PROCESS OPTIMIZATION FOR PRODUCTION OF BIOETHANOL FROM SUGARCANE BAGASSE

By

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Approval Page of M.Sc. Thesis

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DECLARATION

I hereby declare that this Master Thesis entitled "Process optimization for production of bioethanol from sugar cane bagass" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation

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I/we, the advisor(s) of this thesis, hereby certify that I/we have read the revised version of the thesis entitled "Process optimization for production of bioethanol from sugar cane bagass" prepared under my/our guidance by Dula Debela Terefe submitted in partial fulfillment of the requirements for the degree of Mater's of Science in Industrial chemistry.

Therefore, I/we recommend the submission of revised version of the thesis to the department following the applicable procedures.

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ABBREVIATIONS

ASTU----------Adama science and Technology University

CCD---------- Central composite design

DNSA----------Di nitro salicylic acid.

D H_2O ---------- Distilled water

FAO ----------Food and Agricultural Organization

GHG---------- Greenhouse gasses

MCD ---------- Multilevel categorical Design

Na-K tertaret --------- Sodium potassium tertaret

NAOH ---------- Sodium hydroxide

Psi---------- Pascal

RSM---------- response surface metrology

RCBD ……..randomized complete block diagram

SCB----------Sugar cane bagasse

SDG---------- Sustainable development goal.

SHF ----------separate hydrolysis and fermentation.

H_2SO_4----------sulfuric acid

SSF---------- Simultaneous scarification and fermentation

SSCF----------Simultaneous Scarification& Co-Fermentation

%SY---------- Percent sugar yield

YPDA---------- Yeast peptone dextrose adenine

ABSTRACT

Sugarcane bagasse, a byproduct of sugar processing, is a cellulosic biomass that comprises of lignocellulose molecule. Nowadays, it is becoming an increasingly popular, environmentally safe and renewable alternative source of energy to petroleum fuel. The Ethiopian sugar estates produce huge and surplus amount of bagasse annually. However, only 85-90% used for cogeneration while rest quantity was wasted. In order to convert this valuable byproduct to bio-ethanol, pretreatment and process optimization are the primary requirements. In spite of the importance of bio-ethanol and huge wastage of bagasse in Ethiopian sugar estates, there is no research effort made so far on pretreatment and process optimization to valorize and utilize bagasse as alternative source of bio-ethanol energy. Therefore, this study was aimed to optimize pretreatment, hydrolysis and fermentation processes to produce bio-ethanol from sugarcane bagasse. Accordingly, in pretreatment optimization experiment, the effect of NaOH (0.5, 2.5, and 5 %) and pressure (10, 15, 20 Psi) under different reaction times (5, 20, 35 minutes) was evaluated. For hydrolysis experiment, the effects of H_2SO_4 (1, 2 and 3 %), temperature (160,190 and 220 ^{O}C) and hydrolysis time (20, 40 and 60 minutes) were evaluated while the fermentation experiment consists of different incubation temperatures (30, 35 and 40 ^{O}C) and incubation periods (24, 48 and 72 hours) under different initial pH (4, 5 and 6). All the three experiments were arranged in randomized complete block design with three factor factorial. Each treatment was arranged in two replications. In pretreatment experiments, data on cellulose, hemicellulose and lignin, in the hydrolysis experiment data on reducing sugar and on fermentation experiment data on ethanol yield were collected. After quality test, the collected data were subjected to statistical analysis and model optimization using design expert statistical software version 7.0. Results of the statistical analysis on pretreatment optimization revealed that 2.5 % NaOH and 15 psi pressure at 35 minutes gave the maximum extraction of cellulose (81.25) with maximum removal of hemicellulose (8.41) and lignin (6.02%). For pretreated bagasse hydrolysis, 2.05 % H_2SO_4 at a temperature 205.92 ^{O}C within 60 minutes produced maximum yield of reducing sugar (80.89 g/l) while the maximum ethanol produced at optimized conditions (6 initial PH, 30 ^{O}C incubation temperature and 71.83 hours incubation period) was 42.98 g/l. From these results, it can be deduced that the treatments that gave optimum results for pretreatment, hydrolysis and fermentation can be used to produce bio-ethanol from sugarcane bagasse.

***Keywords**: Sugarcane bagasse, bio-ethanol, pretreatment optimization, sugarcane bagasse hydrolysis, fermentation, Saccharomyces cerevisiae*

CHAPTER ONE

1. INTRODUCTION

1.1.Background of the Study

In the 20th century, the world economy has been dominated by technologies that depend on nonrenewable, such as petroleum, coal, or natural gas to produce fuels, chemicals, and materials (Sun & Cheng, 2002). However, due to depletion of stocks of fossil fuels, the increase in oil prices, and growing concerns over global climate change have forced policy makers and researchers to investigate comparably greener (renewable) alternatives of energy (fuels) and technology for the production of biofuels (Rencoret et al., 2017 ; Tang, 2013). Increasing the use of renewable energy sources for biofuels generation purposes is of particular interest nowadays because they allow mitigation of greenhouse gases, provide means of energy independence and may even offer new employment possibilities (Pradhan and Nag, 2007). Moreover, renewable energy is now capturing a good share of the worldwide headlines because of concerns about declining supplies of fossil fuels, escalating population and industrialization triggering ever-increasing demand of fuel (Tursi, 2019). The major types of renewable energy sources applicable for biofuels generation are mainly lipid-based biomasses and sugar orlignocellulosic based biomasses **(**Borjesson, 1996; Robak & Balcerek, 2018).

Lignocellulosic based biomass refers to dry matter of plant representing nearly 70% of the total plant biomass and is one of the most promising sources of renewable raw material for various biotechnological processes (Zhao et al., 2010). It is used mainly in the industrial production of biofuels such as bioethanol, and bio-based chemicals, due to their low economic value and high availability (Vassilev et al., 2013). The leftovers and/or waste obtained from agriculture and industry, such as sugarcane bagasse (SCB), Corn Stover, wheat and rice straw, wood chips, and the like, can be regarded as suitable lignocellulosic materials for bioethanol production (Barahona et al., 2020).

Sugar cane bagasse is a fibrous residue remains in a sugarcane mill after crushing the sugarcane stalk and extracting of its juice (Móczó et al., 2020; Barahona et al., 2020) which accounts 25-30% of the total weight of crushed cane (Sahu, 2018;Vargas Betancur & Pereira, 2010). It is

mainly used as a burning raw material (85%) in sugarcane mill furnaces (for cogeneration), whereas the excess or surplus of bagasse left is deposited on empty field altering the landscape (Birru, 2016). Therefore its existence presents a serious environmental problem which many researchers have performed research work to develop technology, procedure or method that may help to exploit their efficient use (Anggono et al., 2019).

As any lignocellulosic materials, sugarcane bagasse is, mostly constituted two polysaccharideic fractions: cellulose 35.2–50 % (Hajiha & Sain, 2015; Rezende et al., 2011) and hemicellulose (17–38 %)and a polyphenolic macromolecule lignin (19-33%)(Mthembuet al., 2016), which is resistant to enzyme/acid attack and degradation, and thus its content and distribution are recognized as the most important factors determining cell wall recalcitrance to hydrolysis (Halim and Hanim, 2018;Rocha et al., 2012). Due to its complex structure, sugar cane biomass cannot be directly utilized by most bioethanol producers. Hence, utilizing suitable biochemical and thermal methods for the conversion of the polysaccrides found in sugar cane bagasse could significantly improve bioethanol productivity and sustainability (Jaisamut et al., 2013).

Bioethanol production from sugar cane wastes requires steps, including the pretreatment of biomass, enzymatic/acid hydrolysis to produce fermentable sugars and fermentation (Hashmi et al., 2017; Halis et al., 2012)**.** Pretreatment is always been crucial and important step in producing bioethanol because it enhance the efficiency of sugar conversion during hydrolysis process (Halis et al., 2012). The pretreatments act by disrupting the lignocellulosic matrix, reducing the amount of lignin and hemicellulose, modifying the crystalline structure of cellulose and increase the porosity of the material to make it more susceptible to acid or enzymatic attack in hydrolysis processes to increase yield of fermentable sugars released into the liquid medium (Halim and Hanim, 2018; Rocha et al., 2012). However, the effectiveness of the pretreatment stage for maximal sugar production are influenced by many factors, such as; temperature, strength of the chemical used for the pretreatment, the type of chemical, the pretreatment time and the concentration of the substrate (Harmsen & Huijgen, 2010; Tan et al., 2021).

Different pretreatment technologies were applied to lignocellulosic substrates to decrease their recalcitrance and to improve the yields of monomeric fermentable sugars that are liberated by enzymatic/acid hydrolysis (Rezende et al., 2011), these are broadly categorized as physical

(commination, hydro-thermolysis), chemical (acid, alkali, solvents, and ozone), physio-chemical and biological pretreatment methods, and have been examined over the years (Halim and Hanim, 2018). The appropriate pretreatment method can be selected depending on the requirements of hydrolysis and fermentation that have the goals of bioethanol process such as high yields of fermentable sugar and ethanol, low production cost, low toxic compounds and recovery of spent chemicals (Wunna et al., 2017)**.**

Alkaline biomass pretreatment is normally performed at a lower temperature and pressure than other pretreatment methods and is suitable for processing of agricultural residues (Hernández et al., 2017). During alkaline pretreatment, ester bonds, which cross-link lignin and xylan, are degraded and glycosidic linkages in the lignocellulosic cell wall matrix are broken down, resulting in alteration of the structure of lignin to polymeric lignin-like compounds, reduction of the lignin hemicellulose complex, cellulose swelling, and the partial decrystallization of cellulose (Loow et al., 2016). During Alkali pretreatment the organic acids and phenols formed during the process is neutralized the, less sugar is degraded, and fewer inhibitors are formed compared to acid pretreatment (Canilha et al., 2012; Jaisamut et al., 2013 & Paulová et al., 2013). Sodium hydroxide (NaOH) presents the greatest degradation and subsequent fermentation yields when compared to other alkalis, such as sodium carbonate, ammonium hydroxide, calcium hydroxide and hydrogen peroxide (Rezende et al., 2011). In which, sodium hydroxide mediated pretreatment disrupts thesugar cane bagasse cell wall by solubilization of hemicellulose and lignin (Chandel et al., 2014).

Hydrolysis of biomass is essential process for generation of fermentable monomeric sugars which are then converted to ethanol by microbial action (Philippini et al., 2020). Acid and enzyme approach, are employed for biomass hydrolysis and its efficiencies varies depending on treatment conditions, type of biomass and properties of hydrolytic agent (Kucharska et al., 2018). The produced monomeric sugars, hexose (six carbon) can be fermented to ethanol quite easily using *Saccharomyces cerevisiae*, while the fermentation of pentose (five carbon sugars) is only done by fine strains (Cardona et al., 2010). Ethanol fermentation is accomplished using industrial strains of the yeast *Saccharomyces cerevisiae*, which are used due to their fast growth rate, high conversion efficiency, low byproduct production, and tolerance to high ethanol concentrations (Huezo & Shah, 2019). However, here are several factors thataffect the

efficiency of the conversion of sugars into ethanol, ranging between physical (temperature, osmotic pressure), chemical (pH, oxygen, mineral and organic nutrients, inhibitors) and microbiological (species, strain and concentration of yeast, bacterial contamination) (Bonassa et al., 2015).Therefore, the aim of this study was to assess the potential of bioethanol production from Sugar Cane bagasse through process optimization (i.e. the alkali pretreatment conditions, the dilute acid hydrolysis and the fermentation processes).

1.2.Statement of the Problem

The population of the world is projected to reach 8.5 billion by 2030 and 9.7 billion in 2050. Commensurate with the increasing population, the global energy consumption is expected to rise from 575 British thermal units (BTU), as estimated in 2015, to about 736 quadrillion BTU in 2040, which is a 28% increase over a period of 25 years (Baruah et al., 2018). The global dependence on non-renewable fossil fuels for meeting the current energy needs cannot be sustained for long in the face of the depleting fuel reserves and these reserves are limited both in volume and geographical distribution in the light of exploding global energy requirements. Moreover, the effects of this excessive dependence are already evident in the escalation of fuel prices over the past decade and severe environmental impacts like climate change due to emission of greenhouse gasses (GHG) (Hilares et al., 2017). To overcome this very complex problem of the energy resources, there is a critical need to develop energy resources that have the highest net energy yield, the most abundant supplies and meets the requirement of the lowest overall cost socially, economically and environmentally.

Biofuels are considered as one of the widely used renewable energy sources and have been researched in the past years to make it sustainable, safe and efficient energy supply. It is a fuel derived from plant biomass through thermal, chemical, and biochemical conversion and used in solid, liquid, and gaseous forms. Biofuels considered in this study is biofuels synthesized from sugar-based or lignocellulosic biomasses, such as ethanol with low investment cost and minimum environmental pollution. In Ethiopia there are many sources of lignocellulose biomasses. Sugar cane bagasse generated from sugar industries are the promising source of lignocellulose biomass and generated in large quantity in Ethiopia. In Ethiopia about greater than 3.58-4.41 million ton/annum of sugar cane is crushed and in average generate >1.1million tone /annum of bagasse was produced. But about 90% of bagasse was used for solid biofuels to

generate heat and electricity that is consumed by the industry in the process of sugar manufacturing. The rest 10% surplus bagasse disposed elsewhere generates greenhouse gasses due to composting at dump site and fire risk to the industry. Utilization of this surplus raw material to produce bio-ethanol, which add the product variety and maximize the profitability of the sugar industry as well as to minimize import amount of petroleum for machines (increase energy security the country) is important. Although very small researches have been conducted particularly on sugar cane bagasse, this research is aimed to use the opportunity of difference in sugar cane varieties, difference in climate change, and difference in soil type to investigate the potential of sugar cane bagasse in the production of bio-ethanol in Ethiopia.

1.3.Objectives

1.3.1. General objective

The general objective of this study wasoptimizing alkalipretreatment, acid hydrolysis and fermentation of sugarcane bagasse that can be used to produce bio-ethanol from sugar cane bagasse by using the commercially available yeast strain Saccharomyces *cerevisiae.*

1.3.2. Specific objective

- To determine the chemical composition of raw and pretreated sugarcane bagasse in terms of cellulose, hemicelluloses, lignin, ash and extractive
- To evaluate different concentrations of NaOH under different rates of pressure and residence time to optimize the pretreatment process for sugarcane bagasse
- To optimize the hydrolysis process of cellulose to simple sugars by varying acid concentration, residence time and temperature
- To produce bioethanol at optimized fermentation process of sugars by varying initial PH, incubation temperature and incubation periodusing the commercially available yeast strain *Saccharomyces cerevisiae*

1.4.Scope of the Study

This study was focused on optimization of sugarcane bagasse pretreatments, hydrolysis and fermentation processes that can be used to produce bio-ethanol from sugarcane bagasse. As the composition of sugarcane bagasse is affected by several factors. These are climate,

harvesting practices, soil type, sugar cane varieties and factory processing. Thus, pretreatment and process optimization for the bagasse produced by Ethiopian sugar estates is mandatory. Due to the recalcitrance or complex structure of cellulose, hemicellulose and lignin it is difficult to convert cellulose directly to fermentable sugar. Therefore it requires pretreatment to deconstruct the linkage between cellulose, hemicellulose and lignin and to increase the accessibility of cellulose for hydrolysis process. A number of pretreatment mechanisms were available. However, this study was limited to dilute NaOH (0.5-5%) pretreatment optimization owing to the availability, cost, environmental and material required to apply pretreatments and its ability to remove hemicellulose and lignin. In addition, the chemical composition determination of untreated and pretreated sugar cane bagasse in this study was limited to gravimetric method. Even though there are two (acid and enzymatic) methods of cellulose hydrolysis to fermentable sugar this study was limited to dilute acid (1-3%) hydrolysis optimization, because enzymes are highly Costly and difficult to extract. The glucose content determination in this study was limited to spectrophotometric method

A number of factors affecting fermentation conditions such as incubation temperature, initial PH of sugar, ratio of substrate to yeast inoculums, alcohol concentration, nutrient availability, and fermentation period. Even though fermentation conditions are affected by several factors and a number of yeast strains are available for fermenting glucose to ethanol, this study was limited to optimization of initial PH, incubation period and incubation temperature using commercial yeast strain Saccharomyces cervicea because Saccharomyces cervicea tolerates higher alcohol concentration, easily available and cheap. The ethanol content determination of the ferment in this study was limited to alcoholometry method.

1.5.Significance of the Study

- Pretreatment and process optimization to produce bio-ethanol from wasted sugarcane bagasse creates an additional source of income, job opportunity and reduces environmental pollution.
- The use of bio-ethanol blended with fossil fuel help reduce the consumption and pollution from fossil fuel as well as reduce the cost and currency required for fuel importation.

- Bioethanol production from cane bagasse is considered a second-generation biofuels process since it has no direct conflict with food security issues, unlike the case of first generation biofuels produced from agricultural crops, such as corn and soybean oil.
- In general, this study urges the Ethiopian sugar estates to use the annually wasted huge amount of sugarcane bagasse for production of bio-ethanol.
- Furthermore, the result could strength the policies and strategies promoting green economy using bioethanol, as alternative energy source.

CHAPTER TWO

2. LETRATURE REVIEW

Access to energy is a key pillar for human wellbeing, economic development and poverty alleviation (Hussein & Filho, 2012)**.** The world population is estimated to increase from 6.7 billion to 8 billion in 2030 and on other hand global oil production is expected to decline from 25 billion barrels to 5 billion barrels by 2050 (Joshi et al., 2011). Ensuring everyone has sufficient access is an ongoing and pressing challenge for global development. Balancing the challenge between development and environment therefore provides us with an ultimate goal of ensuring everyone has access to enough sustainable energy to maintain a high standard of living (Hafneret al.,2018).

Most of the world's energy demand is currently met using non-renewable energy derived from fossil fuels such as coal, oil and natural gas, which do not regenerate at sustainable rates (Mekala et al., 2014). Factors such as depleted supplies of fossil fuel, regular price hikes of gasoline, rising concerns over national energy security and dependency on foreign oil imports, and environmental damage have necessitated the search for economic and eco-benign alternative to gasoline (Long et al., 2013). These concerns have enforced to explore the alternative means of cost competitive and sustainable supply, and renewable form of energy (Canilha et al., 2012)**,**such as wind, sun, hydro, geothermal, lignocellulosic biomass etc. are important in green energy utilization, Greenhouse Gases (GHG's) emission reduction, lessen global warming and to achieve sustainable development goal (SDG).

Among these sources, lignocellulosic biomass especially agricultural residues have been paid much attention by the researchers due to inexpensive, renewable and abundant source for the production of second generation biofuels (Bari & Fakhruddin, 2018)**.** The development of new technologies for lignocellulosic ethanol production from sugarcane bagasse is of special interest, since it would increase the efficiency of ethanol production without expanding the agricultural areas, avoiding the current conflict produced by change in land use to meet growing energy demands (Zhu et al., 2016).

