as University advisor

Name: Mr. NEGESO B. Signature: _____

Examiner: _____ Signature_____

Chairperson: _____ Signature_____

Project Organizer _____ Signature_____

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Table of Contents

List of figure

List of table

List of Abbreviations

AFEX - Ammonia Fiber Explosion

C_2H_5OH- Ethanol

$(C_6H_{10}O_5)_n$ - Cellulose

$C_6H_{12}O_6$ - Glucose

DC - De Candolle

FT - Fischer-Tropsch

HMF - Hydroxymethyl furfural

IEA- International Energy Agency

LHW- Liquid hot water

MON - Motor octane number

MT - Metric ton

MTBE- Methyl-tertiary-butyl-ether

NREL- National Renewable Energy Laboratory

P.juliflora- Prosopis juliflora

PH - Power of Hydrogen

RON - Research octane number

ABSTRACT

This thesis employs optimization of acid hydrolysis to produce ethanol from *prosopis juliflora*. It was used dilute acid pretreatment, dilute acid hydrolysis, fermentation and distillation processes. The experiment has three factors (temperature, time and acid concentration) which have two level and a replicas (2^3*1=8 experimental runs) was run to optimize acid hydrolysis. Temperature varied from 100 ^{0}c to 115^{0}c, time varied from 5 to 15 min and acid concentration varied from 0.3 to 3%.The screening of significant acid hydrolysis factors were done by using the two-level full factorial design using design expert® 7 software. The statistical analysis showed that the ethanol yield of (40.91% (g/g)) was obtained at optimised acid hydrolysis variables of 0.3%v/v acid concentration, 100.01°C temperature, and 5.00 minutes hydrolysis time.

1. Introduction

1.1. Background

According to the research conducted by Lin and Tanaka, the demand of energy is currently exponentially exceeding the rate of local supply sources, a look beyond the fossils is crucial for long term economic growth and energy security purpose. The researchers had reported that, the volatile situations in the Middle East, where vast reservoirs are creating uncertainties about the availability of the supply. There is also the greater environmental risks associated with exploitation of crude oil (IEA world energy outlook, 2004). As a result of the concerns of sustainability, environmental protection, and national energy security, more and more countries have prioritized the importance of renewable energy sources. Ethanol has once again become attractive in the energy marketplace and, in fact, the demand for ethanol has been increasing in recent years (Lin and Tanaka, 2006; Ford, 2004).

Production of ethanol is historically a well known process and is carried out by fermentation of plant sugars into ethanol using strains of yeast. However, plant biomass is made of sugar polymers, ordered in a matrix called lignocelluloses, which is not as easily fermented. In order to produce ethanol, this material must undergo degradation for the yeast more accessible components for example mono and dimers of sugars. This degradation can be made by hydrolysis of biomass using enzymes called enzymatic hydrolysis (J. Caraballo, August 2005). As the work done by (Tanaka, 2006) approximately 80% of the ethanol produced in the world is still obtained from fermentation; the remainder comes largely by synthesis from the petroleum product ethylene.

One of the fermentation method for ethanol production is obtained from the plant species called *Prosopis juliflora.* It is a tree species native to most countries in the world and also available in hottest regions of Ethiopia. It survives droughts and thrives in sunny arid regions because of its multiple and important potential and actual uses, as well as of its remarkable resistance to

drought, heat, and poor soils. The plant fixes its own nitrogen, requires no seeding, fertilization or irrigation, and grows on dry, nutrient-poor soils (Hailu Shiferraw et al.,2004).

There are two primary routes for the production of cellulosic ethanol - biochemical and thermochemical routes. The biochemical route relies primarily on the use of enzymes and other microorganisms and the thermochemical route relies on the application of heat and chemical synthesis. The biochemical routes can be conducted by the process of acid hydrolysis by the adjustment of the required parameters such as: temperature, time and acid concentration as the method optimization for the production of ethanol from the stem of the *Prosopis juliflora* plant. Therefore, the present study was to optimize the acid hydrolysis process to obtain the highest yield of ethanol from *Prosopis juliflora* plant and to ferment the acid hydrolysis product for ethanol production.

1.2. Statement of the Problem

Ethiopia is currently looking at growing high-yielding crops for the production of bio-fuels as alternatives to traditional fuels (petrol and diesel) to address imminent shortages and reduce impacts of climate change. Owing to such phenomenon, and indeed in view of the recent trends in the escalating price of the traditional petro-fuel, biofuel has been gaining greater attention by the Ethiopian government. But due to the increased cost of food crops, producing ethanol using Prosopis juliflora wood is an alternative feed stock: for one thing, Prosopis juliflora is a fast growing tree species and grows in Ethiopia mainly in arid and semi-arid areas of the Rift Valley. And the other reason is it is a highly invasive exotic tree that is spreading in the pastoralist areas of Ethiopia making vast areas of land unavailable for grazing and it is becoming difficult to remove it.

Thirdly, when the plant is cut, new off springs is grown from the root in a short period (Hailu Shiferaw et al., 2004). Invasion of rangelands by Prosopis juliflora also caused shortage of grazing land for livestock, which resulted in drastic reduction of livestock number as well as product; thorns damage eyes and hooves of camels, donkeys, and cattle then by poisoning eventually lead to death (Senayit et al., 2004). Prosopis juliflora is invading potential croplands forcing local farmers with less capital and machinery to abandon their farmland and settlement

(Senayit et al., 2004). In general, this is a matter of serious concern for the life of the local people as pastoralists depending on livestock for their livelihood (Senayit et al., 2004). Due to the above reasons and as *Prosopis juliflora* is widely available in Ethiopia, we can use Prosopis juliflora as the raw materials for the production of ethanol.

1.3. Objectives

1.3.1 General Objectives

The main objective of this research work is to optimize the acid hydrolysis for the production of high yield ethanol from *Prosopis juliflora.*

1.3.2. Specific objective

The specific objectives are:

- To determine the optimum acid hydrolysis process variables using the parameters such as acid concentration, temperature and reaction time.
- To ferment acid hydrolysis products.

1.4. Significance of the Research

This study has significant in terms of assuring the production of an alternative form of energy from *prosopis juliflora*; which is locally available, increasing and no economic value. if consumption goes in this rate the fossil fuel reserve will be depleted completely within short period. In addition to this, continuous burning of fossil fuel increase emission of green house gasses to the atmosphere and causes global warming. So to sustain the fast depletion of fossil fuel reserves and to solve environmental concerns, the search of an alternative fuel is crucial, and one of the alternatives is ethanol. Therefore, a renewable and non-food competitive feedstock is desirable for the production of alternative fuel such as bioethanol. This study used *Prosopis juliflora* as a feed stock to produce ethanol. The use of ethanol in a conventional petrol engine can also greatly reduce emissions of unburned hydrocarbons, carbon dioxide, carbon monoxide, sulfates, polycyclic aromatic hydrocarbons, ozone-forming hydrocarbons, and

particulate matter by increasing oxygen number. In general, this study is important as *Prosopis juliflora* is a widely available plant and is an alternative feedstock for ethanol production and address problems related with energy security, promote rural development through job creation, promote environmental conservation and decrease greenhouse gas emission.

2. Literature Review

2.1. Biofuel Types

Biofuels, in conjunction to their positive carbon balance with regards to fossil fuels, also represent a significant potential for sustainability and economic growth of industrialized countries because they can be generated from locally available renewable material.

• Biofuels are usually classified as follows:

A. First-generation biofuels; are directly related to a biomass that is generally edible such as sugarcane or corn, are actually used.

B. Second-generation biofuels; are defined as fuels produced from a wide array of different feedstock, ranging from lignocellulosic feedstocks to municipal solid wastes.

C. Third-generation biofuels; are at this point, related to algal biomass but could to a certain extent be linked to utilization of CO_2 as feedstock. The most accepted definition for third-generation biofuels is fuels that would be produced from algal biomass, which has a very distinctive growth yield as compared with classical lignocellulosic biomass (Brennana and Owendea, 2010).

2.2. Ethanol as Fuel in History

Since the humanity exists, the biofuel are in use over the history. Man kind hadrelied on renewable energy resources like wood, windmills, water wheels andanimals such as horses and oxen. Exploration of new energy resources was a major driving force behind technological revolution. In the start of nineteenth century, alcohols were over and over again reported as biofuel with the invention of ignition engines using biofuel. Nikolaus August Otto used ethanol for his spark ignition engine in the 1860s. Henry Ford also marketed his Model T, totally operating on 100% ethanol (Kovarik1998). Ethanol production was widely abolished due to the unbeatably low price of gasoline in the USA. Ethanol as a fuel was revived in the 1970s in Brazil where one of the largest bioethanol industries is located today. Like modern crude oil refinery, the bio-industry for biofuel has a dual purpose in the economy, as it is used as a supply of energy as well as basic chemicals (Zaborsky 1982). The

upcoming “bio refinery” revitalizes the old tradition of a careful thrifty economy and intends to make use of all energy and carbon stored in biomass, feeding byproducts into secondary conversion process or refining them as fuel.

