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OPTIMIZATION OF ACID HYDROLYSIS IN FERMENTABLE SUGAR PRODUCTION FROM CLADODE BY RESPONSE SURFACE METHODO

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MASTER OF SCIENCE IN PROCESS ENGINEERING

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BY

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JULY, 2021

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OPTIMIZATION OF ACID HYDROLYSIS IN FERMENTABLE SUGAR PRODUCTION FROM CLADODE BY RESPONSE SURFACE METHOD

By

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in Partial Fulfillment of the Requirements for the Degree of Master of Science in

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Advisor: Solomon Workneh (PhD)

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BAHIR DAR UNIVERSITY

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THESIS APPROVAL SHEET

I hereby confirm that the changes required by the examiners have been carried out and incorporated in the final thesis

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As member of the Board of Examiners of the Master of Science (M.Sc.) thesis open defense examination, we have read and evaluated this thesis prepared by Mr. Limenew Aschie Demsea entitled "**Optimization of acid hydrolysis in fermentable sugar production from cladode by response surface methodology.**" We here certify that the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Science (M.Sc.) in Process Engineering.

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DECLARATION

This is to certify that the thesis entitled "Optimization of acid hydrolysis in fermentable sugar production from cladode by response surface methodology",

submitted in partial fulfillment of the requirements for the degree of Master of Science in **Process Engineering** under **chemical and food engineering**, Bahir Dar Institute of Technology , is a record of original work Carried out by me and has never been submitted to this or any other institution to get any other degree or certificates. The assistance and help I received during the course of this investigation have been duly acknowledged.

Limenew Aschie Demsea		15/07/2021
Name of the candidate	signature	Date

ABBREVIATION

- ASTM- American standard for testing materials
- BBD- Box Behken design
- CAM -Crassulacean Acid metabolism
- DNSA –Di Nitro Salicylic Acid
- NREL-National Renewable Energy Laboratory
- ODW-Oven Dry Weight
- OPI- Opuntia -ficus-indica
- RPM- revolution per minute
- R-Response
- SRM -Surface Response Methodology
- SSF-simultaneous saccharification and fermentation
- TRS-total reducing sugars
- UV-VIS- ultraviolet–visible spectrophotometer
- XRD- x- ray diffraction

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ABSTRACT

Bioethanol is a significant renewable liquid fuel for automobiles. Bioethanol production from biomass is one technique to minimize crude oil usage while also reducing pollution. Conversion technologies for manufacturing ethanol from cellulosic biomass resources such as forest materials, agricultural residues, and urban wastes are still in the works and have yet to be proven. Because of its Crassulacean Acid metabolism, the prickly pear can be grown in arid settings. Bioethanol is a clean and renewable energy source made from lignocellulose biomass. It is a secondary crop that is extensively distributed in dry and semi-arid zones, and its adaptability as a crop has lately been examined for its potential in the biofuel business. Cladode is a lignocellulosic material source. Lignin, cellulose, and hemicellulose are all present in varying amounts in lignocellulosic materials. Cladode is employed as a feedstock for the production of fermentable sugar in this study, and it comprises 91.8 percent water, 27 percent extractives, 29 percent ash, 6.87 percent protein, 7.43 percent lignin, 11 percent hemicelluloses, and 18.7 percent cellulose after acid pretreatment and acid hydrolysis. It also improved the surface responsiveness and hydrolysis of Opuntia ficus-indica cladodes using diluted acid. Seventeen experimental runs were carried out at temperatures ranging from 109℃ to 121℃, with H_2SO_4 concentrations ranging from 2 to 4 percent and residence times ranging from 10 to 30 minutes. The influence of the parameters was determined by the amount of reducing sugar produced, and the optimization was carried out using the Box-Behnken design process. The results demonstrate that the temperature, time and concentration greatly affected the reducing sugar concentration. The yield of reducing sugar dropped at low temperatures, concentrations, and times, whereas it increased at high value parameters. With an acid concentration of 3.04 percent, hydrolysis duration of 29.2 minutes, and a temperature of 120.16℃, this optimization resulted in the production of 10.33 mg/ml of total reducing sugars (TRS).

1. INTRODUCTION

1.1. Background

Presently, the economy of the world is mainly dependent on fossil energy sources like oil, coal, natural gas, etc which are being used for the generation of fuel, electricity and other goods (Sarkar et al,2012). Large consumption of fossil fuels has resulted in high levels of pollution (Demirbas, 2011). Global energy consumption has increased gradually with the expansion of the human population and the increase in industrial prosperity. The use of transport fuel is affected by limited reserves of fossil fuel in the world (Sarkar *et al*, 2012).

Alternative energy sources must be technically feasible, economically competitive, environmentally acceptable, and readily available (Demirbas, 2011). Bioethanol, biodiesel, methanol, hydrogen, boron, natural gas and liquefied petroleum gas are some of the alternative fuels that have been considered. Bio-ethanol is by far the most popular bio-fuel for transportation in the world (Balat, 2011).

Africa is a large consumer of traditional sources of energy and faces energy insecurity for the majority of its people. The availability and accessibility of socially and environmentally acceptable energy sources is still quite limited, and the disparity between rural and urban areas is still significant. Other energy sources (coal, crude oil, and, more recently, bio-fuels) have been used as major sources of power for the transport and industry sectors in addition to fuel wood (vanzyl, 2011).

Africa will have the largest potential for bio-energy production by 2050 in the world, depending on the level of advancement of agricultural technology (vanzyl, 2011). In order to achieve this, advanced agricultural technologies and practices must be used in a sustainable manner to meet the needs of rural and urban communities, foster industrial development, reduce greenhouse gas emissions, develop agricultural infrastructure, and lead to land restoration and ecologically healthy landscapes in Africa.

Current research is concentrating on low-cost, renewable large-scale technologies utilizing lignocellulosic feedstock derived primarily from agriculture and forest waste.

Agricultural waste has no nutritional value and does not necessitate distinct land, water, and energy needs. (Sarkar, 2012) and (Ricke, 2012). Lignocellulose is the favored biomass for the generation of a range of fuels all over the world. It is the most plentiful and extensive source of carbon in nature, providing enough feedstock to meet the world's energy demands in a renewable manner (vanzyl, 2011). It has a lot of promise for producing cheap ethanol fuel (Zheng and Pan, 2009). Lignocellulose materials are an abundant renewable resource for the production of bio-fuels. The major components of lignocelluloses are cellulose, hemicellulose and lignin, while the minor components are extractive liquids and ash. The major pathway for bio-ethanol production from biomass is biochemical conversion through saccharification and fermentation (Balat, 2011).

The prickly pear cactus, Opuntia ficus-indica, is a drought-resistant plant that can be found in dry and semi-arid climates all over the world. Opuntia. ficus- indica cladodes could be used as a second-generation feedstock for bio ethanol production, without competing for prime agricultural land or considerably displacing natural vegetation (Ginestra, 2009).

Prickly pear cactus is a crop cultivated mainly for its fruits (pears) that are sold fresh or transformed into jellies, juices and other products. Young stems (cladodes) are also sold as vegetables in some world regions (mainly in Mexico). Older cladodes, especially those from varieties with no or few or small thorns can be used as fodder and their fruits and cladodes have a variety of other minor uses including medicinal and cosmetic purposes (Sáenz, 2000). The cladodes contain bioactive chemicals that are well-known for their health related characteristics, despite their rarity in modern nutrition and medicine. Extracted compounds have shown a number of pharmacological actions including emollient and wound-healing effects, hypocholesterolemic effects, and inhibition of stomach ulceration, neuroprotective effects through antioxidant actions and anti-inflammatory effects. Effects on blood glucose control serum lipid levels and antiglycated activities have been related to the hypoglycaemic and anti-diabetic properties of Opuntia cladodes.

Because of its Crassulacean Acid metabolism (high water retention capability, nocturnal stomata opening, and a special CO_2 fixation pathway) prickly pear can be grown in arid environments.Opuntia ficus-indica can tolerate a wide range of environmental and climatic conditions (Russell C, 1987). Opuntia ficus-indica has up to a fivefold higher water usage

efficiency than C_4 plants like corn and sugar cane allowing it to produce up to 50 tons of dry mass ha-1 year-1 while requiring less agronomic input (Inglese etal,2002 and Nobel,2002).There is a growing emphasis on the use of low-cost lignocellulosic biomass for bioethanol production. Cladode yields are heavily reliant on crop management as agricultural operations such as fertilization and weeding are rarely carried out when clumps are the harvestable product. Values of more than 100 tn/ha of fresh cladode can be considered for rain-fed farms in central Mexico with Opuntia ssp. plants that have been in place for five years or longer (López, 2001). In rain-fed semi-arid zones with suitable land management (Cordeiro et al , 2001) observed values of between 22 and 50 tn/ha for two different types of O. ficus-indica. Finally, yields of 3–9 and 15–22.5 tn/ha. of dry matter for O. ficus-indica cultivated in deep and sandy soils in areas with 200 and 400 mm of rain and adequate land management (Le Houerou, 1996).Due to its high content of carbohydrates (about 30% d.m.b not including the holocelullosic fraction) and low content of lignin (< 4%, d.m.b)(Stintzing S,2005). Carbohydrates make up a major component of prickly-pear biomass. According to several authors, non-fibrous carbohydrates might account for 25 to 50 percent of total dry matter in terminal and sub-terminal cladodes whereas their cellulose and hemicellulose content might account for 18 to 34 percent on a dry matter basis (M. Da Silva Vilela, 2010).

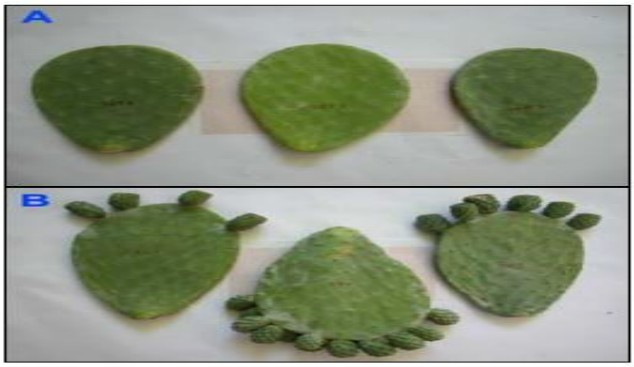


Figure1.1 Cladodes sample (F. Sánchez, 2012)

Initially, prickly pear ethanol production can be thought of as a highly valued by-product of crops primarily intended for food production or as the primary product of crops primarily intended for fuel generation (considering prickly pear as a so-called energy crop).

One fuel source that is gaining global momentum is fuel ethanol derived from crops and that is why our eyes are turning to Brazil which has arguably the most developed alternative fuel infrastructure in the world. Domestically manufactured ethanol from carbohydrate feedstock is giving Brazil a serious opportunity for energy independence, a surprise legacy of the global oil crisis that dominated the 1970s (Sendlium and Johan, 2005). Because Brazil is as subject to the politics and economics of oil as other countries. The Brazilian government launched an ethanol infrastructure initiative that has flowered over decades of dedication. This report will focus on Brazil as a case study for the construction and implementation of a successful ethanol fuel program (Igly, 2006).

There are few studies of hydrolysis on cladodes to obtain sugars that consider the effect of temperature, and there is no study that examines the optimization of this process among the ones that have been completed. As a result, it has become vital to apply and develop strategies targeted at exploiting this potential sugar supply, as well as to include it in the study of so-called second generation bio fuels.

1.2. Statement of the problem

Many individuals throughout the world are currently interested in the development of bio-fuels from agricultural waste for use as a transportation fuel. This is mostly owing to the rise in petroleum prices, which has coincided with a scarcity of foreign currency reserves, as well as their lower carbon emissions when compared to fossil fuels. These factors, as well as the growing energy need for transportation to keep up with economic growth, are prompting many countries, including Ethiopia, to look for alternative energy sources to ensure their energy security.

Cladode is a cheap and readily available lignocellulosic biomass that is predominantly found in the East Gojam region and can be utilized to make bio ethanol. It contains a small quantity of lignin (4%, d.m.b) and high amount of celulose (F.Sánchez, 2008). Because lignin is the most resistant biopolymer in plant cell walls to hydrolysis, i.e less energy is required for lignin removal in saccharification (Yang, 2015).Furthermore, current studies using cladode hydrolysis to get sugar are few and none of the ones that have been completed include an analysis of the process's optimization. As a result, it is required to apply and develop strategies targeted at optimizing this potential resource of sugar. And the majority of the research failed to notice the impact of temperature (Kuloyo, 2012) and (A. Taxco-lopper et al, 2018).

1.3 .Objective

1.3.1. General objective

The general objective of this research is Optimization of acid hydrolysis in fermentable sugar production from cladode

1.3.2. Specific objective

- To characterize the physicochemical properties of cladodes.
- To investigate the effect of temperature, time, acid concentration and their interaction in acid hydrolysis on reducing sugar yield
- To optimize the hydrolysis parameter (temperature, time and acid concentration)

1.4 .significance of the study

Because of its Crassulacean Acid metabolism, Opuntia ficus-indica can tolerate a wide range of environmental and climatic conditions. Opuntia ficus-indica has higher water consumption efficiency than C_4 plants like corn and sugar cane, allowing it to generate up to 50 tonnes of dry mass per hectare. For the generation of bioethanol, the plant requires a modest agronomic input and affordable lignocellulosic biomass.

1.5. Scope of the study

Cladode has been chosen as the cellulosic biomass for the generation of fermentable sugar in this thesis. The major goal of this thesis work was to apply response surface techniques to optimize the acid hydrolysis of cladodes. The impact of operational variables such hydrolysis duration, temperature and acid concentration on the amount of reducing sugar produced was investigated.
It also addresses the investigation of optimum operational parameters

2. LITERATURE REVIEW

The most abundant renewable organic resources are lignocellulosic materials containing cellulose, hemicellulose and lignin as their main constituents. However, due to the recalcitrant nature of lignocelluloses, the pretreatment processes are the rate-limiting step in bioethanol production from lignocellulosic feed stocks. To increase their biodegradability, studies focusing on various pretreatment technologies have been carried out (Zehra Sapci, 2014). A lot of literature has been written about chemical hydrolysis approaches to enhance the ferment-ability of lignocellulosic materials (Etana et al, 2021).Relying on conventional fossil fuels for the entire energy supply is problematic worldwide because of their finite supply, greenhouse gas emissions, global warming and increasing prices. Green house gases in the earth's atmosphere, such as carbon dioxide, cause the biosphere to become warmer and traditional fossil fuels will be used up in a few decades. Therefore, ethanol production from cellulosic waste has been getting more and more attention around the world.

2.1. Structure of lignocellulose and its major components

Second-generation feedstocks for bioethanol production are lignocellulosic materials. These primarily consist of three types of polymers; cellulose $(C_6H_{10}O_5)_n$,hemicelluloses $(C_5H_8O_4)_m$, and lignin $[C_9H_{10}O_3\ (OCH_3)_{\ 0.9–1.7}]_{\ x}$, as well as pectins, extracts, glycosylated proteins, and a variety of inorganic materials, all of which are linked together to form the structural framework of the plant cell wall (Kankia, 2014). Between the layer of lignin on the outside and the layer of cellulose and hemicellulose on the inside are interlaced cellulose and hemicellulose.

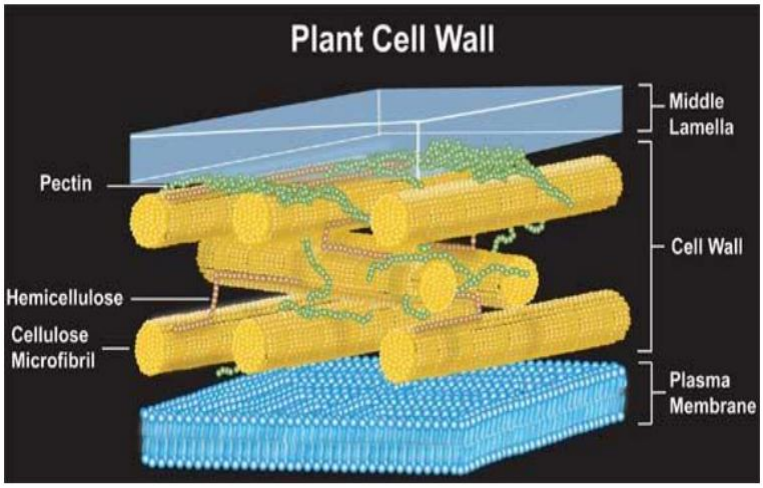


Figure 2 1: Typical cell wall structures (Kankia, 2014).

Cladode is a lignocellulosic material source. Certain percentages of lignin, cellulose, and hemicelluloses can be found in lignocellulosic materials. The cell walls of the cladode contain lignin, cellulose, and hemicelluloses.

In the literature, the chemical makeup of O. Ficus-indica cladodes varies widely. Agronomic variables (such as soil type, climate, and growing circumstances), cultivar type, season, and plant age may all influence carbohydrate content. As a result, statistics on the constituents should not be regarded as absolute values (Stintzing & Carle, 2005). In 100 g (dry wt.) of despined dry cladode biomass, Malainine et al. (2003) found 19.6 g ash, 7.2 g fat and wax, 3.6 g lignin, and 69.6 g polysaccharides but crude protein was not identified.

Table2.1 Mean chemical composition of despined Opuntia ficus-indica cladodes (adapted from Stintzing & Carle (2005)

Constituent	Fresh weight (g/100 g)	Dry weight (g/100 g
Water	88-95	0
Carbohydrate (totalpolysaccharides)	3-7	64-71
Ash	1-2	19-23
Crude fibre	1-2	18
Protein	0.5-1	4-10
Lipid	0.2	1-4

On a dry biomass basis, O. ficus-indica cladodes contain roughly 22% cellulose, 13% hemicelluloses, and 34% acidic polysaccharides (mucilage and pectin's), for a total carbohydrate content of 69 percent (Malainine et al.,2003).

2.1.1. Cellulose.

Cellulose, the most prevalent organic substance on the planet, is made up of 1-4-polyacetal of cellobiose (4-O- β-D-glucopyranosyl-D-glucose) subunits linked by $\beta -1$, 4-glycosidic linkages, as shown in Figure.2.2. Hydrogen and Vander Waal linkages bind the long-chain cellulose polymers together, causing the cellulose to pack into micro fibrils. By breaking the β-1, 4-glycosidic bonds with acid or enzymes, fermentable D-glucose can be generated from cellulose. Lignocellulosic material is made up of both crystalline and amorphous forms of cellulose; the crystalline part is made up of tightly linked parallel organized bundles of cellulosic chains, whilst the amorphous part is less organized and visible (Kankia, 2014).