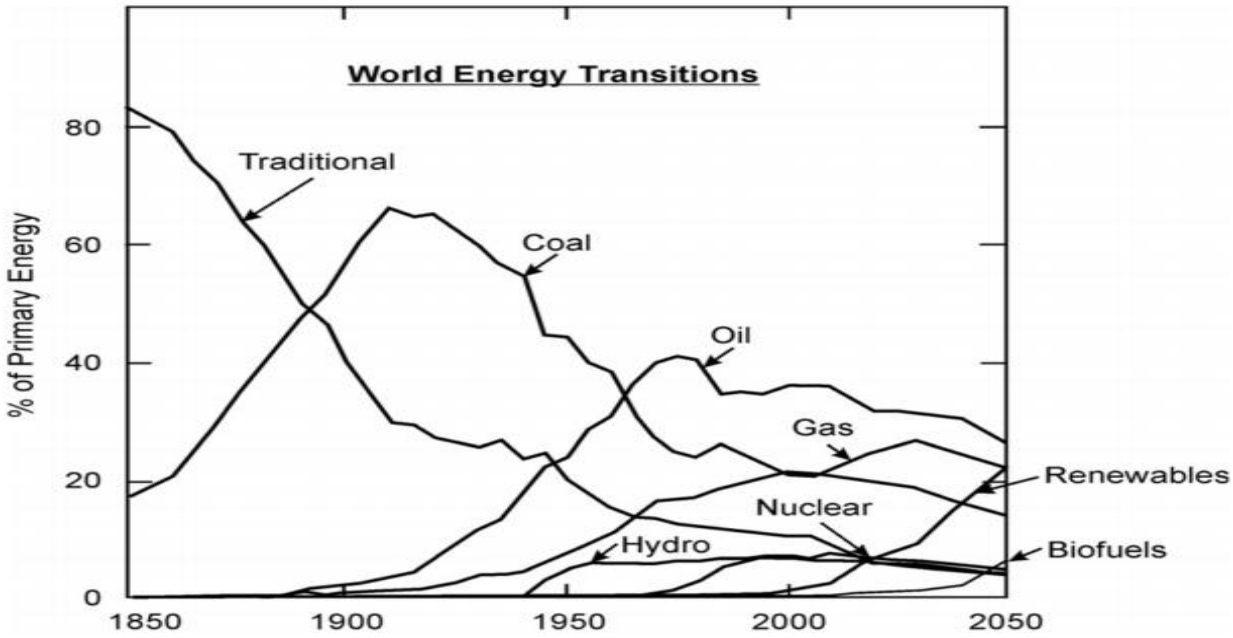


Figure 1: World primary energy shares 1850-2050: future projections based on shell 'Dynamics as usual' scenario (Allen & Hammond, 2019)**.**

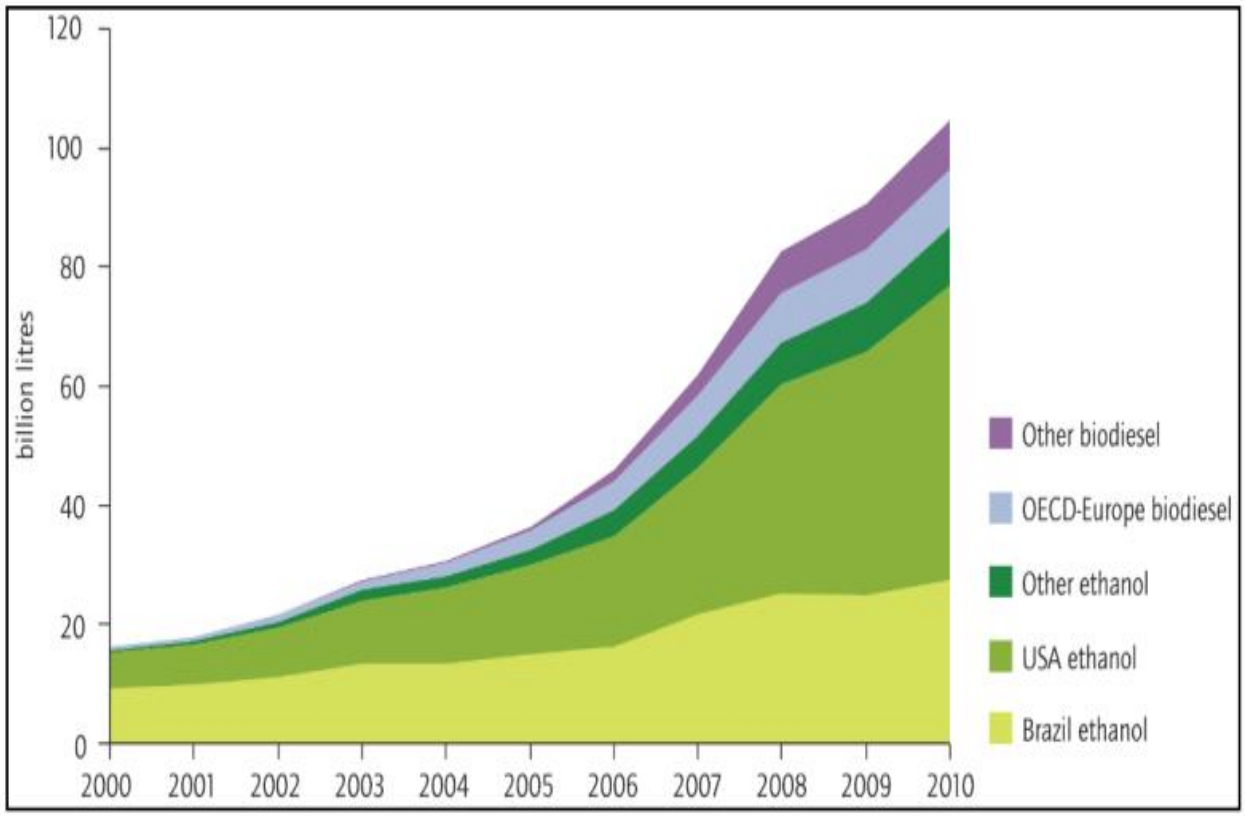


Figure 2: Global biofuel production (Richard Ahorsu, 2018).

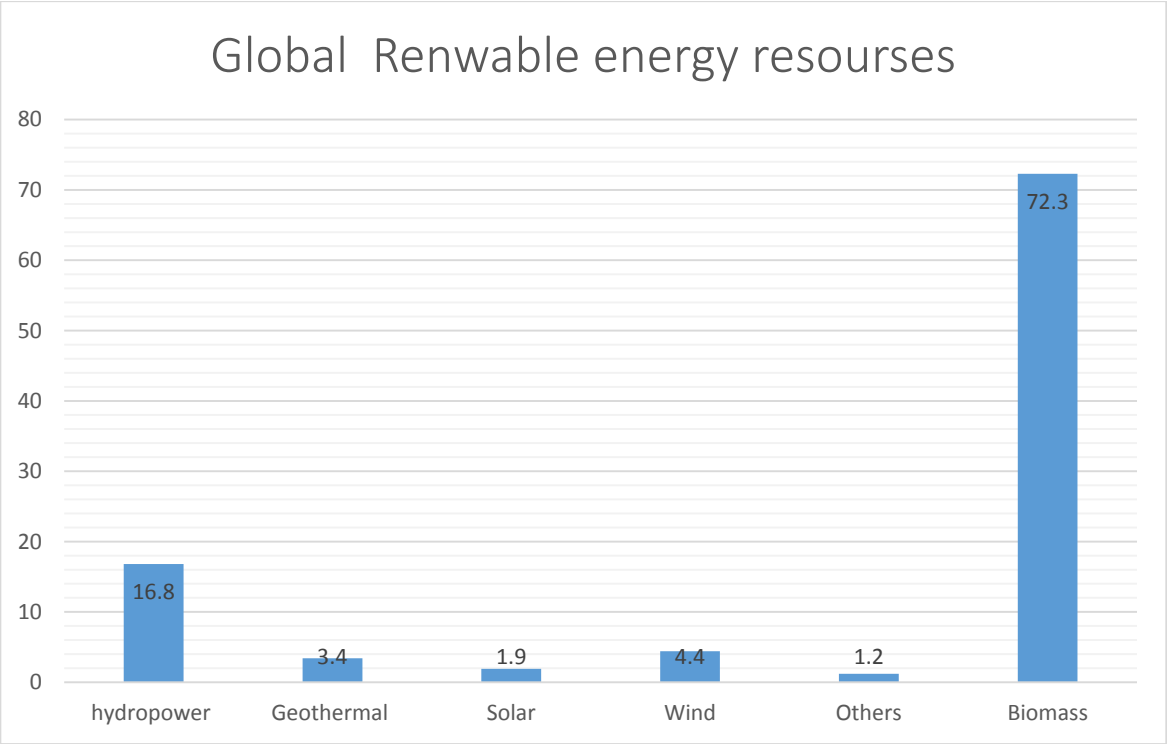


Figure 3: Global Renewable energy recourses (Demirbas, 2006).

2.1.Lignocellulosic Biomass

Lignocellulos biomass is the general term used to describe the plant matter composed of polymeric compounds cellulose, hemicellulose, and lignin (Becer and Isikgor, 2015). Cellulosic feed stocks contain sugars within their cellulose and hemicellulose, but they are more difficult to convert biochemically into ethanol than starch- and sugar-based feed stocks (Lee & Rangaiah, 2009).The exact composition of biomass depends on plant material, however, typical percentage compositions of the two polysaccharide components are: cellulose 35–50%, and hemicellulose 20–35%. Harnessing the carbohydrates from lignocellulosic biomass into bioethanol is not only a ‘nice idea’ but an ‘important necessity’ owing to the increased energy demand globally, safe environment, and sustainable employment. Chemical composition of lignocellulosic feed stocks is a key factor affecting efficiency of biofuel production during the complex conversion process (Chandel et al., 2014).

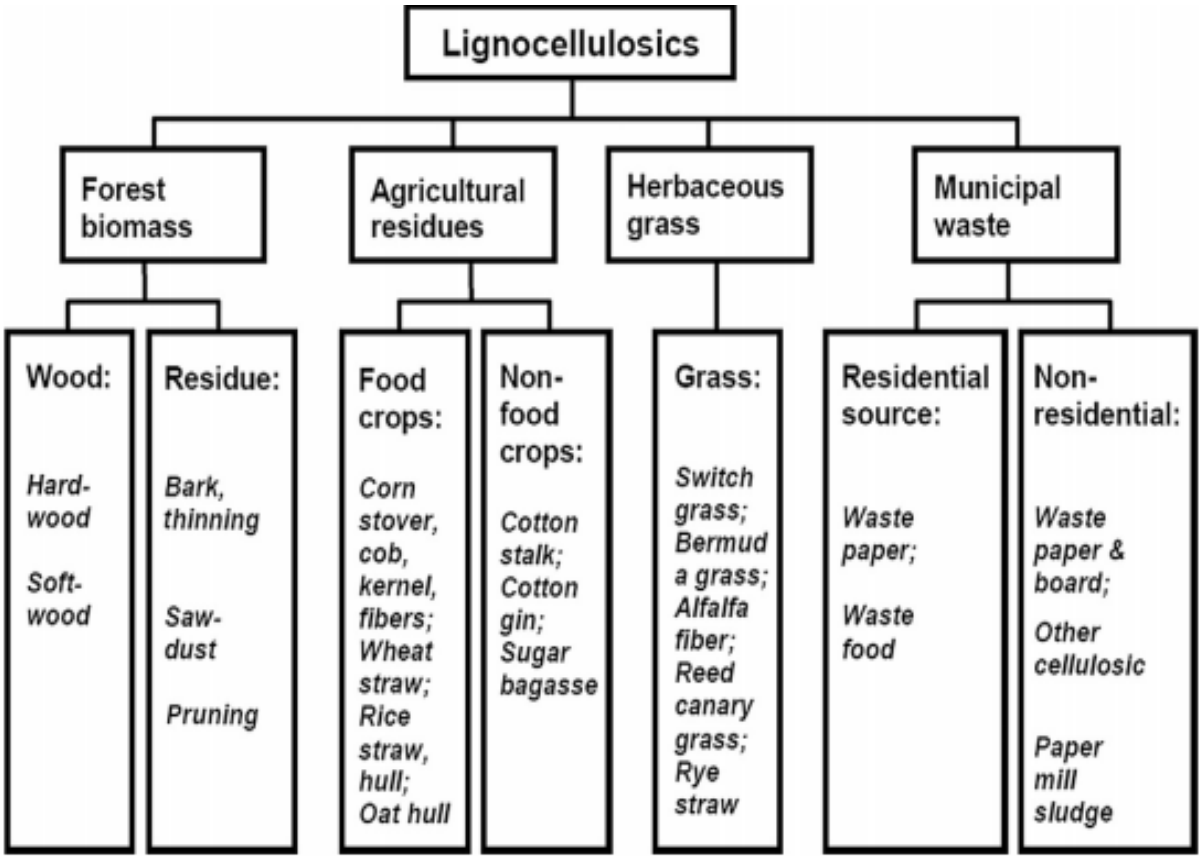


Figure 4**:** Major lignocellulosic feedstock explored for bioethanol production (Mekala et al., 2014)**.**

2.1.1. Chemical composition of lignocelluloses biomass

Chemical composition of lignocellulosic feed stocks is a key factor affecting efficiency of biofuel production during the complex conversion process. The structural and chemical composition of lignocellulosic feed stocks is a highly variable factor, because of genetic and environmental influences and their interactions. The composition of biomass is largely diverse. For example, residues of plant origin are mainly composed of cellulose, hemicellulose, and lignin with varying percentages (Baruah et al., 2018)**.**

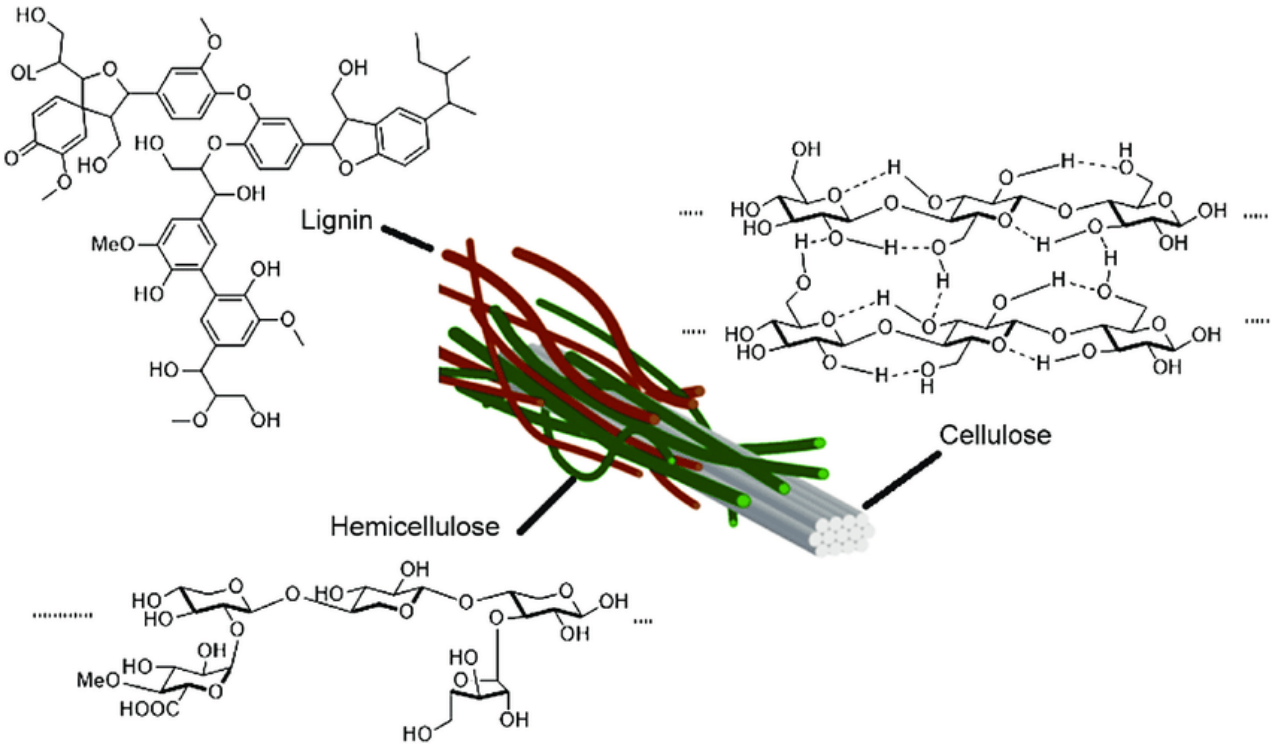


Figure 5: Structure of the composition of lignocellulose (Qiu, 2012)

Table 1: Proportions of the main chemical Compound Groups within various types of Biomass (Kumar et al., 2009)

Lignocellulosic Biomass	**% of total dry weight**		
	Cellulose	**Hemicellulose**	**Lignin**
Bamboo	49-50	18-20	23
Corn Cobs	45	35	15
Corn Stover	35-42	20-28	11-22
Grasses	25-40	35-50	10-30
Hard wood steams	40-50	18-40	18-28
Nut shells	25-30	25-30	30-40
Rice strew	29-41	15-26	8-19
Soft wood steams	34-50	21-35	25-35
Sugar cane bagasse	25-50	24-34	10-26
Switch grass	30-40	10-40	5-20
Wheat strew	31-44	20-25	15-24

2.1.1.1.Cellulose

Cellulose is the most abundant polysaccharide polymer which comprisedof a linear chain of β (1 → 4) linked D-glucose units that generates crystalline regions and consequently increases resistance to the hydrolytic process (Halim and Hanim, 2018).Those linear polymer composed of D-glucose subunits linked by β-1, 4 glycosidic bonds forming the dimmer cellobiose, a glucose-glucose dimer. These linear polymers are linked together by different hydrogen bonds and inter and intra molecular van der Waals forces, which allow them to be packed side by side in planar sheet and bundled into micro fibrils (Figure6). Cellulose is insoluble in water as the hydroxyl groups insugar chains are bonded toeach other, making a hydrophobic scenario.

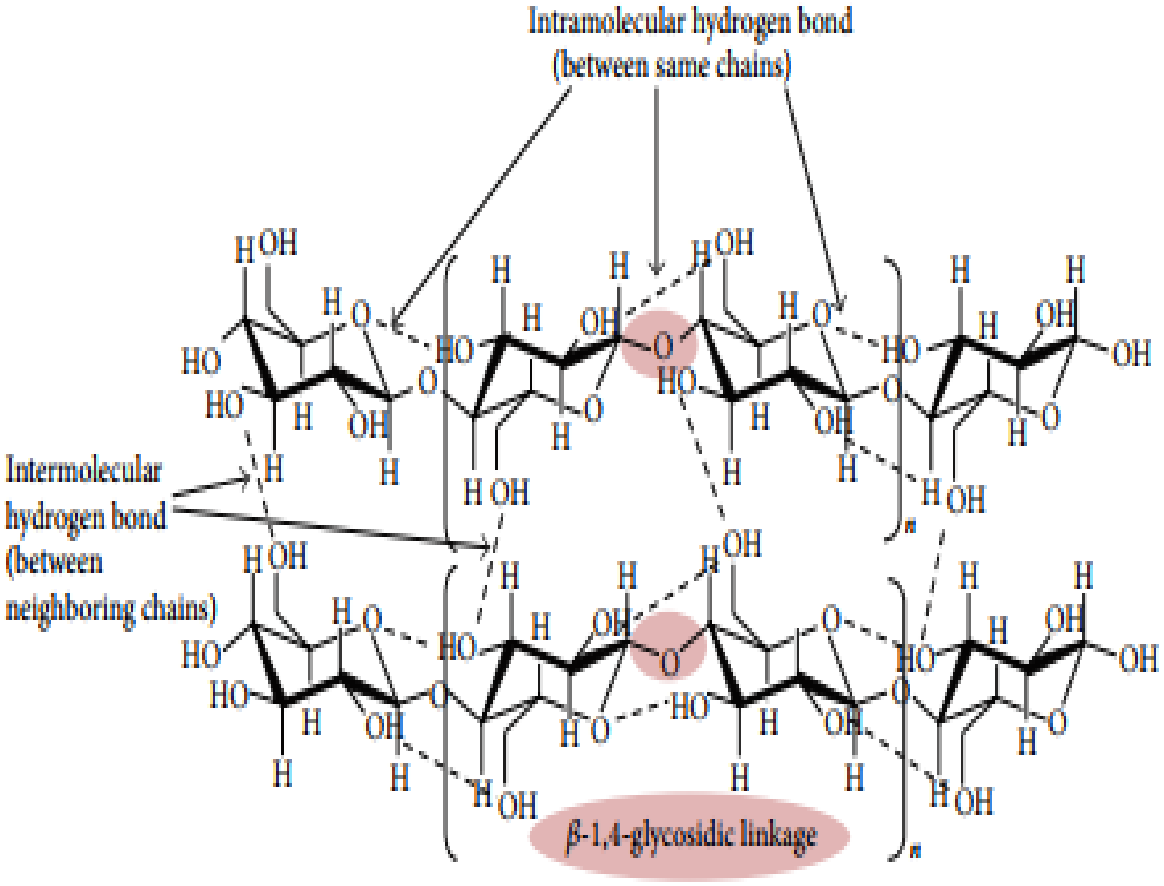


Figure 6: The structural formula of cellulose (Lee et al., 2014).

The reactivity and morphology of cellulose chains are substantially influenced by the intermolecular hydrogen bond between the hydroxyl group on C-3 carbon and the oxygen of the nearby glycosidic ring. The formation of these bonds makes the molecules more stable and

rigid. In some cases, the presence of many intermolecular bonds can generate an orderly crystalline region due to the considerable proximity between the different monomers.

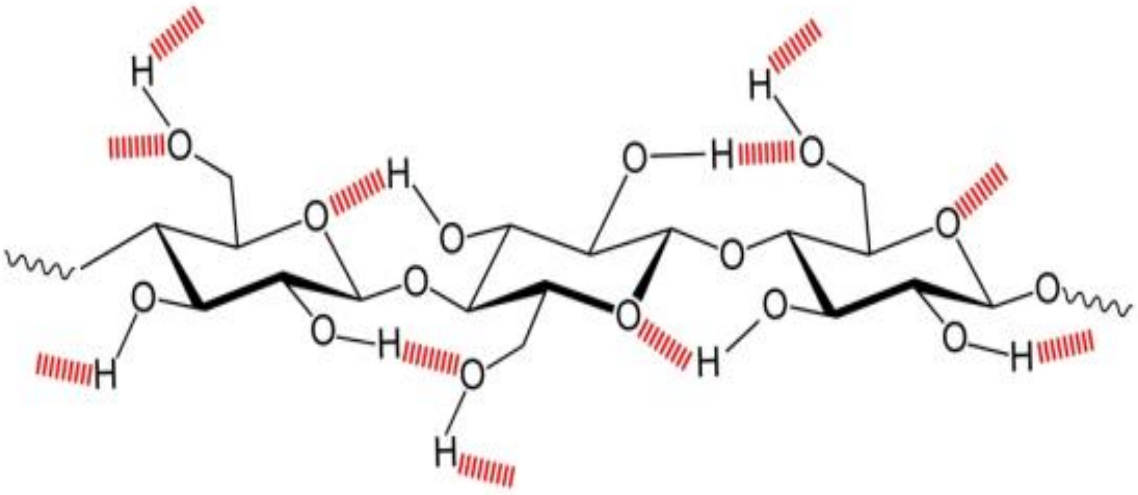


Figure 7: Inter and intra–molecular hydrogen bonding in cellulose (Khazraji & Robert, 2013).