2.3. World economy of ethanol

The economics of biofuel is majorly determined by the value of the feedstock usedfor their production (Elbehri et al. 2013). For first-generation biofuel raw material price accounts approximately between 60 and 90% of the total production (Ho et al.2014; Tan et al. 2013). The price competitiveness of biofuel to petroleum counterparts varies between countries and with the feedstock used (Wesseler and Drabik 2016). The “factory gate” price of Brazilian ethanol remained lesser than the “refinery gate” price of gasoline in last decade (Onal and Nunez2014). Both Brazilian and US ethanol remains expensive than gasoline on an energy equivalent basis.

Sugarcane derived Brazilian ethanol is better competitive than US ethanol, but is still usually more expensive than gasoline. In case of biodiesel, it is more expensive than diesel, even though a liter of biodiesel provides around 14% less mileage than diesel. While the biogas is much completive due to unavailability of natural gas everywhere (Alam and Hasan2017). Brazilian ethanol production cost remains lower than for US corn or European wheat ethanol, due to use of sugarcane bagasse in boilers; to come across on site steam and power demand. Moreover the production of biogas and its utilization in electricity can further lower the production cost. Ethanol production cost from wheat grains can be lowered if the impact value of by-products is considered.

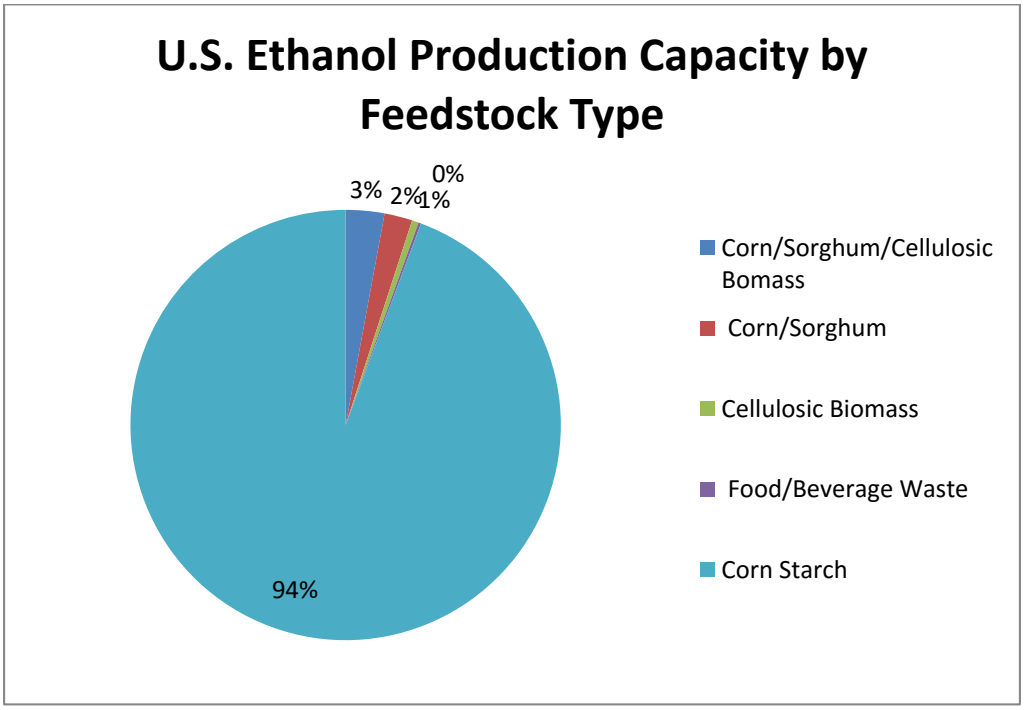


FIG.1 ethanol production capacity

Source: RFA

Likewise, biodiesel production cost will be fall, if main byproduct, glycerin can fetch a market value, which is utilized in the beverages, food and pharmaceuticals industries (Jonker et al. 2015; Losordo et al. 2016). To produce ethanol from sugarcane molasses is cheaper than from sugarcane itself (Castañeda-Ayarza and Table 1.3 The biofuels with possible production route, their use and the applications to engines (Antoni et al. 2007) Biofuel Process Status Engine application Biomethanol Thermochemical/microbial Pilot plant [pure/blend] MTBE/biodiesel Bioethanol Microbial Industrial Pure/blend Biobutanol Microbial Pilot plant/Industrial Pure/blend ETBE Chemical/Microbial Industrial blend Biomethane Microbial Industrial Pure/blend Biohydrogen Microbial Laboratory Pure/blend Pure biodiesel Physical/chemical (enzymatic) Industrial (laboratory) Pure/blend 1 An Overview of Biofuel 7 Cortez2016). Biodiesel from non-food seeds like jatropha largess an interesting, alternative if yields can be improved to commercial level sand if sufficient low-cost labour can be assembled for the highly labour intensive seed collection process (Carriquiry et al.2011). The capital costs of second-generation biofuel account for a higher portion, while the feedstock costs are significantly lower as compared to first-generation biofuel (van Eijck et al. 2014). Overall production costs from

micro-algae appear to be higher presently, but could fall in the future as well as technology improves and production expands (Kern et al. 2017). Biofuel, like fossil fuels, come in a number of forms and meet a number of different energy needs. The present book chapter is an introduction of the major globally used biofuel, biodiesel, bioethanol and biogas. Each type of the biofuel has been explained well.

2.4. Ethanol production and supply in Ethiopia

The worldwide recent awareness for the use of ethanol to replace petroleum and generation of power along with sugar mill plants should have led to setting up of number of ethanol plants and co-generations. Ethiopia has several sugar real estate (Fincha, Metehara and Wonji Shoa) industries which are run and administered by Sugar Development Agency. Among molasses derived products ethanol takes the largest part, but its utilization must attract the attention of the government policy makers in order to utilize as a bioethanol. Bioethanol or biofuel is ethanol based products that can process into liquid fuels for transport purposes (ESDA, 2005) as cited by Nigus Worku, 2010.

2.5. Raw materials of Ethanol

Almost any plant-based material can be an ethanol raw material. All plants contain sugars, and these sugars can be fermented to make ethanol in a process called biochemical conversion. Plant material also can be converted to ethanol using heat and chemicals in a process called thermo chemical conversion. Some plants are easier to process into ethanol than others. Some require few resources to grow, while others need intensive care. Some are used for food as well as fuel, while others are cultivated exclusively for ethanol; even plant-based wastes can become ethanol. Climate and soil type determine the types and amounts of plants that can be grown in different geographic areas. Another important consideration is feedstock logistic is the steps necessary to move feed stocks from fields or collection areas to ethanol production plants. For agricultural and forestry feed stocks,

these steps include harvesting, transportation, storage, and preprocessing (Cellulosic Ethanol, 2010) as cited by Nigus Worku, 2010.

2.5.1. Prosopis juliflora as a raw material of ethanol

This plant was described by De Candolle under the name of Prosopis juliflora. The specific name juliflora, comes from julus meaning whip-like; referring to the long inflorescence, and flora being flower (Havard, 1884).The genus Prosopis was systematically described and organized by Burkart (1976) in to five sections that together contains 44 species and with many varieties (Pasiecznik et al., 2001). Prosopis juliflora belonging to the family Leguminaceae (Fabaceae) and subfamily Mimosoideae, section Algarobia that has six series; specifically it belongs to the series Chilensis that contain eleven species and many varieties. *Prosopis juliflora* is particularly closely connected to *Prosopis pallida*. It is a tree or shrub sized woody perennial plant found mainly in the arid and semi arid regions of the world (Geezing et al., 2004).

Prosopis juliflora landraces often have multi-stemmed, coppiced and prostate shrub forms with long branches and a crown that even touches the ground and have erect, flat topped and decumbent tree forms. *Prosopis juliflora* produced coppices except those stumped at 10 cm below the ground (Hailu Shiferaw et al., 2004).

2.5.2. Prosopis juliflora in Ethiopia

Existing evidence suggests that Prosopis was introduced to Ethiopia in the early 1980s purposely for the sake of tackling the challenge of desertification in over grazed arid and semi-arid areas of Eastern Africa by some multi-national development agencies (Rettberg and Müller-mahn, 2012). Since then in terms of coverage, the areas' most adversely affected nationally include the Afar and Somali Regions in the east and southeast of the country and the area around Dire Dawa city. There are also moderately affected areas in Amhara, Oromia, Southern Nations Nationalities and Peoples (SNNP) and Tigray Regions that is, in the mainly dry lands of Central, East and North Ethiopia (Steele etal., 2009). Above all, the worst thing is its negative impacts on the ecosystem like forming impenetrable shrubby thickets, invading water courses, lowering the water-table and thus

indirectly starving plants of other species of moisture and nutrients, creating what are known as 'green deserts', largely devoid of life, instead of meeting the stated objective (Gordon and Arne, 2013).

The rapid expansion of Prosopis is considered as a major threat mainly for pastoralist livelihood in the environment due to its invasive nature. It can infest pasturelands, irrigated cultivated lands and irrigation canals, finally causing an irreversible displacement of natural pasture grasses as well as native tree species (Kassahun et al., 2004). Its negative effects include inhibiting the ecosystem alone by displacing beneficial native species; encroachment onto paths, villages, homes, water sources, crop- and pastureland; and injuries due to thorns that impacted animal and human health apparently resulting in some human fatalities (Mwangiand Swallow, 2008; Maundu et al., 2009).

2.6. Production Methods of Cellulosic Ethanol

The below process flow diagram (fig. 2.7) shows the basic steps in production of ethanol from cellulosic biomass (Zhu JY et al, 2009).