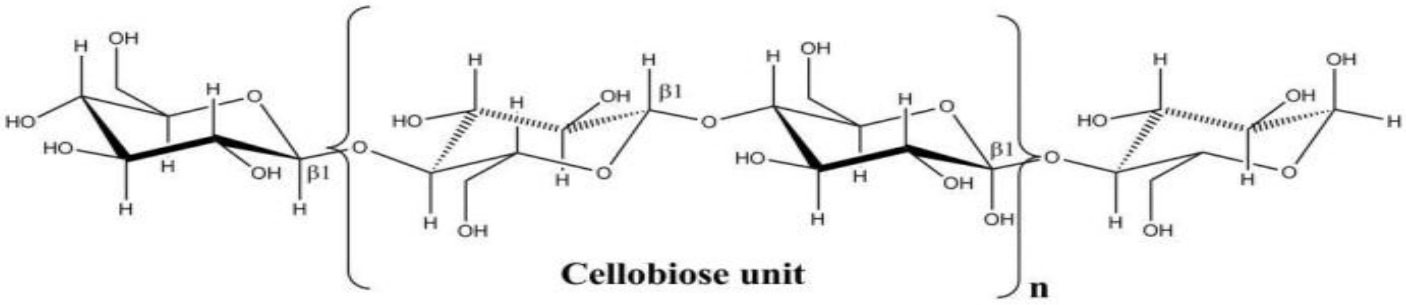


Figure 2 2-Structure of cellulose (Iñaki Gandarias and Pedro Luis Arias, 2013)

As illustrated in Figure 2.3, cellulose is made up of a lot of covalent connections at the molecular level (Cellulose's molecular structure).Covalent C-H, C-O, or O-H bonds are represented by solid lines. Between groups of molecules, the dashed lines represent O-H hydrogen bonds. Amorphous and crystalline cellulose are the two types of cellulose. The gap between the $C_6H_{10}O_5$ groups distinguishes amorphous and crystalline cellulose. Because crystalline cellulose is more densely packed than amorphous cellulose, it is water insoluble, whereas amorphous cellulose is water soluble. Crystalline cellulose also takes longer to hydrolyze than amorphous cellulose. Amorphous cellulose surrounds crystalline cellulose in most cases. Simple glucose molecules can arise once the hydrogen bonds are broken, which S. cerevisiae can utilize for fermentation.

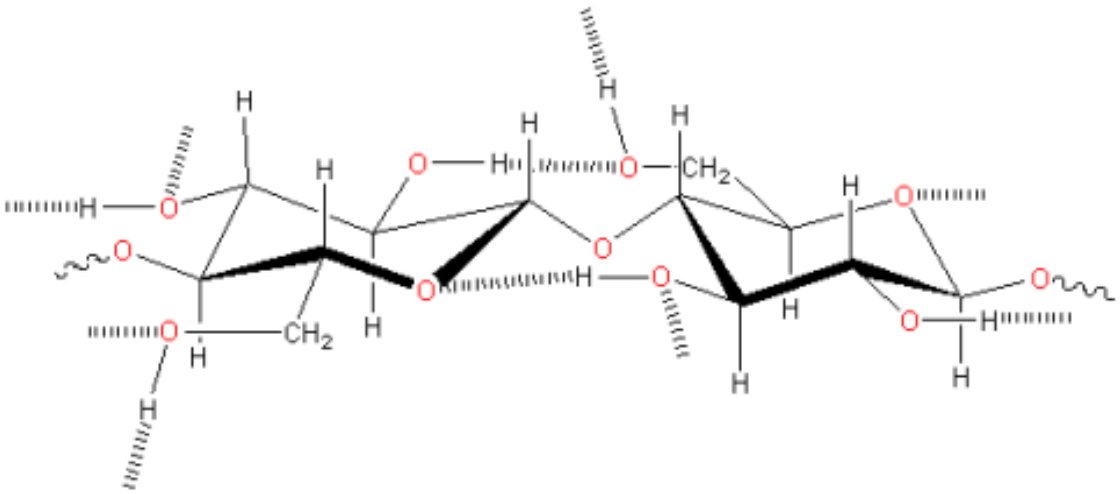


Figure 2 3 .Molecular structure of cellulose.The solid lines are covalent C-H, C-O, or O-H bonds. The dashed lines are O-H hydrogen bonds between groups of molecules (Iñaki Gandarias and Pedro Luis Arias, 2013)

2.1.2. Hemicelluloses

Hemicelluloses are made up of numerous monomers such as pentoses (such as xylose and arabinose), hexoses (such as mannose, glucose, and galactose) and sugar acids, and are the second most abundant natural carbohydrate polymers. Xylan is the primary component of hemicelluloses from hardwood and agricultural plants, such as grasses and straw, whereas glucomannan is the primary component of softwood hemicelluloses. Hemicelluloses have a random, amorphous, and branched structure with little resistance to hydrolysis.

Hemicellulose has a lower molecular weight than cellulose and splits into short lateral chains made up of various sugars. Xylan, the most prevalent kind of polysaccharide in the hemicellulose family, is made up of D-xylopyranose joined together by a β-1, 4-linkage with a molecular weight of less than 30,000 and a polymerization temperature of up to 200 degrees. Hemicelluloses serve as a link between lignin and cellulose fibers, increasing the stiffness of the entire cellulose hemicelluloses–lignin network (Kankia, 2014). Figure 2.4 depicts the molecular structure of hemicelluloses. Hemicelluloses are cellulose-like polysaccharides. As seen in Figure 2.4, hemicelluloses is usually interlaced in between the strands of cellulose in the plant's cell wall and acts as glue between the cellulose and lignin. It is substantially smaller than cellulose since it is made up of 300 to 3000 linked sugar molecules (Bon and Ferrara, 1996). Hemicelluloses, like cellulose, can be broken down into simple, fermentable sugars. The fundamental distinction between cellulose and hemicelluloses is that hemicelluloses degrades into sugar molecules with five carbon atoms, such as xylose (hemicelluloses most prevalent sugar), whereas cellulose degrades into sugar molecules with six carbon atoms, such as glucose. Because hemicelluloses lack the strength of cellulose, it's easier to break it down into simple sugars. This is partly due to the fact that hemicelluloses is virtually usually found amorphous. Overall, hemicelluloses is valuable; having commercial uses for cellulose and hemicelluloses, such as creating a smooth feel to food goods due to hemicelluloses water soluble nature. Another section of hemicelluloses containing C_5 xylan is shown in Figure 2.4.

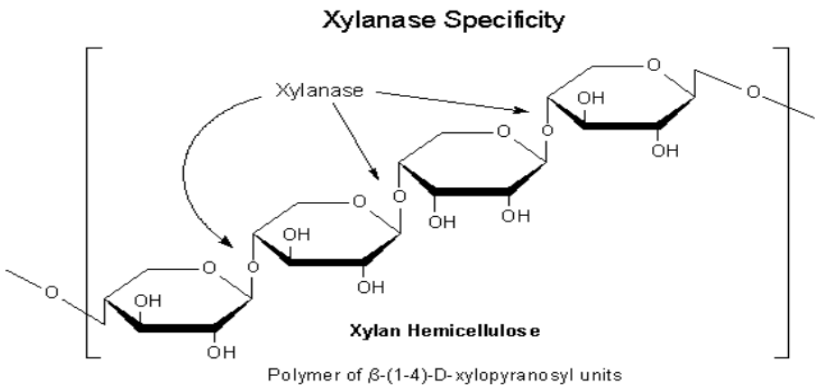


Figure 2 4 A part of hemicelluloses' chemical make-up (Daya Ram Nhuchhen, Prabir Basu and Bishnu Acharya, 2014) Depending on whether section of the hemicelluloses is examined, it has a distinct chemical structure. This section consists of C_5 Xanylans that have been connected together. This component of hemicelluloses becomes xylose after hydrolysis, which can be fermented into ethanol (Daya Ram Nhuchhen, Prabir Basu and Bishnu Acharya, 2014).

2.1.3. Lignin

After cellulose and hemicelluloses, lignin is one of the most abundant amorphous hetero polymers, consisting of three different phenyl propane units held together by various types of linkages to form a complex with hemicelluloses encasing cellulose, making it resistant to chemical and enzymatic hydrolysis. The main purpose of lignin is to give the plant structural support, impermeability, and resistance against microbial attack and oxidative stress. Lignin, with a molecular weight of less than 20,000, is found in relatively larger amounts in hardwood (oak, walnut, maple, poplar, birch) than in soft wood (pine, balsam, spruce, tamarack, fir) and herbaceous plants (grasses, rice and wheat straws) (grasses, rice and wheat straws). During the decomposition of lignin, furan (furfural and hydroxyl methyl furfural) molecules may occur, which may impede fermentation (Kankia, 2014).

Despite its absence from ethanol synthesis, lignin is quite helpful. On the outside of the cell wall, lignin serves as a barrier between the hemicelluloses and cellulose. Lignin holds everything together while maintaining the rigidity of the cell walls. Lignin is not a carbohydrate, unlike hemicelluloses and cellulose. This means it can't be turned into ethanol under any circumstances. Lignin is resistant to microbial development and chemical or biological degradation (Bon and

Ferrara, 1996). Figure 2.5 depicts the chemical structure of lignin, which is exceedingly complicated and disordered. Lignin is the most plentiful renewable resource on the globe, second only to cellulose. Lignin is generated in paper mills in excess of 50 million tons (Kankia, 2014). Because lignin burns so effectively, distilleries use it to generate electricity. Wood adhesive, UV stabilizer and coloring agent, biopolymer additive, surfactant, usage in radical technology, and durability increase (due to lignin's hard nature) are some of the other applications for lignin.

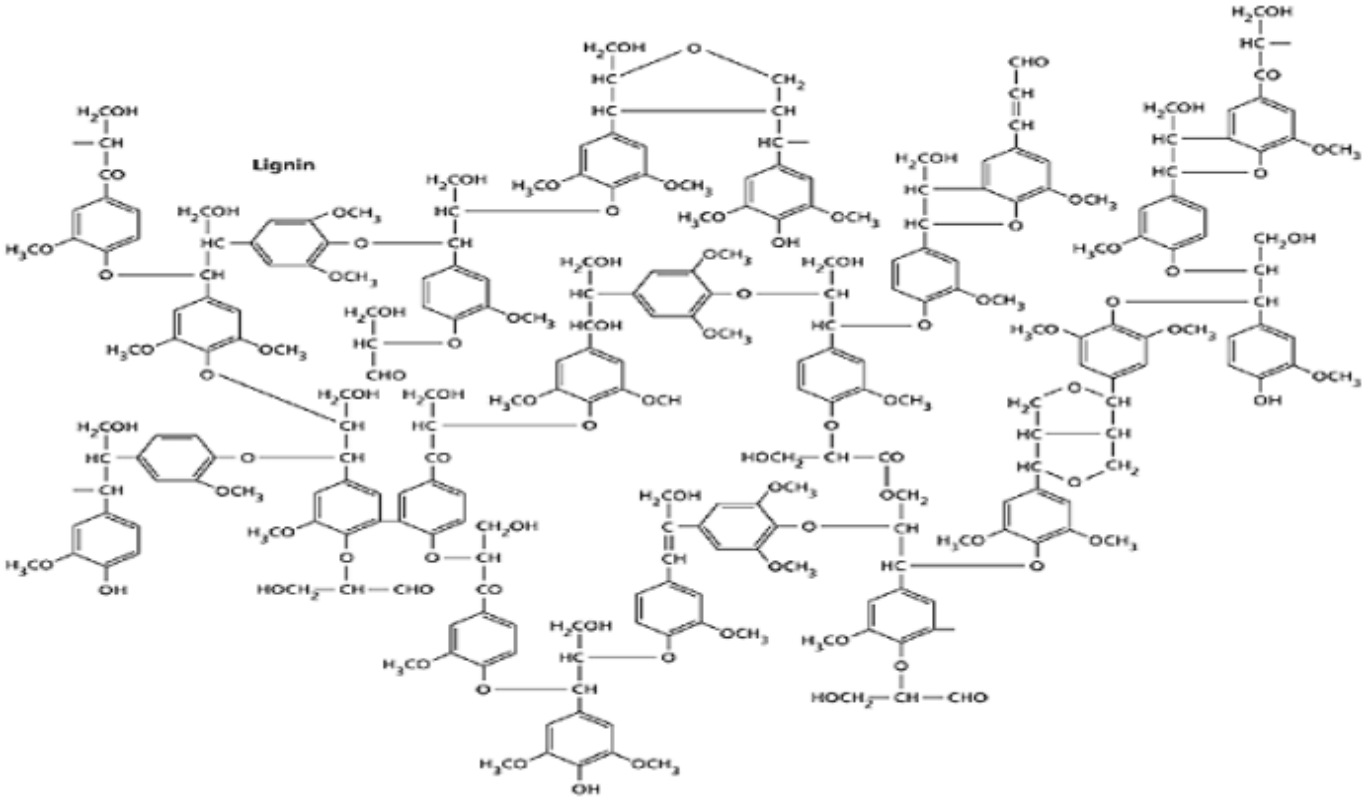


Figure 2 5 Macromolecular polymeric structure of lignin (G.C.Galletti, 1991)

2.1.4. Mucilage/water Extractive

The chemical compositions of Opuntia mucilage have been described by several research groups, with important contradictions in their results. For some authors, the mucilage was neutral with mainly D-galactose and L arabinose residues (Harlay, 1902). Others suggest the mucilage was acidic and contained L-arabinose, D-galactose, L-rhamnose and D-galacturonic acid (Anderson, Sands, & Sturgis, 1925). More recently (Parikh & Jones, 1965, 1966) have reported that the mucilage of Opuntia fulgida consisted of a backbone of β(1-3)-linked galactose units with

branches on carbon C-6 containing D-galacturonic acid, D-galactose, D-xylose, L-rhamnose and L-arabinose units. The mucilage of OFI Mill was analyzed by (Amin et al., 1970) and they found that it was neutral and contained arabinose, galactose, rhamnose and xyloseresidues

2.2 .Ethanol production from lignocellulosic biomass

All of the sugars present in the cellulose and hemicellulose must be available to the fermenting organism for a lignocellulosic ethanol process to be economically competitive with starch or sugar-based methods (Hahn-Hägerdal et al., 2007a). However, due to the above-mentioned structure and content of the plant cell wall, this process is much more difficult, resulting in high ethanol production costs (Cardona & Sánchez, 2007). The conversion of lignocellulose to ethanol is divided into three phases: (1) pretreatment, (2) acid/enzymatic hydrolysis, (3) bacteria, yeasts, or filamentous fungi fermentation of the resultant hydrolysate (Balat, 2009). Analysis of the structure and composition of the prospective biomass is required before any of the aforementioned stages may be taken. Information gained from such analysis aids the design of biomass conversion/ethanol production processes that are specific to the feedstock.

Table2.2 Numerous studies on ethanol production from cladodes have been conducted.

Authors	Title	Parameter	Result	Limitation
O.Kuloyo (2014)	Opuntiaficus-indica cladodes as feedstock for ethanol production By Kluyveromyces marxianus and Saccharomyces cerevisiae	K.marxianusand S.cerevisiae	Ethanol concentrations of up to 19.5 and 20.6 g/l,	enzymatic hydrolysis, very expensive
Hawa Myovelaetal (2018)	Enhancement of anaerobic batch digestion of spineless cacti (Opuntia ficus indica)	Time (untreated and 72 h aerobic pretreated batch bioreactors)	The amount of reducing sugar is 12 and 59 g/L	Don’t see the effect of temperatu

	feedstock by aerobic pre-treatment			re
F. Sánchezetaletal (2012)	Variability of carbohydrate content in prickly-pear OFI(Cladodes harvest date presence absence of fruits, or cladode size and age)		Suggestions for reducing sugar (8.7, 4.7, 13.1) (9.3,9.0) (8.0 ,10.3 ,7.1) (9.2,9.0)	it is difficult to determine the age of the cladodes
present work	Optimization of acid hydrolysis in fermentable sugar production from cladode by response surface methodology	Temperature of hydrolysis (120.16^{o}C), residence time (29.2 minutes), and acid concentration (3.04%)	10.3217mg/ml of TRS	plant varieties not determine

2.3 Conversion Process

2.3.1. Pretreatment

The biochemical conversion of lignocellulosic materials into bioethanol requires pretreatment. Monosaccharides are not commonly available for bioconversion in lignocellulosic materials. Polysaccharides, such as cellulose and hemicelluloses, are present instead. Pretreatment is mostly used to change the structure of cellulosic biomass in order to make cellulose more accessible to enzymes that convert carbohydrate polymers to fermentable sugars (Chen et al., 2007).Pretreatment is the most expensive processing step in the conversion of cellulosic biomass

to fermentable sugars, and many studies have concentrated on it to produce a cost-effective conversion of cellulose to bioethanol. Preserving maximum hemicelluloses fractions so that they can be converted into fermentable sugar, which can then be converted into ethanol, minimizing inhibitor formation due to degradation products, limiting carbohydrate loss, minimizing energy input, and ensuring that the process is both economically and environmentally friendly are all characteristics of an effective pretreatment. When evaluating various pretreatment solutions, all of the aforementioned characteristics should be taken into account as a whole in order to achieve the best possible end product (Kumar et al., 2009).

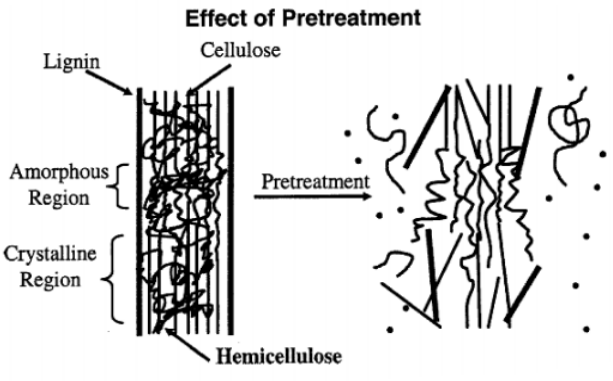


Figure 2 6 Schematic of the effect of pretreatment on lignocellulosic biomass (Mosier et al, 2005)

According on the varied forces or energy consumed during the pretreatment process, they can be classed as biological, physical, chemical, or physicochemical pretreatments (Hendriks ATWM, 2009).

2.3.1.1. Physical pretreatment

Physical preparation of biomass can be accomplished using a variety of mechanical and non-mechanical methods. To reduce biomass size and cellulose crystalline, mechanical procedures use chipping, grinding, and milling to minimize biomass combinations (Kumar et al., 2009). The amount of energy required for mechanical pretreatment is determined by the ultimate particle size as well as the properties of the biomass. In most situations, however, the energy spent exceeds the theoretical energy present in the biomass (Kumar et al., 2009 and Sun & Cheng, 2002). Irradiation and other non-mechanical methods have also been tried. The breaking of β-1,

4-glycosidic linkages occurs when cellulose is irradiated with gamma rays, resulting in a greater surface area and reduced crystalline. However, this method is far too expensive to be employed in a large-scale procedure and questions regarding its viability persist (Galbe & Zacchi, 2007) and (Kumaret al., 2009). Pyrolysis has also been studied as a means of physical pretreatment. When biomass is heated over 300°C, cellulose decomposes quickly into gaseous compounds and residual char. Decomposition is significantly slower at lower temperatures and less volatile compounds are generated. Pyrolysis, on the other hand, is an exceedingly expensive approach due to the high temperatures employed and the system's cooling expenses (Bridgwater et al., 1999).