Alternation of the crystalline and amorphous regions of cellulose could affect the accessibility of its functional groups, which are involved in reactions. In fact, the reactivity of cellulose depends on the reactive capacity of its primary and the secondary hydroxyl groups, emerging from the glycosidic rings. In particular, the primary hydroxyl groups have a higher reactivity than the secondary ones due to lower steric impediment (Khazraji & Robert, 2013).

2.1.1.2.Hemicellulose

Hemicellulose is the second most abundant polysaccharide after cellulose and is a short and highly branched polymers which comprised of pentose (xylose and arabinose) and hexose (mannose, glucose, and galactose) sugars. It possesses a heteropolysaccharide composition that varies according to the source. Sugarcane bagasse hemicellulose is composed of heteroxylans, with a predominance of xylose. Hence, it can be chemically hydrolyzed more easily than cellulose (Halim and Hanim, 2018). Those sugars are linked together by β-1, 4- and sometimes by β-1, 3-glycosidic bonds. In nature, hemicellulose is amorphous and has adhesive properties, with a high tendency to toughen when it is dehydrated. It is almost entirely consists of sugars with five carbon atoms (xylose and arabinose) and six carbon atoms (glucose, galactose, mannose, and rhamnose) with an average molecular weight of <30,000 amu. The different

groups of molecules making up hemicellulose include xylans, mannans,galactans, and arabinogalactans (Chen et al., 2015).

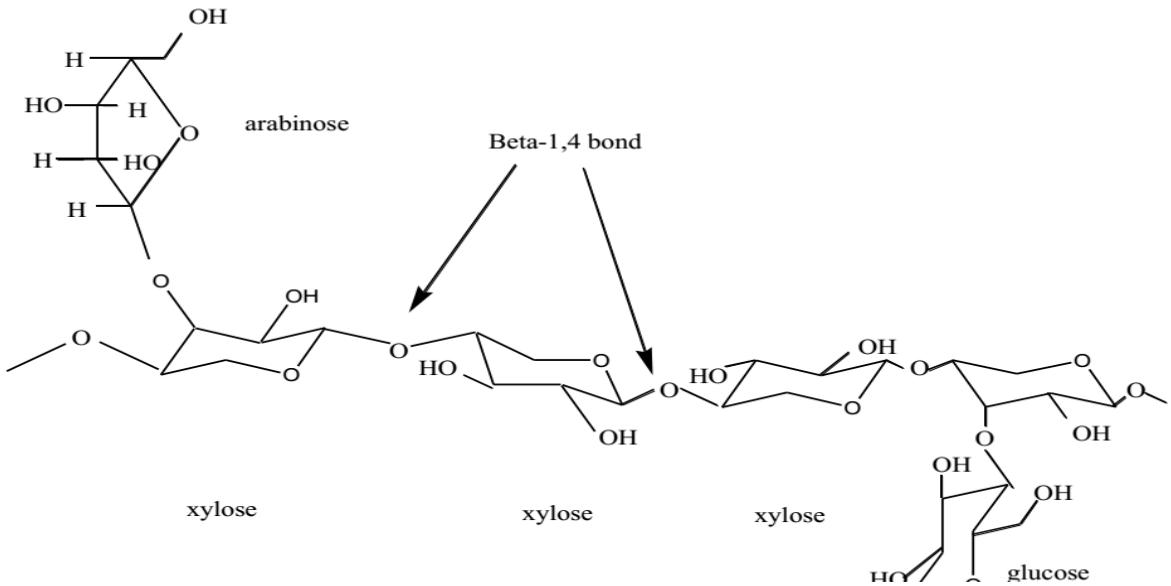


Figure 8: The structural formula of Hemicelluloses (Chen et al., 2015).

2.1.1.3. Lignin

Lignin is a complex molecular structure formed by the polymerization (cellulose-hemicellulose-lignin matrix) of aromatic alcohols or cross-linked polymers of phenolic monomers, confers structural support, impermeability and resistance against microbial attack (enzymatic) and oxidative stress (chemical) (Vargas Betancur & Pereira, 2010; Halim and Hanim, 2018). Among the components of lignocellulose, it is the most recalcitrant. The presence of lignin in lignocellulosic biomass is the main obstacle of biomass recalcitrance during separation process. Lignin acts as a protective barrier for plant cell and prevents plant cell destruction. Lignin is formed from three precursor alcohols shown on (figure 9): especially phydroxycinnamyl (coumaryl) alcohol, which forms p-hydroxyphenyl units in the polymer; 4-hydroxy3-methoxycinnamyl (coniferyl) alcohol, the guaiacyl units; and 3, 5-dimethoxy-4-hydroxycinnamyl (sinapyl) alcohols, and the syringyl units. Free radical copolymerization of these alcohols produces the heterogeneous, optically inactive, cross-linked and highly polydisperse polymer (Lee et al., 2014).

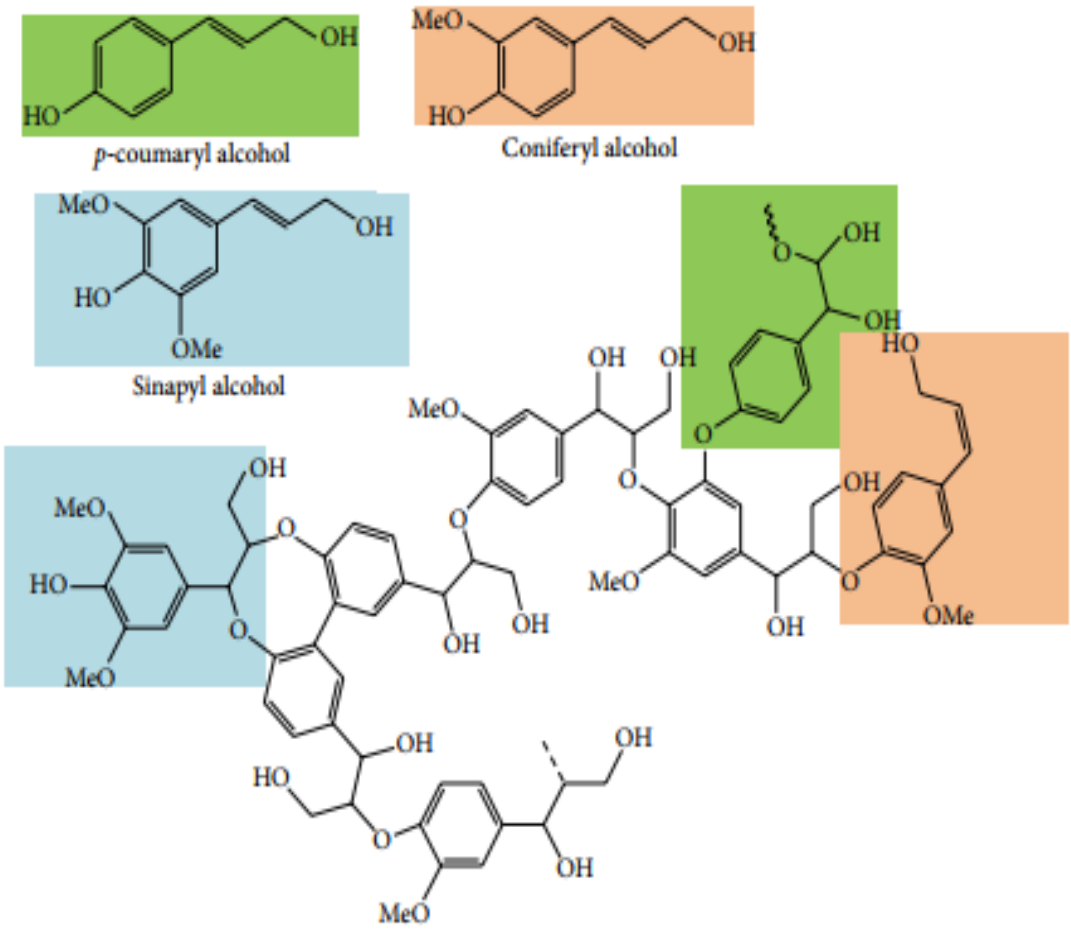


Figure 9: Chemical structures of lignin and its precursors (*p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol) (Lee et al., 2014).

2.2.Sugar Cane Bagasse

Sugarcane is a perennial grass belonging to the *Saccharin officinarum* genus, grown in tropical and subtropical regions.Sugarcane is one of the preferred crops for ethanol production due to high biomass yields and high fermentable sugar content (Benjamin et al., 2013). Production of bioethanol from SCB has a major advantage, like its less carbon intensive, than fossil fuel which reduces air pollution. The bioethanol produced from lignocellulosic materials is named as second-generation (2G) ethanol or cellulosic ethanol, while the first generation ethanol is produced from sucrose (juice extracted from sugarcane, sugar beet, or sweet sorghum) or starch (typically extracted from grains) (Halim and Hanim, 2018; Souza et al., 2012). The world's total sugar cane production was estimated at 2057.85 million tons (Daniel & Fabio, 2020) and the total sugar cane production of Ethiopia is 3.58-4.41milliontons (Khan et al., 2020). Sugarcane

bagasse (SCB), which is an abundant waste fibrous pulp material, results after the juice extraction from sugarcane stalks. It amounts approximately 25-30 % of the sugarcane mass (Ruiz et al., 2020). Sugar cane bagasse is mainly composed of two polysaccharide fractions (cellulose and hemicellulose) and a polyphenolic macromolecule (lignin) (Souza et al., 2012). Bagasse can be used to produce a number of value added products such as pellets, electricity, ethanol, paper board, specialty chemicals such as vanillin, furfural and animal feed (Rocha et al., 2012; Varshney et al., 2018).

Table 2: Chemical composition (%w/w, dry bases) of sugar cane bagasse reported in literature

SN	Components					References
	Cellulose	**H. cellulose**	**Lignin**	**Ash**	**Extractives**	
1	40-50%	20-35%	20-35%	-	-	(Tana et al., 2016)
2	33-36%	28-30%	17-24%	-	-	(Halim and Hanim, 2018)
3	38.59± 3.45	27.89±2.68	17.79±0.62	8.80 ± 0.02	2.72±1.23	(Guilherme et al., 2015)

From table 2 above the more abundant component is cellulose (33-50 %). Hemicellulose is the second predominant fraction (20-35%), and it possesses a hetero polysaccharideic composition that varies according to the source. These sugar monomers can be used as substrates (building-blocks) for biotechnological and chemical processes (Steinbach, 2017).

SCB is an attractive feedstock for the large-scale biological production of bioethanol, because of the abundance and concentration of low-cost raw materials, contributing to the reduction of greenhouse gas emissions and the improvement of food security (Hernawanet al., 2018)**.** Bioethanol produced from sugarcane residues is one of the most suitable alternatives for partial replacements of fossil fuels because it provides energy that is renewable and less carbon intensive than gasoline. Bioethanol reduces air pollution and also contributes to mitigate climate change by reducing greenhouse gas emissions (Mekalaet al., 2014). In general, the biological process converting the lignocellulose biomass to fuel ethanol involves three main steps (Obeng et al., 2019): pretreatment either to remove lignin or hemicellulose to liberate cellulose; (2) depolymerization of carbohydrate polymers to produce free sugars by enzyme/acid mediated action; (3) fermentation of hexose and/or pentose sugars to produce ethanol (Qiu, 2012)

2.2.1.Pretreatment of Sugarcane Biomass

Pretreatment is aimed to break (deconstruct) the lignocellulosic complex, solubilize (remove) the lignin and hemicellulose) but preserve the materials for further valorization, reduce cellulose crystallinity, and increase the porosity of the materials for subsequent depolymerization process (Baruah et al., 2018). The recalcitrance (resistance of plant cell walls to deconstruction) is a major obstacle in the separation of cellulose, hemicellulose, and lignin for different application (Rezende et al., 2018).The main goal of pretreatment is to overcome the recalcitrance which is targeted to alter the size and structure of biomass through separation of cellulose from the matrix polymers and create access for hydrolysis to turn cellulose into monomers by acid or enzyme **(**Kumar et al., 2009). Pretreatment must meet the following requirements: (1) production of highly digestible solids that enhances sugar yields during hydrolysis. (2) avoid the degradation or loss of carbohydrate, (3) avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes, and (4) to be cost effective by operating in reactors of moderate size and by minimizing heat and power requirements (Brodeur et al., 2011).

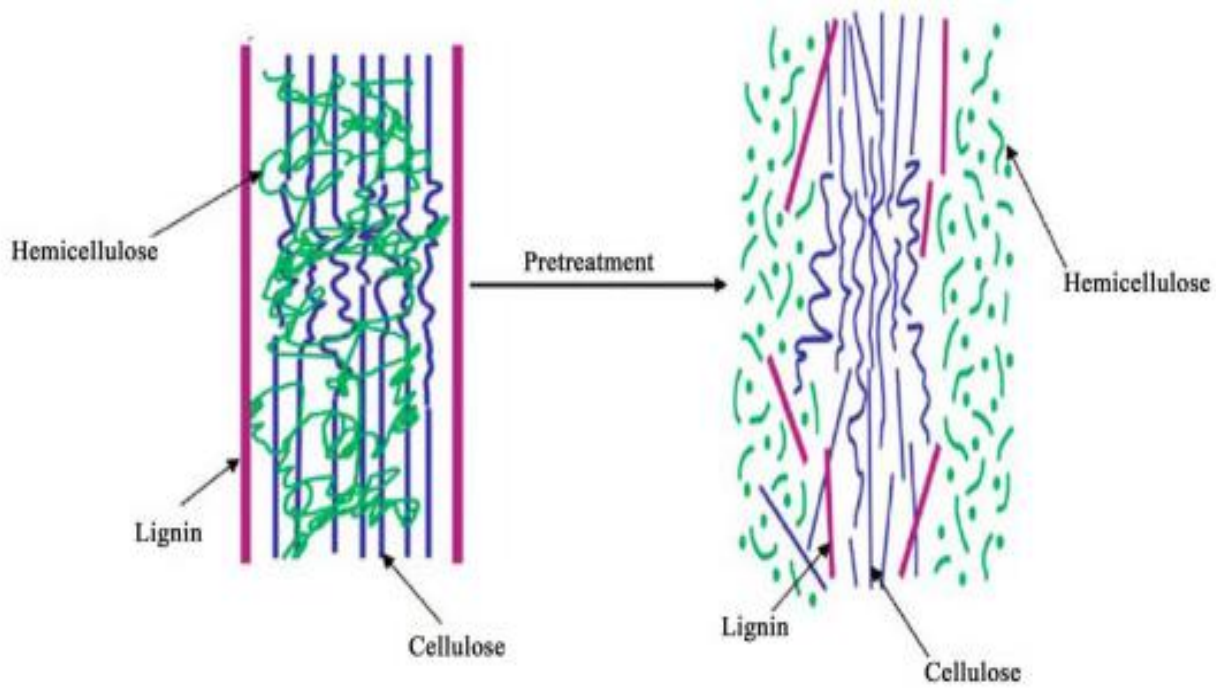


Figure 10: Pretreatment for deconstruction of lignocelluloses into cellulose, hemicellulose, and lignin) of the rigid structure of biomass (Kumar et al., 2009).

2.2.1.1. Sugar cane bagasse pretreatment methods

Pretreatment methods, which disrupt the highly-ordered cellulose structure and the lignin-carbohydrate complex, remove lignin, and increase the surface area accessible to enzymes, promote the hydrolysis, and increase the rate and extent of hydrolysis of cellulose in various lignocellulosic residues (Mahamud & Gomes, 2012). Several types of pretreatment methods that are used to open bio matrix structures are categorized into the following: (i) physical (milling and grinding); (ii) chemical (alkaline, dilute acid, oxidizing agents, and organic solvent); (iii) biological and (iv) Multiple or combinatorial pretreatment of physical and chemical techniques (steam pretreatment/auto hydrolysis, hydrothermolysis, and wet oxidation). Among the biomass pretreatment, chemical pretreatment proved to be the most efficient method and cost effective for biomass deconstruction with low pretreatment severity. Thermal methods are less efficient and consume more energy than chemical methods, while enzyme for biological pretreatment is expensive and it takes longer pretreatment duration (Lee et al., 2014).

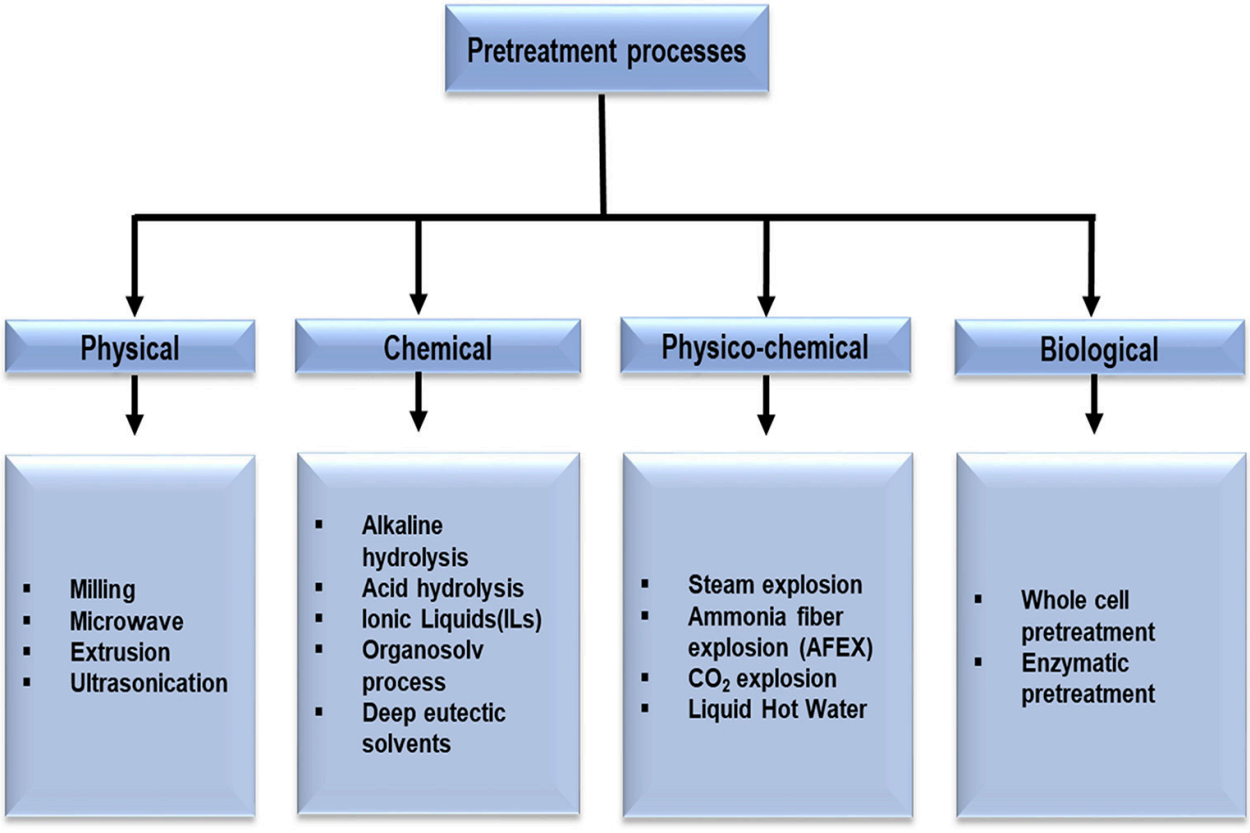


Figure 11: Flow chart diagram of pretreatment processes (Baruah et al., 2018).

2.2.1.1.1. Physical methods

The main aim of physical pretreatment is the reduction of the size of the biopolymer particles by means of fragmentation, grinding, milling, and hacking, rolling and mechanical interactions. Physical pretreatment also includes such methods as microwave radiation, sonication, spray drying, gamma radiation and pyrolysis. Pore size and surface area of sugar cane bagasse can be increased, whereas crystallinity and degree of polymerization of cellulose can be decreased with the application of physical methods (Quintero et al., 2011).

2.2.1.1.1.1. Milling

Reduction of particle size is often needed to make material handling easier and to increase surface/volume ratio. This can be done by chipping, milling or grinding. Mechanical pretreatment is usually carried out before a following processing step, and the desired particle size is dependent on these subsequent steps. For mechanical pretreatment factors like capital costs, operating costs, scale-up possibilities and depreciation of equipment are very important (Aslanzadeh et al., 2014).