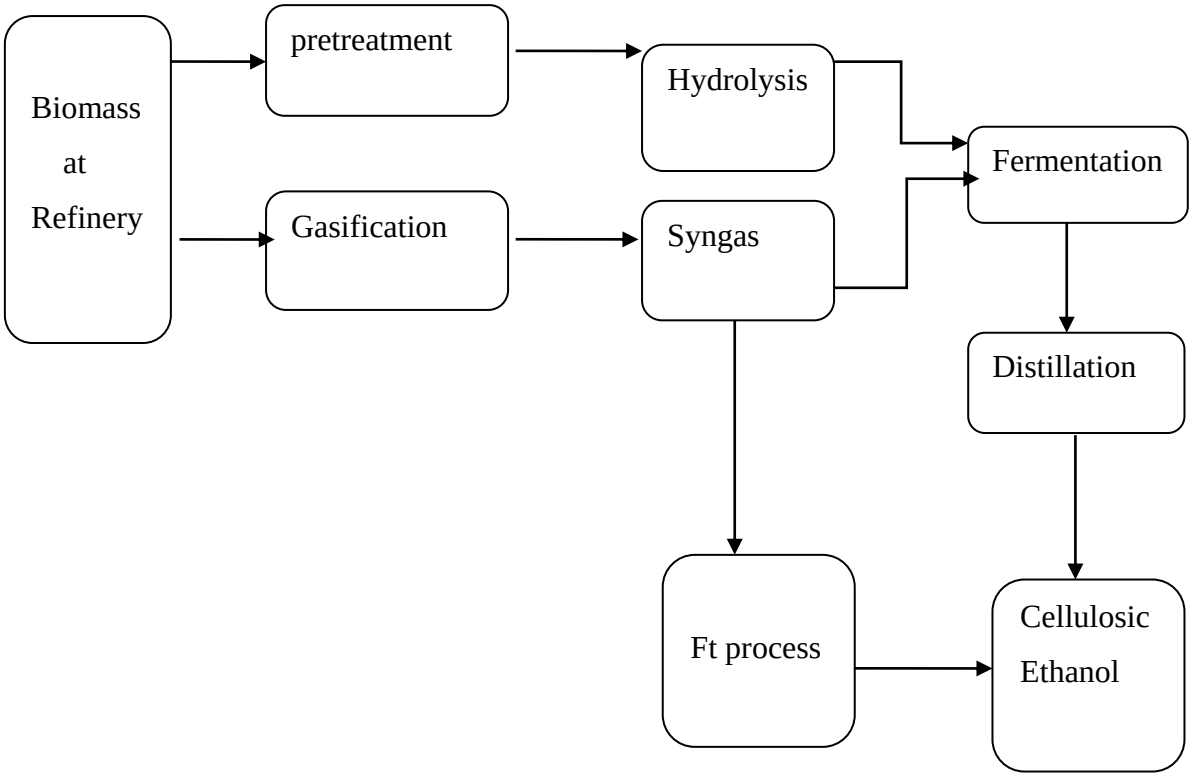


Figure 2.: Schematic Diagram of Ethanol productions from lignocellulosic feedstocks (Zhu JY et al., 2009).

2.6.1. Pretreatment

Pretreatment refers to the solubilization and separation of one or more of the four major components of biomass: hemicellulose, cellulose, lignin, and extractives to make the remaining solid biomass more accessible to further chemical or biological treatment. The effect of pretreatment of lignocellulosic materials has been recognized for a long time. The purpose of the pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, 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 enzymatic hydrolysis, avoid the degradation or loss of carbohydrate, avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes, and be cost effective (Graf A and Koehler T, 2000).

Pretreatment	Process Benefits	Drawbacks
Biological	Lignin and hemicellulose can be decomposed easily Least energy needed	Hydrolysis reaction is much sluggish
Milling	Crystal structure of cellulose can be relaxed	Additional power required
Steam explosion	Lignin can be converted to its component seasily Better release of glucose molecules	Discharge of toxic compounds Partial degradation of Hemicelluloses
CO_2explosion	Much surface area exposed Better in terms of cost No toxic compounds generation	High pressure needed
Wet oxidation	Better abstraction of lignin Reduced formation of inhibitors	High cost
Organosolv	Efficient decomposition of lignin and hemicellulose	Drainage can cause environmental issues

Diluted acid	Lesser corrosion issues as compared to concentrated acid	Byproducts are formed
Concentrated Acid	Much better glucose production	Acid recovery is essentia

Table 2.1 Advantages and disadvantages about different methods for pretreatment of lignocellulose materials (Harmsen et al. 2010).

2.6.1.1. Lignin removal, hemicellulose hydrolysis

In its function of making the cellulose feedstock more digestible by enzymes, this step is often classed as pre-treatment: the surrounding hemicellulose and/or lignin are removed, and the cellulose micro fibre structure is modified. By chemical, physical or biological treatment, lignin and all or part of the hemicellulose is solubilised. Subsequently, when water or steam is added, the free hemicellulose polymer is hydrolysed to monomeric and oligomeric sugars. The soluble sugar products are primarily xylose, and further mannose, arabinose, and galactose. A small portion of the cellulose may already be converted to glucose. However, the cellulose bulk will be converted in a separate step. The product is filtered and pressed, solids (cellulose + lignin) go to the cellulose hydrolysis, and liquids (containing the sugars) go to a fermenting step.

Chemical pretreatment methods: Common chemical pre-treatment methods use dilute acid, alkaline, ammonia, organic solvent, sulphur dioxide, carbon dioxide or other chemicals. We discuss the most important approaches: Acid catalyzed hydrolysis: Acid pretreatment involves the use of concentrated and diluted acids to break the rigid structure of the lignocellulosic material. The most commonly used acid is dilute sulphuric acid (H2SO4), which has been commercially used to pretreat a wide variety of biomass types switch grass , corn stover , spruce (softwood) , and poplar (B. Du et al., 2010).

2.6.2. Hydrolysis

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 (Mosier et al. 2005). 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 (Prasad et al., 2007).

Where lignin removal and hemicellulose hydrolysis are classed as pre-treatment, cellulose hydrolysis is abbreviated to hydrolysis: it is considered the major hydrolysis step. In hydrolysis, the cellulose is converted into glucose sugars ($(C_6H_{10}O_5)\ n + nH_2O \rightarrow n\ C_6H_{12}O_6$).This reaction is catalysed by dilute acid, concentrated acid, or enzymes (cellulase). Hydrolysis without preceding pre-treatment yields typically less than 20 %, whereas yields after pre-treatment often exceed 90 %. After pretreatment, there are two types of processes that convert the lignocellulosic material into its monomeric sugars, commonly known as acid (dilute or concentrated) and enzymatic hydrolysis. The process involves cleaving the cellulose and hemicellulose into their monomer. Complete hydrolysis results in glucose, whereas the complete hydrolysis of the hemicellulose gives rise to pentoses and hexoses (Galbe and Zacchi, 2002).

Acid hydrolysis

Acid hydrolysis can be performed with various types of acids including sulfuric, sulfurous, hydrochloric, phosphoric, nitric acid, etc. Acid hydrolysis is subdivided into concentrated and dilute acid hydrolysis. Acids have been used to catalyze (speed up) the hydrolysis of starch in "starch cookers" operating at temperatures of 50 to 150°C, a process referred to as acid hydrolysis. Acid pretreatment for ethanol production was developed in Germany in the 19th century. In the United States, the U.S. Forest Service, Forest Products Laboratory (FPL) conducted extensive research using acid pretreatment for ethanol production from wood (Zhu et al., 2008)

Concentrated acid: process has a very high sugar yield (90 %), can handle diverse feedstock, is relatively rapid (10-12 h in total), and gives little degradation. Critical for the economical viability of this process is to minimize the amount of acid, by cost effectively separating the acid for recycling. Early (1948) membrane separation already achieved 80% acid recovery. Continuous ion exchange now recovers over 97% of the acid; 2% of the sugar is lost. Furthermore the required equipment is more expensive than for dilute acid (Graf A and Koehler T, 2000; US DOE, 2003).

Dilute acid: Dilute acid hydrolysis with 1% to 5% sulfuric acid is generally considered the most

cost-effective means of hydrolyzing wood and agricultural residues.Yields of hemicellulosic

sugars can be 80% to 95% of theoretical. Yields of glucose from cellulose are generally less than 50% but can approach 55% at elevated temperatures (Burden, 2008). Most dilute acid processes are limited to a sugar recovery efficiency of around 60 to 75%.The reason for this is that at least two reactions are part of this process. 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. Thus, once the molecules are broken apart, the reaction proceeds rapidly to break down the sugars into other products most notably furfural, a chemical used in the plastics industry. Not only does sugar degradation reduce sugar yield, but the furfural and other degradation products can be poisonous to the fermentation microorganisms (Nigus Worku, 2011). (Mohammad, 2008) the optimum results of ethanol yield from bagasse were obtained at a temperature of 102^{o}C, 1.5 % sulfuric acid concentration, and 18.2 min retention time.