2.3.1.2 .Chemical Pretreatment

To release lignin and breakdown hemicelluloses, chemical pretreatment uses a variety of chemical agents such as ozone, acids, alkalis, hydrogen peroxide, and organic solvents (Sánchez & Cardona, 2008).

Alkaline Pretreatment

The alkali pretreatment process frequently employs bases such as hydroxides, calcium, sodium, ammonia, and potassium. Pretreatment with bases causes ester and glycosidic side chains to degrade, resulting in cellulose expansion, partial de crystallization of cellulose, removal of lignin and partial salvation of hemicelluloses. Pretreatments with alkaline solutions improve cellulose digestibility and lignin solubilization. Compared to acid or hydrothermal procedures, alkaline pretreatment causes less cellulose and hemicelluloses solubilization. The disruption of cross linkages in NaOH processed lignocellulosic material was raised from 14 to 55 percent using alkaline pretreatment. The amount of lignin in the body has decreased from 55% to 20%. This impact was only detected in hard wood (lignocellulosic material) and not in softwood (kumar.p et al, 2009). The hydrolysis of straws with a low lignin concentration of 10–18 percent was aided by a dilute alkaline (NaOH) pretreatment. Many researches on the usefulness of sodium hydroxide pretreatment on hardwoods, wheat straw and switch grass have been published. However, data demonstrate that alkaline pretreatment from lignocellulosic materials removes less lignin in softwoods (Zhao X, 2010).

Acid Pretreatment

Acid hydrolysis or pre hydrolysis is the hydrolysis of the hemicelluloses fraction with dilute acids (usually sulphuric, hydrochloric, or acetic acid, typically 1–10 percent weight) at a moderate temperature (in the range 100–150°C). Concentrated acids, though being potent cellulose hydrolysis agents, are poisonous, corrosive, and hazardous, necessitating corrosion-resistant reactors. The entire process necessitates specialist assembly in the form of non-metallic or noncorrosive materials such as ceramic or carbon brick lining for this purpose. It is connected with environmental concerns when compared to other pretreatment processes, particularly dilute acid hydrolysis. High operational expenses are associated with the usage of intense acid hydrolysis. On a large scale, all of these disadvantages limit interest. However, dilute acid pretreatment outperforms intense acid hydrolysis when it comes to difficulties like acid recovery, toxicity, and acid and special care for corrosion-resistant materials. Several biomass feed stocks, such as herbaceous materials (grass), hardwoods, and agricultural wastes, can benefit from acid pretreatment. The majority of substrates improve performance by solubilizing hemicellulose (Sun FB, 2007).

Hydrochloric Acid (HCl) Pretreatment: Environmental Impact and Corrosiveness Hydrochloric acid's characteristics severely restrict its use. Hydrochloric acid, on the other hand, can be used to detoxify lignocellulosic materials such sorghum straw, ryegrass, and palm oil waste. Pretreatment with an acid Phosphoric acid can be used alone or in combination with alkaline treatments. The interest in the use of H_3PO_4 after alkaline pretreatment was beneficial because salt (sodium phosphate) is formed. Microorganisms utilize this salt as nutrition, thus it can stay in the form of a hydrolysate. As a result, no filter operation is frequently required, resulting in increased process profitability (by eliminating salt removal and lowering the amount of nutrients required for fermentation) and a positive environmental impact (the salt created is not a waste) (Gamez S, 2006).

Sulphuric Acid Pretreatment: Sulphuric acid pretreatment can be used on a variety of lignocellulosic materials, including wheat straw, paddy straw and sugarcane bagasse, with positive results. Sugarcane bagasse hydrolyzed with 2% sulphuric acid provided the maximum xylose recovery of 80 percent in 60 minutes. Sulphuric Acid Pretreatment: With positive results,

sulphuric acid pretreatment can be employed on a variety of lignocellulosic materials, such as wheat straw, paddy straw, and sugarcane bagasse. In 60 minutes, sugarcane bagasse hydrolyzed with 2% sulphuric acid yielded the highest xylose recovery of 80 percent.

Dilute-acid pretreatment

The use of dilute acid for lignocellulose pretreatment has proven to be successful (Sun & Cheng, 2002). Most studies have employed dilute H_2SO_4 pretreatment, especially at concentrations below 4% w/w, because it is affordable and can generate high reaction rates (Galbe & Zacchi, 2007). Also tested were nitric acid, hydrochloric acid, and phosphoric acid (Azzam, 1987) and (Xiao & Clarkson, 1997). The hemicelluloses component of lignocellulosic biomass is effectively hydrolyzed by dilute acid and the majority of it is recovered as monomeric sugars. The removal of hemicelluloses improves cellulose digestibility in the residual solids, and when the hemicelluloses is entirely hydrolyzed, glucose yields of up to 100% can be obtained (Kumaret al., 2009). There are two types of dilute acid pretreatment processes: I a high temperature (> 160^{o}C) continuous-flow process for low solids loadings (i.e. weight of solids/weight of reaction mixture equals 5-10%), and (ii) a low temperature (160^{o}C) batch method for high solids loadings of roughly 10-40 % (Karimi, 2008). Recently developed dilute acid procedures use less harsh conditions to achieve high xylan to xylose conversion. Because xylan accounts for about 30% of the total carbohydrate in many lignocellulosic materials, this is critical for overall process economics (Sun & Cheng, 2002). Despite the fact that dilute acid pretreatment can considerably improve cellulose hydrolysis, the hydrolysate has been proven to be difficult to ferment due to the presence of hazardous compounds (Galbe & Zacchi, 2007). Furthermore, the costs of creating non-corrosive reactors, applying high pressures, and neutralizing and conditioning the hydrolysate prior to hydrolysis and fermentation all add up to make dilute acid pretreatment more expensive than, say, steam explosion or the AFEX method (Kumar et al., 2009).

Ozonolysis

Many lignocellulosic materials, such as wheat straw, bagasse, green hay, peanuts, pine, cotton straw, and poplar sawdust, can be treated with ozone to decompose lignin and hemicellulose.

The degradation was largely limited to lignin and hemicellulose, which were both mildly harmed, whereas cellulose was untouched.

Pretreatment with ozonolysis provides the following benefits:

(1) It successfully eliminates lignin;

(2) It leaves no harmful leftovers for downstream operations; and

(3) The reactions take place at ambient temperature and pressure. After ozonolysis pretreatment, the enzymatic hydrolysis yield of certain agricultural residues, such as wheat straw and rye straw, increased (Garcia AMP, 2006). Despite some promising results, more study on ethanol generation from lignocellulosic materials processed with ozone is needed. The massive volumes of ozone required, which can make the process economically unviable, is a key disadvantage to consider (Sun Y, 2002).

2.3.1.3. Physico-chemical pretreatment

Steam pretreatment and ammonia fibre explosion (AFEX) are the most prevalent treatments in this category, which combine purely physical and chemical approaches (Galbe & Zacchi, 2007).

Pretreatment with steam

This method of pretreatment is extensively employed in the pretreatment of various lignocellulosic materials. It's actually a chemical process that's very similar to dilute acid hydrolysis. In this process, dry matter lignocellulosic material is subjected to high pressure. For 1–15 minutes, the appropriate material is normally immersed in high-pressure saturated steam at temperatures ranging from 180 to 260°C. The pressure is released after 15–20 minutes (Amores I, 2013). CO_2 was used as an impregnating chemical at high pressure to impregnate sugarcane bagase and sugarcane leaves. When sugarcane bagasse was pretreated at 205°C for 15 minutes, glucose yield was around 85 percent, while glucose yield was 94 percent when sugarcane leaves were subjected to a high temperature of 226°C for 15 minutes.

Ammonia fibre explosion (AFEX)

Ammonia fibre explosion is a pretreatment technology that includes working at high pressures, similar to steam pretreatment (Balan et al., 2009). AFEX comprises exposing the material to liquid ammonia (1-2 kg ammonia per kg of dry biomass) at temperatures below 100°C and pressures more than 3 MPa for 10-60 minutes, following which the pressure is abruptly lowered (Galbe & Zacchi,2007) and (Sun & Cheng, 2002). Only a little amount of hemicelluloses is solubilized after pretreatment and the lignin is not eliminated. The hemicellulose is degraded into oligomeric sugars and de acetylated, which could explain why there is no solubilization. The pretreatment, on the other hand, modifies the structure of the material, resulting in higher water retention capacity and improved digestibility(Galbe & Zacchi, 2007).Herbaceous and agricultural residues, such as alfalfa, wheat straw, maize Stover, municipal solid waste, switch grass, and sugar cane bagasse, have all been pretreated using the AFEX process. This approach, however, is only moderately efficient on hardwoods and ineffective on materials with higher lignin content, such as newsprint and aspen chips, which contain up to 25% lignin (Mosier et al., 2005) and(Sun & Cheng, 2002). Because AFEX does not produce inhibitors that could interfere with downstream processes, there is no need for a water wash after pretreatment, and the biomass does not need to be in microscopic particle sizes to be effective (Sun & Cheng, 2002).

2.3.1.4. Biological pretreatment

Biological pretreatment techniques take advantage of microorganisms' ability to decompose lignin and hemicellulose, such as brown, white, and soft-rot fungi (Sun & Cheng, 2002). White and soft rots attack both cellulose and lignin, but brown rots attack only cellulose. For biological preparation of lignocellulosic materials, white-rot fungi are the most effective. White-rot fungi degrade lignin by the action of lignin-degrading enzymes such as peroxidases and laccase (Lee et al., 2007). In response to carbon or nitrogen limitation, the white rot fungus Phanerochaete chrysosporium produces lignin peroxidases and manganese-dependent peroxidases as secondary metabolites (Sun & Cheng, 2002). Because biological pretreatment uses no chemicals and is carried out at low temperatures, it is regarded to be ecologically benign and energy efficient. However, because these organisms may consume lignocellulose to some extent, the rate of hydrolysis is sluggish and some material is wasted (Hsu, 1996). Biological pretreatment, on the other hand, might be used as a step before using some of the other types of pretreatment procedures (Galbe & Zacchi, 2007).

In general, a pretreatment procedure should include as many of the following characteristics as possible:

(1) Chemicals for pretreatment, neutralization, and subsequent conditioning are inexpensive.

(2) Waste production is kept to a minimum.

(3) Limited size reduction due to the energy-intensive and expencive nature of biomass milling

(4) Minimize pretreatment reactor expenses by using fast reactions and/or noncorrosive chemicals.

(5) To keep fermentation reactor size at a reasonable level and promote downstream recovery, the concentration of hemicellulose sugars from pretreatment should be greater than 10%.

(6) With minimal conditioning cost, pretreatment must encourage high product yields in future enzymatic hydrolysis or fermentation activities.

(7) Hydrolysis conditioning prior to following biological stages should not result in products that are difficult to treat or dispose of.

(8) A low enzyme loading should be sufficient to provide better than 90% digestibility of pretreated cellulose in fewer than 5 days, ideally 3 days.

(9) Pretreatment should make it easier to extract lignin and other elements for conversion to valuable co-products and downstream processing (Quintero-Ramirez, 2008).

Table2.3 Summary of the advantages and limitations of various pretreatment methods (kuloyo, 2012)

Method	Pretreatment Process	Advantages	Limitations

Physical Pretreatment	Mechanical	Reduces cellulose crystalline and increases biomass surface area	The amount of energy required is usually greater than the amount of energy inherent in biomass.
	Combination		
	Pyrolysis	Produces gas and liquid Products	High temperature; ash Production
Chemical Pretreatment	Ozonolysis	Reduces lignin content; toxic substances are not produced.	Expensive;ozone required in large amounts
	Dilute acid	Hydrolyzes hemicellulose to xylose and other sugars, causing lignin structure to change.	High cost; equipment corrosion; formation of inhibitors
	Alkali	Removes hemicellulose and lignin; increases biomass surface area	Long residence times; Irrecoverablesalts formed and incorporated into biomass; not effective on softwoods
	Hydrogen Peroxide	It dissolves lignin and produces inhibitors.	Hydrogen peroxide decomposes at high temperatures, causing a Decrease in lignin and hemicellulose solubilization
	Organosolv	Hydrolyses lignin and hemicelluloses	High cost; solvents need to be recovered and recycled
Physicochemical	Steam Pretreatment	Causes hemicellulose degradation and lignin transformation; short residence time; cost effective	Destroys a portion of the xylan fraction;incomplete destruction of the lignin carbohydrate matrix;

Pretreatment			formation of toxic compounds
	AFEX	increases biomass surface area, removes some hemicellulose and lignin, and prevents the formation of inhibitory compounds	Ammonia is expensive and hazardous; it is ineffective for biomass with high lignin content.
Biological Pretreatment	Fungaldelignification	Degrades lignin and hemicellulose; low energy required	Slow reaction rate; loss of Cellulose

2.3.2. Hydrolysis

Hydrolysis is the next step in the lignocelulose conversion process. This is the process of breaking down complex curbs into simple sugars by adding water. Because cellulose and hemicelluloses hydrolysis is a time-consuming process, either an acid or an enzyme is usually used as a catalyst. Water has difficulty penetrating the tight bonds of cellulose (especially crystalline cellulose), which is another reason why an enzyme or an acid is needed (Wang, 2007).

2.3, 2.1.Acide hydrolysis

One method for hydrolyzing cellulose is acid hydrolysis. To maximize output, more research is being done to identify which acid to use, at what dose, and for how long. One of the most difficult aspects of acid hydrolysis is determining the proper concentration. A high acid concentration dissolves the bonds between simple sugars, but it destroys the lignocellulosic materials. Although a little concentration of acid does not damage the substance, it is unable to penetrate the crystalline cellulose's strong bonds. Acid hydrolysis is not the most effective method of glucose production. According to one study, the greatest actual yield of glucose from cellulose acid hydrolysis is always less than 70 % (Quintero-Ramirez, 2008). When the incorrect acid is employed, hazardous byproducts can result. Because the hydrogen bonds between sugar groups are weaker than those between glucose groups in cellulose, the acid can be diluted while

the process is yet completed. Enzyme hydrolysis is another option for completing the hydrolysis process. Instead of acid, an enzyme is introduced to the pretreated cladodes in the presence of water or steam.

2.3.2.2. Enzymatic hydrolysis of cellulose

Currently, highly specialized cellulases are used to hydrolyze cellulose under mild circumstances (pH 4-5, temperature 45-50^{o}C) (Sun & Cheng, 2002). Glucose and other reducing sugars are the end products, which yeasts and bacteria can ferment into ethanol (Sun & Cheng, 2002). Cellulases are enzymes that belong to one of three groups: I endoglucanase or cellobiohydrolase (CBH,β -1,4-D-glucan cellobiode hydrolase), which degrades the cellulose further by removing cellobiose units from the free chain ends; (ii) exoglucanase or cellobiohydrolase (CBH, β-1,4-D-glucan cellobiode hydrolase), which degrades the cellulose further by removing cellobiose units from the free chain ends(iii) -glucosidase, which breaks down cellobiose into two glucose molecules (Prasad al., 2007).

Commercial cellulases are mostly made from Trichorderma reesei and, to a lesser extent, Aspergillus niger (Sánchez & Cardona, 2008). Sclerotium, Schizophyllum, and Penicillium species are among the fungi that have been identified to produce cellulases. Clostridium, Cellulomonas, Bacillus, Thermomonospora, Ruminococcus, Bacteroides, Erwinia, Acetovibrio, Microbispora, and Streptomyces are examples of bacteria that can create cellulases with high specific activity but low enzyme titres(Sun & Cheng, 2002) .The enzymatic hydrolysis of cellulose can be influenced by a number of circumstances. The overall glucose yield would be poor if the substrate concentration was low (Hamelinck et al., 2005). An increase in the substrate concentration would result in a higher glucose yield and a faster reaction rate. A high substrate concentration, on the other hand, can generate substrate inhibition, which reduces the rate of hydrolysis significantly, and the level of substrate inhibition is determined by the ratio of total substrate to total enzyme. A high cellulase dosage would also increase process costs dramatically (Prasad et al., 2007). The structural characteristics of cellulosic substrates, such as cellulose crystallinity, degree of polymerization, surface area, and lignin content, influence their susceptibility to enzymatic hydrolysis (Sun & Cheng, 2002) & (Karimi,2008). Lignin acts as a barrier to hydrolysis, blocking celluloses from accessing cellulose and hemicelluloses, resulting

in longer reaction times and lower conversion rates. Furthermore, lignin adsorbs a considerable percentage of the cellulase in an irreversible manner, rendering it inaccessible for further cellulose hydrolysis (Qing et al., 2010). As a result, eliminating lignin during pretreatment is crucial for raising the hydrolysis rate significantly (Prasad et al., 2007). Furthermore, removing hemicelluloses raises the substrate's mean pore size, improving cellulase accessibility to cellulose (Hendriks & Zeeman, 2009). The irreversible adsorption of cellulases on cellulose causes cellulase activity to decrease during hydrolysis (Sun & Cheng, 2002). For laboratory research, an enzyme dosage of roughly 10 FPU/g (filter paper units per gram cellulose) is commonly employed since it produces excellent yields in a fair amount of time (48-72 hours) and at a reasonable cost (Sun & Cheng, 2002). Non-ionic surfactants, non-catalytic protein (e.g. bovine serum albumin), and polymers (e.g. polyethylene glycol) have been shown to significantly improve the enzymatic conversion of cellulose into fermentable sugars while reducing the number of enzymes required for hydrolysis (Qing et al., 2010). Surfactant addition is thought to have a favorable effect on lignocellulose through the following mechanisms:

I. changing the substrate structure to make it more accessible to enzymes,
II. stabilizing enzymes and preventing de naturation during hydrolysis,
III. Boosting surface interaction between substrates and enzymes, and
IV. Decreasing nonproductive enzyme absorption. However, a consistent mechanism for how surfactants promote enzymatic hydrolysis has yet to be discovered (Qinget al., 2010).

Improved hydrolysis yields and reaction rates have also been achieved using cellulase mixes from several organisms or a combination of cellulase and other enzymes (Sun & Cheng, 2002). The extent of cellulose and hemicellulose conversion is also significantly increased when hemicellulases or pectinases are combined with cellulases (Sun & Cheng, 2002). Even though T. reesei cellulases include some -glucosidase, the enzyme responsible for hydrolyzing the generated cellobiose into glucose, its activity is modest. Unfortunately, when cellobiose is produced during enzymatic hydrolysis, end-product inhibition of cellobiohydrolases occurs. To complement the action of the cellulases from this fungus, -glucosidase from other sources should be introduced (Sánchez & Cardona, 2008). Inhibition of intermediate and end products can also be decreased by utilizing larger enzyme concentrations, ultra filtration to remove sugars during

hydrolysis, or simultaneous saccharification and fermentation. Enzymes can also be recovered and recycled to save money on enzymes, albeit the quality of the enzymes will deteriorate with each recycling process (Hamelin et al., 2005). Enzyme hydrolysis can be quite efficient, with some studies indicating a yield of over 95% on fermentable sugars (Quintero-Ramirez 2008). However, because of the actual challenges in the production process, as well as the limited reusability of past stock, enzyme manufacture is quite expensive. According to some research, enzyme hydrolysis can account for up to 40% of the cost of manufacturing ethanol (Miyamoto, 1997).