2.2.1.1.1.2. Mechanical extrusion

When materials that can pass through a defined cross section die, it appears out with the fixed definite profile. This is the extrusion process which is known for sugar recovery from biomass. Adaptability to modifications, no degradation products, controllable environment, and high throughput are few advantages related to mechanical extrusion pretreatment process. Single screw extruder and twin screw extruder are two types of extruders. Different parameters like speed of screw, temperature of barrel, and compression ratio can significantly affect recovery of sugars. Short-time extruders provide fast heat transfer, proper mixing, and increased shear. When material passed through the extruder barrel, structure of biomass is disturbed, exposing more surface for enzymatic/acid hydrolysis (Farooq et al., 2016)**.**

2.2.1.1.2. Chemical pretreatments

2.2.1.1.2.1. Acid pretreatment

There are two types of acid pretreatments either using concentrated acid or diluted acids. Concentrated acid hydrolysis can be performed at a low temperature (30–60^{O}C) using acid with the concentration around 40–80%. High sugar yield can be obtained using this method,

however, requires large volumes of acid which are toxic and corrosive. Thus, corrosion resistant reactors are needed if concentrated acid is employed. Furthermore, the acid concentration must be recovered after hydrolysis to make the process economically feasible. The development of effective acid recovery technologies has made this process renewed its interest. On the other hand, dilute acid hydrolysis is the most widely used and has been considered to be one of the treatment methods with greater potential for wide-scale application. This process can be performed using diluted acids in the range of 0.5–6% and high temperatures from 120–170°C, with variable treatment times from minutes up to an hour. Dilute acid pretreatment has received numerous research interests, and it has been successfully developed for pretreatment of lignocellulosic biomass. Dilute acid pretreatments are normally used to degrade the hemicellulosic fraction and increase the biomass porosity, improving the enzymatic hydrolysis of cellulose. The dilute acid pretreatment is important to weaken the glycosidic bond in the hemicellulose and lignin-hemicellulose bond and the lignin bond. This will lead to the dissolution of the sugar in the hemicellulose and also increase the porosity of the plant cell wall for effective enzyme digestibility.

Acid pretreatment is a very commonly used technology for biomass to ethanol conversion due to its low cost and the fact that the used acids are easily available. However, acid pretreatments can cause side effects such as the formation of furan and short chain aliphatic acid derivatives, which are considered strong inhibitors in microbial fermentation.The generation of inhibitory products in the acid pretreatment, renders it less attractive for pretreatment option. Furfurals, aldehydes, 5-hydroxymethylfurfural, and phenolic acids are the inhibitory compounds that are generated in huge amount in acid pretreatment (Halim and Hanim, 2018).

2.2.1.1.2.2. Alkali pretreatment

Alkaline pre-treatment is a highly effective pretreatment which makes lignocellulose swollen and porous, mainly through hemicellulose solubilization, and/or redistribution of lignin (Ahmadi et al., 2015).Alkaline pretreatment is basically a delignification process. It disrupts the cell wall of SCB by (1) dissolving hemicelluloses, lignin, and silica, (2) hydrolyzing uronic and acetic esters, and (3) swelling cellulose under mild conditions. This process results in two fractions, a liquid (hemicellulose oligomers and lignin) and a solid fraction (cellulose) (Halim and Hanim, 2018). As compared to other pretreatment methods, alkali treatment requires less

pressure and temperature and ambient condition, but alkali pretreatment needs time in days and hours**.**Ammonium, sodium, calcium, and potassium hydroxides are used for alkaline pretreatment, but among these sodium hydroxide is the most commonly used alkaline pretreatment agent. Crystallinity index increases in lime pretreatment because of the removal of lignin and hemicellulose. Structural features resulting from lime pretreatment affect the hydrolysis of pretreated biomass (Chandel et al., 2014).

2.2.1.1.2.3. Organosolvent

Aqueous organic solvents like methanol, acetone, ethanol, and ethylene glycol are used in this method with specific conditions of temperature and pressure. Organosolvent pretreatment is usually performed in the presence of salt catalyst, acid, and base. The biomass type and catalyst involved decide the temperature of pretreatment, and it can go up to 200°C. This processes used to remove (extract) lignin. Cellulose fibers are exposed when lignin is removed, which leads to more hydrolysis. During Organosolvent pretreatment, fractions and syrup of cellulose and hemicellulose, respectively, are also produced.

There are certain variable factors like catalyst type, temperature, and concentration of solvent and reaction time which affects the characteristics of pretreated biomass like crystallinity, fiber length, and degree of polymerization. Inhibitor formation is triggered by long reaction, high temperature, and acid concentrations. Organosolv is not a cost-effective pretreatment process because of the high cost of catalysts, but it can be made cost-effective by recovering and recycling of solvents. For sugarcane bagasse ethanosolvat 195^{O}C for 60 min, and results showed formation of 29.1% sugars from 30% ethanol (Farooq et al., 2016).

2.2.1.1.2.4. Ionic liquids

Ionic liquids containing cations or anions are a new class of solvents with high thermal stability and polarity, less melting point, and negligible vapor pressure. Normally large organic cations and small inorganic anions compose ionic liquids. Factors like degree of anion charge delocalization and cation structure significantly affect physical, biological, and chemical ionic liquid properties. Interactions between ionic liquids and biomass get affected by temperature, cations and anions, and time of pretreatment.

Ionic liquids actually compete for hydrogen bonding with lignocellulosic components, and in this competition disruption of network occurs. 1-Ethyl-3- methylimidazolium diethyl phosphate-acetate, 1-butyl-3-methylimidazoliumacetate, cholinium amino acids, cholinium acetate, 1-ethyl-3-methylimidazolium diethyl phosphate-acetate, 1-allyl-3-methylimidazolium chloride, and chloride are ionic liquids used for the treatment of rice husk, water hyacinth, rice straw, knife powder, poplar wood, wheat straw, and pine. It removes lignin at temperature of 160°C for 3 hours. Results showed 62.9% lignin removal enhanced enzymatic digestibility, and reduced cellulose crystallinity was reported. Ionic liquid pretreatment is less preferred over other techniques because of high thermal and chemical stability, less dangerous conditions for processing, low vapor pressure of solvents, and retaining liquid state at wide range of temperature. Ionic liquids can be recycled easily and are non-derivatizing. Disadvantage of using ionic liquid pretreatment is that non compatibility of cellulase and ionic liquids results in the unfolding and inactivation of cellulase. High temperatures trigger more side reactions and negative side effects like reducing ionic liquid stability (Farooq et al., 2016).

2.2.1.1.2.5. Ozonolysis

Ozone pretreatment is a great option for lignin content reduction. Inhibitors are not formed in this pretreatment which is a great advantage because other chemical pretreatments produce toxic residues. In ozone pretreatment, ozone acts as an oxidant in order to break down lignin. Ozone gas is soluble in water and being a powerful oxidant, by breaking down lignin, releases less molecular weight, soluble compounds. It is highly reactive towards the compounds incorporating conjugated double bonds and functional groups with high electron densities. Therefore, the most likely biomass constituent to be oxidized is lignin due to its high content of C=C bounds. Wheat straw, bagasse, cotton straw, green hay, poplar sawdust, peanut, and pine can be pretreated with ozone in order to degrade lignin and hemicellulose; however, only slight changes occur in hemicellulose, whereas almost no changes occur in cellulose. Ozone mass transfer is limited at less water concentration, which ultimately affects its reactivity with biomass. Longer residence time of ozone is caused by the blockage of pores by water film. During Ozonolysis, pH of water decreases because of the formation of organic acids. Alkaline media trigger delignification because it removes lignin’s that are bonded to carbohydrates. About 49% lignin degradation was observed when with Ozonolysis (Canilha et al., 2012).

2.2.1.1.3. Physicochemical pretreatment

2.2.1.1.3.1. Ammonia fiber expansion (AFEX)

In the AFEX process, biomass is treated with liquid ammonia at high temperature and pressure. After a few seconds, pressure is swiftly reduced. A typical AFEX process is carried out with 1-2 kg ammonia/kg dry biomass at 90 ^{O}C during 30 minute. It reduces the lignin content and removes some hemicellulose while decrystallising cellulose. The cost of ammonia and especially of ammonia recovery drives the cost of the pre-treatment, although ammonia is easily recovered due to its volatility (Ribeiro et al., 2020).

2.2.1.1.3.2. Steam explosion (Auto hydrolysis)

Steam explosion is the most promising method under physicochemical pretreatment. Lignocellulosic materials are subjected to high-pressure saturated steam (0.69–4.83 MPa) and temperature (160–260°C) for a short period of time like to several seconds to minutes and then suddenly the pressure is reduced to atmospheric pressure.

Hemicelluloses and lignin materials can be released through stream pretreatment. Residence time, temperature, biomass size, and moisture content are the most influencing parameters in such process. Due to some attractive features such as low environmental influence, utilization of less harsh chemicals, and high-energy efficiency, steam explosion has now been widely used before lignocellulosic hydrolysis. The main drawback of this process is the production of inhibiting aromatic compounds or byproducts, which are required to be detoxified through vigorous water washing or other methods (Saratale et al., 2013)

2.2.1.1.3.3. Carbon dioxide explosion

In this process, supercritical carbon dioxide is used that behaves like a solvent. Supercritical fluids are compressed at room temperature above its critical point. When carbon dioxide is dissolved in water, carbonic acid is formed which causes less corrosiveness due to its special features. During the process, carbon dioxide molecules enter into small pores of lignocellulosic biomass due to its small size. Carbon dioxide pretreatment is operated at low temperature which helped in prevention of sugar decomposition by acid.Cellulosic structure is disrupted when carbon dioxide pressure is released which ultimately increased the accessibility of the substrate

to the cellulolytic enzymes for the process of hydrolysis. Carbon dioxide pretreatment for alfalfa and observed 75% theoretical release of glucose (Saratale et al., 2013).

2.2.1.1.3.4. Liquid hot water (LHW)

Hot compressed water is another terminology used for this method of treatment. High temperature (160–220°C) and pressure (up to 5 MPa) are used in this type of pretreatment in order to maintain the liquid state of water. In this method, water in liquid form remains in contact with lignocellulosic biomass for about 15 min. In this treatment pressure is used to prevent its evaporation, and sudden decompression or expansion in this pretreatment process is not needed. This method has proved to be very effective on sugarcane bagasse, wheat and rye straw, corncobs, and corn Stover. Disadvantage of liquid hot water pretreatment is high energy consumption requirement for downstream process because of the involvement of large amount of water. However, the advantage of this process is that chemicals and catalysts are not required and no inhibitor is formed (Kumar et al., 2009).

2.2.1.1.3.5. Wet oxidation

The wet oxidation process occurs in the presence of oxygen or catalyzed air, where the most used catalyst is the sodium carbonate. Wet oxidation allows obtaining high yields of biomass conversion into monosaccharaides with low formation of furan and phenolic aldehydes. In the wet oxidation process, the delignification is reported with the increasing of aliphatic acids. This pretreatment is considered expensive. The major advantage of this pretreatment is the combination with alkalis where it is possible to achieve released sugars without generation of furfural and 5-hydroxymethylfurfural, undesirable compounds for fermentation (Canilha et al., 2012).

2.2.1.1.4. Biological pretreatment

Conventional methods for chemical and physical pretreatments require expensive reagents, equipment, and high energy. On the other hand, biological pretreatment requires live microorganisms for the treatment of lignocellulosic material, and this method is more environments friendly and consumes less energy. There is certain microorganism present in nature that exhibit cellulolytic and hemicellulolytic abilities. White-rot, soft-rot, and brown fungi are known for lignin and hemicellulose removal with a very little effect on cellulose.

White rot is able to degrade lignin due to the presence of lignin degrading enzymes like peroxidases and laccases. Cellulose is commonly attacked by brown rot, whereas white and soft rot target both lignin and cellulose contents of plant biomass.Generally, microorganisms degrade untreated bagasse slowly; therefore, isolation of efficient strains is regarded as an important research area for lignin degradation in SCB (Cardona et al., 2010)**.**

Table 3: Advantages and limitations of various pretreatment strategies (Paulová et al., 2013)**.**

Pretreatment method	Advantages	Disadvantages
Ball milling	Increases the surface area of biomass; decrease in crystallinity and degree of polymerization of cellulose; no chemical requirement; functional groups are not generated	High energy requirement and is not economically feasible
Wet disk milling	reduction in particle size, increase in surface area, pore volume, reduced crystallinity index of cellulose, no release of fermentation inhibitors	High energy consumption
Irradiation	does not involve use of solvents in large quantities, recovery or recycling, downstream steps of cooling and neutralization after biomass pretreatment are not required	During technology development, the commercial implementation is a costly affair, safety regulations are to be followed while using radiations to avoid health hazards associated
Alkaline Pretreatment	Saponification of intermolecular ester bonds cross-linking hemicellulose and lignin. Decrease in the degree of polymerization of cellulose and causes swelling of cellulose leading to an increase in its internal surface area	irrecoverable salts and incorporation of salts into the biomass during the pretreatment reactions makes the alkaline pretreatment challenging issue, high cost of catalyst
Ozonolysis	Low generation of inhibitory furfural and HMF (which might hinder following downstream stages). Selective lignin degradation with minimal effects on cellulose and hemicellulose. Operation at ambient temperature and pressure	Highly reactive, flammable, corrosive and toxic characteristics of ozone, leading to potentially dangerous processes. cooling systems are required for exothermic processes
Organosolv	easy process, possibility of recovery of solvent and the effectiveness of organic solvent to break the internal bonding of lignin with hemicellulose	expensive, so they should be recovered as much as possible, solvents need to be drained from the reactor, evaporated, condensed, and recycled, this causes an increase in energy consumption
Hydrothermal pretreatment	reduces the downstream pressure by making cellulose more accessible to the enzymes, minimizes the formation of degradation products, catalyst is not needed	LHW pretreatment pattern selected for a certain lignocellulosic biomass and combined with other pretreatments would overcome its disadvantages of high water consumption and energy input
Ionic liquids	achieve high sugar yields with low biomass loading, mild process conditions, makes cellulose to amorphous, when combined with other methods can efficiently process a wide range of lignocellulosic feedstock's	Expensive, recovery of solvents besides, as they are recycled and reused, the efficiency of the ILs for pretreatment decreases
Steam explosion	hemicellulose solubilization and lignin transformation; cost-effective technique; high yield of cellulose and hemicellulose in a two-stage process	Partial destruction of hemicellulose, incomplete disruption of the lignin-carbohydrate matrix; generation of compounds inhibitory to microbes
AFEX	increases accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for downstream processes	AFEX not efficient for biomass with high lignin content, high cost of ammonia, recycling of ammonia
Biological pretreatment	low impact on the environment, increased yield of the product, mild reaction conditions, few side reactions, less energy demand, decreased reactor necessities to resist pressure and corrosion and reduces the formation of inhibitors since there are degradation of sugar compounds	Longer duration time

2.2.2.Hydrolysis of Cellulose (scarification)

Hydrolysis is the chemical reaction that converts the complex polysaccharides in the raw feedstock to simple sugars.Cellulose obtained from pretreatment should be degraded into glucose (scarification) using acids or enzymes (Cardona et al., 2010)**.** Hydrolysis of cellulose is very critical for bio-ethanol production, because only glucose, not cellulose, can be consumed by the bacteria/yeast used in fermentation to produce bio-ethanol. Hydrolysis is conducted in the presence of enzymes (exoglucanases, endo-glucanases and cellobiases) or mineral acids and releases glucose units from the cellulose molecules (Karp et al., 2013). In terms of cost, chemical hydrolysis is more advantageous than enzymatic because of the high cost of producing enzymes (Megawati et al., 2020).

$$C_6H_{10}O_5 \text{ (Cellulose)} + nH_2O \longrightarrow n\ C_6H_{12}O_6 \text{ (Glucose)}$$

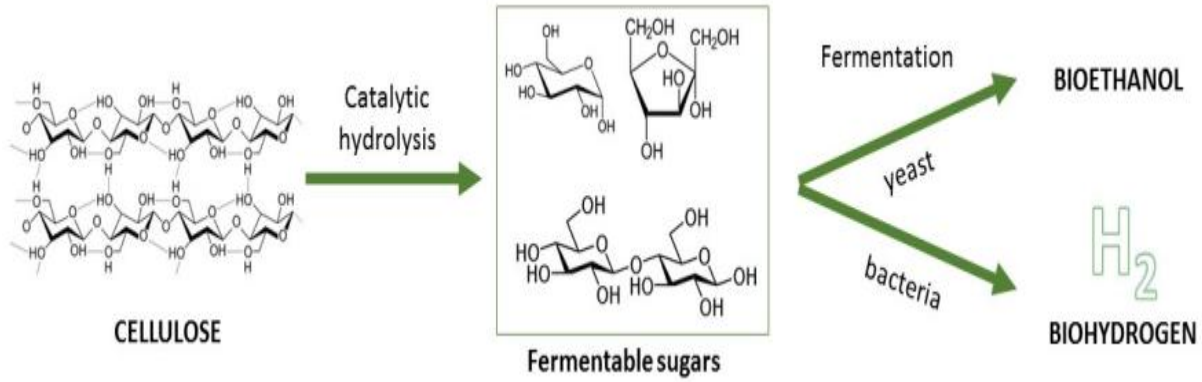


Figure 12**:** Conversion of biomass to biofuels (Medina, 2018)

2.2.2.1. Acid hydrolysis

Acid hydrolysis can be classified into two categories: concentrated and dilute. Diluted acid hydrolysis and concentrated acid hydrolysis are two conventional techniques where lignocellulose materials are exposed to diluted acid or concentrated acid for a specific period of time and specific temperature for the conversion of simple sugars. From the various types of acids used, hydrolysis using sulfuric acid is more efficient, because it has many advantages; the price is cheaper and available in large quantities (Megawatiet al., 2020).

2.2.2.1.1. Dilute acid hydrolysis

According to (Cardona et al., 2010) dilute acids (H_2SO_4 and HCl) are employed, temperatures of 200–240 ^{O}C at 1.5% acid concentrations are required to hydrolyze the crystalline cellulose, but the degradation of glucose into HMF and other non-desired products is unavoidablee under these conditions. One variant of the acid hydrolysis is the use of extremely low acid and high-temperature conditions during batch processes.

According to(Vinícius et al., 2012**;** Gurgel et al., 2012) dilute acid concentration (up to 3–4%) is used at temperatures of 100–240 °C. Dilute acid hydrolysis has many advantages compared with concentrated acid and enzymatic hydrolysis, such as reduced corrosion, shorter reaction times, and higher reaction rates

Several acids can be used, such as HCl, H_2SO_4, H_3PO_4 and HNO_3. At temperatures between 110–140 °C hemicellulose is hydrolyzed, while crystalline cellulose remains practically unchanged up to 170 °C and its hydrolysis takes place up to 240 °C. The difference between these two parts dominates the design of a two-stage process. The separate hydrolysis of the hemicellulose and cellulose parts has already been studied for wood biomass concerns initial hemicellulose hydrolysis in low temperature (120–150 °C) and then cellulose hydrolysis at higher temperatures of up to 240 °C. During pre-hydrolysis the lignin-hemicellulose complex is broken down, facilitating the hydrolysis of hemicellulose and the production of sugar, mainly xylose, under relatively mild conditions. However at increased temperature xylose is broken down and undesirable byproducts are formed. Sugar removal is thus required before the activation of the second stage. The next step is the application of higher temperatures (>170 °C) and potentially increased acid concentrations, so that the cellulosic part will be hydrolyzed. The two stage process has several advantages such as:

- It allows the production of useful byproducts such as xylitol and arabitol
- it increases the cellulose breakdown during hydrolysis and consequently the sugar yield
- It is more economical than the concentrated acid reaction because it requires cheaper equipment
- Important environmental problems related to the use of strong acids are avoided in its general design and management is less complicated than that of enzymatic hydrolysis.

However, it is noted that the operating conditions need to be carefully selected in order to avoid high concentrations of byproducts with significant inhibitory effect during the fermentation. Also, before the fermentation process, the hydrolyzates' pH should be regulated, in order not to suspend the metabolism of the fermentation microbial cultures.

2.2.2.1.2. Concentrated acid hydrolysis

Concentrated acid hydrolysis requires reactors that are resistant to corrosion, and the acid must be recovered after hydrolysis to make the process economically feasible (Science, 2014). Diluted acid hydrolysis is comparatively more advantageous as it facilitates the step-by-step hydrolysis for the separation of hemicellulose and cellulose compounds, whereas concentrated acid (70–90%) hydrolysis mainly facilitates the liberation of hemicellulose sugars. Minimum sugar degradation and achieving maximum sugar yield up to 100% are the most positive features of such process, although the high cost of acid consumption and environmental and corrosion problems make the process unsuitable for commercial applications (Mekala et al., 2014).

2.2.2.2. Enzyme hydrolysis

Enzymatic hydrolysis requires pretreatment of lignocellulosic material to improve accessibility and large investments in genetic engineering to develop modified microorganisms that are capable of synthesizing hydrolytic enzymes on a large scale at low cost.Enzymatic hydrolysis comes up with 100% selective conversion of hemicellulose materials to simple sugars with fewer requirements of energy and mild environmental conditions. The recalcitrance nature of lignocellulose materials due to the presence of lignin, high surface area, and cellulose crystallinity makes the enzymatic hydrolysis very slow. Mild environmental conditions (temperature 40–50°C, pH 4–5), less corrosion properties, high yield (75–85%) of reactions, and selective operations make the enzymatic hydrolysis more suitable than acid hydrolysis.

Enzymatic hydrolysis is substrate specific, as cellulase enzymes break down bonds of cellulose molecules and hemicellulose enzymes are only responsible for hemicellulose molecules. Cellulases are group of enzymes that synergistically hydrolyze cellulose to glucose monomers.