The biggest advantage of dilute acid processes is their fast rate of reaction, which facilitates continuous processing. The biggest disadvantage is their low sugar yield. For rapid continuous processes, in order to allow adequate acid penetration, feedstocks must also be reduced in size so that the maximum particle dimension is in the range of a few millimeters. 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 process conditions to recover the 5-carbon sugars while the second stage is conducted under harsher conditions to recover the 6-carbon sugars. Unfortunately, sugar degradation is still a problem and yields are limited. The most important advantage of dilute acid processes is their fast rate of reaction, which facilitates continuous processing. Their biggest disadvantage is the low sugar yield. For rapid continuous processes, in order to allow adequate acid penetration, feedstocks must also be reduced in size so that the maximum particle dimension is in the range of a few millimeters (Cellulosic Ethanol, 2010).

Enzymatic hydrolysis: In the South Pacific during World War II, a fungus broke down cotton clothing and tents. This fungus, Trichoderma Reesei, in fact produced cellulase enzymes, which hydrolyses cellulose (US DOE, 2003). The first application of these enzymes for wood hydrol ysis in an ethanol process was to simply replace the cellulose acid hydrolysis step

with a cellulase enzyme hydrolysis step. This has several advantages: the very mild process conditions give potentially high yields, and the maintenance costs are low compared to acid or alkaline hydrolysis (no corrosion problem). The process is compatible with many pretreatment options, although purely physical methods are typically not adequate (Sun Y and Cheng J, 2002).Many experts see enzymatic hydrolysis as key to cost effective ethanol production in the long run (US DOE, 2003). Although acid processes are technically more mature, enzymatic processes have comparable projected costs and the potential of cost reductions as technology improves (Lynd LR, 1996). Hydrolysis is negatively influenced by structural features such as crystallinity, degree of cellulose polymerisation, and lignin content, and positively by surface area (Sun Y and Cheng J, 2002). A low substrate concentration gives low yield and rate, and a high cellulase dosage may increase the costs disproportional. However, the substrate/enzyme ratio should not be too high (inhibition). Hydrolysis can be enhanced by adding certain surfactants (to facilitate desorption of cellulase after reaction), by using mixes of cellulase from different organisms, and by adding other enzymes (e.g.
pectinase). Nearly complete saccharification of steam exploded chips is possible (Sun Y and Cheng J, 2002).

To improve the yield and rate of the enzymatic hydrolysis, research focuses both on enhancing enzyme activity in distinctive hydrolysis and fermentation process steps (Sun Y and Cheng J, 2002) as well as combining the different steps in less reactors. Intermediate and end products of the hydrolysis, cellobiose and glucose, inhibit the cellulase activity. This can be avoided by supplying extra enzymes during the reaction, or by taking away the product by ultrafiltration or by simultaneous fermentation in the same reactor. Enzymes can be recovered and recycled, so that the enzyme concentration can be higher against lower enzyme cost, although the enzyme quality decreases gradually (Sun Y and Cheng J, 2002).

2.6.3. Fermentation

A variety of micro-organisms generally bacteria, yeast, or fungi, ferment carbohydrates to ethanol under oxygen free conditions (Lynd LR, 1996). They do so to obtain energy and

to grow. According to the reactions, the theoretical maximum yield is 0.51 kg ethanol and 0.49 kg carbon dioxide per kg sugar:

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO*$$

Methods for C6 sugar fermentation were already known (at least) 6,000 years ago, when Sumerians, Babylonians and Egyptians began to perfect and describe the process of making beer from grain (starch). After it became possible to free the C6 sugars in lignocellulosic crops (end 19The century), conversion of the C5 sugars became interesting. They represent a high percentage of theavailable sugars, the ability to recover and ferment them into ethanol is important for the efficiency and economics of the process. Only in the 1980s research on xylose fermentation began to bear fruit, when a number of wild type yeast were identified that could convert xylose to ethanol (US DOE, 2003). Bacteria have drawn special attention from researchers because of their speed of fermentation. In general, bacteria can ferment in minutes as compared to hours for yeast. All micro-organisms have limitations: either in the inability to process pentoses and hexoses, the low yields of ethanol, or the coproduction of cell mass at the cost of ethanol. Furthermore, the oxygen free condition of fermentation slowly exterminates the microorganism population (Lynd LR, 1996). Therefore, in early processes, the different sugars were fermented in different sequential reactors. There is a tendency towards combining reaction steps in fewer reactors. When hydrolysis and fermentation reactions are connected directly, intermediate inhibitive products are avoided, and the yield is potentially higher. Also, genetic engineering and new screening technologies will bring bacteria and yeast that are capable of fermenting both glucose and xylose, although fermentation of xylose and arabinose remains problematic (US DOE, 2003).

Near-term fermentation using genetically engineered yeast or bacteria may even utilize all five of the major biomass sugars glucose, xylose, mannose, galactose and arabinose. Mid to long term technology will improve the fermentation efficiency of the organism (yielding more ethanol in less time), as well as its resistance, requiring less detoxification of the hydrolysate (Wooley R et al, 1999; Graf A and Koehler T, 2000). The fermenting bacteria and yeast are grown in series of aerated seed reactors. These consume a side streamed carbohydrate fraction (9 % of the cleaned hydrolysate, and some protein nutrients (Wooley R et al., 1999).Yeast are facultative unicellular microorganisms under which is the Saccharomyces cerevisiae falls, which is

considered the most important in ethanol production due to its rapid fermentation, high yields, and uniformity. The Saccharomyces cerevisiae strains are the most commonly used ethanologens for starch and cellulosic biomass sugars and have several advantages for bioethanol production including the efficient fermentation of glucose to ethanol with essentially no side products, superior ethanol tolerance, and rapid fermentation rates under acidic conditions. In an aerobic setting, glucose is fermented to produce yeast cell mass, while anaerobic conditions are used to ferment glucose to ethanol (Graf A and Koehler T, 2000). Most fermenting yeasts have an optimal temperature around 30-35^{o}C while hydrolyzing enzymes show optimal activities around 50 ^{o}C (Kadar et al., 2004). The National Renewable Energy Laboratory (NREL) recently developed an advanced strain of its patented Zymomonas mobilis bacterium that could lead to more efficient fermentation of cellulosic materials. The bacterium is able to ferment both five and six carbon sugars simultaneously, expanding the amount of biomass material that can be converted into ethanol by up to 40 percent (Graf A and Koehler T, 2000).

2.6.4. Distillation

Distillation is one of the steps of the purifications. 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. This means that proportion between two materials, in this case ethanol and water can be changed, still, there are two materials in both layers, the liquid and the vapor layers (Onuki, 2005) (as cited by Nigus worku ,2011).Whatever method of preparation is used, the ethanol is initially obtained in admixture with water. The ethanol is then extracted from this solution by fractional distillation. Although the boiling point of ethanol, 78.3^{0}C, is significantly lower than the boiling point of water, 100^{0}C, these materials cannot be separated completely by distillation. Instead, an azeotropic mixture (i.e. a mixture of 95% ethanol and 5% water) is obtained, and the boiling point of the azeotrope is 78.15^{0}C. 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 vapour has exactly the same composition as the liquid; towards distillation, then, 95% alcohol behaves exactly like a pure compound (Jackman, 1987). Since the ethanol contains 95% alcohol it is a binary azeotrope. Azeotropes having boiling points higher than those of their components are known as maximum boiling mixtures (Jackman, 1987).

3. Materials and Methods

3.1. Time and Place of the Study

The study was conducted from December to January 2020 at Wolkite university College of Engineering and technology chemical engineering department.

3.2. Materials

The materials used to run all experiments are listed below:

Chemicals

- Sodium Hydroxide (NaOH, min. assay 98% BDH Chemicals Ltd Poole England)- used to adjust the PH of pretreatment (soluble hemicellulose and/or cellulose) and hydrolysis (soluble cellulose) sugar solution mixture.
- Sulphuric Acid (H_2SO_4, (98%, England)) - used to pretreat and hydrolysis prosopis juliflora and as a reagent to determine whether the mixtures of pretreatment and hydrolysis products are sugars or not.
- Dextrose sugar - used as a nutrient in the media preparation
- Urea - used as a nutrient in the media preparation
- $MgSO_4.7\ H_2O$ -used as a nutrient in the media preparation
- yeast (Saccharomyces cerevisiae) (manufactured in France by S.I. Lesaffre with the strainsaf-instant‟)

Equipments

- Pycnometer - for density measurement
- pH-Meter -to adjust the pH of the hydrolyzates before fermentation
- Shaking incubator Vertical Autoclave – for hydrolysis and sterilization of equipments
- Cutting mill
- Autoclavable bio Reactor
- Shaker
- Ovens- Loading model 100 -800, Beschikung
- Funnel
- Sieves (mesh size of 2.0 mm, Sortmks-3332, PFEUFFR, Germany)

- Digital balances (model = Sartorius with 0.01 mg sensitivity, and model = EP214C)
- Vacuum Filter (model = BN 3 STAATLICH, Berlin)
- Rotary Evaporator (model = D79219, Staufen, Germany)

3.3. Methods

3.3.1. Prosopis juliflora Sample collection area

Prosopis juliflora sample was collected from the town of Dire Dawa , Eastern region of Ethiopia. The land has an elevation of 650 meters above sea level; mean temperature of 29^{o}C and annual mean rainfall varies from 250-500 mm.

Prosopis juliflora shrub was collected at their vegetative stage. Since the plant has two type appearance: the tree types of Prosopis are more than 2 meter in which they were used for production of charcoal in some Afar, Dire Dawa, Somalia region and they are not suitable for harvest. On the other hand, the shrub type of this plant has 0.5 meter to a maximum 1.79 meter height and much branched which makes it a lot easier to harvest.5 kilogram 100% wood of the vegetative stage shrubs are selected. These samples were taken from the targeted place with a random collection with a plastic bags. The plants were cut with a handsaw.