2.3.3. Fermentation

Fermentation is the next step after hydrolysis. Fermentation has remained mostly unchanged over time. The goal of current research is to figure out the most efficient and cost-effective ways to ferment simple carbohydrates. The best yeast to employ in fermentation, the best means to transport the ethanol/water mixture from fermentation to distillation, and the most effective way to retain yeast after fermentation are all goals of future study.

Fermentation takes occur in big fermentation vats in a typical mill. The procedure might be carried out in stages or in a continuous manner. Because the same strain of yeast can create ethanol from both types of carbon sources, C_5 and C_6 (five and six carbon molecules, respectively) can be blended in the same vat. These vats can be open to the atmosphere (aerobic) or confined to the atmosphere (anaerobic) (mostly anaerobic). The advantages of a closed vat include increased efficiency, as the simple sugars are fermented rather than respired (anaerobic process), and the ability to recover the evaporated alcohol using carbon dioxide. The disadvantage of a closed vat is that it is more costly to construct and maintain. In a 2 to 1 ratio, the simple sugar/water mixture is incubated with yeast. Fermentation takes 4 to 12 hours and takes place at a temperature of 32 degrees Celsius. The vats must be cooled, usually by water circulation, because the fermentation process is exothermic.

The efficiency of the fermentation process can be influenced by a variety of factors. The effectiveness of the fermentation process is influenced by the sugar content. There is too much water in the broth if there isn't enough sugar, which means additional distillation and wasted time

and money because more alcohol could be produced in the same amount of time. A high sugar concentration can increase osmotic pressure in the cells, lowering fermentation efficiency significantly. The most common sugar percentage is 16 to 18 percent (Guar, 2006).

The temperature of the broth can also have a significant impact on its efficiency. Sugars are typically fermented at 25-35 degrees Celsius in a typical mill. Too high a temperature might reduce cell viability and output (Guar, 2006). The broth heats up on its own due to the exothermic nature of fermentation. As a result, several mills have discovered that keeping the soup cool is more cost-effective because it saves resources. Although the volume of alcohol produced is lower than usual, the expenses of manufacturing are lower, saving money for many mills. The efficiency can also be affected by pH. Because the pH drops during the fermentation process, this is critical. If the pH isn't kept under control, it might have a negative impact on the ethanol yield. All three of these factors contribute to a more efficient fermentation process, allowing mills to generate a liter of ethanol for the least amount of money (kuloyo, 2012).

Fermentation and hydrolysis are done in the same bioreactor, which saves money in the long run. In addition, the presence of ethanol during hydrolysis minimizes the risk of contamination, which is especially important in commercial operations .SSF operation, on the other hand, has been found as a large contributor (> 20%) to the cost of producing ethanol from biomass, as well as having the major disadvantage of having different optimum temperatures for saccharification (50°C) and fermentation (35°C). On the plus side, sugarcane has been discovered as a viable cellulose substrate (Krishna et al., 2000), and using sugarcane leaves (agro-residues burned after harvesting the crop) could help with pollution abatement.

3. MATERIALS AND METHOD

3.1. Equipment required:

Table3.1 Lists of materials, models and their purposes utilized to conduct this research

Equipment	Types of equipment/model	Purpose
Bag	Plastic bag	To collect and transport samples
Knife	Any knife	for chopping up the sample
Oven	202-OA, Germany	For drying
Crusher		To mill dried sample
Sieve	250m particle size, No. 60mesh	To sieve the crushed sample to the particle size of 1mm or less
Balance	HCB1002-ADAM, UK	To weigh samples
Digital pH meter	3505-JENWAY, UK	To measure the pH of the hydrolysates
Graduated cylinders	250-ml volumetric flask	for volume measurement
Autoclave	HV-110 autoclave	For pretreatment and hydrolysis
VISspectrophotometer	Biochrom Libra S11^{TM}	To ascertain reducing sugar
Filter paper	Whatman # 1 filter paper	For filtering material
Refrigerator		For frozen samples at-18ºC until analyzing
Centrifuge	Thermo Fisher Scientific, USA	Supernatants must be separated.

3.2. Chemicals required

Different chemicals were utilized in order to achieve the best result and arrived at the set objective

Table3. 2 List of chemicals used

Chemical	Types of chemicals	Purpose
sulfuric Acid	diluted sulfuric Acid	For Hydrolysis
Sodium hydroxide/NaOH		To adjust ph and DNSA reagent preparation
Water	Distilled water	For delusion
Sodium sulfite		used for DNS reagent preparation
potassium and sodium tartrate		used for DNS reagent preparation
Phenol		used for DNS reagent preparation
3,5-dinitrosalicylic acid		used for DNS reagent preparation

3.3. Experiment methods

The overall activities for this thesis are depicted below in a block diagram, beginning with raw materials and ending with the final activities.

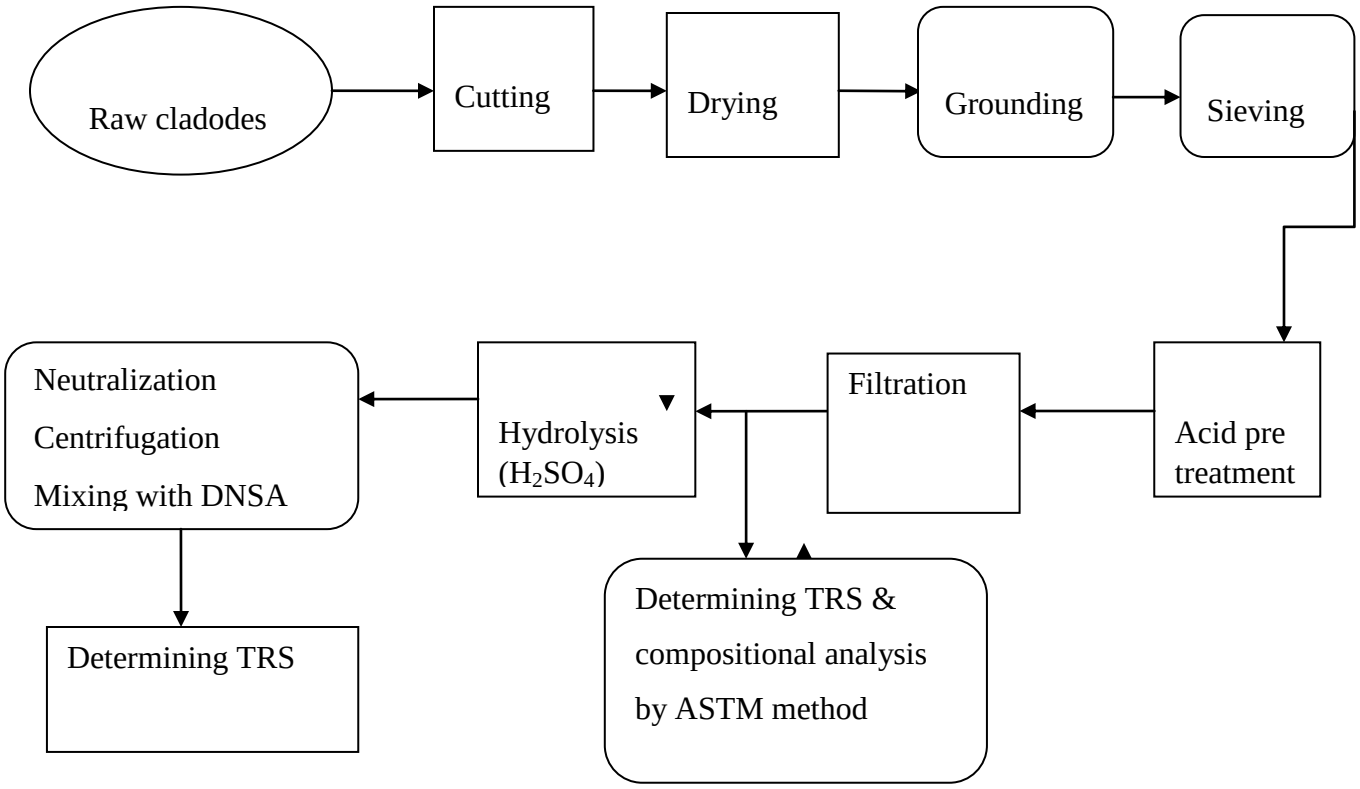


Figure3.1 Steps involved with determination of reducing sugar from cladode

3.3 1.Sample preparation

Raw material and compositional analysis

A large number of yang Opuntia ficus indica cladodes (sprouts ranging in length from 18 to 25 cm) were collected in Burie (northwestern Ethiopia), at longitude 34° 4' east, latitude 10° 42’N, and an altitude of 2091 m. Cladodes first appeared in March 2020.The cladodes were cut into strips with a mechanical shredder (knife), sun-dried, and then hammer milled to a particle size of

1 mm. To ensure representative samples, the dried and milled cladode biomass was properly mixed and stored in a sealed container at room temperature until further usage. The total solids, ash, cellulose, structural carbohydrates, lignin, and extractives of the cladode were determined using conventional biomass analytical methodologies defined by the National Reliability Energy Laboratory (2012).

Figure3.2 Mechanical pretreatment of O. ficus-indica cladodes

Compositional analysis by standard ASTM methods

Preparations of water extractives from biomass

A powdered biomass sample of 10g with a particle size of 600μm or less was used to prepare extractives. After that, 200 mL of distilled water was added to the sample. For 3 hours, the solution was maintained in a boiling water bath at 80°C. It was then filtered through a 100-meter-man filter paper. After that, the liquid filtrate was kept at -4°C in a sterile container. The filtered biomass residue was dried at 105°C in a hot air oven until a constant dry weight was obtained. (Vijayanand c. et al, 2016)

A. Determination of hemicelluloses in biomass

To measure the hemicelluloses present in the biomass, a 1g sample of dried extractive was obtained and 10 ml of 0.5 mol NaOH solutions were added to it. The solution was then

maintained at 80°C for 3.5 hours in a boiling water bath. The pH was then neutralized by washing it with distilled water. 20g of NaOH was dissolved in 1 liter of distilled water to make a 0.5mol NaOH solution(Vijayanand c. et al, 2016).

$$\text{Hemi cellulose} = \text{Weight}_{\text{Final}} - \text{Weight}_{\text{initial}}$$

B. Lignin determination in biomass

The national renewable energy laboratory (NREL) method will be used to determine the amount of acid soluble lignin (ASL) and acid insoluble lignin (AIL). 150 mg of pretreatment and milled sample is combined with 1.5 ml of 72 percent sulfuric acid and incubated for one hour at 30 degrees centigrade in a water bath. The acid will then be diluted to 4 percent H_2SO_4 acid with 42 ml of distilled water. It was then autoclaved for one hour at 121 degrees centigrade. After drying and converting the final mixture, the filtrate is collected for ASL determination and the residue is taken for AIL determination.

% AIR= (weigh of crucible plus AIR-weight of crucible)/ODW sample/100, an acid-insoluble residue (AIR)

% AIL = AIR-% ash-% protein spectrometer analysis is used for acid soluble lignin. This will be carried out at 240absorbance.

% ASL= (UV abs * volume filtrate * dilution)/ (ε * ODW sample * path length) * 100

Where UV abs is UV absorbance, ε is absorptive coefficient of biomass at wave length, ODW is weight of sample

Dilution= (volume of sample + volume of diluting solvent)/(volume of sample)

From the above lignin determination

% Extractive free lignin=% ASL+AIL

C. Determination of ash content in biomass

The ASTM D 482 standard technique was used to determine the ash content of the paddy straw biomass. A 2g biomass sample from the oven dried sample was deposited in a crucible and heated in a muffle furnace at 575.25°C for 4 hours, then cooled to room temperature in desiccators and weighted. After that, it was dried in a muffle furnace to a consistent weight. The percentage of ash in the sample was determined using an expression, (Vijayanand c. et al, 2016)

Ash (%) = [weight of crucible with ash) – (weight of crucible)/oven dry weight of sample] x 100

D. Determination of Protein Content in Biomass

Protein content was determined using the automatic Velp Scientifica Kjeldahl analyzer instrument (UDK 159), according to AOAC International, (2010) with official Method 920.87. Briefly, cladode flour sample (0.5 g) was mixed with a catalyst (K_2SO_4 and $CuSO_4$.$5H_2O$) and digested in 12 ml of concentrated sulfuric acid at 420 °C for 60 min to liberate the organically bound nitrogen in the form of ammonium sulfate. The ammonia in the digested ammonium sulfate is then distilled off with reagents (50 ml distillation water, 50 ml NaOH, and 30 ml H_3BO_3) added and then automatically titrated with standard hydrochloric acid (0.1 N). Finally, a conversion factor of 6.25 is used to convert the total nitrogen content to determine the crude protein of the samples, as shown below

% protein = % nitrogen ×NF

Where: NF = nitrogen factor = N-Facto=6.25

E. Determination of cellulose

After considering the extractives, lignin, hemicelluloses removal, protein, and ash of the sample, the amount of cellulose that remained after pretreatment was estimated. (Vijayanand, et al., 2016)

100- (Lignin + hemicellulos + extractives + protein + ash)

3.4. Biomass pretreatment

First, 200 g of cladode flour was mixed with 672 ml of a 1.5 percent (w/w) solution of H_2SO_4 to achieve a solid loading of 30 percent (w/v) in a Hiclave HV-110 autoclave (Hirayama, Saitama, Japan) for autoclaving the vessel containing the slurry. A central composite design was used to optimize the pretreatment conditions, which included a dilute acid concentration (1.5 percent), time (50 minutes), and a temperature of 120 degrees Celsius (Kuloyo, 2012). The slurry was washed with 100 mL of demonized water after cooling and vacuum filtered through a wire mesh to extract the solid fraction (WIS) and liquid fraction (WIF) (prehydrolysate).The WIS fraction was oven-dried for 24 hours at 40°C before being employed in acid hydrolysis studies. A part of the WIS was preserved for structural component analysis, while the liquid fraction was tested for sugar content.

3.5. Acid hydrolysis of cladodes and optimization by RSM

Because of its inexpensive price (in comparison to other inorganic acids), ease of handling, and high effectiveness in the hydrolysis reaction, dilute inorganic acid with sulphuric acid (H_2SO_4) is one of the most commonly used reagents. The use of sulfuric acid has been shown to remove up to 100% of the hemicelluloses present in various residues (Taherzadeh and Karimi, 2008).Mild acid hydrolysis facilitates the conversion of soluble oligomers to their respective monomers.

A stainless steel autoclave with 1 atm and temperatures of 109°C, 115°C, and 121°C was utilized to carry out the hydrolysis, and flasks were placed inside with aluminum lids to prevent leakage during the process (A. Texco-loper, 2018).

The solid-to-liquid ratio was set at 1:15. (Cladode: dilute sulphuric acid solution of 1 g/ ml). This ratio was chosen because it is close to the water content in immature OFI cladodes, according to various reports (Malainine et al., 2003),(Ginestra et al., 2009) , (Kuloyo et al., 2014) and (Yang et al., 2015). The hydrolysis was carried out (in triplicate) under these circumstances for 10, 20, and 30 minutes with the following concentrations of sulphuric acid solutions: 2, 3, and 4%. The total reducing sugars were measured at the end of the hydrolysis and the treatment that released the highest TRS was chosen. The hydrolysates were neutralized with NaOH and centrifuged for 10 minutes at 8000 rpm. (A. Texco-loper, 2018).

The data was examined using the Design Expert® application to create an experimental design for optimizing hydrolysis. Temperature, acid percentage, and residence duration were chosen as the optimization factors, with a constant solid: liquid (S: L) ratio as the response variable. As the response variable, the TRS was obtained. We continued to validate the already optimized model after finishing the design

3.6 Determination of Reducing Sugars

Using the dinitrosalicyclic acid method established by Miller et al. (1959) using glucose as a reference, the concentration of reducing sugars was measured.

Estimation of reducing sugars by DNS method

To create the DNSA reagent, combine 0.5g of 3, 5-dinitro salicylic acid with 0.125g of sodium potassium tartarate, 0.1g of phenol, and 0.025g of sodium sulphate in a 50 ml volumetric flask. The mark was filled with fresh water and 10ml of NaOH solution (2mole/l) was added, which took 1:30 hours to dissolve. For the determination of reducing sugars, 1 ml of extractive solution was mixed with 5 ml of distilled water in each test tube, followed by 1 ml of DNS reagent. The solution was heated for 5 minutes in a boiling water bath, and then cooled before being examined in a spectrometer with a 540nm band width.

3.7. Experimental Design

In trials with two or more factors, the box behken design is commonly utilized. It will be able to investigate the relationships between the variables in depth and in a methodical manner. Furthermore, it identifies important elements. Design Expert Software created the experimental factors of arrangement and interactions in this investigation. Hydrolysis temperature, duration, and acid concentrations were employed as independent input process variables in this experiment. Experiments (3 factors, 3 levels and 2 replications) were employed, and analysis was performed to determine the interaction effects of the components and their impact on the determination of reducing sugars. The study's actions were expressed by determining the project's level and factors.

Table3.3 Factors and the corresponding levels of the experimental design

Factors	Level		
Sulfuric acid concentration (%)	2	3	4
Hydrolysis temperature (℃)	109,	115,	121
Hydrolysis time (mint)	10	20	30
Replication =3			
Single run	12 and 5 center points=17		
Total run	41		

3.8. Data Analysis

Process parameter/factor interaction effects on the response were analyzed using design expert software by box behken design 3Factors and levels analyzed

4. RESULTS AND DISCUSSION

4.1. Composition of the raw material

The OFI cladodes have high water content (91.8%), low lignin content (7.9%) and a high content of water-soluble chemicals (mucilage) (27%) according to their physicochemical analysis .The mucilage of OFI was analyzed by Amin et al., 1970 and they found that it was neutral and contained arabinose, galactose, rhamnose and xylose residues. The raw material yielded S: l ratio of 15 (A.Texco liper et al, 2018) which is comparable to other research. Plants that use CAM metabolism have high moisture content. These plants create hydrocolloids in their tissues for the purpose of storing water. This characteristic is critical for the survival of plants that grow in arid environments (Yang et al., 2004). The content of the mucilage is also influenced by factors such as the age of the cladodes. Mucilage enhances cell adhesion in older plants, lowering its concentration in the plant (Chang et al., 1994)and (Habibi et al., 2004). The moisture content, on the other hand, fluctuates according to the season. The moisture content of the cladodes increases dramatically during the rainy season, but has minimal effect on the carbohydrate content (Ribeiro et al., 2010). The results revealed a sugar concentration that differed from that reported by other writers. The biggest changes discovered were in lignin content, which were most likely attributable to the age of the cladodes employed in this study.

Table 4.1 compares the results of the current study to those reported by other writers. There are variances in the values, as can be seen. Climate conditions, plant kinds and other factors may all play a role in these variances (A.Texco-lopper et al, 2018). According to Stintzing and Carlem (2005) and Ribeiro et al. (2010), edaphic variables and crop location, among other things, have a significant impact on the composition of OFI

Table4.1.Physicochemical characteristics of OFI cladodes Results are presented on a dry basis, with the exception of water content (n.r. not reported).