Such enzymatic mechanism is carried out by endo-glucanases, exoglucanases, or cellobiohydrolases and β-glycoside enzymes. Endo-glucanases hydrolyzes intermolecular β-1, 4-glucosidic bonds of cellulose chains randomly to produce new chain ends, whereas exoglucanases suitably cleave cellulose chains at the ends to release soluble cellobiose or glucose and β-glycosides hydrolyze cellobiose to glucose. Hemicellulases are the complex mixture of at least eight enzymes that hydrolyze hemicellulose to xylose and arabinose as five-carbon sugars and galactose, glucose, and mannose as six-carbon sugars (Brunner, 2014).

Table 4: Difference between acid and enzymatic hydrolysis (Jahnavi et al., 2018).

Comparing variable	**Dilute/concentrated acid hydrolysis**	**Enzymatic hydrolysis**
Hydrolysis	Temperature used is high (100-240)	Carried out at mild conditions at 40-50oC
Yields	Higher sugar recovery is not possible in dilute acid hydrolysis	High yield of sugars can be obtained
Inhibiters	Results in the formation of inhibiters	No inhibiters formation
Product inhibition during hydrolysis	No	Yes
Cost of catalyst	Law	High
Time of hydrolysis	Occurs in short time periods (from minutes – hour)	Takes longer duration (from hours to days)

Due to the high cost and unavailability an enzyme cellulase and hemicelulase for this work the conventional dilute acid hydrolysis operating conditions will be optimized.

2.2.3.Detoxification

During pretreatment of lignocellulosic, in addition to the sugars, aliphatic acids (acetic, formic and levulinic acid), furan derivatives furfural and HMF, and phenolic compounds are formed. The existence of these substances is more probably when acid and/or high-temperatures are used. These compounds are known to affect ethanol fermentation performance. Furfural could be generated as a degradation product from pentoses. It was found that furfural contents increase with the concentration of the acid catalysts such as H_2SO_4. Several detoxification methods like neutralization, over liming with calcium hydroxide, activated charcoal, ion exchange resins and enzymatic detoxification using laccase (are known for removing various inhibitory compounds from lignocellulosichydro lysate (Cardona et al., 2010)**.**

2.2.3.1. Over-liming

Over liming the hydrolyzates has been effective as a detoxification process due to partial removal of toxic inhibitors, such as furfural and 5-hydroxymethylfurfural, although the whole mechanism is not well understood. During over liming, sulphuric acid is removed from the initial hydrolyzates by adding lime to adjust the pH and precipitation as gypsum. However, it has been observed that the concentrations of acetic acid before and after the detoxifying treatment were not altered significantly; however it is effective in removal of furans (45.8%) and phenolics (35.87%) (Martinez et al., 2001).

2.2.3.2. Adsorption with activated charcoal

Charcoal adsorption decreases the concentrations of both acetic acid and phenolics derived from the SCB hydrolyzates. Treatment with activated charcoal caused 38.7%, 57% and 46.8% reduction in furans, phenolic and acetic acid, respectively (Cardona et al., 2010).

2.2.3.3. Ion exchange resins

Ion exchange treatment has demonstrated to be an efficient method for removing furans (63.4%), total phenolics (75.8%) and acetic acid (85.2%) from SCB hydrolyzates (Cardona et al., 2010).

2.2.3.4. Enzymatic detoxification

Treatment with the enzymes like laccase, obtained from the ligninolytic fungus Trametes vesicular, has been shown to increase the ethanol productivity in a hemicellulose hydrolyzates of SCB (Chandel et al., 2014). The laccase treatment led to selective removal of total phenolics by 77.5% without affecting furans and acetic acid content of the hydrolyzates(Jönsson et al., 2013; Walker&Stewa**rt**, 2015 and Karimi et al., 2007).

2.2.3.5. Electro dialysis

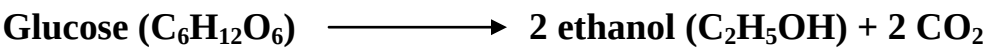
Another detoxification method more currently used is electro dialysis (ED), which is an electrochemical separation process in which electrically charged membranes and an electrical potential difference are applied to separate ionic species from an aqueous solution and other uncharged components.

Volatile compounds, such as furfural, were stripped by boiling, while acetic acid and sulfuric acid were removed by electro dialysis. After treatment by electro dialysis, 90% of acetic acid in hydrolyzates was removed (Cardonaet al., 2010).

2.2.4.Fermentation

Fermentation is a series of chemical reactions that convert sugars to ethanol. A wide variety of bacteria and fungi have the ability to ferment sugars into ethanol, but only a few are suitable for the challenging conditions encountered in industrial applications. The production of alcoholic beverages from fermentable carbon sources by yeast is the oldest and most economically important of all biotechnologies. The yeast species that dominates in the production of alcoholic beverages worldwide is *Saccharomyces cerevisiae* whichis regarded as ethanol genic yeast that can readily ferment glucose, fructose, mannose, galactose, sucrose, maltose and maltotriose into ethanol and carbon dioxide (Walker & Stewart, 2016).It is regarded as the best microorganism for industrial ethanol production due to its high specific ethanol productivity, and its high tolerance to ethanol and osmotic pressure from substrates and salts (Osman & Elhussieny, 2014).

Saccharomyces cerevisiae is used extensively in batch fermentations to convert sugars to ethanol for the production of beverages and biofuels(Dombek & Ingram, 1987). According to (Bonassa et al., 2015), there are several factors that affect the efficiency of the conversion of sugars into ethanol, ranging between physical (temperature, osmotic pressure), chemical (pH, oxygen, mineral and organic nutrients, inhibitors) and microbiological (species, strain and concentration of yeast, bacterial contamination). These factors may influence the efficiency of alcoholic fermentation and, thus, the efficacy of the conversion of sugar into ethanol.



2.2.4.1. The methods of fermentation

2.2.4.1.1. Simultaneous scarification and fermentation (SSF)

The pretreated SCB substrate is hydrolyzed to monosaccharide and then directly converted to ethanol in the same vessel while the scarification is still in process.

SSF process is a method to combine the scarification of pretreatment biomass with simultaneous fermentation of reducing sugars formed from hydrolysis that could not only utilize substrate more efficiently and increase product yield but also reduce inhibition and deactivation of Cellulases that occurs due to the high concentration of released sugars in a single reactor. The main feature of this process is that once sugars are produced in cellulose hydrolysis, they are rapidly converted into biofuels (Lü and Wang,2021)

Figure 13: Schematic representation of the SSF.

2.2.4.1.2. Separate hydrolysis and fermentation (SHF)

SHF is the traditional design, in which the hydrolysis of the cellulose, after pretreatment of the raw material, is made prior to fermentation. In this form of conduction, hydrolysis of the biomass is initially performed. After hydrolysis, the medium is centrifuged, the unhydrolyzed solid is discarded, and in the liquid medium the fermentative microorganism is inoculated. The process continues until the sugars in the medium are consumed. An advantage of this process is the possibility of performing each step, hydrolysis and fermentation, under the optimal conditions. Is more commonly adopted, since it allows the maximum optimization of each process individually. Because scarification and fermentation usually require different optimal conditions for highest efficiency, the simultaneous process tends to result in inferior system performance (Fernando Santos et al., 2020).

Figure 14: Separate hydrolysis and Fermentation (SHF)

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Descriptions of the Study Site

This study was carried out at sugar technology laboratory of Wonji Research center of the Ethiopian sugar corporation.The experimental material used for this study was sugarcane bagasse.The bagasse samples were collected from Wonji sugar factory at specific place called Dodota. About two kilograms of bagasse was taken and dread in an oven (model 30-1060, Gmbh, Germany) at 105 ^{O}C for 24 hour. After drying, it was grinded using mill grinder and sieved with sieve having mesh size of 4 mm. The grinded sample was stored in polyethylene plastic bag to keep sample dry until used. Wonji sugar factory is located 110 kms from Addis Ababa in East shoa zone of Oromia national regional state, at 10 kms from Adama town in south-east direction. It is situated at 8.38^{o}N latitude and 39.30^{o}E longitude with an altitude range of 1223 and 1553m above sea level. The area has a semi-arid climate with long year's average rainfall of 70mm with mean maximum and minimum annual temperature of 27^{O}Cand 12 ^{O}C, respectively. The soils of the area used for sugarcane cultivation are predominantly Andsols, Fluvisols, Laptosols and Phaezemes.

3.2. Chemicals and Equipment Used

All the chemicals and reagents used in this study were analytical grades and includes: Sulfuric acid (H_2SO_4)(AR, Assay 98%), Sodium Hydroxide (NaOH) (AR, Assay 98%), DNS (dinitrosalicylic acid or 2-hydroxy-3,5-dinitrobenzoic acid), Sodium sulfate(Na_2SO_4), Na-K tartrate (KNaC4H4O6·4H2O)(AR, Assay 99.5%), Potassium Bromide (KBr), Acetone, yeast extract, Dry yeast (Saccharomyces cervicea), Peptone, Glucose ($C_6H_{12}O_6$), Urea, Activated charcoal, milk of lime ($Ca(OH)_2$), Ammonium sulfate ($(NH_4)_2SO_4$), citrate buffer, magnesium sulfate pent hydrate ($MgSO_4.5H_2O$) (AR, Assay 99.5%), potassium phosphate mono basic (KH_2PO_4) (AR, Assay 99.5%), sodium phosphate mono hydrogen (Na_2HPO_4), Zinc Sulfate heptahydrate ($ZnSO_4.7H_2O$).

In this study equipment's used were: Vertical autoclave ,Vacuum filtration pump, Uv-Visible spectrophotometer, Water bath (Sr.N. 106544116, Germany), microkajeldhal digestion apparatus, incubator, HANNA pH meter (model pH 211, Romania), Thermo denly Horizontal shaker (Sr.N. 321, Germany), Sieve, memmert Oven, electronic Ebullio meter, mill grinder (Sr.N. 129070704D, Germany), electronic analytical balance(model: WB1160078, kern & sohn Gmbh, Germany), digital thermometer, muffle Furnas (model: 683, UK), Hot Plate with magnetic stirrer (model: RHB2, IKA) , condenser, relaxing bottle, Centrifuge.

3.3.**Experimental Design and Treatments**

3.3.1. The effect of dilute NaOH treatment on Sugarcane bagasse

The effects of dilute NaOH under different reaction pressure and time on extraction of cellulose, hemicellulose, lignin, ash and extractive from sugarcane bagasse were evaluated. Accordingly, different concentrations of NaOH (0.5, 2.5 and 5 %) under different reactions pressure (10, 15 and 20 Psi) and time (5, 20 and 35 minutes) with a total of 3*3*3 = 27 treatment combinations were investigated. The diluted NaOH pretreatment on sugarcane bagasse was carried in a vertical autoclave (model VSL-52, India), equipped with digital temperature and time controller while the amount of solid (sugarcane bagasse) to liquid (NaOH) ratio was kept constant at 1:10(g/ml). The experiment layout was completely randomized design with three factor factorial treatment arrangements (NaOH, pressure and time). Each treatment was replicated twice. Treatment without NaOH (0%) and without autoclaving was used as control treatment. After cooking, the contents were transferred to a stainless steel sieve of 150 mesh size and washed toughly with distilled water until neutral pH. Then, data on the amount of extracted cellulose, hemicellulose, lignin, ash and extractive were collected. The percentage of each of the collected data were computed and used for statistical analysis to determine the optimum treatment combination. In addition, a regression model between the optimum response (as dependent variable) and optimum treatment combination (as independent variables) wasdeveloped. The optimum treatment combination of dependent and independent variables were modeled by equation: 3.4.1 (Medhanit, 2020).

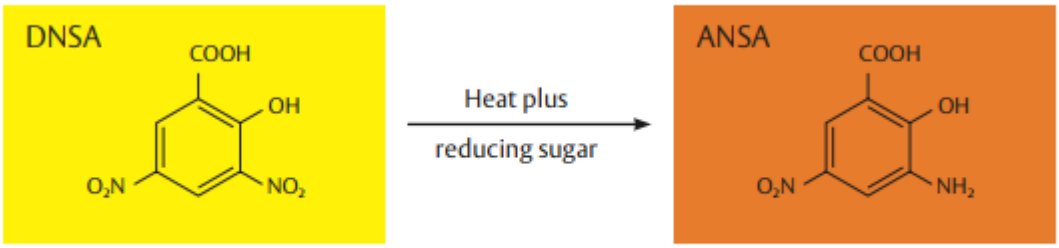
$$Y=\beta_o+\sum_{i=1}^{3} BiXi + \sum_{i=1}^{3} BijXiXj \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq.\ 3.4.1$$

Where Y is the response, βo, βi, βii and βij are an intercept, linear, quadratic and interaction coefficients, respectively and Xi and Xj are the independent variables.

3.3.2. Impact of diluted H_2SO_4 on Sugarcane bagasse cellulose hydrolysis.

To carry out this experiment, DNSA reagent was prepared with mixing 1 g DNSA in 20 ml 2M NaOH and named reagent I. Then, in 100 ml volumetric flask 30 gram of sodium potassium tartrate was added and marked to make the final volume 100 ml and named as reagent II. Finally solution I and IIwere mixed according to the procedure used by Garriga et al., (2017).

A standard glucose solution (0.25g/l solution) was prepared by mixing 0.025 g of glucose powder to 100 ml volumetric flask and marked with DW. Form preparedstock standard glucose solution, take 0, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 4.5 ml to a test tube; and to other test tubes 1 ml of each unknown sample was pipetted. Then, test tube containing the standard solutions and unknown were marked to a total volume of 4.5 ml with DW.Then after, 1 ml DNSA reagent prepared before was mixed with all standards and unknowns. The standards and unknown samples in a test tube was boiled in water bath at 95 ^{O}C for 5 minute and cooled at room temperature. On heating the color of the reagent changes from yellow to orange or red, depending upon the concentration of reducing sugar present.



Finally, the optical density of both the sample and the standard were measured with spectrophotometer at wave length off 540 nm after which the curve was plotted using the standard glucose solution verses its optical density to determine the concentration of reducing sugar in unknown sample.

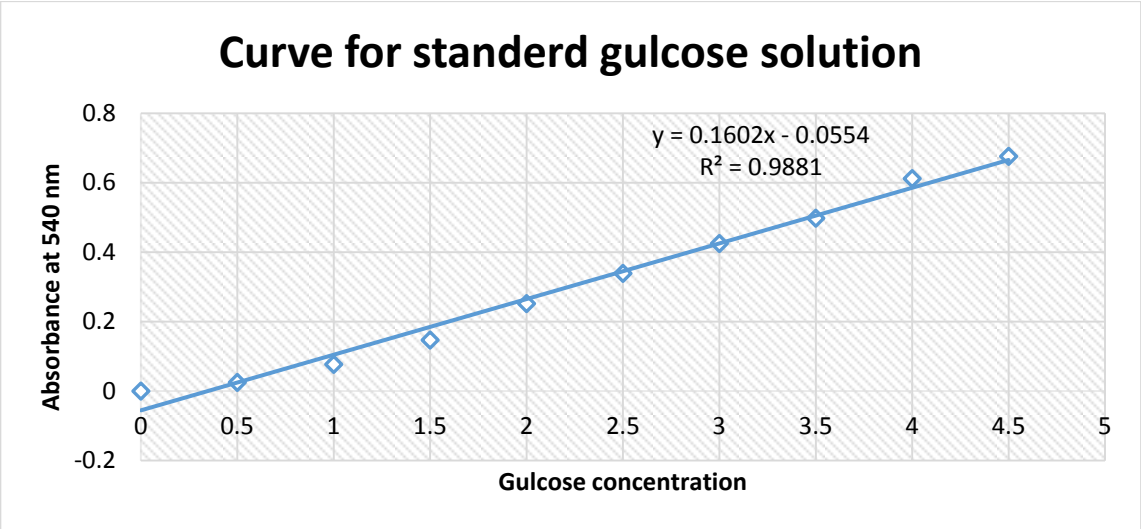


Figure 15: Glucose standard curve for determination of unknown sugar inhydrolyzates

Then, the developed curve (figure 15), was used to determine the unknown concentration of reducing sugar from hydrolysis of sugarcane bagasse cellulose.

The effects of dilute H_2SO_4 under different reaction temperature and time on reducing sugar yield were evaluated. The optimum treatment combination obtained from experiment one was used to lay this experiment. Accordingly, different concentrations of H_2SO_4 (1, 2 and 3 %) under different reactions temperature (160, 190 and 220 ^{O}C) and time (20, 40 and 60 minutes) were used as initial levels. These initial levels for factor were re-leveled using central composite design under response surface methodology. Then, the adjusted concentrations of H_2SO_4 (0.32, 1, 2, 3 and 3.68 %), reaction temperature (139.55, 160,190,220 and 240.45 ^{O}C) and reaction time (3.36, 20, 40, 60 and 73.64 minutes) were used for the study. The diluted H_2SO_4 on sugarcane bagasse cellulose hydrolysis was carried out with Kjeldahl digestion block (Sr.N. 008-1027, Germany). The amount of solid (sugarcane bagasse cellulose) to liquid (H_2SO_4) ratio was kept constant at 1:20(g/ml). The experiment arrangement was completely randomized design. Each treatment was replicated twice. Then, after separation of the hydrolyzates from suspended solid using vacuum filtration pump (Sr.N. 31338601, Germany) the optical density of the hydrolyzates was measured at 540 nm wave length using spectrophotometer (Sr.N 923-384, Cambridge, England)method validated by (Garriga et al., 2017); (Biotechnol, and Tripathi, 2018) with slight modifications. The optimum treatment combination of dependent and independent variables were modeled by equation: 3.4.2 (Medhanit, 2020).

$$Y=\beta_o+\sum_{i=1}^{3} BiXi + \sum_{i=1}^{3} BiiXi2 + \sum_{i=1}^{3} BijXiXj \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq.3.3.2$$

Where Y is the response, βo, βi, βii and βij are an intercept, linear, quadratic and interaction coefficients, respectively and Xi and Xj are the independent variables.

3.3.3. Optimization of fermentation process for sugarcane bagasse hydrolyzates using *Saccharomyces cervicea.*

For this experiment, yeast (*Saccharomyces cervicea*) inoculums and media preparation are necessary and prepared according to method used by (Sarah *et al*., 2020; Turhan *et al*., 2010). Yeast extract and peptone (YP) 10× were prepared by mixing 50 g of yeast extract and 100 g of peptone into distilled water (DW), to a total volume of 0.5 L. This solution was autoclaved at 121 ^{O}C for 30 min and used as a stock solution for media preparation. Glucose solution (50%) was prepared by diluting 125 g of glucose in DW to a total volume of 0.25 L. YP-glucose (5%) was prepared by mixing 100 mL of YP 10× and 100 mL of 50% glucose solution in 800 mL of DW. Citrate buffer (pH 4.5, 1 M) was prepared by adding 192 g of anhydrous citric acid to DW to a total volume of 1 L; the solution was titrated to a pH of 4.3 with a solution of sodium hydroxide 10 M (NaOH). The yeast pre-culture media was prepared by mixing 1/2 L of YP-glucose 5%, 100 mL of citrate buffer, and adding 3.07 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), 1.80 g of potassium phosphate monobasic (KH_2PO_4), 4.87 g of sodium phosphate monohydrate ($Na_2HPO_4 \cdot H_2O$), 0.32 g of zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$), and DH_2O to a total volume of 750 ml. To this, 1.0 g of dried *Saccharomyces cerevisiae* was added. The yeast inoculum was incubated at 32 ◦C in an incubator for 19 h in using horizontal shaker at 180 rpm to a viable yeast concentration of ~1 × 10^8 cells/ml. Before incubation of the media was started, the hydrolyzates was detoxified (conditioned) using $Ca(OH)_2$ and activated charcoal according to the method used by (Hajar et al., 2017; Canilha et al., 2012). Then, the prepared inoculums and media were incubated in an incubator (model ES120, London, UK) with 5:1 ratio and at the end the incubation period, the fermented sample was centrifuged using centrifugal (Sr.N. 5696/03/013, UK) to separate the supernatant solution and yeast cell and finally, the bio-ethanol content were determined using *electronic* Ebullio meter (Sr.N. E60172 CON Treading, USA) after which the samples were used for this experiment.

In this experiment, the effects of temperature, initial pH of the hydrolyzates and incubation time on fermentation of sugar from sugarcane bagasse hydrolysis (experiment 2) was evaluated using *Saccharomyces cervicea.* The optimum treatment combination obtained from experiment 2 was used to lay this experiment**.** Accordingly, initial temperature of 30, 35 and 40 ^{O}C, initial pH of the hydrolyzates at 4, 5 and 6; and incubation time of 24, 48 and 72 hours were selected. Then, based on guidance of central composite design under response surface methodology, the adjusted temperature at 26.95, 30, 35, 40 and 43.41^{O}C, with the adjusted initial pH of 3.32, 4, 5, 6 and 6.68; and incubation time of 7.64, 24, 48, 72 and 88.36 hours were used for this experiment. The amount of hydrolyzates to yeast inoculums ratio was kept constant at 5:1 (ml/ml).