3.3.2. Sample Preparation

Sample preparation process include: manual size reduction (Knife cutting), drying, grinding and sieving. Grinding of Prosopis juliflora into powder form gives the surface area of the sample increased which enhance the contact between hemicellulose and cellulose with dilute acid to reduce cellulose crystallinity.

Steps involved in sample preparation were:

- After collection, Prosopis juliflora originally had 36 percent moisture, but it was air-dried by spreading on the laboratory floor to approximately less than 8 percent moisture prior to storage to prevent spoilage
- About 5 kg of Prosopis Juliflora (100 % wood) was taken and cut by knife into pieces of about 2-3 cm length for easily drying and grinding.

- Sample was dried using oven to remove all the moisture present in it and to get easily crushable material.
- After drying, the samples were grinded in cutting mill into 2mm size.
- Then samples greater than 2 mm were separated using sieve analysis and ground until all
- sample size became 2mm or less.
- The sample was then kept at low temperature until the next stage of experiment.

3.3.3. Pretreatment of Prosopis Juliflora

Acid pretreatment involves the use of concentrated and diluted acids to break the rigid structure of the lignocellulosic material. The most commonly used acid is dilute sulphuric acid (H_2SO_4), which has been commercially used to pretreat a wide variety of biomass types switch grass , corn stover , spruce (softwood) , and poplar (B. Du et al., 2010). In this study dilute sulfuric acid pretreatment method with 1.2% concentration was used. The powder Prosopis juliflora was pretreated inside autoclave and heated at temperature of 135^{o}C for 30 minutes. Prosopis juliflora powder was fed as batches and every batch contains 100g of screened Prosopis juliflora powder with a ratio of 10:1(v/w) water to the sample. In sample pretreatment for all batches acid concentration of 1.2%, temperatures of 135^{o}C and retention time of 30 minutes were used.

Steps involved in dilute acid Pretreatment were:

- 100g of grinded Prosopis juliflora sample was added in to three numbers of 250ml conical flasks.
- 750ml of 1.2 % dilute sulfuric acid concentration was added to the sample (750ml to each flask).Then the conical flasks capped with the help of rubber plugs.
- All the samples were heated to 135^{o}C pretreatment temperature for 30 minutes of pretreatment time in a vertical auto clave.
- Then the samples in the autoclave were removed and cooled after the given pretreatment time and pretreatment temperature.
- The soluble portion was separated from the non soluble portion by filtration.
- The soluble portion was put in another conical flask and the non soluble part was taken in the next step for hydrolysis.

3.3.4. Hydrolysis

The cellulose molecules composed of long chains are broken down to "free" the sugar, before it is fermented for alcohol production. Though hydrolysis is of many types, dilute acid hydrolysis is an easy and productive process. Also the amount of alcohol produced in case of acid hydrolysis is more than that of alkaline hydrolysis. Concentrated acid hydrolysis is not used as it is a hazardous and corrosive process and also acid has to be separated out after hydrolysis for the experiment has to be feasible (Nigus Worku, 2011).

The 2 level full factorial experimental design method using Design expert®7 software was chosen to optimize acid hydrolysis in ethanol production from Prosopis juliflora and to determine the effect of four operating variables of the acid hydrolysis, including acid concentration, temperature (T), and time, at aconstant solid fraction and a level of two, with one replica (2^3*1 =8experiment) and one response variables which were yield of ethanol. Selection of the factors and range of the variables were based on the operating condition, which has a significant influence on the acid hydrolysis process according to previous works (Grohmann et al. 1995; Vaccarino et al. 1989b), with 0- 3.5%(v/v) acid concentration, 5%(w/w) solid concentration,100-200 °C temperature, and 5-15 minutes time.

No. Factor	Variables	Units	Low level (-)	High level (+)
1	Acid concentration	% v/v	0.3	3.5
2	Temperature	^{0}C	100	115
3	Time Minutes	Min	5	15

Table3. Maximum and minimum values of variables of acid hydrolysis in ethanol production from Prosopis juliflora

Exper. Run	Codified levels of variables			Non codified levels of variables		
	X_1	X_2	X_3	Acid con. (%)	T(°C)x	Time(min)
1	-1	-1	+1	0.3	100	15
2	+1	-1	+1	3	100	15
3	-1	+1	-1	0.3	115	5
4	-1	+1	+1	0.3	115	15
5	+1	+1	+1	3	115	15

6	+1	+1	-1	3	115	5
7	+1	-1	-1	3	100	5
8	-1	-1	-1	0.3	100	5

Table 3.1: Codified (x_1 ,x_2 and x_3) and Respective no codified Levels (Acid%, T, and Time)

in experimental design for optimization of dilute acid hydrolysis of the Prosopis juliflora by two level full factorial experimental design method,

Steps involved in dilute acid hydrolysis were:

- Three numbers of 250ml volume measuring conical flasks were taken and with a solid fraction of 5% w/w 150ml of dilute sulfuric acid concentration of 0.3% v/v and 3% v/v was added to the non soluble portion of pretreated Prosopis juliflora according to the experimental design for acid hydrolysis shown in table 3.2.
- In all experiments all the samples were left to be soaked to dilute sulfuric acid concentrations of 0.3 % v/v and 3% v/v for 24 hrs.
- The bottles were then capped with the help of rubber plugs.
- Then the samples were put in a vertical autoclave for hydrolysis temperature of (100and 115^{O}C) and time of (5 and 15 minutes) according to the design of the experiments for dilute acid hydrolysis in ethanol production from Prosopis juliflora wood.
- Then all samples were removed from auto clave and allowed to cool.
- The soluble portion (cellulose) was separated from the non soluble portion (lignin) using filter media (vaccum filter).

- Finally the soluble portions of hydrolysis and pretreatment steps were mixed and taken to the next step.

3.3.5. pH adjustment

Before addition of any micro-organism to the above prepared samples, pH of these samples has to be adjusted. Otherwise the micro-organism will die in hyper acidic or basic state. A pH of around 5-5.5 is maintained.

Steps involved in pH adjustment were:

- All 250 ml flask containing mixture of 0.3% v/v and 2 % v/v dilute sulfuric acid hydrolyzed Prosopis Juliflora and pretreated solution were taken and their pH were checked with the help of a pH meter.
- The pH was 1.7 for mixture of 0.3% acid concentration hydrolysis and pretreatment sugar solution and 0.8 for mixture of 3% acid concentration and pretreatment sugar solution.
- As samples are acid hydrolyzed, a highly basic solution was added to bring the pH in the range of 5-5.5.
- For this purpose, a highly concentrated NaOH solution was prepared by mixing water with Na pellets.
- This NaOH solution was added drop wise to all beaker containing mixture of 0.3% v/v and 2 % v/v acid concentration hydrolysis and pretreatment sugar solution with constant stirring until the pH reaches 5.2.
- If suppose the pH goes beyond 5.5, concentrated H_2SO_4 acid was added drop wise to maintain the pH in the range.
- The above steps were repeated for all the experiments.

3.3.6. Fermentation

Microorganism: All fermentations were carried out using yeast (Saccharomyces cerevisiae) manufactured in France by S.I. Lesaffre with the strain „saf-instant“) in an anaerobic condition.

Fermentation Medium: One liter of production medium was prepared according to the requirements of Saccharomyces cerevisiae, containing 100 gm dextrose, 2gm dry yeast extract, 10 gm Urea, 1gm $MgSO_4.7\ H_2O$ and 1000 ml make up distilled water. It was added in all experimental sample for fermentation process of soluble hydrolysates for a distillation.

3.3.7. Distillation

Distillation is the method used to separate two liquids based on their different boiling points. However, to achieve high purification, several distillations are required. In this study separation are made by rotary evaporator at a temperature of 85 ^oC.

3.4. Data Analysis

The resulting data for optimization of acid hydrolysis in ethanol production from prosopis juliflora and the effect of four operating variables of acid hydrolysis were analyzed using Design expert® 7 software. Significance of the result was set from analysis of variance (ANOVA).

4. RESULTS AND DISCUSSION

In this section, the results of the experiment carried out on P. juliflora plant wood for ethanol potential through acid hydrolysis in different parameters such as: the effect of acid concentration, temperature, time on the ethanol yield and their relationship with ethanol product was investigated and discussed here under.

4.1. Statistical Analysis of the Experimental Results

In this study, the experimental design techniques were used to determine the effects of acid concentration, temperature and reaction time on acid hydrolysis in ethanol production from prosopis juliflora. A total of 8 experiments were conducted for optimization of acid hydrolysis purpose and the effect of each factor was analyzed by taking minimum and maximum values from operating conditions which has significant influence on acid hydrolysis taking the methods of the work done by (Grohmman et al. 1995; Vaccarino et al.1989b) and modifying some parameters. The ethanol yields obtained from experiments were used as a response parameter for optimization and Table 4.1 shows experimental results of each run.