Author	Water contain (%)	Extractive (%)	Ash (%)	Protein	Lignin (%)	Hemicelluloses (%)	Cellulose (%)
In this work	**91.8**	**27**	**29**	**6.87**	**7.43**	**11**	**18.7**
A. Texco-Lóper et al 2018	94.7	63.1	15	n.t	2.7	n.t	18.9
Malainine et al. 2003	n.r	48	19.6	n.r	3.6	n.t	21.6
Kuloyo et al. 2014	88-95	24.3	16.8	7.5	7.9	n.t	23.1
Yang et al. 2015	93.9	25.0	23.7	7.4	12.3	18.5	13.1
Ginestra et al. 2009	n.r	17.3	23.7	6.42	16	n.r	13.5
Miciteka,2008	n.t	n.t	22.5	5.5	11.8	9.1	6.8

4.2. Model Fitting and Response Surface Methodology

From the model alternatives (linear, two factor interaction (2FI), quadratic, and cubic polynomial), the RSM under design expert provided a "suggested quadratic model" as shown in appendix 3 with a good agreement between the adjusted R^2 and Predicted R^2 (i.e. the difference is less than 0.2.) was chosen for the study due to its highest order polynomial.

Analysis of variances (ANOVA) was used to evaluate the quadratic model fitness and significance, as well as individual terms and their interaction effect on the responses, in the statistical analysis of sugar production from cladodes of OFI employing H_2SO_4 as a catalyst, as shown in appendix-4. The ANOVA result's probability of error value (p-value) was used to evaluate the quadratic model's relevance as well as the significance of each model term. The

greater the significance, the lower the p value; p-values less than 0.05 indicate the statistical significance of a given model component, otherwise insignificant (P > 0.05). (Lee et al.2011).

P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC BC, A^2, B^2, C^2 are significant model terms. The "Lack of Fit F-value" of 2.73 with P-value of 0.1782 implies the Lack of Fit is not significant relative to the pure error. There is a 17.82% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good because we want the model to fit. In short, the p-value of the lack of fit parameter is greater than 0.05, and even from 0.1, demonstrating the behavior of good fitting between experimental data and the model. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The Model F-value of 158.23 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

The Predicted R^2 of 0.9217 is in reasonable agreement with the Adjusted R^2 of 0.9888; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 41.209 indicates an adequate signal. This model can be used to navigate the design space

The regression model equation in terms of coded factors (A-temperature, B, time, and C-acide concentration) could be used to make predictions about the response, and the final equation can be expressed as:

$$TRS=8.3+2.62A+1.19B+0.3038+0.0575AB-0.0175AC-0.3BC-1.38A^2-0.4075B^2-0.9825C^2$$

The positive sign before the phrases indicates a synergistic effect in raising the reducing sugar concentration, whereas the negative sign indicates an antagonistic effect in lowering the yield (Lee et al., 2011). As a result, the positive coefficients of A, B, C and AB imply that the sugar concentration, increment, has a linear effect on TRS. However, as their value increases, the terms AC, BC, A^2, B^2, C^2 and have a negative influence on diminishing the yield.

Below are the factors and levels of work (Table 4.3). ‘This design was broken down into 12 experimental components, each with five center points. All of the tests were done in triplicate.

However, the terms AC, BC, A^2, B^2, C^2 and have a negative effect on decreasing the yield as their value increases further.

The factors and levels of work are shown below (Table 4.3). From this design, it was analyzed into 12 experimental units) with 3 replication and 5 central points.

Table4.2 3Factors and levels analyzed by box behken design

Variables	Symbol used for the variable	Low	Center	High
Temperature	A(0c)	109	115	121
Time	B(min)	10	20	30
acid concentration	C (%)	2	3	3

Table 4 3 Design obtained by the program Design Expert® (TRS mean values of 3 replication)

Run	A:tepretur (Oc)	B:time (Min)	C: acid coc (%.)	TRS. (mg/ml)
1	115	20	3	8.30
2	115	30	2	8.00
3	121	30	3	10.33
4	121	20	4	8.65
5	115	20	3	8.30
6	109	20	2	3.20
7	109	10	3	2.82
8	109	30	3	4.92
9	109	20	4	3.50

10	115	30	4	8.25
11	121	10	3	8.51
12	121	20	2	8.42
13	115	10	2	4.87
14	115	20	3	8.30
15	115	20	3	8.30
16	115	20	3	8.30
17	115	10	4	6.42

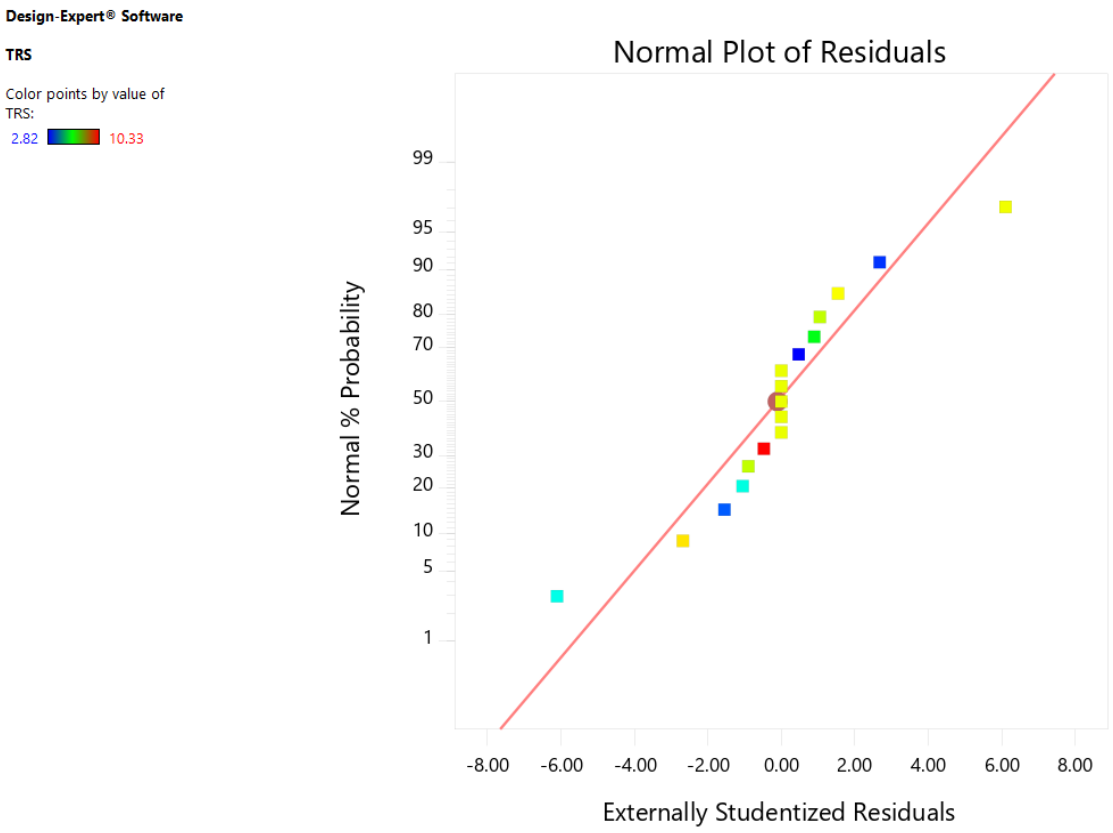
4.2.1. Model Adequacy Checking and Diagnostic plot

It's crucial to double-check the model's accuracy in describing the fluctuation of the reduced sugar concentration as a function of the chosen process variable. As a result, the value of coefficients of determination (R^2), which reflects the fluctuation of the dependant variable by the predictor variable, can be used to verify its correctness/fitting. R^2 (measures the amount of variation around the mean explained by the model), Predicted R^2 (measures the amount of variation in new data explained by the model), and Adeq precision (signal-to-noise ratio).

A higher R^2 value around unity, better projected R^2 values with fair agreement with the Adjusted R^2 with a difference of less than 0.2, and a value of appropriate precision of more than 4 are all desirable for a model to demonstrate that the experimental data and the model are well-fitting. The absence of several important terms from the model or the presence of unusually large residuals (difference between the observed and predicted response) resulting from fitting the model indicate that the model does not adequately describe the relationship between the dependent and independent variables (Dharma et al., 2016). As a result, the decreased standard deviation (0.2401) and C.V (3.42) show that the model is more precise and reliable. The greater R^2 (0.9951), which is close to unity, indicates that 99.51 percent of the response variance can be attributed to the independent variable, while only 0.49 percent of the overall variation is unaccounted for by the model. A good agreement with a minor difference between Adjusted R^2 (0.9888) and Predicted R^2 (0.9257) values, as well as a bigger value of Adeq Precision

(41.2089), suggests that the model is significant in showing great correlation between the dependent and independent variables.

A diagnostic plot of a model (Figure 4-1) uses a normal probability plot of the residuals and an actual versus predicted value plot to illustrate its adequacy. Externally, the student zed residuals plot is a more sensitive diagnostic plot that looks for outliers by mapping all the individual normal distributions to a single standard normal distribution, and it was selected to facilitate the satisfactory fit of the developed model. The normal probability plot of residuals shows the normality of data (Dharma et al., 2016), in which the errors are normally distributed in a straight line and insignificant (Lee et al., 2011). A non-linear pattern suggests that the error term is not normal. The plot depicts an almost linear distribution of data points near and on the normal line in this case. The plot of actual against anticipated values, on the other hand, is used to identify a value or collection of values that the model cannot easily predict. As a result, all predicted points are reasonably close to being on a straight line, implying adequate agreement with a high correlation (R^2 = 0.9951) between the actual reducing sugar concentration and the model's anticipated value. The contribution of x to predicting y is measured by R^2, which indicates how much variance in the dependent variable is explained by the independent variable.R^2= 1: Perfect match between the line and the data points and R^2= 0: There are no relationship between x and y.



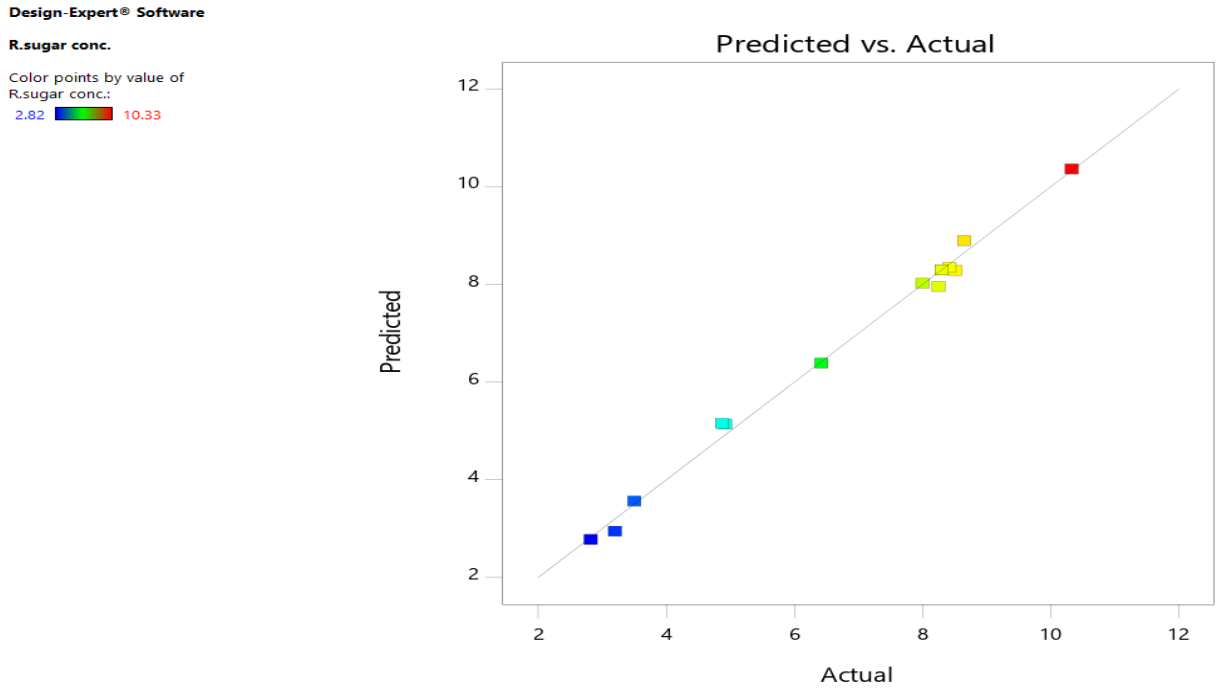


Figure4.1.Diagnostic plots for the proposed model

4.2.2. Single factor effects on the TRS concentration

According to the findings, the significant hydrolysis process variables under investigation (A-temperature, B-time, and C-acid concentration) and their interaction under the stated operating conditions can influence the reduced sugar concentration. The minimum and highest reducing sugar concentrations from the hydrolysis variables combination were (2.82mg/ml) and (10.33mg/ml), respectively, according to the experimental results.

The best result was obtained at the design point of the predictor variables (right edge/121^0c, 30 min, 3 percent (H_2SO_4) in the experiment. The following is a discussion of the effects of the selected hydrolysis parameters on the response based on a 2D plot of graphical observation illustrating the effects of changing the level of a single factor.

Effects of temperature

The influence of reaction temperatures on TRS yield was examined in the hydrolysis of cladodes under mild acid treatment at temperatures ranging from 109^0C to 121^0C, as shown in Fig. 4. 2. The yield of TRS tends to increase with increasing temperature from 109^0C to 121^0C, as shown in Figure 4-2; the maximum yield of TRS was reported to be 10.33 mg/ml at 121^0C and 30 minutes of reaction time.When the temperature was elevated above 121°C, however, the concentration dropped (Etana, 2021), indicating that sugar was being degraded into non-fermentable compounds such as hydroxyl methyl furfural (HMF) and furfural, both of which are harmful to microbial cells (R. Sindhu, 2014).

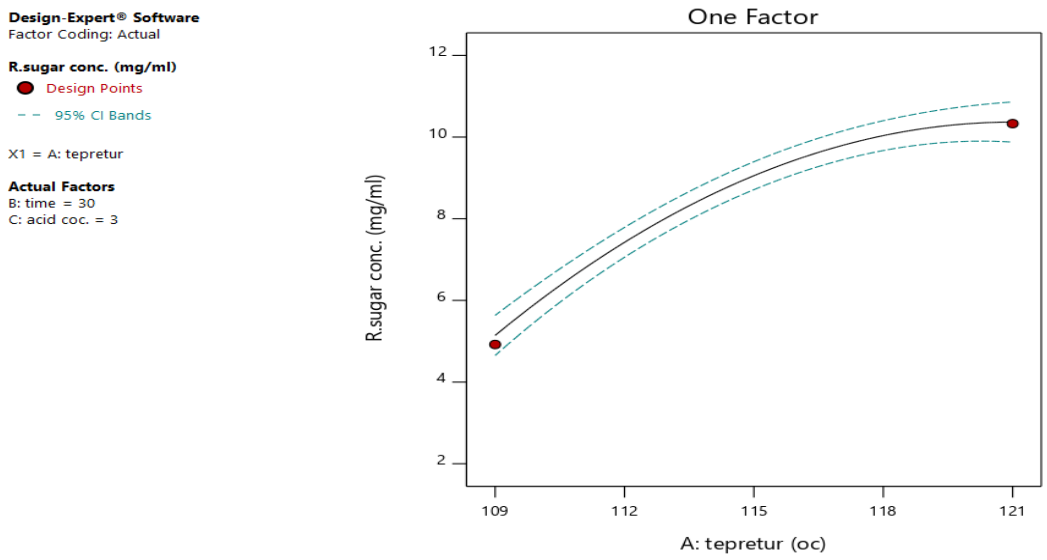


Figure4. 2 Effect of temperatures on reducing sugar concentration

Effects of time

The hydrolysis of the cladode flour was seen (figure 4.3) to take place in a very short length of time and to stabilize between 10 and 30 minutes. Part of this occurrence could be explained by the fact that the mucilage in its hydrocolloid is made up of hydrolyzable neutral polysaccharides like arabinose, rhamnose, galactose, and xylose (Wyman et al., 2005). The findings imply that the TRS released is primarily derived from cladode hydrocolloids and hemicellulose. Soft acid hydrolysis affects the methylated derivatives of Opuntia mucilage, releasing sugars such as arabinose, xylose, galactose, and a wide variety of oligosaccharides, according to some authors. Other sugars, such as rhamnose and glucose, are produced under more severe hydrolysis conditions (McGarvie and Parolis, 1981). The absence of oligosaccharides and the type of sugars found indicated that the circumstances utilized allowed access to the crystalline matrix of the cellulose of Opuntia cladodes.