The experiment arrangement was completely randomized design. Each treatment was replicated twice. Then, the optical density of the hydrolyzates was measured at 540 nm wave length using spectrophotometer method validated by (Garriga et al., 2017; Biotechnol and Tripathi, 2018) with slight modifications. The optimum treatment combination of dependent and independent variables were modeled by equation: 3.4.2 (Medhanit, 2020).

$$Y=\beta_o+\sum_{i=1}^{3} BiXi + \sum_{i=1}^{3} BiiXi2 + \sum_{i=1}^{3} BijXiXj \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq.\ 3.3.3$$

Where Y is the response, βo, βi, βii and βij are an intercept, linear, quadratic and interaction coefficients, respectively and Xi and Xj are the independent variables.

3.3.4. Response variables collected

For the first experiment, data on compositional analysis of untreated and alkali pretreated sugarcane bagasse cellulose,hemicellulose, lignin,ash and extractive were collected gravimetrically based on method developed by (Chesson, 2016). Based on this method, one g of dry sample (a) was added with 150 ml H_2O and refluxed at 100 ^{O}C in water bath for 1 h. The result was filtered, and the residue washed with 300 ml hot water. The residue was dried until constant in an oven then weighed (b). Residue was added 150 ml of 1 N H_2SO_4 then refluxed in a water bath temperature of 100^{O}C for 1 h. The results were filtered to neutral (300 mL) and dried (c). Dried residue was added with 10 ml of 72% H_2SO_4 and soaked at room temperature for 4 h. Residue then added 150 ml of 1 N H_2SO_4 and refluxed on a water bath for 1 h.

Residue was filtered and washed with H_2O to neutral (400 ml) and then dried with a temperature of 105 ^{O}C and the results weighed (d), and ashed in the furnace (e): Then, the compositions were computed using the following formula.

$$\%\ Liginin\ content = \left[\frac{d-e}{a}\right] * 100\%$$

$$\%\ Cellulose\ content = \left[\frac{c-d}{a}\right] * 100$$

$$\%\ Hemicellose\ content = \left[\frac{b-c}{a}\right] * 100\%$$

The computed percentages for each response variable were used for statistical data analysis.

For the second experiment (experiment 2) data on determination of reducing sugar was collected through spectrophotometer by measuring the optical density at 540 nm. Then the optical density was converted to concentration using the standard curve (Y= 0.1602X – 0.0554, where Y is the optical density and X is the concentration of unknown) obtained from known concentration. Then, the value for the concentration of reducing sugar obtained from curve was used for statistical data analysis.

Similarly, for the third experiment, data on the percentage of bio-ethanol was directly measured usingelectronic Ebullio meter. The obtained data was converted to gram per liter units and used for statistical data analysis.

Unit conversion used between percent and g/L was calculated by

$$1\% = \frac{10000\text{mg}}{\text{L}} = \frac{10\text{g}}{\text{L}}$$

3.4.**Statistical data analysis**

The collected data from experiments were subjected to statistical data analysis using Design expert version 7. Specifically experiment 1 was subjected multilevel categorical design while for data from experiment 2 and 3 were computed using central composite design under response surface methodology.

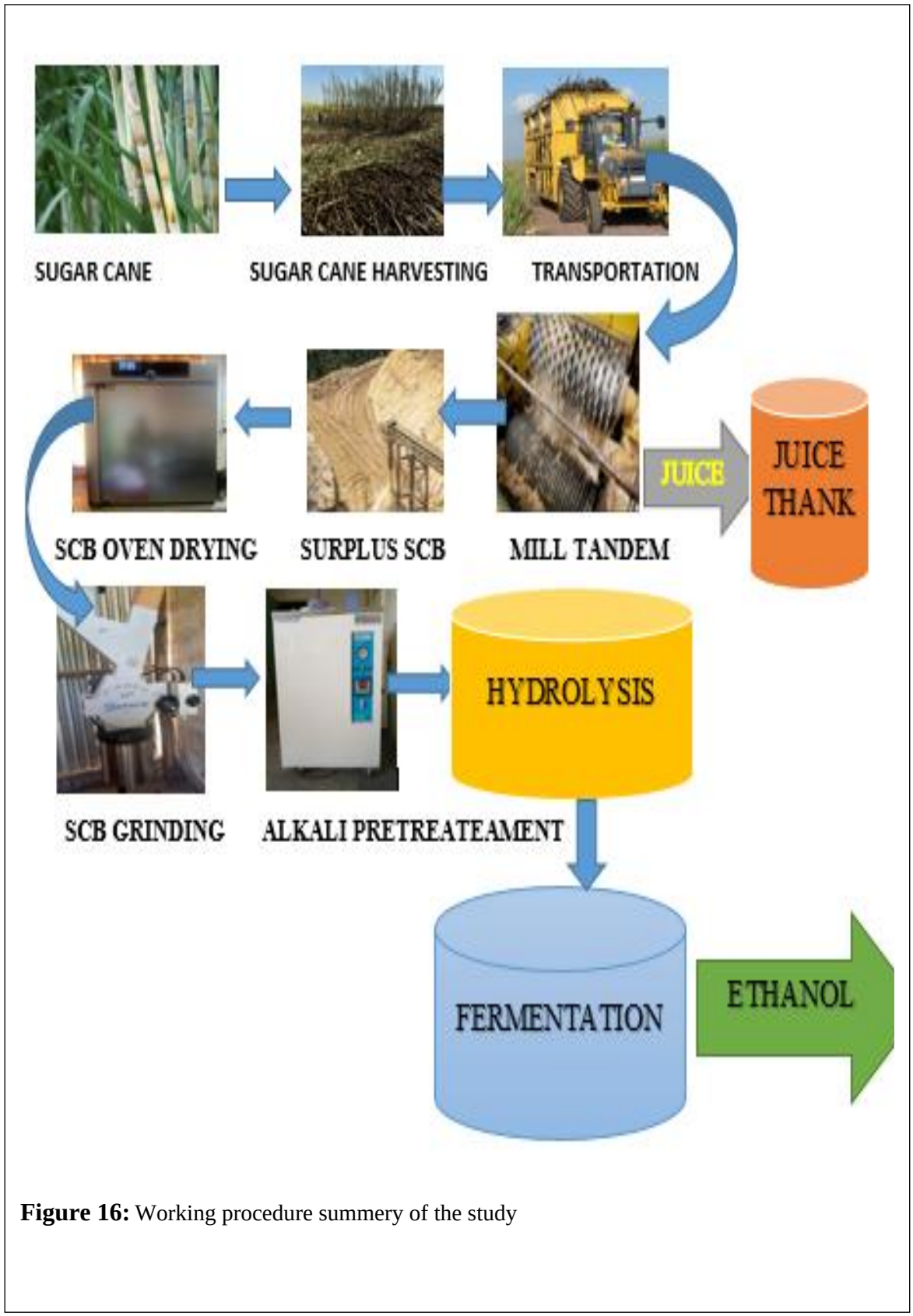


Figure 16: Working procedure summery of the study

CHAPTER FOUR

4. Result and Discussion

4.1.The effect of dilute NaOH treatment on Sugarcane bagasse

Analysis of variance revealed that**,** except on the percentage of hemicellulose, pressure has a significant effect on both cellulose and lignin percentages (Table 5). The other main factors, NaOH and time have a significant impact on all the response variables tested. *I.e*. cellulose (%), hemicellulose (%) and lignin (%). Similarly, the interaction of pressure (psi) and NaOH (%) affected all the response tested. The interactions of autoclave pressure and time affected only the percentage of hemicellulose and lignin while NaOH and time showed a significant effect only on cellulose and hemicellulose percentage. The result also proved that the interaction effects of the three factors have a significant effect on all of the responses tested and hence the mean separations of these interactions were considered to determine the optimum treatment combination.

Table 5. ANOVA summary for the effects of NaOH, pressure and time on Cellulose, hemicellulose and lignin

Source of Variation	**DF**	**Mean squares**		
		Cellulose (%)	**Hemicellulose (%)**	**Lignin (%)**
Model	26	60.68*	13.38*	12.21*
A	2	145.12*	2.29ns	2.76*
B	2	414.47*	66.05*	76.10*
C	2	62.17*	28.12*	22.81*
A*B	4	12.88*	9.75*	8.96*
A*C	4	5.59ns	4.96*	11.82*
B*C	4	20.52*	13.05*	1.49ns
A*B*C	8	23.05*	5.48*	3.11*
CV		8.39	10.81	8.39
R^2		0.96	0.8296	0.9414
Adj. R2		0.93	0.66	0.89
Pred. R2		0.86	0.097	0.77

Remark: A= Pressure B=NaOH; and C=Time. '' indicates the effect of the specific factor has a significant effect on the specified response variable at alpha =5% while 'ns' indicates non significance at same alpha value. CV = coefficient of variation. R^2 is the coefficient of determination.*

From the results obtained from the interactions of the three factors (A*B*C), the control treatment (unautoclaved treatment without NaOH), gave 36.8 ± 4.4, 23.7±0.4 and 21.4±3.9 respectively, for cellulose, hemicellulose and lignin percentages. Keeping the autoclave time at 5 minutes and NaOH concentrations at 0.5%; and increase in pressure from 0 psi to 10 psi increased the cellulose percentage from 36.8 ± 4.4 to 61.37±1.5 while reducing the percentages of hemicellulose and lignin from 23.7±0.4 to 15.94±1.22 and 21.4±3.9 to 14.89±0.1, respectively. However, at the same (constant) levels of NaOH and time, increase in pressure from 15 to 20 psi showed a decline in cellulose percentage (Table 8). Again, maintaining the autoclaving time at 5 minutes and NaOH at 2.5%, increase in pressure from 10 psi to 15 psi increased the percentage of cellulose from 68.63±0.56 to 71.15±0.89 while the hemicellulose and lignin percentages remain unchanged.

Generally, keeping the autoclaving time and concentrations of NaOH constant and increasing pressure from 10 to 15 increased the percentage of cellulose while further increase from 15 to 20 psi showed a declining trend. In the contrary, the percentages of the other responses (hemicellulose and lignin) have no smooth changing trends (Table 6). Among the different treatment combinations of the three factors, NaOH at 2.5% under 15 psi pressures for 35 minutes gave the optimum cellulose (81.25±0.59), hemicellulose (8.41±.0.16) and lignin (6.02±0.03). Furthermore, holding the autoclaving time at 35 minutes and increase in both NaOH concentration as well as the pressure fails to give higher cellulose percentage with lower values of hemicellulose and lignin (Table 6). The graphical descriptions for the optimum treatment combinations were also detailed in Fig. 17, 18 and 19.

Table 6: Separation of treatment means on the effects of NaOH, pressure and time on cellulose, hemicellulose and lignin

SN	Treatments			Responses (mean ± SD)		
	A	B	C	Y1	Y2	Y3
Check	0	0	0	36.8 ± 4.4	23.7±0.4	21.4±3.9
1	10	0.5	5	61.37±1.5	15.94±1.22	14.89±0.1
2	15	0.5	5	64.21±0.6	14.855±0.88	13.70±0.22
3	20	0.5	5	62.02±0.3	14.535±1.35	12.03±0.30
4	10	2.5	5	68.63±0.56	13.35±1.6	9.01±0.12
5	15	2.5	5	71.15±0.89	13.855±2.1	8.12±0.31
6	20	2.5	5	70.19±1.35	13.52±.11	8.24±0.05
7	10	5	5	65.50±1.32	11.22±0.62	11.54±0.44
8	15	5	5	70.43±082	13.10±0.55	10.63±0.38
9	20	5	5	64.50±0.23	11.30±0.92	12.94±0.06
10	10	0.5	20	67.23±1.1	12.32±0.84	10.80±0.08
11	15	0.5	20	68.00±.55	13.15±1.3	13.00±1.2
12	20	0.5	20	62.73±0.56	14.32±1.1	8.63±.099
13	10	2.5	20	70.92±0.91	10.76±0.52	8.42±0.09
14	15	2.5	20	78.91±0.26	10.55±0.5	6.21±0.52
15	20	2.5	20	76.74±0.62	10.72±0.8	6.15±0.55
16	10	5	20	66.65±0.53	10.75±0.5	7.35±0.70
17	15	5	20	73.20±0.44	12.45±0.3	10.50±0.10
18	20	5	20	65.00±1.44	14.35±0.22	9.92±0.33
19	10	0.5	35	58.00±.09	14.39±0.34	10.22±0.18
20	15	0.5	35	71.78±0.61	12.05±0.85	10.46±0.26
21	20	0.5	35	67.71±0.22	12.14±1.55	14.30±0.88
22	10	2.5	35	73.83±0.66	14.32±1.3	10.46±1.0
23	15	2.5	35	81.25±0.59	7.12±.0.16	6.02±0.03
24	20	2.5	35	73.82±0.13	11.20±1.3	8.97±0.79
25	10	5	35	67.85±0.89	11.20±0.88	10.03±0.22
26	15	5	35	67.50±	12.45±0.11	9.16±0.41
27	20	5	35	62.02±	15.63±0.6	13.44±0.11

Remark: A= pressure (psi); B=NaOH (%); C=Time (min.); Y1= Cellulose (%); Y2=Hemicellulose (%); Y3=Lignin (%)

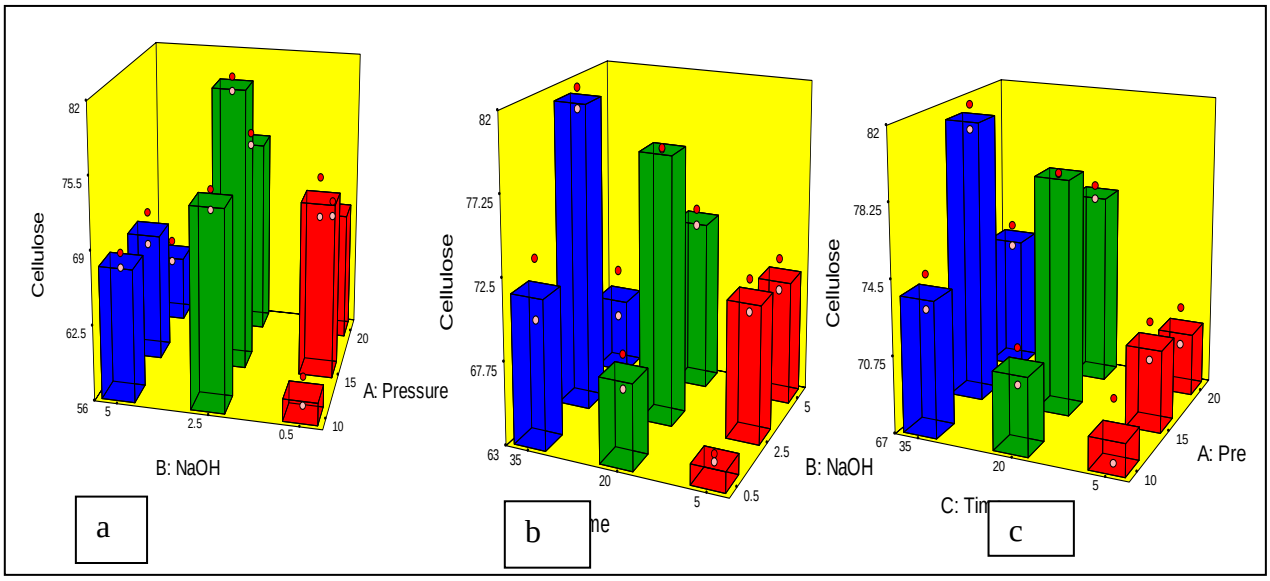


Figure: 17.a, b, and c: Multilevel categorical design plots of cellulose extraction under variable condition of pretreated of SCB a) at 15psi pressure b) at 2.5% NaOH concentration c) at 35min of time

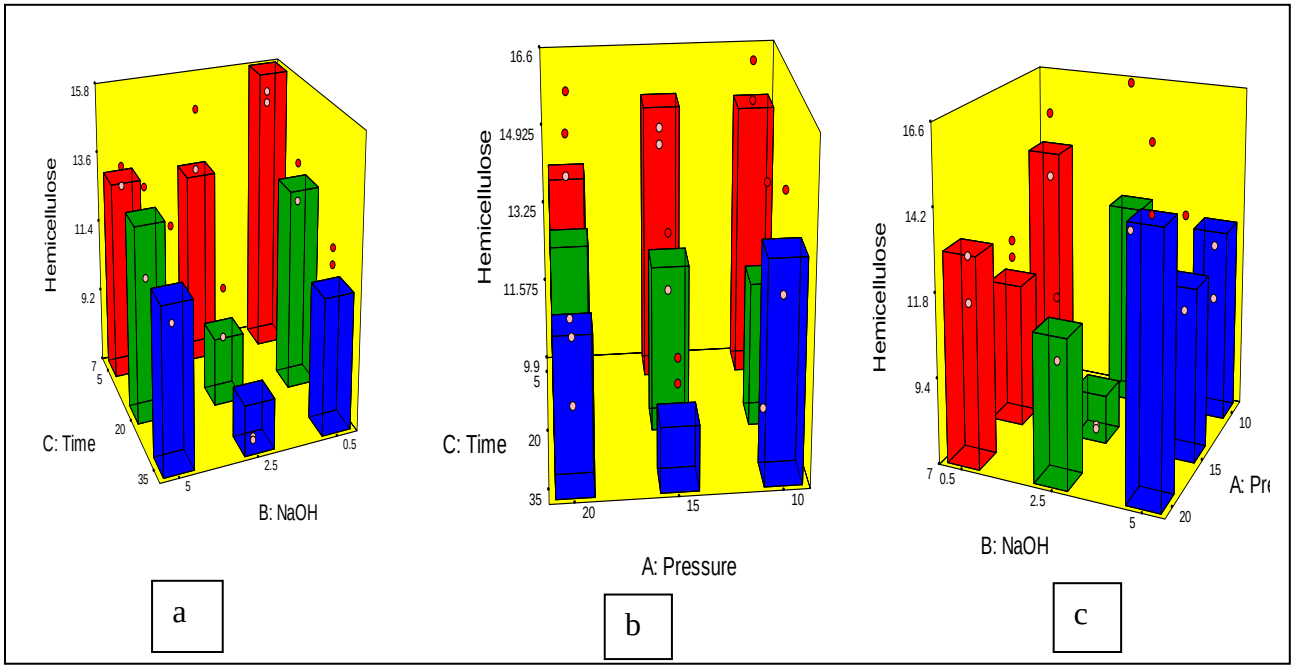


Figure:18 a, b and c: Multilevel categorical design plots of Hemicellulose removal under variable condition of pretreated of SCB a) at 15 psi pressure b) at 2.5 % NaOH concentration c) at 35 min of time

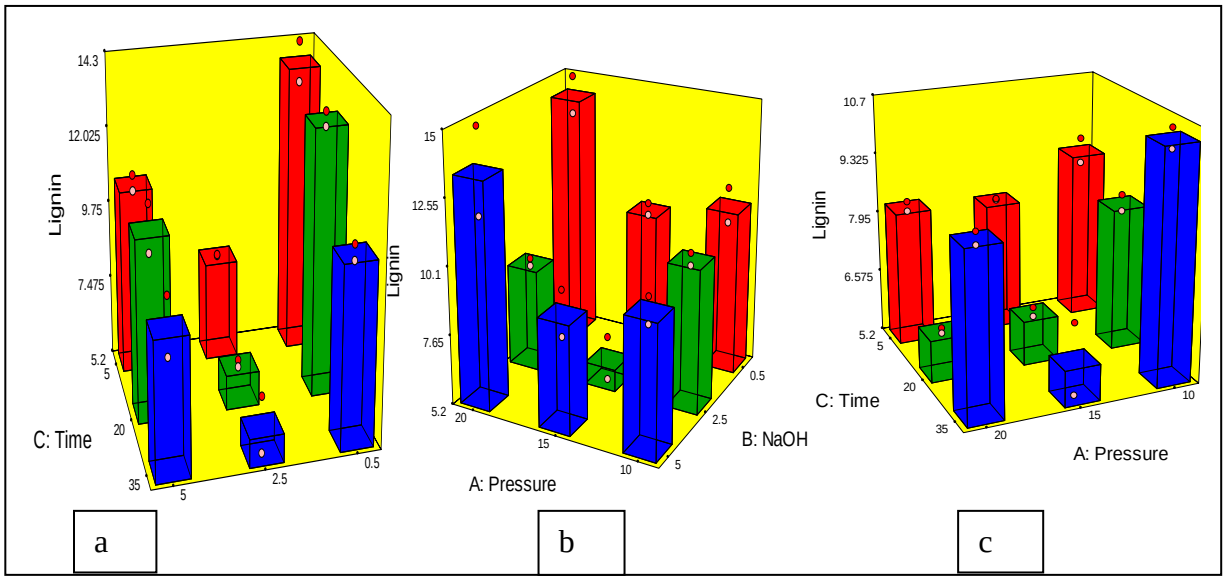


Figure: 19 a, b and c: Multilevel categorical design plots of lignin removal under variable condition of pretreated of SCB a) at 15 psi pressure b) at 2.5 % NaOH concentration c) at 35 min of time

For the optimum treatment combinations with high percentage of cellulose and lower levels of hemicellulose and lignin, the fitted regression models are as follows.