Run no.	Acid conc. (% v/v)	Temp. (^oC)	Time (minute)	Volume (cm^3)	Density (g/cm^3)	Concentr. (Volume %)	Yield (%)
1	0.3	100	15	38.7	0.87	70.00	39.82
2	3	100	15	37.9	0.88	64.03	45.86
3	0.3	115	5	39.9	0.86	72.3	42.74
4	0.3	115	15	40.3	0.90	56.00	38.98
5	3	115	15	40	0.85	69.6	44.86
6	3	115	5	36.5	0.89	66.00	43.21
7	3	100	5	40.2	0.86	70.00	46.25
8	0.3	100	5	39	0.91	50.00	39.85

Table 4-1 above shows that run number 7,2,5,and 6 gives high yield of ethanol in decreasing order.

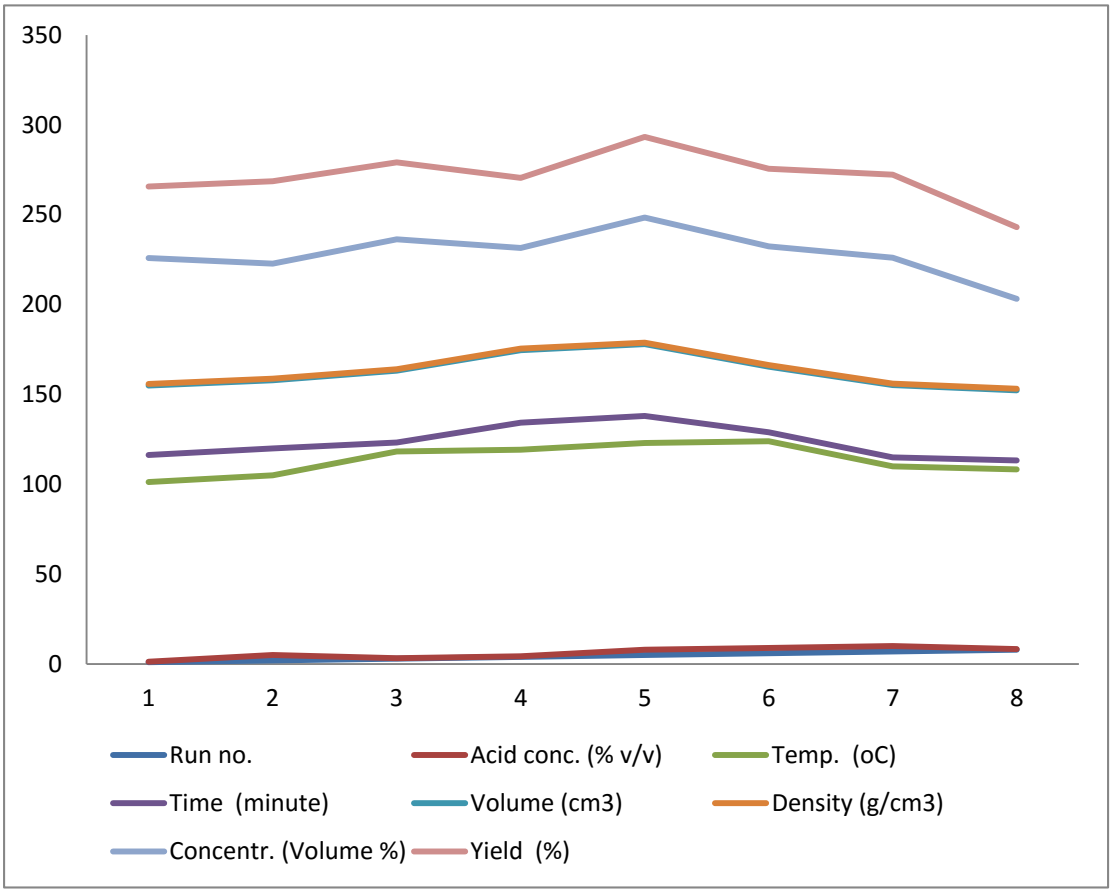


Figure 4.1Ethanol yields and factors of acid hydrolysis in ethanol production

The resulting data, Table 4.1, were analyzed using exile to determine the effects of acid concentration, temperature and time. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

4.2 Interaction effects

Acid hydrolysis is influenced by different factors and the ethanol yield has a complex relationship with independent variables that contain first, second and third-order polynomials and may have more than one maximum point. The best way of expressing the effect of any parameter on the yield within the experimental space under investigation was to generate response surface plots of the equation. The three dimensional response surfaces, contours and interactions were plotted in figures (4.6), (4.7), (4.8), (4.9) and (4.10) as a

function of the interactions of any two of the factors by holding the other two at average value. In the interaction plots the black line represents low level of variables and the red line represents high level of variables.

Design-Expert® Software

Yield

46.25

38

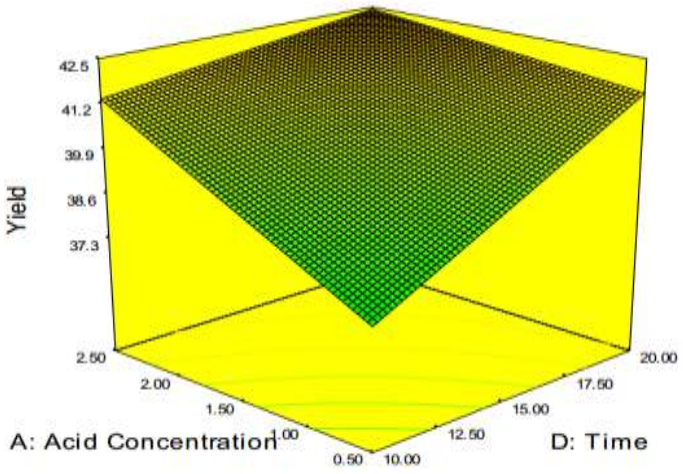
X1 = D: Time

X2 = A: Acid Concent.

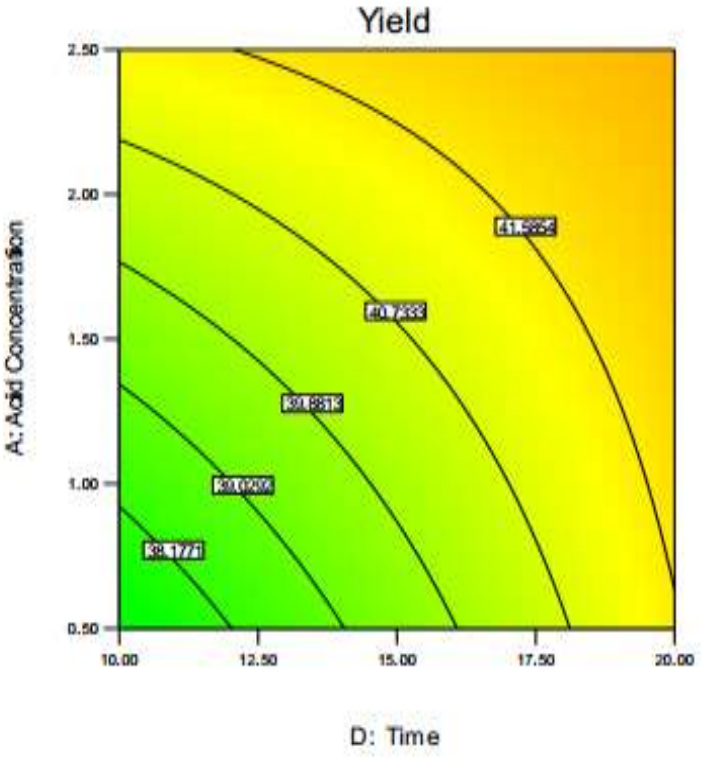
Actual Factors

B: Solid Fraction = 7.50

C: Temperature = 115.00



(a)



(b)

Design-Expert® Software

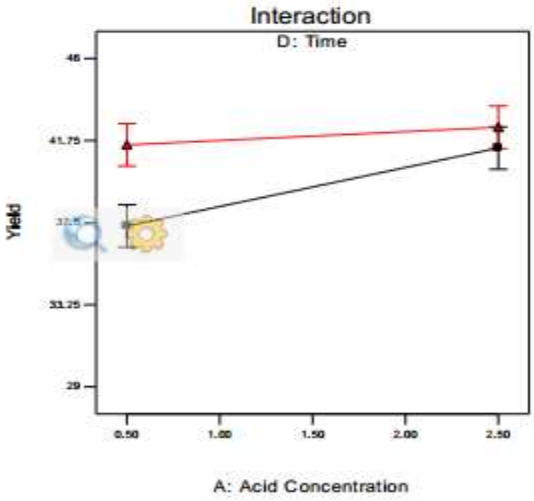
X1 = D: Time

X2 = A: Acid Concent.