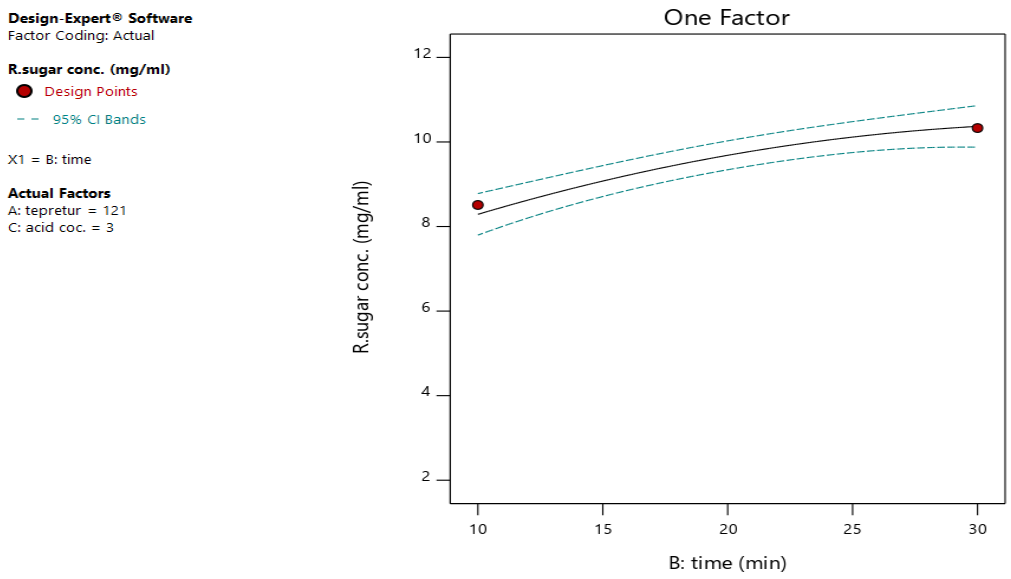


Figure4.3 Effects of time on reducing sugar concentration

Effects of acid concentration

The results of the treatments with various concentrations reveal that there is a relationship between the amount of acid employed and the release of TRS, with no more TRS being collected from the samples despite increased acid concentration. This could be due to acid hydrolysis of hemicellulose, proteins, lipids, and other biomolecules like polyphenols, leaving crystalline cellulose, which is difficult to hydrolyze (Wyman et al., 2005). Excessive exposure of lignocellulosic material to acid conditions, on the other hand, can increase carbohydrate decomposition by creating chemicals such as furfurals,which are harmful to fermentative processes (Chandel et al., 2007). Similar chemical hydrolysis procedures with barley straw have shown that reducing particle size significantly increases the process (Quintanar-Gómez et al., 2012) found that increasing the surface area makes more holocellulose accessible, which enhances TRS release

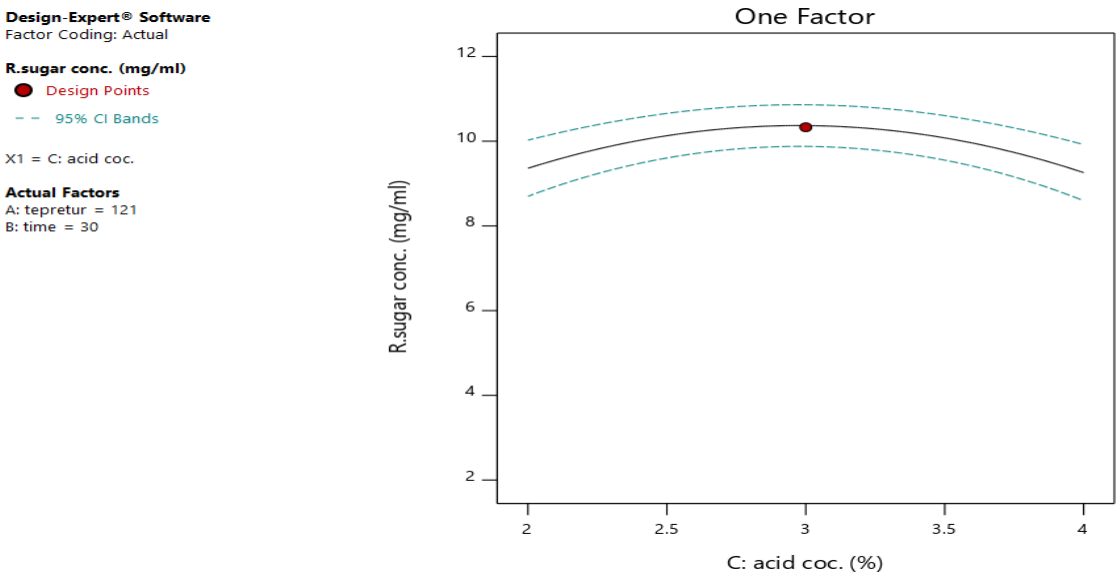


Figure4.4 Effects of acid concentration on reducing sugar concentration

4.2.3. Interaction Effect of independent Variables on reducing sugar concentration

RSM allows for the interaction of hydrolysis parameters that could have a big impact on the response variable. As a result, the three-dimensional response surface plot was used to investigate the combined impact of two variables on reducing sugar concentration while keeping the third variable constant. The process variables were discovered to have substantial positive interaction effects based on the ANOVA results and model equation. The interaction plots of temperature with time, temperature with acid concentration, and time with acid concentration on a 3-D response surface are shown from Figure 4-5 to Figure 4-7, and the counter plot of interaction appendix 5-7, respectively.

Interaction between temperature and time

The 3D plots, counter plot interaction in appendix 5 and 2D plot in apedex8 created as a function of temperature and time are shown in Figure 4.5, with the acid concentration kept at the center point to enhance sugar concentration. An increase in reduced sugar was noticed when the hydrolysis time and the temperature was raised. However, at lower temperatures and for longer periods of time, the reducing sugar levels decrease. This is observed when cellulose is exposed to high temperatures; the sugar obtained from it is degraded into non-fermentable products. (Etana, 2021)

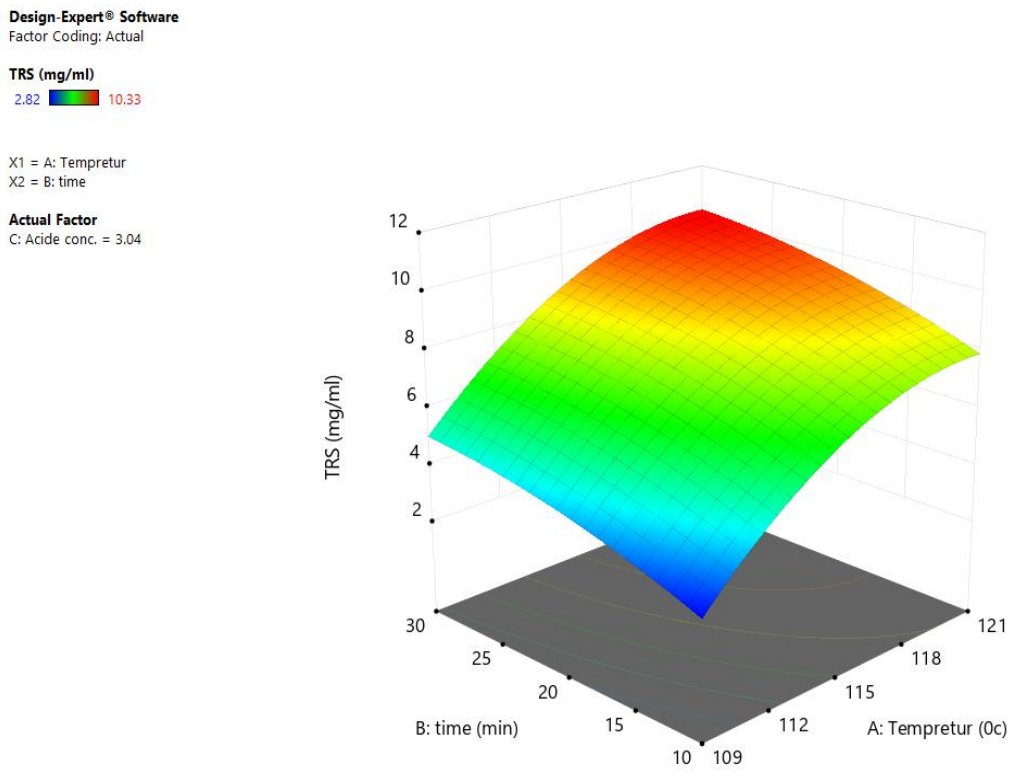


Figure4.5 3D plot of interaction between temperature and time

Interaction between temperature and acid concentration

On reducing sugar, the interaction impact of acid concentration and temperature over time was depicted (Figure.4. 6, Counter plot of interaction appendix 6 and 2D plot of interaction in appendix 9). The sugar output increases with the hydrolysis temperature and acid concentration up to a peak at a specific time. However, as the acid concentration was increased, the yield gradually decreased; this is because sugar degrades to the poisonous hydroxyl methyl-furfural (HMF), resulting in a reduction in glucose yield. (Etana; 2021)

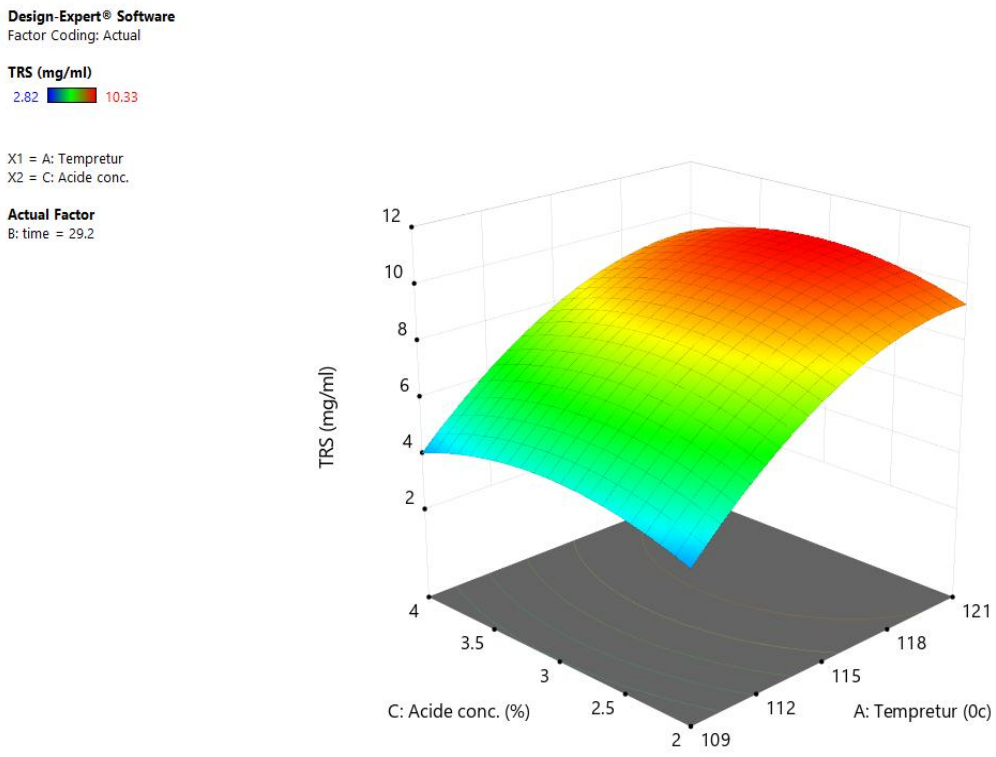


Figure4.6 3D plot of interaction between temperature and acid concentration

Interaction between time and acid concentration

Figure 4.7, the counter plot of interaction in appendix 7 and 2D plot of interaction in appendix 10 demonstrate the effect of acid concentration and hydrolysis time when the temperature was designated at around 120^0c. At high hydrolysis duration and around midway point acid concentration (3%), higher sugar yield was obtained. Since the possible creation of additional particles instead of glucose creation increases with rising acid content and time, the yield of reducing sugar increases. (Etana; 2021)

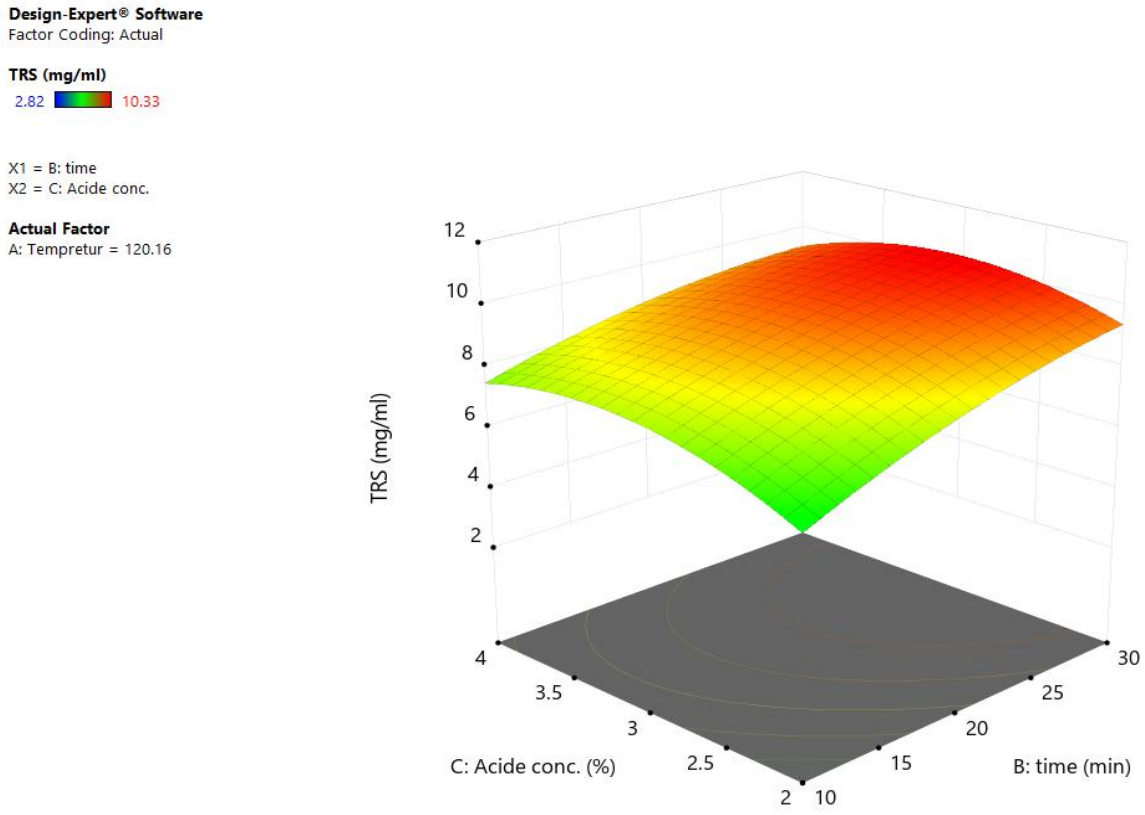


Figure4.7 3D plot of interaction between time and acid concentration

4.3. Hydrolysis optimization and validation of TRS Yield

The procedure was improved to maximize the release of the TRS based on the prior results. Temperature, duration, and the acid percentage of hydrolysis were chosen as variables for optimization and box behken design utilizing response surface methodology for this procedure (A. Texco-loper et al, 2018)

Finally, based on the response surface results, a box behken design was presented to corroborate the observed curvature and identify the best circumstances of TRS release, which was 10.33 ml/mg. The best conditions for TRS release were discovered with the acid at 3.04 percent, a hydrolysis period of 29.20 minutes, and a temperature of 120.16℃ at 0.93.

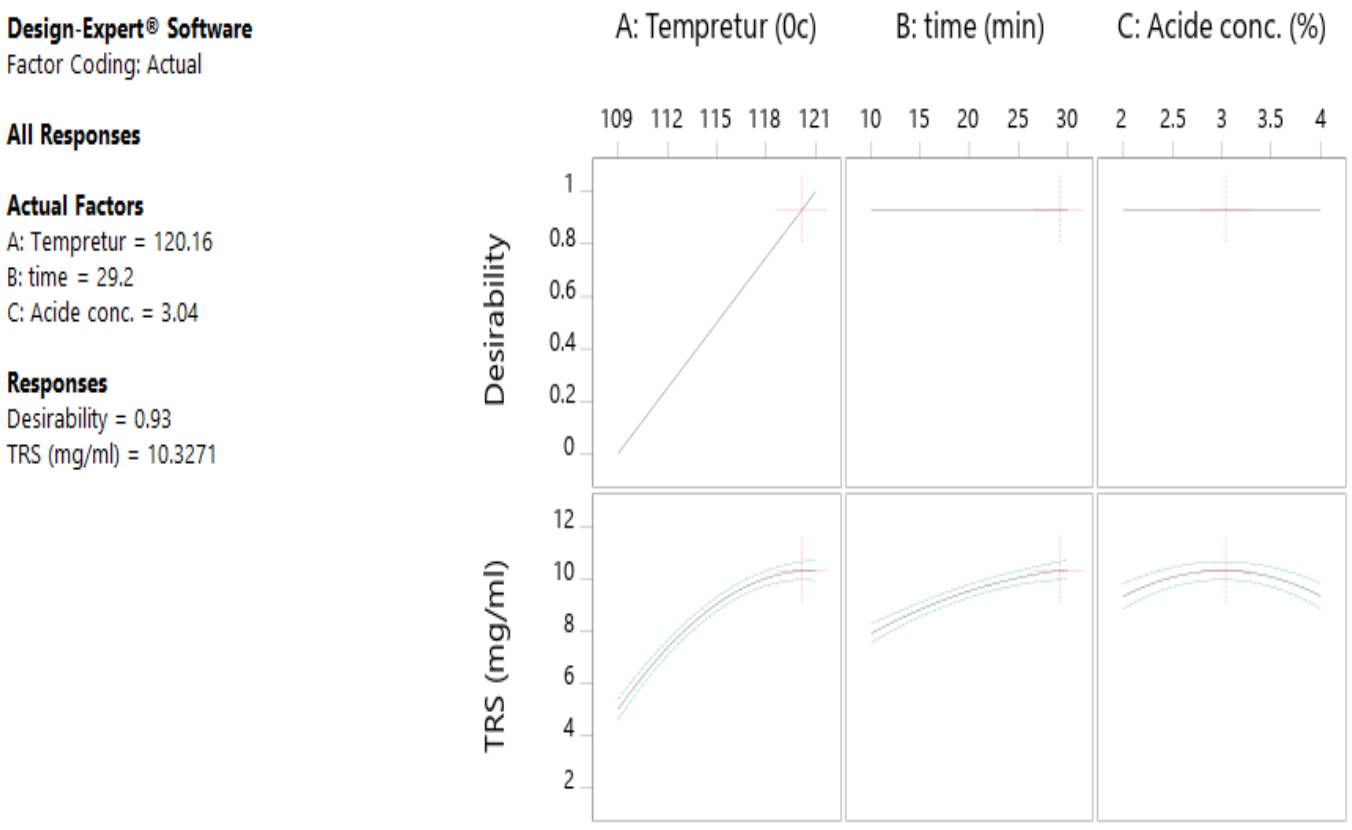


Figure4. 8 A, B.C, optimization of the response surface using the bbd model for the release of TRS when actual factor is acid concentration, time, and temperature respectively.

A triple hydrolysis was performed with the proportion of acid, time, and temperature specified by the model to test this given point and confirm the model acquired.

The TRS release was found to be 10.10 mg/ml, with a standard deviation of 0.023 mg/ml. It is confirmed that the theoretical and experimental data is reproducible at this value. Following this phase, it was noticed that when comparing the results of this study to those of others, it is critical to consider the S/l ratio, temperature, time, and acid proportion employed.

Because adjusting these numbers changes the quantity and thus the concentration of biomass and polysaccharides to be hydrolyzed, the findings are inconsistent. In the work of Kuloyo et al. 2014, hydrolysis of OFI cladodes yielded 12.6 mg mL of TRS with a S/l ratio of 3.36 (50 min, 120 °C, 1.5 percent H_2SO_4), implying working with cladodes with a high amount of biomass in a lower volume of work. Santos et al. 2016 created three TRS cultivars with S: l ratio of 13.33 under the following hydrolysis conditions: 9.18 mg/ml (O. ficus indica Var.Round palm), 12.87 mg/ml (Nopalea cochenilliferavar. palm) and 16.36 mg/ml (O. ficus indica Var. palm) (Ofivar. Giant palm).The results reveal that there is a difference between the types of varieties in terms of the sugars that can be obtained after 60 minutes at 121°C and 1 percent H_2SO_4, indicating that there is a difference between the sugars that can be obtained after 60 minutes at 121°C and 1 percent H_2SO_4.It should be noted that this could be independent of age, soil variables and harvesting time (Stintzing and Carle, 2005).According to the findings, the TRS obtained by SRM optimization in this study is a few differences in previous research, and the deference is due to variety of OFI and cladode to acid ratio and other edaphic factors.

So, modifying the variety of OFI and cladode flour to acid ratio could improve the quantity of TRS obtained by SRM optimization.