% Cellulose (Y1) = 68.56 - 1.90*A1 + 3.26*A2 - 3.78*B1 + 5.38B2 - 2.12*C1 + 1.37*C2 - 0.69A1B1 - 0.052*A2B1 - 0.92*A1B2 - 0.099A2B2 + 0.62*A1C1 + 1.11*A2C1 + 0.23*A1C2 + 0.17A2C2 - 0.13*B1C1 - 1.83*B2C1 + 0.17A1C2 + 0.22*B2C2 + 0.80 * A1B1C1 - 0.42 * A2B1C1 + 0.83* A1B2C1 - 0.89 * A2B2C1 + 3.60* A1B1C2-1.37 * A2B1C2 - 2.02* A1B2C2 + 0.046* A2B2C2……..…eq 4. 1

% Hemicellulose (Y2) = 12.65 + 0.044 * A1 - 0.47 * A2 + 1.10* B1 - 0.94* B2 + 0.87* C1 - 0.50 * C2 + 0.43*A1B1 + 0.082*A2B1 + 1.05 *A1B2 - 0.73* A2B2 - 0.060*A1C1 + 0.89 *A2C1 - 0.92 *A1C2 + 0.37* A2C2 + 0.50 * B1C1+ 1.00* B2C1 + 0.017* B1C2 - 0.54 *B2C2.…….eq4.2 and

% Lignin (Y3) =10.19 + 0.11 * A1 - 0.44 * A2 +1.81 * B1 - 2.23 * B2 +1.04* C1 - 1.19* C2 - 0.14* A1B1 + 0.82 * A2B1 + 1.23 * A1B2 - 0.74* A2B2 + 0.47* A1C1 + 0.018 * A2C1 - 0.25* A1C2 + 1.34* A2C2 + 0.50* B1C1-0.54 * B2C1 - 0.001667* B1C2+0.16* B2C2 + 0.91 * A1B1C1 - 0.24* A2B1C1 -1.26* A1B2C1 + 0.82* A2B2C1 + 0.28* A1B1C2 + 0.47 * A2B1C2 + 0.40* A1B2C2 - 0.89*A2B2C2……eq.4. 3

The optimum result obtained in this experiment (81.25±0.59 cellulose, 7.12±.0.16 hemicellulose and 6.02±0.03) at 15 psi pressure, 2.5% NaOH and 35 minutes was in close agreement with the findings of (Iram *et al*. 2018). Iram and his collogues reported the maximum cellulose extraction of 81% and delignification of 68.5 % using 2.5 % NaOH for 30 minutes steaming time. The slight difference in time of autoclaving might be due to the difference in the composition of the sugarcane bagasse.

4.2. The effect Dilute H_2SO_4, hydrolysis temperature and reaction time on hydrolysis of celluloses.

Physiochemical parameters that affect the hydrolysis of cellulose include hydrolysis temperature, dilute H_2SO_4 concentration and the hydrolysis time. A total of 20 experiments were performed using varied combinations of the inputparameters as per the central composite design. Experimental data (Table 8) were used to derive a coded polynomial equation (Eq. 3.3.2), describingreducing sugar yield as a simultaneous function of hydrolysis temperature, dilute H_2SO_4 concentration and hydrolysis time. In the model development only significant terms were used.The positive regression coefficient indicates that there is a synergistic effect, while the negative coefficient value indicates the effect of an inverse relationship (D Sartika, 2019).

Reducing sugar (Y) = 63.79 + 4.94 * A + 3.56* B + 16.32* C - 3.99 * A^2 - 8.87* B^2 - 0.40 * C^2 - 1.22 * A * B - 0.64 * A * C - 2.04* B * C …….eq.4.2

In this experiment, the quadratic model was assessed using the Analysis of Variance (ANOVA) and the results are presented in (Table 7). The developed hydrolysis model displayed a high F value (22.75) and low p-value (< 0.0001), this indicates the model was significance (Table 7).

The "Lack of Fit F-value" of 1.38 and *p* value of 0.3664 implies the Lack of Fit is not significant relative to the pure error. There is a 36.64% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good.The factorial factors revealed that all the main factors: hydrolysis temperature (^{O}C), H_2SO_4 Concentration (%) and hydrolysis time (minutes) have a significant effect on yields of reducing sugar (g/L) (Table 7). Nevertheless, the two factor factorial interactions: hydrolysis temperature *H_2SO_4 concentration; hydrolysis temperature * hydrolysis time; and H_2SO_4 concentration * hydrolysis time showed no significant effect on the response tested (reducing sugar yield). The statistical analysis result also proved that the square of temperature of hydrolysis (^{O}C) and H_2SO_4 concentration (%) showed significant quadratic effects on the yields of reducing sugar. However, the square of hydrolysis time did not show a significant effect on reducing sugar yields (Table 7). Hence, the two factor factorial treatment combination showed no significant variation on the response tested, the mean of the main factors were considered to determine the optimum treatment combination that gave the maximum result.

Table 7: ANOVA summary for the effects of temperature, H_2SO_4 and time on hydrolysis of cellulose

Source of variation	DF	Mean Square	F value	P value	Coefficient of Estimation
Model	9	608.39*	22.75	<0.0001	63.79
A	1	333.16*	12.45	0.0055	4.94
B	1	172.86*	6.47	0.0292	3.56
C	1	3639.32*	136.09	<0.0001	16.32
A*A	1	229.49*	0.44	0.5214	-3.99
B*B	1	1132.72*	0.12	0.7345	-8.87
C*C	1	2.31^{ns}	1.24	0.2917	-0.4
A*B	1	11.81^{ns}	8.58	0.0151	-1.22
A*C	1	3.26^{ns}	42.35	<0.0001	-0.64
B*C	1	33.13^{ns}	0.086	0.7748	-2.04
Residual	10	26.74			
Lack of Fit	5	31^{ns}	1.38	0.3666	

Std. Dev .=5.17 *C.V= 9.45* R2 = 0.9534 Pred R2 =0.7648
Mean = 54.73 *Press + 1350.96* R2 Adj = 0.9115 Adeq precision= 18.676

Remark: A= hydrolysis temperature (^{O}C); B= H_2SO_4 concentration; C= hydrolysis time (minute); std. dev= standard deviation; C.V= coefficients of variation R2 = coefficients ofdetermination; prid. R2= predicted coefficients of determination and R2 adj. = adjusted coefficients of determination.

4.2.1. The effect of hydrolysis temperature, H_2SO_4 concentration and hydrolysis time on reducing sugar yield

The reducing sugar yield obtained for the 20 experimental runs was shown in (Table 8).Reducing sugar yield for the experiments ranged from 26.98 to 87.78 g/L. The hydrolysis process gave high reducing sugar yields 63.79 g/l when all the input variables were maintained at their middle values (std. order 15-20). A minimum hydrolysis temperature (160 ^{O}C), minimum H_2SO_4 concentration (1%) and minimum hydrolysis time (20 minutes) gave a minimum ethanol yelled (26.98 g/L). However, the median hydrolysis temperature (190 ^{O}C), a median H_2SO_4 concentration (2%) and maximum hydrolysis time (73.64 minutes) gave a maximum reducing sugar yelled of 87.78g/L.

From table 8, maintaining the temperature of hydrolysis at the center points (190^{O}C) and simultaneous increasing the concentration of H_2SO_4 from (1-3%) and time of hydrolysis time from (20-60 minutes) shows increasing the reducing sugar yield from (26.98-67.57 g/l). However, future increase H_2SO_4 concentration from (3-3.68 %) and time of hydrolysis time from (60- 73.64 minutes) shows inverse effect on reducing yield (Table8). This means, keeping time of hydrolysis time at minimum points and increasing the concentration the concentration H_2SO_4 from the maximum design points to higher axial values from (3-3.68%) shows slightly decrease in reducing sugar yield from (47.28-43.48 g/l) (Table 8) and (Figure 20A). However, increasing the design point of hydrolysis time to the maximum axial points from (60-73.64) shows increase of reducing sugar yield from (74.9-87.78 g/l) (Table 8) and (Figure 20C).

This result shows future increase hydrolysis time beyond the maximum point increase the reducing sugar yield but increase of concentration of H_2SO_4shows inverse effect. Similarly, keeping the concentration of H_2SO_4 at center point and simultaneous increase of hydrolysis temperature from (160-220 ^{O}C) and time of hydrolysis from (20-60 minutes) increase of reducing sugar yield from (26.98-71.23 g/l). However, future increase temperature from (220-240.45^{O}C) and time of hydrolysis time from (60 - 73.64 minutes) shows inverse effect on reducing yield (Table 8).

This means, keeping hydrolysis temperature at minimum point and increasing the hydrolysis time from the maximum design points to higher axial values from (60-73.64 minutes) shows

increase in reducing sugar yield from (74.9-87.78 g/l) (Table 11) and Figure 20C). However, increasing the design point of temperature to the maximum axial points from (220-240.45 ^{O}C) shows decrease of reducing sugar yield from (71.23-57.48 g/l) (Table 8) and (Figure 20B). This result shows future increase hydrolysis time beyond the maximum point increase the reducing sugar yield but increase of temperature shows inverse effect.

Finally, maintaining the time of hydrolysis at center points and simultaneous increase temperature (160-220 ^{O}C) and H_2SO_4 (1-3%) concentration shows increasing in reducing sugar yield from (38.05-71.23 g/l). However, future increase of temperature (220-240.45^{O}C) and (47.28-43.48 g/l) H_2SO_4 concentration (3-3.68%) shows decrease on reducing sugar yield from and (71.23-57.48 g/l) respectively (Table 8) and (figure 20C).The optimum points prediction for the three factors using CCD under design expert version 7 showed the optimum yield (80.88 g/l reducing sugar) with desirability of 89% at 205.98 ^{O}C temperature, 2.05 % H_2SO_4 concentration and 60 minute of hydrolysis time (Table 9) and (figure 21 A) .

Table 8: Design matrix and responses of cellulose hydrolysis for optimization and modeling.

SN	Variables			Responses (reducing sugar g/l)	
	Temperature (^{O}C)	H_2SO_4 (%)	Time (min.)	Experimental result	Predicted value
1	160	1	20	26.98	21.82
2	220	1	20	38.05	35.4
3	160	3	20	36.31	35.44
4	220	3	20	47.28	44.16
5	160	1	60	61.62	59.81
6	220	1	60	74.9	70.85
7	160	3	60	67.57	65.29
8	220	3	60	71.23	71.46
9	139.55	2	40	40.56	44.19
10	240.45	2	40	57.48	60.81

SN	Variables			Responses (reducing sugar g/l)	
	Temperature (^{O}C)	H_2SO_4 (%)	Time (min.)	Experimental result	Predicted value
11	190	0.32	40	26.98	32.73
12	190	3.68	40	43.48	44.69
13	190	2	6.36	30.56	35.2
14	190	2	73.64	87.78	90.11
15	190	2	40	66.89	63.79
16	190	2	40	69.95	63.79
17	190	2	40	63.28	63.79
18	190	2	40	57.05	63.79
19	190	2	40	60.28	63.79
20	190	2	40	66.45	63.79

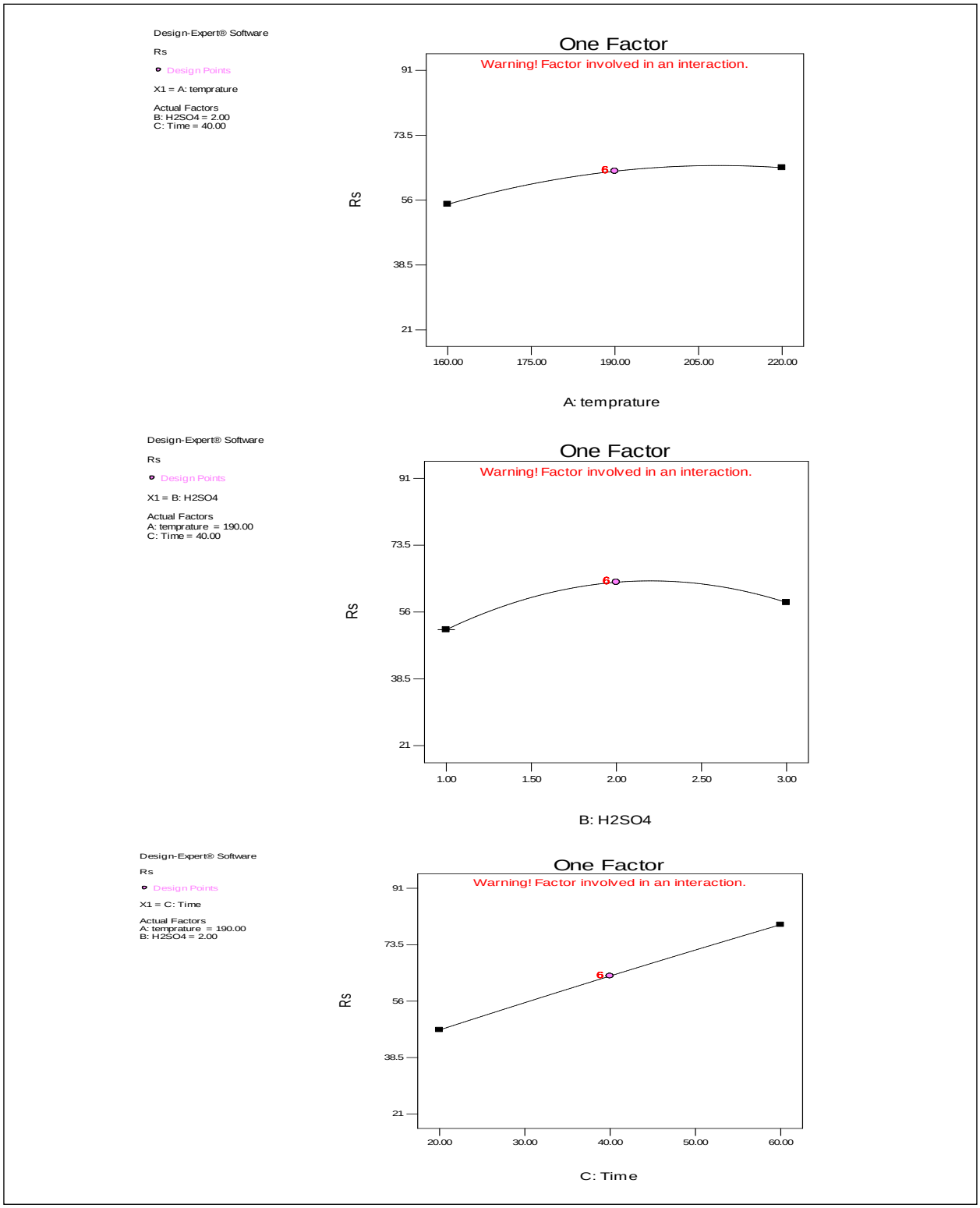


Figure 20. A-C: The effect of temperature, H_2SO_4 concentration and time on reducing sugar

4.2.2. Optimization of the effect of temperature, H_2SO_4 concentration and Time on reducing sugar yield

As shown from table 9 and figure 21 A, surface response plot, the maximum reducing sugar was 80.89 g/L at a temperature 205.92 ^{O}C, H_2SO_4 concentration 2.05 % and reaction time of 60 minutes. The desirability function value was found to be 0.89 for these optimum conditions.The result found in this study showed better result as compared to other findings such as (Theochars Tsoutsos 2011) who reported an optimum reducing sugar yield (28g/l glucose and 22g/l xylose) of 50 g/l from wheat strew hydrolysis with 0.8% H_2SO_4, 208 ^{O}C and 100 minutes of hydrolysis time. (Dussán et al., 2014) , reported a maximum glucose yield of 22.74g/l was obtained at optimum temperature of 155 ^{O}C, 2% H_2SO_4 and 10 minutes of hydrolysis time from hydrolysis of sugar cane hydrolysis. (Roni et al., 2019), reported the 19.16% (191.6 g/l) reducing sugar at optimum hydrolysis condition of 2% H_2SO_4 and 90 minutes of hydrolysis time.

Table 9: Optimum conditions and solutions for reducing sugar yield at optimum conditions.

SN	Temperature (^{O}C)	H_2SO_4 (%)	Time (min.)	Reducing sugar (g/l)	Desirability	
1	205.98	2.05	60	80.8882	0.89	Selected
2	206.2	2.05	60	80.8877	0.89	
3	205.17	2.05	60	80.8855	0.89	

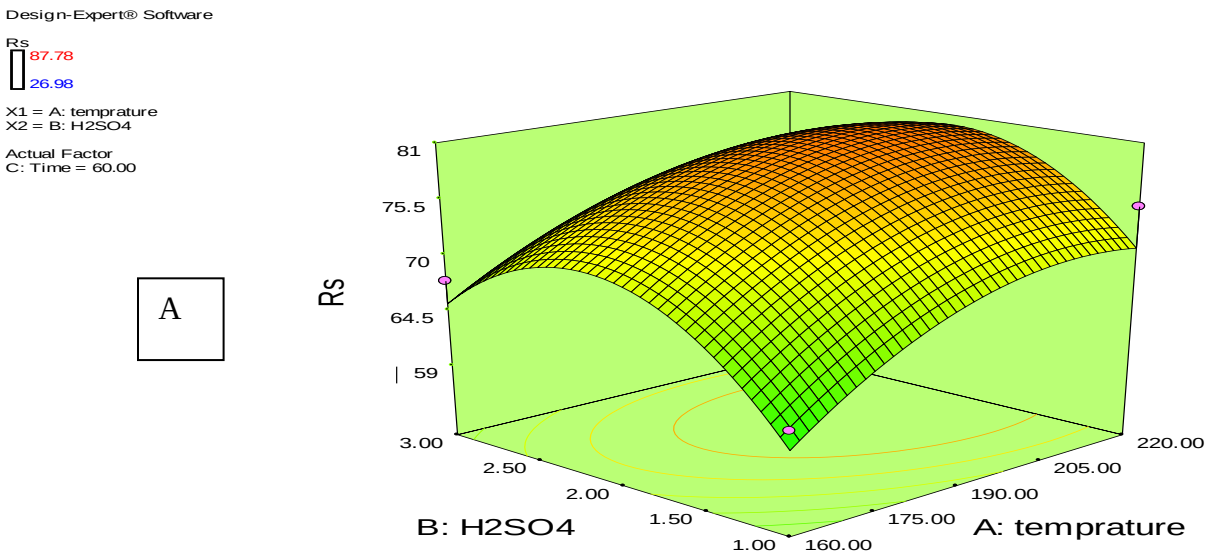


Figure 21A:The desirability 3D response surface plot for optimum Reducing sugar yield

4.3.The effect of incubation temperature, incubation time and Initial pH of fermentation process optimization and modeling

Physiochemical parameters that affect the production of bioethanol include incubation temperature, incubation time and the initial pH. A total of 20 experiments were performed using varied combinations of the input parameters as per the central composite design. Experimental data (Table 11) were used to derive a coded polynomial equation (Eq. 3.3.3), describing bioethanol yield as a simultaneous function of incubation temperature, Incubation time and Initial pH. In the model development only significant terms were used. The positive regression coefficient indicates that there is a synergistic effect, while the negative coefficient value indicates the effect of an inverse relationship (D Sartika, 2019).

Ethanol yield = 13.94 - 2.37* A + 11.80* B + 7.23 * C - 0.97* A * B + 0.16* A * C + 2.20* B *C - 0. 0056* A^2+2.18* B^2+2.81 * C^2

In this experiment, the quadratic model was assessed using the Analysis of Variance (ANOVA) and the results are presented in (table 13). The developed fermentation model displayed a high F value (1033.76) and low p-value (< 0.0001), this indicates the model was highly significance (Table 10). The Lack of Fit value of 0.2263 was not significant suggesting that the experimental data obtained were in agreement with the model. The factorial factors revealed that all the main factors: incubation temperature (^{O}C), incubation time (hour) and initial pH have a significant effect on yields of ethanol (g/L) (Table 10). Similarly, the quadratic the two factor factorial interactions: incubation temperature *incubation time; incubation temperature * initial pH; and incubation time * initial pH shows significant effect on the response tested (reducing sugar yield).

The statistical analysis result also proved that the square of incubation time (hour) and initial pH showed significant quadratic effects on the yields of reducing sugar. Nevertheless, the square of incubation temperature did not show a significant effect on reducing sugar yields (Table 10). However, the two factor factorial treatment combination showed significant variation on the response tested, the mean of the main factors were considered to determine the optimum treatment combination that gave the maximum result.

Table 10: ANOVA summary for the effects of incubation temperature, incubation time and initial PH of the hydrolyzates on ethanol yield.