Actual Factors

B: Solid Fraction = 7.50

C: Temperature = 115.00



(c)

Design-Expert® Software

Yield

D- 10.000

D+ 20.000

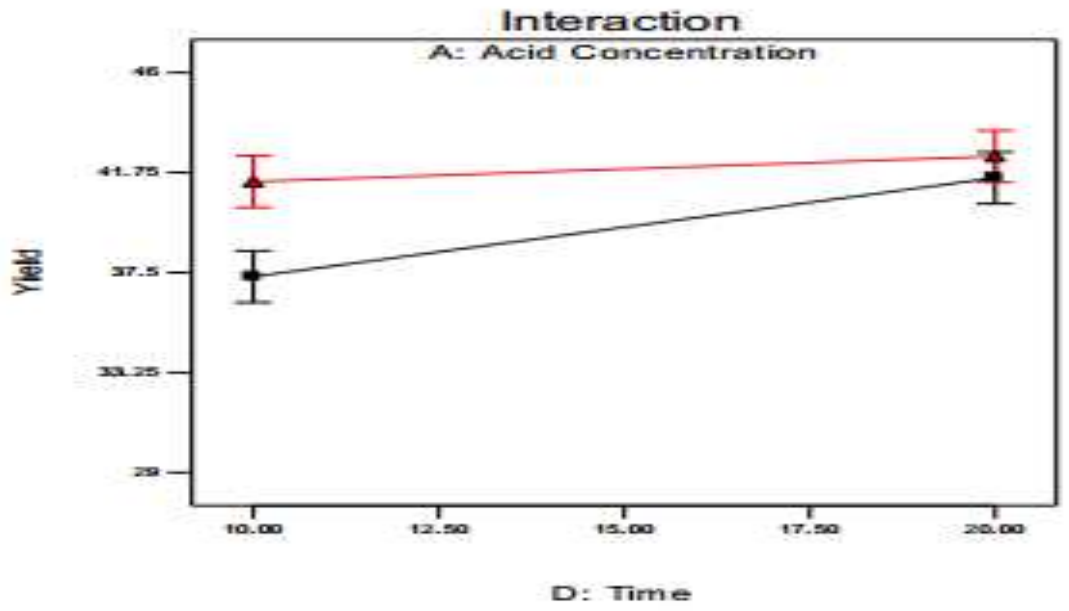
X1 = A: Acid Concent.

X2 = D: Time

Actual Factors

B: Solid Fraction = 7.50

C: Temperature = 115.0



(D)

Design-Expert® Software

Yield

A- 0.500

A+ 2.500

X1 = D: Time

X2 = A: Acid Concent.

Actual Factors

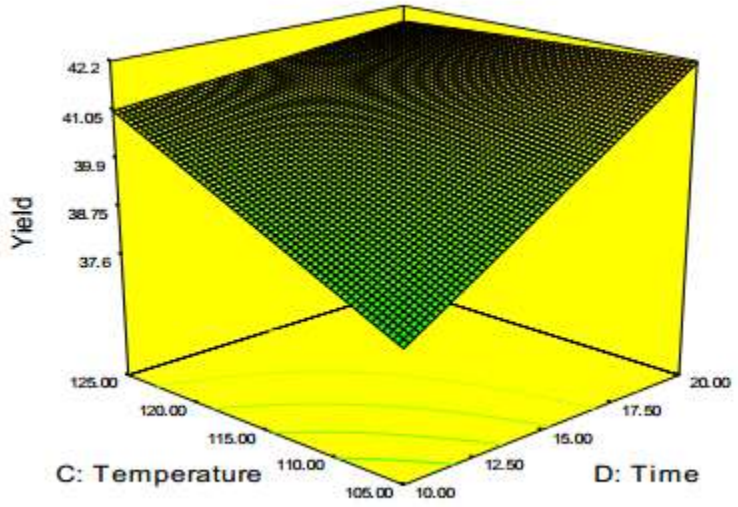
B: Solid Fraction = 7.50

C: Temperature = 115.00

Figure 4.2 Response surface plot (a), contour plot (b) and interaction plot (c)and (d) of ethanol yield as a function of acid concentration and time

The response surface, fig. 4.2(a), obtained from acid concentration and time is slightly flat and sloppy which has one up warded edge at one end suggesting that there is well defined optimum operating conditions and the surface is symmetrical. Moreover, the surface is somewhat flat near the optimum which meant that the response optimized value based on combined effects acid concentration and time may not vary widely from the single variable optimized

conditions.The contour plot, fig. 4.2(b), shows that the interaction between the acid concentration and time is strong on the yield of ethanol. As both acid concentration and time increase, the yield of ethanol increases.The interaction of acid concentration and time on the yield of ethanol is shown in figs. 4.3(c) and (d). Fig 4.3(c) shows at high level of time increasing acid concentration resulted a slight increase in the yield of ethanol ,while at low reaction time increase in acid concentration results i n sharp increase in the yield of ethanol. Similarly fig.4.3 (d) shows that, at high acid concentration increase in reaction time resulted in a slight increase in the yield of ethanol and at low acid concentration increase in reaction time results in sharp increase in the yield of ethanol.



Design-Expert® Software

Yield

45.2

29.7

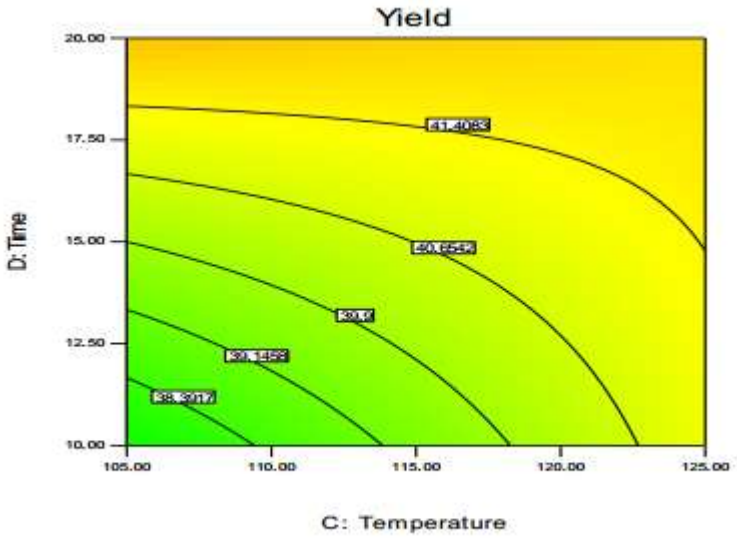
X1 = D: Time

X2 = C: Temperature

Actual Factors

A: Acid Concent. = 1.50

B: Solid Fraction = 7.50



Design-Expert® Software

Yield

45.2

29.7

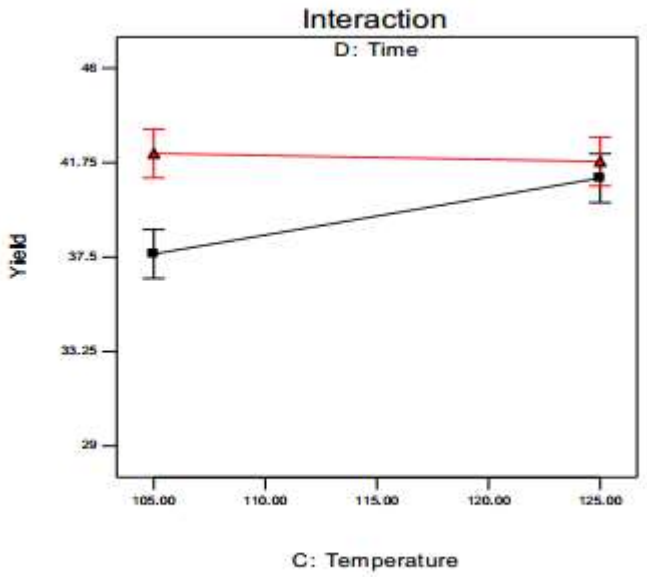
X1 = C: Temperature

X2 = D: Time

Actual Factors

A: Acid Concent. = 1.50

B: Solid Fraction = 7.50



Design-Expert® Software

Yield

D- 10.000

D+ 20.000

X1 = C: Temperature

X2 = D: Time

Actual Factors

A: Acid Concent. = 1.50

B: Solid Fraction = 7.50

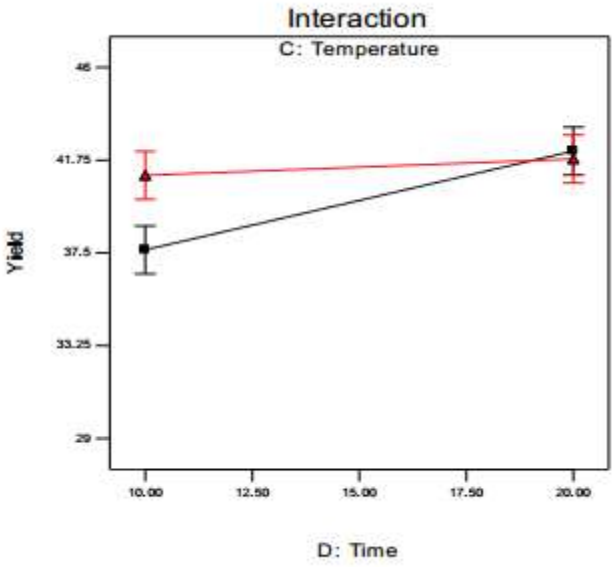


Figure 4.3 Surfaces of possible optimum solutions

Design-Expert® Software

Yield

C- 105.000

C+ 125.000

X1 = D: Time

X2 = C: Temperature

Actual Factors

A: Acid Concent. = 1.50

B: Solid Fraction = 7.5

Figure 4.3 Response surface plot (a), contour plot (b) and interaction plot (c)and (d) of ethanol yield as a function of time and temperature.

The response surface, fig. 4.3(a), resulted from hydrolysis reaction time and temperature is faintly flat and sloppy which has one up warded edge at one end suggesting that there is well defined optimum operating conditions and the surface is symmetrical. Moreover, the surface is somewhat flat near the optimum which meant that the response optimized value based on combined effects reaction time and temperature may not vary widely from the single variable optimized conditions.