CONCLUSION

Cladode is used as a feedstock for the production of fermentable sugar in this study, and after acid pretreatment and acid hydrolysis, it contains 91.8 percent water, 27 percent extractives, 29 percent ash, 6.87 percent protein, 7.43 percent lignin, 11 percent hemicelluloses, and 18.7 percent cellulose. Using diluted acid, it also increased the surface responsiveness and hydrolysis of Opuntia ficus-indica cladodes. Seventeen experiments were conducted at temperatures ranging from 109°C to 121°C, with H_2SO_4 concentrations ranging from 2% to 4% and residence periods ranging from 10 to 30 minutes. The amount of reducing sugar produced determined the influence of the parameters, and the optimization was carried out using the Box-Behnken design process. The data shows that temperature, time, and acid concentration all had a significant impact on the reducing sugar concentration. Through the optimization of independent factors, the manufacture of monosaccharides from lignocelluloses biomass was successful. According to the current study, RSM with BBD provided a consistent and precise process for maximizing reduced sugar production from cladodes. Hydrolysis temperature, hydrolysis time, and acid concentration were independent variables that influenced individual reducing sugar concentrations and interacted with one another. Temperature, hydrolysis time, and acid concentration, in order of single effect, had the greatest influence on the production process. At low temperatures, concentrations, and durations, the yield of reduced sugar was decreased, whereas it was raised at high value parameters. The optimization produced 10.33 mg/ml of total reducing sugars with an acid concentration of 3.04 percent, a hydrolysis time of 29.2 minutes, and a temperature of 120.16°C (TRS) with a desirability of 0.93.

RECOMMENDATION

Other processes, including as pretreatment, fermentation, must be optimized. So, more research is required to optimize acid pretreatment and fermentation processes. Finally, the fermentable sugar produced is used to make bioethanol.

REFERENCE

A. Texco-lópez, a. Cadena-ramírez, j. Álvarez-cervantes, x. Tovar-jiménez, c.a., gómez aldapa(2018). Ptimización de la hidrólisis ácida de cladodios de opuntiaficus-indica por la metodología de superficie de respuesta

Amin, E. S., Awad, O. M., & El-Sayed, M. M.(1970). The mucilage ofOpuntia ficus indica Mill.Carbohydrate Research, 15, 159 –161

Amores I,et al.Electronic Journal of Energy & Environment. 2013;1: 25–36p.

Anderson, E.,Sands, L., & Sturgis, N. (1925). Some plant gums of the southwestern United States. American Journal of Pharmacology, 97,589– 592

AOAC (2005) Chapter 4: Animal Feed. In: Harwitz W, Latimer GW(eds) Official methods of analysis of AOAC international, 18thedn. AOAC International, Arlington, pp 12–21

Azzam, A. M. (1987). Saccharification of bagasse cellulose pretreated with ZnCl2 and HCl. Biomass 12, 71-77.

Balan, V., Bals, B., Chundawat, S. P., Marshall, D.& Dale, B. E. (2009). Lignocellulosic

Balat M (2011) Production of bioethanol from lignocellulosic materials via the biochemical pathway: a review. Energy Conver Manag 52:858–875 bioethanol production. Adv Biochem Eng Biotechnol 108, 41-65.Biological pretreatment of soft wood Pinus densiflora by three white-rot fungi. J Microbiol Biomass pretreatment using AFEX. Method Mol Biol 581, 61-77.

Bioresour Technol 96, 673-686; Chemistry, technology, and uses. Mol Nutr Food Res 49, 175-194.

Bridgwater, A. V., Meier, D.& Radlein, D. (1999).An overview of fast pyrolysis of biomass. Org Geochem 30, 1479-1493.

Cardona, C. A. & Sánchez, O. J. (2007). Fuel ethanol production: Process design trends and integration opportunities. Bioresour Technol 98, 2415-2457.

Chandel, A.K., Kapoor, R.K., Singh, A., Kuhad,R.C. (2007). Detoxification of sugarcanebagasse hydrolysate improves ethanolproduction by Candida shehatae NCIM 3501.Bioresource Technology 98, 1947-1950. doi:10.1016/j.biortech.2006.07.047.

Chang, K.C., Dhurandhar, N., You, X., Miyamoto,A. (1994). Cultivar/location and processingmethods a ect yield and quality of sunflowerpectin. Journal of Food Science 59, 602-605. doi11.1111/j.1365-2621.1994.tb05572.x

Chen ,Y., Sharma-Shivappa, R. & Chen, C. (2007). Ensiling agricultural residues for bioethanol production. Appl Biochem Biotechnol 143, 80-92.

Como orraje." Estudio FAO Productión y Protectión Vegetal nº169. Rome.

Cordeiro, D., Gonzaga, S. (2001) "Opuntia como forraje en el oeste semiárido de Brasil". In "El nopal

Daya Ram Nhuchhen1, Prabir Basu1 And And Bishnu Acharya2. (2014). A Comprehensive Review On Biomass Torrefaction. International Journal Of Renewable Energy & Biofuels .

Demirbas A, Demirbas MF (2011) Importance of algae oil as a source of biodiesel. Energy Conver Manag 52:163–170

Dharma et al.,(2016).Optimization of biodiesel production process for mixed *Jatropha curcas–Ceiba pentandra* biodiesel using response surface methodology

Dias, Marina, Fabian Diaz Mateus,Maria Regina Maciel, Rubens Maciel Filho, and Carlos Rossell. Anhydrous Bioethanol Production in One Side Stream Distillation Column Using Bioglycerol as Solvent. State University of Campinas.

Etana,BennyMalengier1,YasinAhmed,Hundessa Daba Nemomssa and Lieva Van Langenhove1 (2021).Statistical Modeling and Optimization of Reducing Sugar Production for Enzymatic Digestibility of Bamboo Grass by Box-Behnken Design Methodology.

F. Sánchez(1), M.D. Curt(1), J. Fernández(1), J.M. Agüera(2), M. Uceda(2), G.Zaragoza(2). (2009). Bioethanol production from prickly pear (opuntia ficus-indica (l) mill.) Cladodes Hamburg, Germany

F. Sánchez(1), M.D. Curt(1), M. Barreiro (1), M. Uceda(2), G. Zaragoza(2), J.M. Agüera(2), J.Fernández (1).(2012). Variability of carbohydrate content in prickly-pear (opuntia ficus-indica (l.) mill.) cladodes Milan, Italy

F. Sánchez, M.D. Curt, Fernández, J., J.M. Agüera, M. Uceda, G. Zaragoza . Bioethanol Production from prickly pear (Opuntia ficus-indica(L) Mill.) cladodes. Proceedings of the 17thEuropean Biomass Conference & Exhibition, 29 June – 3 July 2009, Hamburg (Germany).

G.C.Galletti.(1991).Production And Utilization Of Lignocellulosics: Plant Refinery And Breeding ... British.

Galbe, M. & Zacchi, G. (2007). Pretreatment of lignocellulosic materials for efficient

Gamez S, et al. Journal of Food Energy.2006; 74: 78–88p.

Garcia AMP, et al. Appl Biochem Biotechnol. 2006; 129(1–3): 278–288p.

Ginestra, G., Parker, M.L., Bennett, R.N., JimRobertson, J., Mandalari, G., Narbad, A.,Lo-Curto, R. B., Bisignano, G., Faulds, C.B., Waldron, K. W. (2009). Anatomical,chemical, and biochemical characterization ofcladodes from prickly pear [Opuntia ficusindica (L.) Mill.]. Journal of Agriculturaland Food Chemistry 57, 10323-10330. doi:10.1021/jf9022096.

Guar,Kadambini. "Process optimization for the Production of Ethanol via Fermentation." Diss. Thaper Institute of Engineering and Technology, 2006.

Habibi,Y.,Heyraud,A.,Mahrouz, M.,Vignon, M.R. (2004).Structural featuresof pectin polysaccharides from the skinof Opuntia ficus-indica prickly pear fruits.Carbohydrate Research 339, 1119-1127. doi:10.1016/j.carres.2004.02.005.

Hahn-Ha¨gerdal B, Galbe M, Gorwa-Grauslund MF, Lide´n G, ZacchiG (2006) Bio-ethanol - the fuel of tomorrow from the residues oftoday. Trends Biotechnol 24:549–556. doi:10.1016/j.tibtech.2006.10.004

Hamelinck, C. N., van Hooijdonk, G. & Faaij, A. P. C. (2005). Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long term. Biomass Bioenerg 28, 384-410

Harlay,V.(1902).Sur le mucilage du cactus a` raquettes (Opuntia vulgaris Mill.) Journal of Pharmaceutical Chimica,16 (6), 193 –198.

Hendriks ATWM, Zeeman G. Bioresource Technol. 2009; 100: 10–18p

Hernandez SJM, et al. Bioresource Research & Reviews: Journal of Agricultural Science and TechnologyVolume 5, Issue 2

Hsu, T. A. (1996**)**. Pretreatment of Biomass. In Handbook on bioethanol: production and utilization, p. 179.Edited by C. E. Wyman. Washington, DC: Taylor & Francis. Indigenous biomass

Iñaki Gandarias And Pedro Luis Arias. (2013). Hydrotreating Catalytic Processes For Oxygen Removal In The Upgrading Of Bio-Oils And Bio-Chemicals.

Inglese P, Basile F, Schirra M (2002) Cactus pear fruit production. In: Nobel PS (ed) Cacti: Biology and Uses. University of CaliforniaPress, Berkeley, pp 163–184

J. Arditti, A. Dunn. Experimental plant physiology: Experiments in cellular and plant physiology.(1969). Winstonhttp://www.cori.unicamp.br/centenario2008/2007/compl

Kankia. (2014). Characterization And Fermentation Of Bamboo Leaf Litter Biomass For Ethanol Production. Punjab, India: Thapar University.

Kankia. (2014). Characterization And Fermentation Of Bamboo Leaf Litter Biomass For Ethanol Production. Punjab, India: Thapar University

Krishna,S. H, T. J. Reddy, and G. V. Chowdary. "Simultaneous saccharification and fermentation of lignocellulosic wastes to ethanol using a thermotolerant yeast." Diss.Andhra University, 2000.

Kuloyo OO (2012) Ethanol production by yeast fermentation of anOpuntia ficus-indica biomass hydrolysate. Dissertation, University of the Free Stat

Kuloyo, O.O., du Preez, J.C., García, A.M del P.,Kilian, S.G., Steyn, L., Görgens, J. (2014).Opuntia ficus-indica cladodes as feedstockfor ethanol production by Kluyveromycesmarxianus and Saccharomyces cerevisiae. World Journal Microbiology and Biotechnology30, 3173-3183. doi: 10.1007/s11274-01417456

Kumar P, et al. Indian Engineering Chemistry Resource.2009; 48: 3713–3729p.

Kumar, P.,Barrett, D. M., Delwiche, M. J. & Stroeve, P. (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind Eng Chem Res 48, 3713-3729.

Le Houerou, H,(1996) " The role of cacti (Opuntia.spp.) in erosion control, land reclamation, rehabilitation and agricultural development in the Mediterranean Basin" Journal of Arid Environments. 33(2), 135-139.

Lee et al., (2011).A measure of glucocorticoid load provided by DNA methylation of Fkbp5 in mice

Lee, J. W., Gwak, K. S., Park, J. Y., Choi, D. H., Kwon, M. & Choi., I.-G. (2007).

Limayem A, Ricke SC (2012) Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects. Prog Energy Combust Sci 38:449–467

López, J.J., Fuentes, J.M., and Rodriguez, A. (2001) "Producción y uso de Opuntia como forraje en el centro-norte de Mexico". In"El nopal (Opuntia ssp.)

Lu XB, et al. Chem Eng Technol. 2007; 30: 938–944p.

M. Da Silva Vilela, M. De Andrade Ferreira, M .De Azevedo, E. Cristina Modesto, I. Farias, A. Vasconcelos Guimarães, S. Valenc Bispo. Effect of processing and feeding strategy of the spineless cactus (Opuntia fícus-indicaMill.) for lactating cows: Ingestive behaviour. Applied Animal Behaviour Science 125 (2010)

Malainine, M.E., Dufresne, A., Dupeyre, D.,Mahrouz, M., Vuong, R., Vignon, M.R. (2003).Structure and morphology of cladodes andspines of Opuntia ficus-indica. Celluloseextraction and characterisation. CarbohydratePolymers 51, 77-83. doi: 10.1016/S0144-8617(02)00157-1.

Marquini, M.F.,D.C. Mariani, C.M.G. Andrade, Antonio J.A. Meirelles, O.A.A. Santos, and L.M.M. Jorge. Simulation and Analysis of an Industrial Ethanol Distillation Columns System Using the Hysys Simulator. n.d. n.d.

Martin C, et al. Enzyme Microb Technol. 2007; 40: 426–432p.

McGarvie, D., Parolis, H. (1981). The acid labile,peripherical chains of the mucilage of Opuntia ficus-indica. Carbohydrate Research 94, 57-65.doi: 10.1016/S0008-6215(00)85595-0.

Mciteka(2008).ermentation characteristics and nutritional Value of opuntia ficus-indica var. Fusicaulis Cladode silage

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31, 426-428. doi:10.1021/ac60147a030

Miyamoto,Kazuhisa.Renewable Biological Systems for Alternative Sustainable Energy Production. 1997. Food and Agriculture Organization of the United Nations.

Mosier, N.,Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M. & Ladisch, M**. (2005)**

N. Retamal, J.M. Durán, J. Fernández. Ethanol Production by Fermentation of Fruits and Cladodes of PricklyPear Cactus (Opuntia ficus – indica(L.) Miller). *Journal of Science Food Agriculture* 40 (1987), pag. 213 – 218.

National Renewable Energy Laboratory(2012) Standard BiomassAnalytical Procedures. www.nrel.gov/biomass/analytical_procedures.htmlAccessed 25 Sept 2013

Parikh,V. M., & Jones, J. K. N. (1965), Structure of Cholla Gum (Opuntiafulgida).Journal of Polymer Science, IIc, 139–148.

Parikh,V. M., & Jones, J. K. N. (1966). Cholla Gum: I. Structure of thedegraded cholla gum. Canadian Journal of Chemistry, 44, 327–333.

Prasad,S., Singh,A. & Joshi, H. C.(2007). Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resour Conserv Recy 50, 1-39.

Qing, Q.,Yang, B. & Wyman, C. E. (2010). Impact of surfactants on pretreatment of corn stover. Bioresour Technol 101, 5941-5951.

Quintanar-Gómez, S., Arana-Cuenca, A.,Mercado-Flores, Y., Gracida-Rodríguez,J.N., Téllez-Jurado, A. (2012). E ect of particle size and aeration on the biological delignification or corn strawusing Trametes sp. Bioresources 7,32734 http://stargate.cnr.ncsu.edu/index.php/BioRes/article/view/BioRes_07_1_0327_QuintanG_CFR_Particle_Size_Aeration_Bio_Delignification/1309.

Quintero-Ramirez,Rodolfo.Hydrolysis of lignocellulosic biomass. 2008. Autonomous Metropolitan University Cuajimalpa Unit.

R. Sindhu, M. Kuttiraja, P. Binod, R. K. Sukumaran, and A. Pandey (2014) Bioethanol production from dilute acid pretreated Indian bamboo variety (Dendrocalamus sp.)By separate hydrolysis and fermentation. Ind Crops Prod 52: 169-176

Ribeiro E.M.O., Silva N.H., Lima F.J.L., Brito J.Z.,Silva M.PC. (2010). Study of carbohydratespresent in the cladodes of Opuntia ficus-indica (fodder palm), according to age and season.Ciência eTecnología de Alimentos 30, 933-939.doi: 10.1590/S0101-20612010000400015.

Rideg,Thomas and Michael Smith. "Brazil's Ethanol: Big Potential."Latin Business Chronical14May2007.http://www.latinbusinesschronicle.com/app/article.aspx.

Rossell, Carlos Eduardo Vaz. "Lignocelluloses hydrolysis." Ciencia e Tecnologia, CGEE, NIPE, and UNICAMP, Brazil.

Russell C, Felker P (1987) The prickly-pears (Opuntia spp.,Cactaceae): a source of human and animal food in semiaridregions. Econ Bot 41:433–445 Inglese P, Basile F, Schirra M (2002) Cactus pear fruit production. In:Nobel PS (ed) Cacti: Biology and Uses. University of CaliforniaPress, Berkeley, pp 163–184

Sáenz, C et al. Arias, E (Coord). (2006) "Utilización Agroindustrial del Nopal". Estudio FAO Producción y Protección Vegetal nº 162. Rome

Sáenz, C. (2000) "Processing technologies: an alternative for cactus pear (Opuntia spp.) fruits and cladodes". Journal of Arid Environments 46: 209–225

Sánchez, O. J. & Cardona, C. A. (2008).Trends in biotechnological production of fuel Ethanol from different feed stocks. Bioresour Technol 99, 5270-5295.

Santos, T.N., Dutra, E.M., do Prado, A.G.,Leite, F.C.B., de Souza, R.F.R., dos Santos,D.C., de Abreu, C.A.M., Simoes, D.A.,de Morais, M.A., Menezes, R.S.C. (2016).Potential for biofuels from the biomass of prickly pear cladodes:Challenges for bioethanol and biogas production in dry areas.Biomass and Bioenergy 85, 215-222. doi:10.1016/j.biombioe.2015.12005.

Sarkar N, Ghosh SK, Bannerjee S, Aikat K (2012) Bioethanol production from agricultural wastes: an overview. Renew Energy 37:19–27

Sendelius, Johan. Steam Pretreatment Optimisation for Sugarcane Bagasse in Bioethanol Production. 2005. Lund Institute of Technology.

Serafim and Igly,. "Brazil: Brazilian Biofuels Industry." USA Department of Commerce Sept. 2006.

Stintzing, F.C., and Carle, R. (2005) "Cactus stems (Opuntia spp.): A review on their chemistry, technology, and uses" Molecular Nutrition Food Research. 49, 175 – 194.

Sun FB, Chen HZ. J Chem Technol Biotechnol.2007; 82:1039–1044p.

Sun Y,Cheng J. Bioresource.Technol. 2002; 83: 1–11p.

Sun,Y. & Cheng, J. (2002**)**. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83, 1-11.

Sun,Y. & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanolproduction: a review. Bioresour Technol 83, 1-11.

Taherzadeh, M. J. and Karimi, K. (2008).Pretreatment of lignocellulosic wastes toImprove ethanol and biogas production:A review. International Journal ofMolecular Sciences 9, 1621-1651. doi:10.3390/ijms9091162

Van Zyl WH, Chimphango AFA (2011) Next-generation cellulosic ethanol technologies and their contribution to a sustainable Africa. Interface Focus 1:196–211

Vijayanand c.*, kamaraj s., karthikeyan s. And sriramajayam s. October 24, 2016. Characterization of biomass

Wang,Nam Sun. Cellulose Degradation. 2007. University of Maryland.

Wyman, C.E., Decker, S.R., Brady, J.W., Viikari,L., Himmel, M.E. (2005). Hydrolysisof cellulose and hemicellulose. In:Polysaccharides. Ed. Dumitriu S. CRC Press.doi: 10.1201/9781420030822.ch.43.

X. Xiao, J. Bian, M. Li, H. Xu, B. Xiao, and R. Sun (2014) Enhanced enzymatic hydrolysis of bamboo (Dendrocalamus giganteus Munro) culm by hydrothermal pretreatment. Bioresour Technol 159: 41-47

Xiao, W. & Clarkson, W. W. (1997**).**Acid solubilization of lignin and bioconversion of treated newsprint to methane. Biodegradation 8, 61-66.