Source variance	Df	Mean Square	F Value	p-value	Coefficient of Estimation
Model	9	323.14	1033.76	< 0.0001	13.94
A	1	76.44	244.55	< 0.0001	-2.37
B	1	1902.49	6086.23	< 0.0001	11.80
C	1	713.37	2282.14	< 0.0001	7.23
AB	1	7.55	24.15	0.0006	-0.97
AC	1	0.19	0.62	0.4502	0.16
BC	1	38.65	123.64	< 0.0001	2.20
A^2	1	0.000455	0.00146	0.9703	0.0056
B^2	1	68.32	218.55	< 0.0001	2.18
C^2	1	114.11	365.06	< 0.0001	2.81
Residual	10	0.31			
Lack of Fit	5	0.42	2.04	0.2263	
Pure Error	5	0.21			
Cor Total	2911.42	19			
C.V=3.22; R2=0.9989; adj. R^2=0.9980; pre. R^2=0.9936					

Remark: A= incubation temperature; B= Incubation time and C=initial PH of the hydrolyzates. '' indicates the effect of the specific factor has a significant effect on the specified response variable at alpha =5% while 'ns' indicates non significance at same alpha value. CV = coefficient of variation. R^2 is the coefficient of determination; Adj. R^2= adjusted coefficient determination; Pre. R^2= predicted coefficient determination.*

4.3.1. Interactive effect of process variables on bioethanol yield

The bioethanol yield obtained for the 20 experimental runs is shown in (Table 11), which ranged from 0.8 to 42.95 g/L. The fermentation process gave high ethanol yields in the range of 13.21–14.41 g/l when all the input variables were maintained at their middle values (std. order 15-20). A high incubation temperature (40 ^{O}C), minimum incubation time (24hour) and minimum initial pH 4 gave a minimum ethanol yelled (0.81g/L). However, minimum incubation temperature (30 ^{O}C), Maximum incubation time (72 hour) and maximum initial pH 6 gave a maximum ethanol yelled (42.95g/L).

Figure 22 a–c: Shows the response surface plots that illustrate the interactive effects of the input variables on the bioethanol yield. The interaction between incubation temperature and incubation period while the initial pH was maintained at the center value (5) is shown in Figure 22a.

As shown from plot increasing incubation temperature and incubation period simultaneously shows an inverse effect on bioethanol yields. That means, an increases in the incubation temperature from 30 to 40 ^{O}C shows a decrees in bioethanol yield from 25 -17.86 g/l keeping incubation period at 72 hour. In the same way, as the incubation time increase from 24-72 hour keeping incubation temperature at 30^{O}C shows an increase in bioethanol yield from 13.39-42.95 g/l ethanol (Table 11).

The interaction between incubation temperature and initial pH while the incubation period kept at center values 48 hours was shown in Figure 22 b. As shown from the plot increasing incubation temperature and initial pH simultaneously shows an inverse effect on bioethanol yields. The increase of incubation temperature from 30 to 40 ^{O}C the bioethanol yield decrees from 25 -17.86 g/l keeping incubation period at 72 hour and initial pH at 4. Meanwhile, an increase of initial pH of the media from 4-6 pH keeping the incubation temperature and at 30 ^{O}C and incubation period at 24 hour shows an increase in bioethanol yield from 3-13.2g/l (Table 11).

The interaction between incubation time and initial pH while the incubation temperature kept at middle values 35 ^{O}C hours was shown in Figure 22c. As shown from the plot increasing incubation time and initial pH simultaneously shows an increasing effect on bioethanol yields. The increase of incubation time from 24 to 72 hour the bioethanol yield increase from 3.1 - 25.28 g/l keeping incubation temperature at 30^{O}C and initial pH at 4. Meanwhile, an increase of initial pH of the media from 4-6 pH keeping the incubation temperature and at 30 ^{O}C and incubation period at 24 hour shows an increase in bioethanol yield from 3-13.2g/l (Table 11).Generally, increasing incubation temperature decrease bioethanol yield. Similarly, increasing incubation time and initial pH of the media shows an increase in bioethanol yields.

Table 11: Design matrix and response variables for fermentation Process optimization and modeling.

SN	Run ord.	A=Incubation temperature (^{O}C)	B=Incubation Period(hour)	C=Initial pH	Experimental Ethanol yield (g/L)	Predicted ethanol yield (g/L)
1	14	30	24	4	3.0935	3.64
2	13	40	24	4	0.8127	0.54
3	18	30	72	4	25.277	24.79
4	6	40	72	4	17.8625	17.81
5	2	30	24	6	13.2252	13.39
6	19	40	24	6	10.3175	10.91
7	7	30	72	6	42.9525	43.33
8	15	40	72	6	37.4075	36.97
9	1	26.59	48	5	18.2056	17.90
10	20	43.41	48	5	9.7844	9.94
11	16	35	7.64	5	0.803175	0.24
12	12	35	88.36	5	39.535	39.94
13	5	35	48	3.32	9.5289	9.74
14	10	35	48	6.68	34.4111	34.05
15	11	35	48	5	13.905	13.94
16	4	35	48	5	13.7023	13.94
17	17	35	48	5	13.9464	13.94
18	3	35	48	5	13.2141	13.94
19	8	35	48	5	14.4111	13.94
20	9	35	48	5	14.4111	13.94

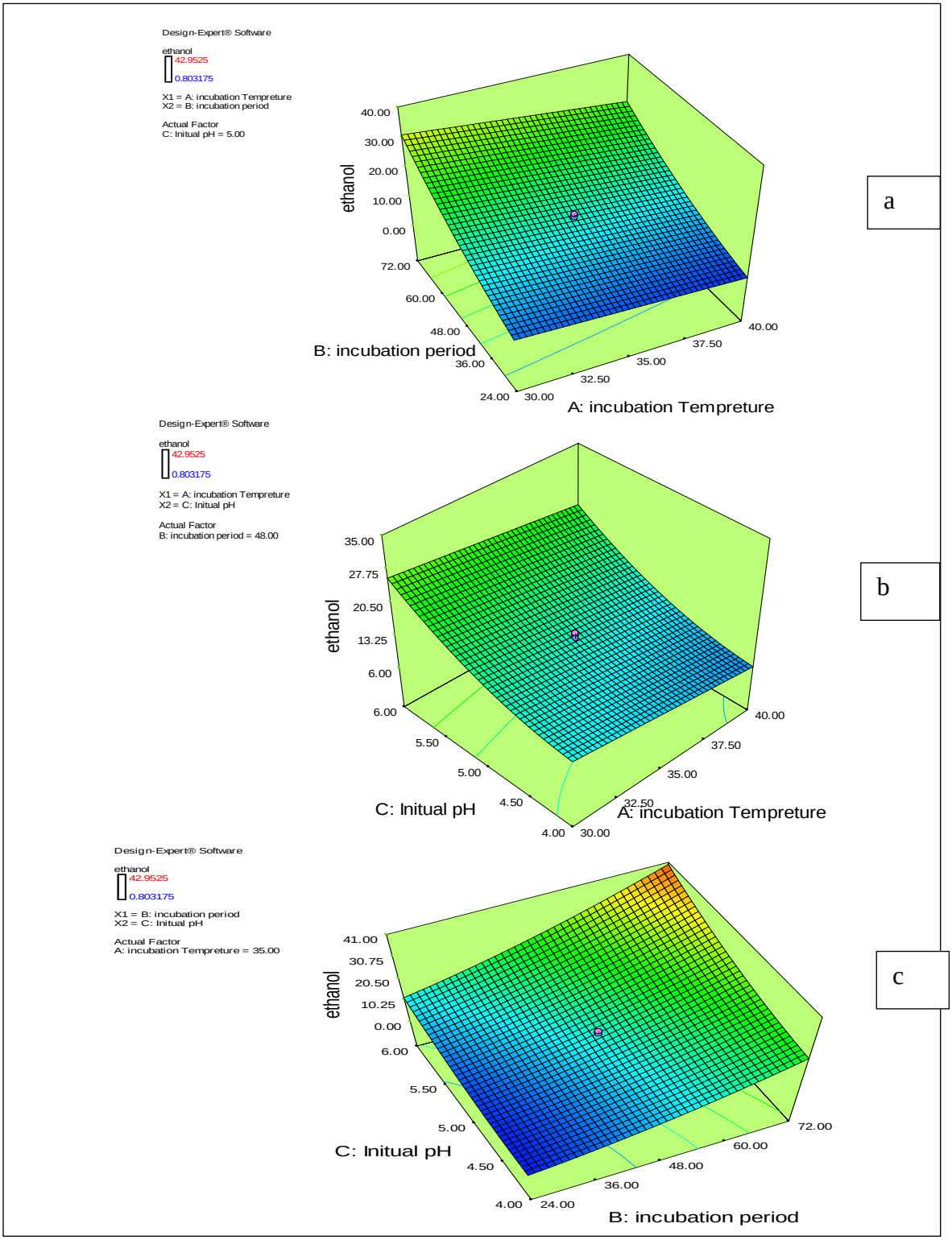


*Figure 22.a-c: Response surface plots showing the interaction of: A= incubation temperature (^{O}C) * incubation time (hour), B= incubation temperature (°C) * initial pH (B), and C= incubation time (hour) * initial pH on the bioethanol yield*

4.3.2. Optimization of the effect of incubation temperature, incubation period and initial pH of the media on bioethanol yield.

The optimum working conditions and respective bioethanol yield were presented in Table 12 and figure 23. As shown in table 12, Response plot figure 23, the maximum bioethanol yield was 42.98 g/L at incubation period of 71.83 h, initial pH 5.99, and incubation temperature 30.08 ^{O}C. The desirability function value was found to be 1(one)for these optimum conditions.

This study showed that there was slightly agreement when compared with the result reported by (Magda et al., 2017), in which the optimum yield of 41.5 g/l bioethanol were obtained from fermentation using the same yeast strain at a temperature of 30 ^{O}C, in 3 day incubation period and initial pH of 6**.** However, this result was in contrary to the findings of (Turhan et al. 2010), whoreported 34.24 g/l ethanol at pH of 5.5 with incubation temperature of 30 ^{O}C and inoculums size of 3%.

Table 12: Optimum conditions and solutions for reducing sugar yield at optimum conditions.

SN	Incubation temperature (OC)	Incubation time (hour)	Initial pH	ethanol Yield (g/L)	Desirability	Remark
1	30.08	71.83	5.99	42.98	1	Selected
2	30.15	71.97	6	43.19	1	
3	30.34	71.91	6	42.97	1	

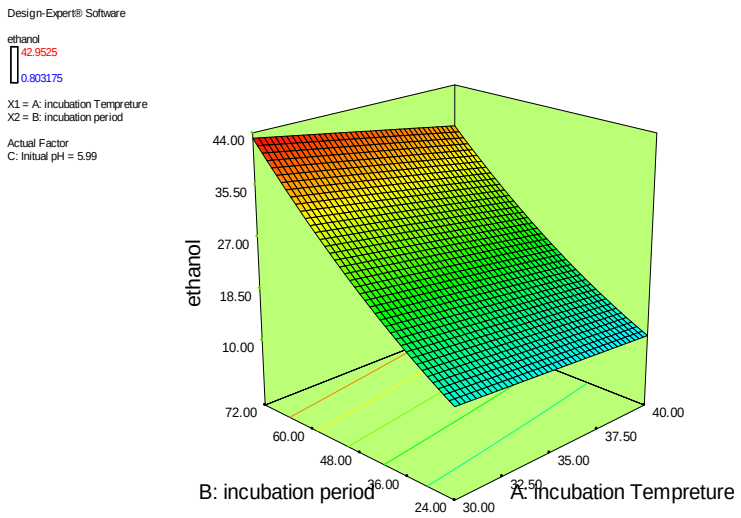


Figure 23: 3D response surface plot for optimum Bioethanol yield

CHAPTER FIVE

5.Conclusions and Recommendation

Nowadays there is a growing interest in bioethanol production from sugar-based or lignocellulosic biomasses in the world since existing energy resources are limited both in volume and geographical distribution as well as increasing emission of GHGs from these sources that cause global climate change.

However, conversion of SCB to bioethanol requires several intermediate steps which are time consuming, expensive, and relatively ineffective. Hence this study aimed at optimizing process conditions for efficient conversion of sugar cane bagasse to bioethanol.

The study of this result confirmed that:

- Maximum cellulose concentration was obtained when sugar cane bagasse was pretreated at 15 psi pressure, 2.5 % NaOH and 35 min. At this optimum treatment combinations maximum cellulose extraction of 81.25%, with removal of hemicellulose (8.71%) and lignin (6.02%) was obtained with desirability value of 91.6%.
- Similarly, the treatment combination containing a temperature of 205.98 ^{O}C, at 2.05 % H_2SO_4 concentration and reaction time of 60 min. gave the maximum reducing sugar yield of 80.88g/l from the pretreated SCB with maximum cellulose hydrolyzed.
- In addition, maximum ethanol yield of 42.98g/l was obtained when the hydrolyzates with maximum reducing sugar fermented with initial pH of 5.99, incubation temperatures of 30.08 ^{O}C and incubation time 71.83 hours.

Further investigation would consider the analysis of the composition of untreated and pretreated SCB, and determine the type and quantities of sugars and inhibiters released during hydrolysis process for better understanding. Therefore analyzing the hydrolyzates contents and amount by HPLC is better.

Moreover, future line work it also important to analyze the composition of sugar cane bagasse based on the cane varieties as one the factors affecting the composition of sugar cane bagasse is the verities of the cane used.

In addition in order to attain the optimum actual yield, the treatment and hydrolysis time levels should be evaluated beyond the end 35 minutes 60 minutes respectively.

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Appendixes

a. Design Summary for pretreatment process optimization.

Study Type	Factorial	**Runs**	54
Initial Design	Full Factorial	**Blocks**	No
Center Points	0		
Design Model	2FI		

Name	**Units**	**Type**	**Low Actual**	**High Actual**	
A Pressure	psi	Categoric	10	20	**Levels:** 3
B NaOH	%	Categoric	0.5	5	**Levels:** 3
C Time	Min	Categoric	5	35	**Levels:** 3

Study Type	Factorial	**Runs**	54
Initial Design	Full Factorial	**Blocks**	No
Center Points	0		
Design Model	2FI		

Factor	**Name**	**Units**	**Type**	**Low Actual**	**High Actual**	
A	Pressure	psi	Categoric	10	20	**Levels:** 3
B	NaOH	%	Categoric	0.5	5	**Levels:** 3
C	Time	Min	Categoric	5	35	**Levels:** 3

Response	**Obs**	**Analysis**	**Min.**	Max.	**Mean**	**SD**	Ratio	**Trans**	**Model**
Cellulose	54	factorial	56.70	81.88	68.56	5.51	1.44	none	3FI
H. cellulose	54	factorial	7.06	16.56	12.65	2.21	2.35	none	2FI
Lignin	54	factorial	5.20	15.21	10.19	2.50	2.93	none	3FI

EFFECTES

Term	DOF	SumSqr	MeanSqr	F Value	Prob>F	%Contribtn
Intercept						
A-Pressure	2	290.235	145.118	< 0.0001	66.3434	17.7317

B-NaOH	2	822.936	411.468	< 0.0001	188.111	50.2766
C-Time	2	124.35	62.175	< 0.0001	28.4245	7.59705
AB	4	51.3389	12.8347	0.0016	5.86766	3.13651
AC	4	22.3728	5.59319	0.0616	2.55704	1.36685
BC	4	82.0921	20.523	< 0.0001	9.38252	5.01535
ABC	8	184.435	23.0543	< 0.0001	10.5398	11.2679
Lack Of Fit	0	0				0
Pure Error	27	59.0589				3.60816
Residuals	27	59.0589	2.1873			

b. Design Summary for hydrolysi process

Study Type Response Surface **Runs** 20
Initial Design Central Composite **Blocks** No Blocks
Design Model Quadratic

Factor	**Type**	**Low Actual**	**High Actual**	**Mean**	**Std. Dev.**
temprature (Oc)	Numeric	160.00	220.00	190.00	24.79
H2SO4 (%)	Numeric	1.00	3.00	2.00	0.83
Time (min.)	Numeric	20.00	60.00	40.00	16.53

Response	**Obs**	**Minimum**	**Maximum**	**Mean**	**Std. Dev.**
Red. Sugar(g/l)	20	21.82	90.11	54.73	16.55

	StdLeverage	**Point Type**
1	0.6698	Fact
2	0.6698	Fact
3	0.6698	Fact
4	0.6698	Fact
5	0.6698	Fact
6	0.6698	Fact
7	0.6698	Fact
8	0.6698	Fact
9	0.6073	Axial
10	0.6073	Axial
11	0.6073	Axial
12	0.6073	Axial
13	0.6073	Axial
14	0.6073	Axial
15	0.1663	Center
16	0.1663	Center
17	0.1663	Center

18	0.1663	Center
19	0.1663	Center
20	0.1663	Center
	Average =	**0.5000**

Watch for leverages close to 1.0. Consider replicating these points or make sure they are run very carefully.

Response 1 Rs Transform: None
***** WARNING: The Cubic Model is Aliased! *****

Sequential Model Sum of Squares [Type I]

Source	Sum of Square	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	59918.40	1	59918.40			
Linear vs Mean	4145.3	3	1381.78	16.61	< 0.0001	
2FI vs Linear	48.24	3	16.08	0.16	0.9194	
Quadratic vs 2FI	1282.54	3	427.51	6.048E+007	< 0.0001	Suggested
Cubic vs Quadratic	6.585E-005	4	1.646E-005	20.44	0.0012	Aliased
Residual	4.832E-00	6	8.054E-007			
Total	65394.54	20	3269.73			

"Sequential Model Sum of Squares [Type I]": Select the highest order polynomial where the additional terms are significant and the model is not aliased.

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Linear	1330.79	11	120.98		
2FI	1282.54	8	160.32		
Quadratic	7.069E-005	5	1.414E-005		
Cubic	4.832E-006	1	4.832E-006		
Pure Error	0.000	5	0.000		

"Lack of Fit Tests": Want the selected model to have insignificant lack-of-fit.

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	9.12	0.7570	0.7114	0.6207	2076.96	
2FI	9.93	0.7658	0.6577	0.4810	2842.17	
Quadratic	2.659E-003	1.0000	1.0000	1.0000	5.564E-004	Suggested
Cubic	8.974E-004	1.0000	1.0000	1.0000	1.065E-003	Aliased

"Model Summary Statistics": Focus on the model maximizing the "Adjusted R-Squared"

and the "Predicted R-Squared".

c. Design Summary Fermentation optimization

Study Type	Response Surface	**Runs**	20
Initial Design	Central Composite	**Blocks**	No Blocks
Design Model	Quadratic		

Name	**Type**	**Low Actual**	**High Actual**	**Mean**	Std. Dev.
Incu. Tempreture (^{o}c)	Numeric	30.00	40.00	35.00	4.13
incu. period(hr)	Numeric	24.00	72.00	48.00	19.83
Initual pH	Numeric	4.00	6.00	5.00	0.83

Response	**Units**	**Obs**	**Minimum**	**Maximum**	**Mean**	**Std. Dev.**
ethanol	g/l	20	8.03	62.95	27.57	13.63

Measures Derived From the $(X'X)^{-1}$ Matrix

	StdLeverage	**Point Type**
1	0.6698	Fact
2	0.6698	Fact
3	0.6698	Fact
4	0.6698	Fact
5	0.6698	Fact
6	0.6698	Fact
7	0.6698	Fact
8	0.6698	Fact
9	0.6073	Axial
10	0.6073	Axial
11	0.6073	Axial
12	0.6073	Axial
13	0.6073	Axial
14	0.6073	Axial
15	0.1663	Center
16	0.1663	Center
17	0.1663	Center
18	0.1663	Center
19	0.1663	Center
20	0.1663	Center
	Average =	**0.5000**

Watch for leverages close to 1.0. Consider replicating these points or make sure they are run very carefully.

Response **1** **ethanol**
Transform: **None**
***** WARNING: The Cubic Model is Aliased! *****

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	15199.31	1	15199.31			
Linear vs Mean	3390.78	3	1130.26	55.27	< 0.0001	
2FI vs Linear	110.54	3	36.85	2.21	0.1355	
Quadratic vs 2FI	180.66	3	60.22	16.74	0.0003	Suggested
Cubic vs Quadratic	29.78	4	7.45	.22	0.0178	
Residual	6.19	6	1.03			
Total	18917.28	20	945.86			

"Sequential Model Sum of Squares [Type I]": Select the highest order polynomial where the additional terms are significant and the model is not aliased.

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	326.15	11	29.65	144.19	< 0.0001	
2FI	215.61	8	26.95	131.07	< 0.0001	
Quadratic	34.95	5	6.99	33.99	0.0007	Suggested
Cubic	5.16	1	5.16	25.11	0.0041	Aliased
Pure Error	1.03	5	0.21			

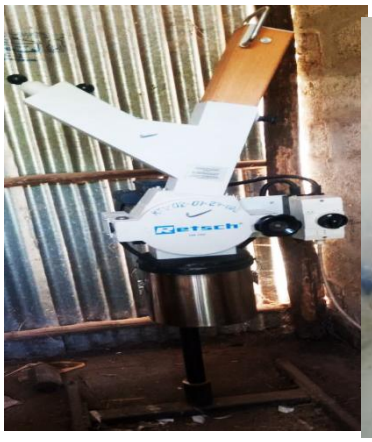
"Lack of Fit Tests": Want the selected model to have insignificant lack-of-fit.

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	4.52	0.9120	0.8955	0.8529	546.96	
2FI	4.08	0.9417	0.9148	0.8124	697.60	
Quadratic	1.90	0.9903	0.9816	0.9228	287.13	Suggested
Cubic	1.02	0.9983	0.9947	0.6935	1139.59	Aliased

"Model Summary Statistics": Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

d. Instruments used in analysis process.



Leif Mill

Analytical Balance

Oven

Vertical autoclave

Furnace

Desiccator

Water bath with reflex

Microkajelhal digestion block

Centrifuge

Spectrophotometry

pH meter

Hote plate & magnetic stirrer

Shaker

Incubator

Vacuum pump

Ebullio meter