The contour plot, fig. 4.3(b), shows the interaction between hydrolysis reaction time and

temperature on the yield of ethanol and indicates that at high hydrolysis time increase in temperature resulted high yield of ethanol.

The interaction plot of time and temperature shown in fig. 4.10 (c) indicates that at high hydrolysis time increase in temperature resulted in a slight decrease in the ethanol yield, while at low level of time increase in temperature resulted in a sharp increase in ethanol yield. Similarly figure 4.3(d) shows at high temperature level increase in hydrolysis time resulted in a slight increase in ethanolyield, while at low temperature increase in hydrolysis time resulted in a sharp increase in the yield of ethanol.

4.3. Optimization

The optimum acid concentration, solid fraction, temperature and time for maximum ethanol yield are 0.50%v/v,5.00%w/w,105.01^{o}C and 10.00 minutes respectively with 40.9 % ethanol yield.

The optimization criteria used are summarized in table below.

Name	Goal	Lower Limit	Upper Limit
Acid concentration	Minimize	0.3	3
Temperature	Minimize	100	115
Time	Minimize	5	15
Ethanol yield	Maximize	38	46.25

Table 4.2 Optimization criteria

The optimum possible solutions for acid hydrolysis in ethanol production and the corresponding contour and surface plot are shown below.

Solution number	Acid concentration	Temperature	Time	yield	Desirability
1	0.3	100.01	5.00	40.91	0.9
2	0.3	100.00	5.14	40.90	0.9
3	0.36	100.01	5.00	40.98	0.9
4	0.31	100.00	5.14	40.93	0.9
5	0.30	100.00	5.13	40.89	0.9
6	0.30	100.19	5.25	40.92	0.9
7	0.50	100.06	5.00	40.93	0.9
8	0.50	100.80	5.00	40.95	0.9

Table 4.3 Optimum possible solutions

Design-Expert® Software

Desirability

Design Points

1

0

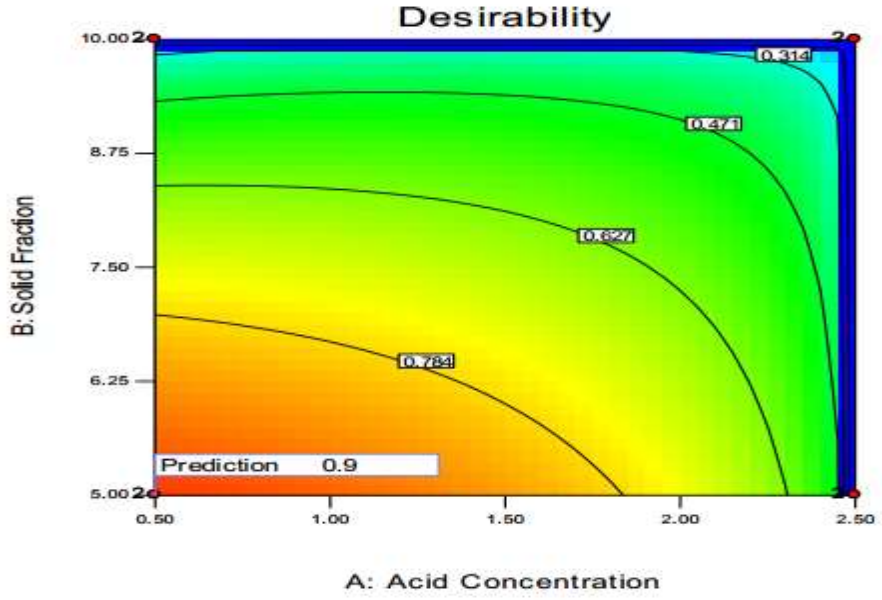
X1 = A: Acid Concent.

X2 = B: Solid Fraction

Actual Factors

C: Temperature = 105.01

D:Time=10.00



Desirability Figure 4.5 optimization contours on ethanol yield

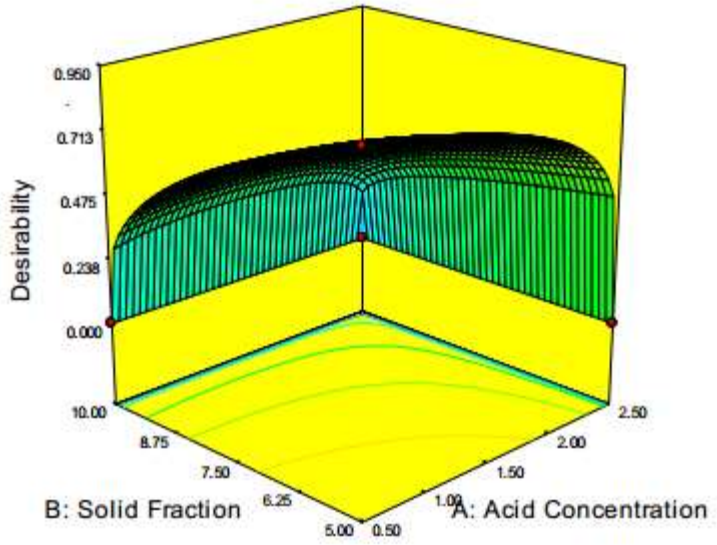


Figure 4.6 Surfaces of possible optimum solutions

Design-Expert® Software

Desirability

1

0

X1 = A: Acid Concent.

X2 = B: Solid Fraction

Actual Factors

C: Temperature = 105.01

D: Time = 10.00

4.4. Model validation

As determined by the 2-level factorial design result using Design-Expert® v.7 software, an experiment with acid concentration , temperature and time was conducted to carry out the effect of the design used. The optimal values test factors were 0.3 % v/v, 100.01°C and 5.00 minutes (obtained from table 4.3). The experiment was carried out at the optimized conditions. Ethanol yield of 40.91 (average) obtained and was in good agreement with the predicted one. Therefore the model is considered to be accurate and reliable for predicting the yield of ethanol.

5. Conclusion and Recommendation

5.1Conclusion

Due to the diminishing of fossil fuel resources, production of ethanol from lignocellulosic material has acquired significance as a fuel for the future. This study examines the possibility of *prosopis juliflora* wood for ethanol production. The conversion of *prosopis juliflora* wood to ethanol was carried out with dilute acid pretreatment, dilute acid hydrolysis, fermentation and distillation process steps.

In this study, 2 level full factorial experimental design was used for the optimization of acid hydrolysis process conditions as well as to investigate interaction between acid hydrolysis process factors using Design Expert® 7 software. The effects of acid hydrolysis variables, namely acid concentration, temperature, and time on the ethanol yield were investigated

It is concluded that the assumed cubic polynomial models satisfactorily explained the effects of the above-mentioned variables on the ethanol yield. Ethanol yield of 40.91% was obtained when optimum conditions were acid concentration 0.3%, temperature of 105.01^0Cand time of 5 minute, which indicates that at this condition no inhibitors (furfural and HMF) are produced that inhibit the fermentation process steps. Validation experiments verified the availability and the accuracy of the model with desirability 50 %. The predicted value was in agreement with the experimental value (40.91 wt. %). Based on this study, it is evident that the chosen method of optimization was efficient, and reliable.

5.2 Recommendations

Producing ethanol from renewable resources is becoming an important issue for the whole world. Therefore, the work needs to be continued for further development of ethanol production from *prosopis juliflora*.

It is also, recommend that in this study acid hydrolysis variables are optimised; future studies should include optimisation of pretreatment process, optimisation of fermentation process and optimisation of distillation process variables to obtain maximum yield of ethanol from prosopis juliflora wood. Additionally, it is recommend that preliminary design of pilot plant, process development and scale up has to be performed.

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Appendix1: The phenol-sulfuric acid method

The Phenol-Sulfuric acid method is widely used to determine the total concentration of carbohydrates present in foods and to decide whether the solution is carbohydrate or not. A clear aqueous solution of the carbohydrates to be analyzed is placed in a test-tube, and then phenol and sulfuric acid are added.

The solution turns a yellow-orange color as a result of the interaction between thecarbohydrates and the phenol (http://wwwnix.oit.umass.edu/~mcclemen/581Carbohydrates.html).

Appendix2: Density Measurements using pycnometer

Specific Gravity: Specific Gravity means the ratio of the mass of a sample to that of an equal volume of a standard substance. In this test, the specific gravity (d' t) d means the ratio of the weight of the sample at t'oC to that of an equal volume of distilled water at t^{o}C. When the specific gravity only is given, unless otherwise specified, it means the ratio of the weight (d ^{20}20) of the sample at 20^{o}C to that of an equal volume of distilled water at 20^{o}C.

Steps involved in specific gravity measurement by Pycnometer

- Weigh accurately a pycnometer, previously cleaned and dried, and note the weight W.
- Remove the cap, fill the pycnometer with the sample, wipe the outside surface thoroughly, weigh accurately, and note the weight W1
- Perform the same procedure, using the same pycnometer containing distilled water; note the weight W2 at the specified temperature (t^{o}C). Calculate the specific gravity (d't) by the formula:

  d' tt = W1-W/ W2-W

  where: W- weight (g) of empty pycnometer

  W1- weight (g) of pycnometer and sample

  W2- weight (g) of pycnometer and water

Appendix3: Laboratory work pictures

Prosopis juliflora wood

Soak sample in hydrolysis process