Yang et al (2015). Biomass characterization of AgaveandOpuntiaas potential biofuel feedstocks

Zehra Sapci, M. M. (2014). Effect of Different Thermal Pretreatment Techniques on Biogas Production from Lignocellulosic Biomass: Wheat Straw and Salix. Norwegian Institute for Agricultural and Environmental Research, 1-6.

Zhao X, et al. Review Article.bioresource.com. 2010; 5(3): 1565–1583p.

Zheng Y, Pan Z (2009) .Overview of biomass pretreatment for cellulosic ethanol production.In t J Agric Biol Eng 2:51–68

APPENDIX

Appendix1-The amount of reducing sugar released in the pretreatment stage at optimum point

Rune	Acid concentration (%)	Temperature(0c)	Time (mint)	Reducing sugar concentration (mg/ml)
1	1.5	120	50	3.91

Appendix 2 the effect of hydrolysis temperature, time and acid concentration on reducing sugar concentration

Rune	Acid concentration (%)	Temperature(0c)	Time (mint)	Reducing sugar concentration (mg/ml)
1	4	121	30	8.71
2	4	115	10	6.5
3	4	109	20	6.3
4	4	121	20	8.65
5	4	121	10	7.5
6	4	115	30	7.65
7	4	115	20	6.4
8	4	109	30	6.42
9	4	109	10	4.32
10	3	121	30	10.33

11	3	115	10	6.72
12	3	109	20	6.51
13	3	121	20	8.71
14	3	121	10	7.60
15	3	115	30	7.70
16	3	115	20	7.3
17	3	109	20	6.5
18	3	109	10	5.2
19	2	121	30	8.4
20	2	115	10	6.42
21	2	109	20	6.2
22	2	121	20	7.9
23	2	121	10	7.6
24	2	115	30	7.5
25	2	115	20	7.12
26	2	109	30	5.30
27	2	109	10	2.82

Appendix 3 Model Fit Summary

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2
Linear	< 0.0001		0.7863	0.7319
2FI	0.9544		0.7308	0.5320

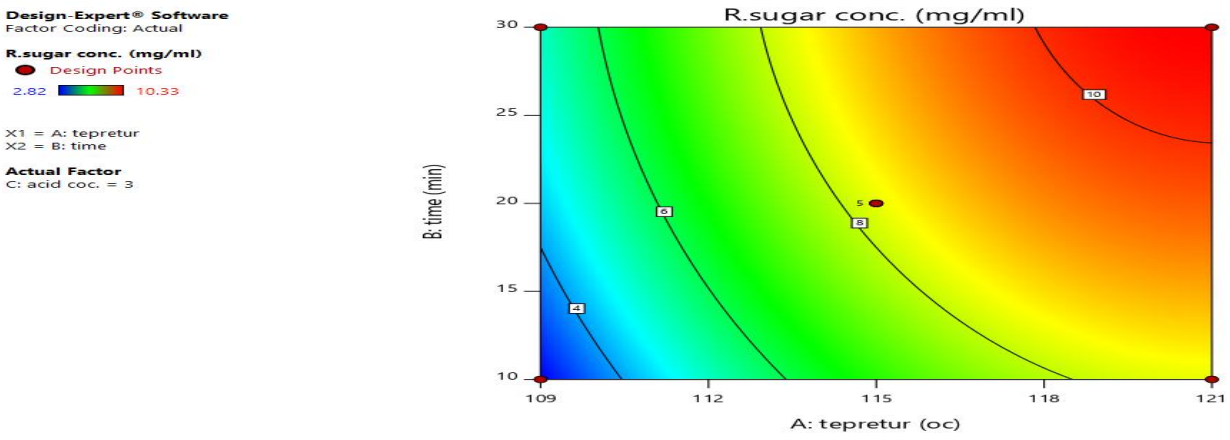
Quadratic	< 0.0001	0.9888	0.9217	Suggested
Cubic		1.0000		Aliased

Appendix.4-ANOVA for Quadratic model for reducing sugar determination

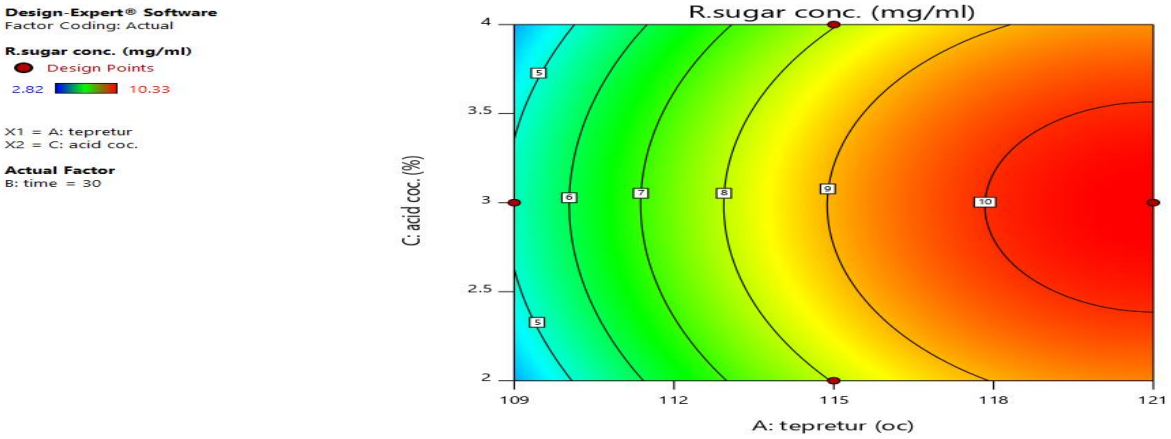
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	82.07	9	9.12	158.23	< 0.0001	Significant
A-tepretur	57.62	1	57.62	999.79	< 0.0001	**
B-time	9.86	1	9.86	171.03	< 0.0001	**
C-acid coc	0.6786	1	0.6786	11.77	0.0110	*
AB	0.0196	1	0.0196	0.3401	0.0481	*
AC	0.0012	1	0.0012	0.0213	0.0382	*
BC	0.4225	1	0.4225	7.33	0.0303	*
A^2	7.10	1	7.10	123.23	< 0.0001	**
B^2	0.5344	1	0.5344	9.27	0.0187	*
C^2	4.72	1	4.72	81.90	< 0.0001	**
Residual	0.4034	7	0.0576			
Lack of Fit	0.4034	3	0.1345	2.73	0.1782	Not significant
Pure Error	0.0000	4	0.0000			
Cor Total	82.47	16				
Std. Dev.	0.2036	R^2	0.9951			
Mean	7.02	Adjusted R^2	0.9888			
C.V. %	3.42	Predicted R^2	0.9217			
		Adeq Precision	41.2089			

**very significant P < 0.01, *significant P≤ 0.05, and Insignificant when P> 0.05

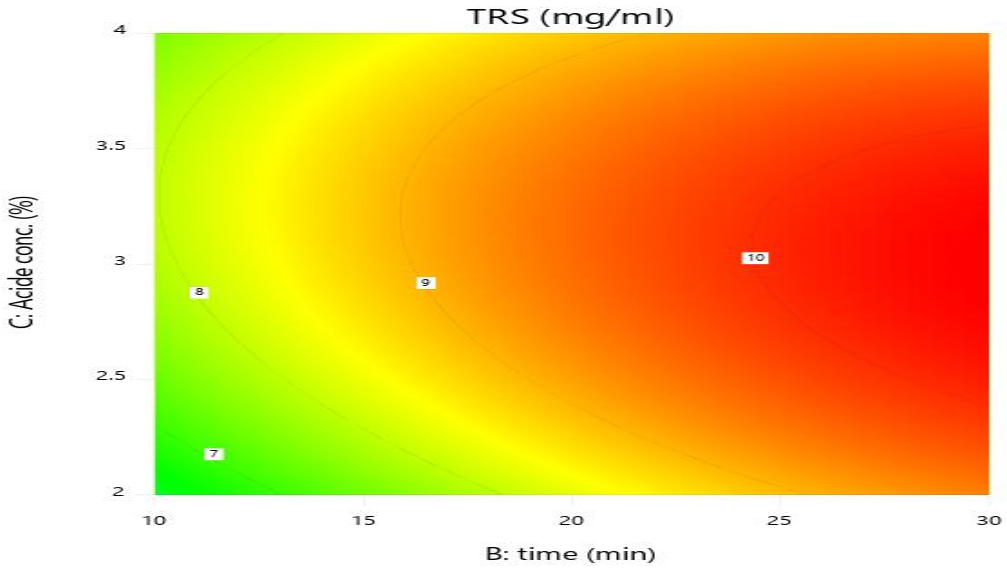
Appendix5 -Counter plot of interaction between temperature and time



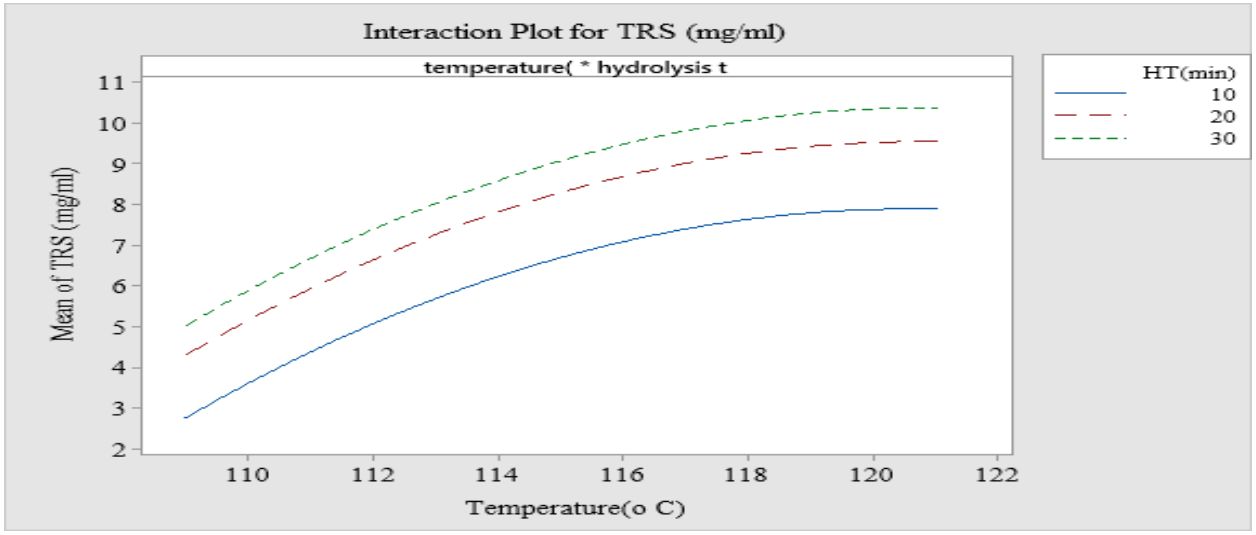
Appedix6- Counter plot of interaction between temperature and acid concentration



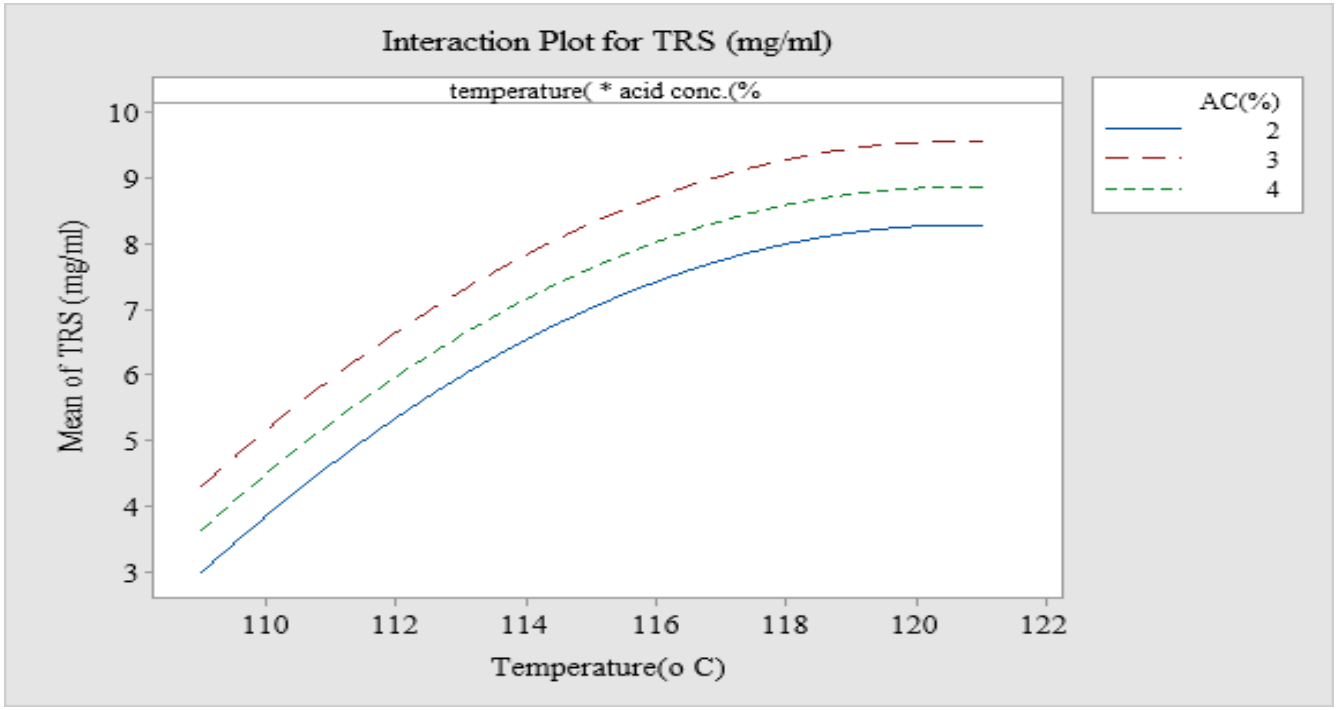
Appendix7 -Counter plot of interaction between time and acid concentration



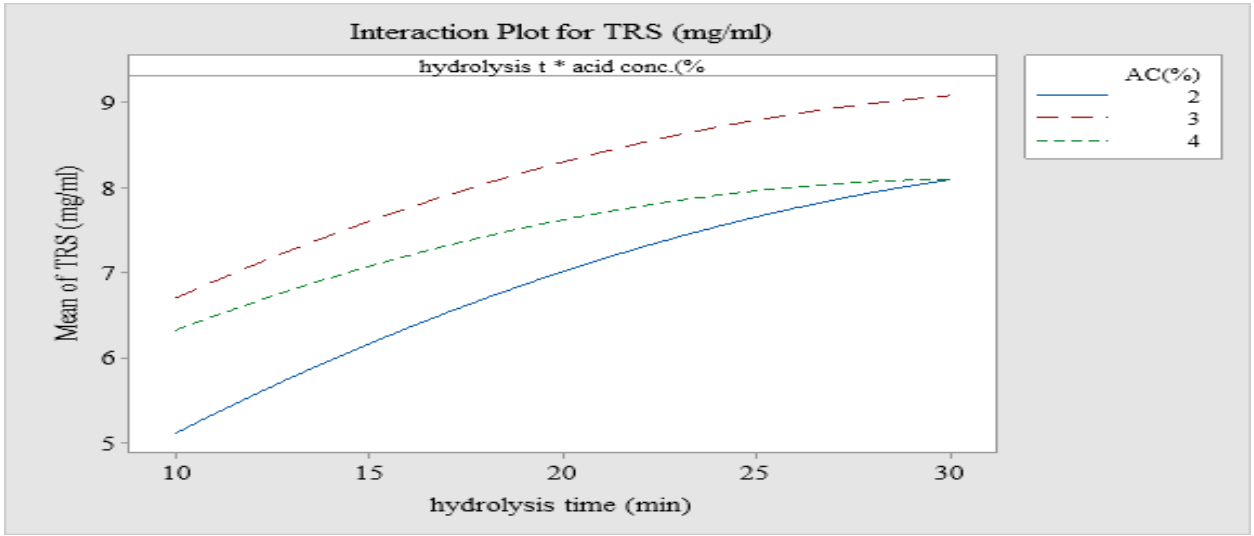
Appendix8 -2D plot of interaction between temperature and time



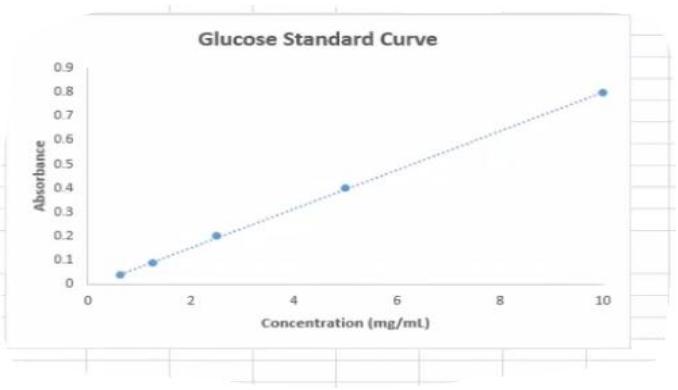
Appedix9-2D plot of interaction between temperature and acid concentration



Appendix10 -2D plot of interaction between time and acid concentration



Appendix 11- Standard curve for reducing sugar estimation by DNSA Method

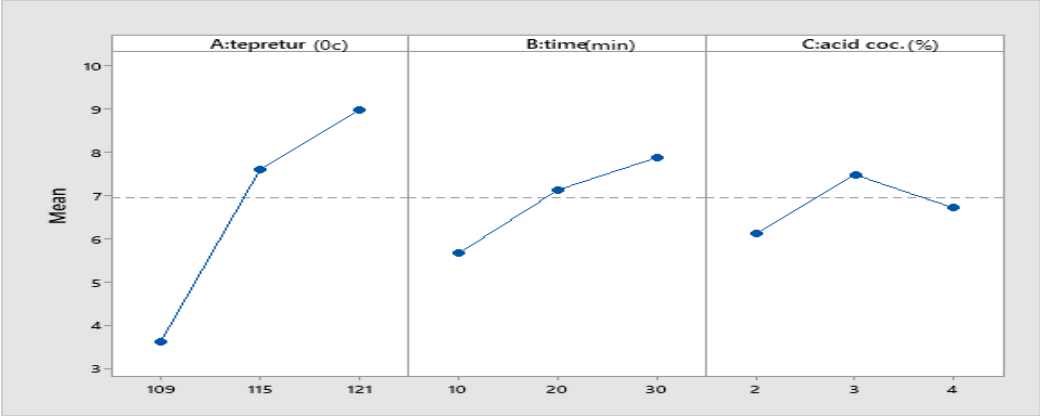


Appendix 12-esteam pressure and temperature relation in autoclave

Run	1	2	3	4	5
Pressure(bar)	5	10	15	20	25
Temperature(oc)	109	115	121	126	130

Appendix 13-Main Effects Plot for TRS by Minitab software

Main effects of temperature, time and acid concentration on TRS by Minitab software



Appendix 14-Bar graphs (chart of) TRS Concentration